MODULATION OF A 5-HYDROXYTRYPTAMINE-RELATED BEHAVIOUR BY NORADRENALINE AND GABA

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Dedicated to My Parents

without whom this work could not have been undertaken.

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Summary

The modulation of 5-hydroxytryptamine (5-HT) mediated head-twitch behaviour by drugs acting at beta-adrenoceptors and at receptor sites related to gamma-aminobutyric acid (GABA) was examined in mice and rats.

Both $beta_1$- and $beta_2$-adrenoceptor agonists potentiated the head-twitching induced by a 5-HT precursor in mice and quipazine in rats. The pattern of selective antagonist potency against this effect in mice and the effect of neurotoxin lesions of locus coeruleus (LC) in rats indicated that the relevant $beta_1$-adrenoceptors are probably located postsynaptically and the $beta_2$-adrenoceptors presynaptically. The potentiating potencies of beta-adrenoceptor agonists on head-twitch response were considerably reduced after 48h withdrawal from chronic but not acute pretreatment of mice with two antidepressants, desmethylimipraimine and iprindole.

Results with drugs acting at the GABA and related receptors indicated that $GABA_A$ receptors are facilitatory to head-twitch and $GABA_B$ receptors are inhibitory and both effects appear to depend on the presence of functional noradrenergic systems probably originating from the LC.

The relationship between these effects and their implications for understanding the pharmacology of centrally acting drugs is discussed.

Key Words

Head-twitch, beta-adrenoceptors, antidepressants, GABA, locus coeruleus.

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1. General

The 5-hydroxytryptamine (5-HT) mediated behaviours in rodents are studied because they allow the examination of the effect of drugs on the function of 5-HT and the interaction of various other neurotransmitters with this monoamine in living animals. Drugs may alter the biochemistry of 5-HT (for example, its metabolism and receptor number) as measured by *in vitro* techniques, without necessarily having any functional consequences in the whole animal. The 5-HT behavioural models allow examination of whether or not these changes have modified the response to 5-HT *in vivo*.

Head-twitching in mice and wet-dog shake (WDS) behaviour in rats are induced by a wide variety of drugs, the vast majority of which are hallucinogens and, furthermore, antagonists of this behaviour are often antihallucinogenic (Corne and Pickering, 1967; Bednarczyk and Vetulani, 1978). Head-shaking behaviour has therefore, been proposed as an index of potential hallucinatory drug effects.

The work reported in this thesis examined the effect of drugs which modulate 5-HT mediated head-twitch response in mice and WDS in rats by altering noradrenaline and gamma-aminobutyric acid (GABA) neurotransmission. In an attempt to understand how drugs acting at beta-adrenoceptors, and GABA and related receptors, can modify the 5-HT-mediated behaviour, the biochemistry of these and of 5-HT receptors is discussed. Their

presence in various regions of the brain and their anatomical localisation are also considered, in order to understand the mode of action of drugs acting at these receptors. In the later stages of this Introduction the pharmacology of 5-HT mediated head-shaking behaviour is discussed and is compared with that of the 5-HT hyperactivity syndrome.

2. The anatomical distribution of 5-HT in the central nervous system

Significant amounts of 5-HT have been found in the mammalian brain (Twarog and Page, 1953), especially in the mesencephalon (midbrain) and diencephalon (hypothalamus and thalamus), the highest concentration being found in the hypothalamus and the caudate nucleus, the lowest in the cerebellum (Amin et al., 1954; Bertler and Rosengren, 1959).

The advent of fluorescence histochemistry (Falck et al., 1962) enabled mapping of 5-HT containing neurones. A recent introduction of several other techniques has led to a more detailed visualization of the fine structure and organization of 5-HT neurones in the central nervous system (CNS; see Steinbusch, 1981; Steinbusch and Nieuwenhuys, 1983). The 5-HT containing neurones are largely confined to the nine defined raphe nuclei lying in or near the midline of the pons and upper brain stem.

The more caudal B_1-B_3 groups project desending pathways to the medulla and spinal cord. The more rostral 5-HT cell groups B_7-B_9

(raphe dorsalis, raphe medianus and centralis superior) provide innervation of the striatum, mesolimbic forebrain, cortex, hippocampus, thalamus and hypothalamus.

3. 5-HT receptors

3.1 Binding studies

On the basis of binding studies, the sites for 5-HT were sub-classified into 5-HT_1 and 5-HT_2. The 5-HT_1 sites were characterised by the use of [^{3}H]-5-HT as the ligand, with non-specific binding being measured by displacement with 5-HT; and 5-HT_2 characterised by use of [^{3}H]-spiperone (a neuroleptic used in dopamine receptor studies) as the ligand and LSD as the displacing drug for the measurement of non-specific binding (Peroutka and Snyder, 1979).

Evidence suggests that both 5-HT_1 (Pedigo et al., 1981; Monroe and Smith, 1983) and 5-HT_2 (Kendall and Nahorski, 1983) binding sites are heterogeneous. Autoradiographic techniques revealed three subpopulations of 5-HT_1 sites characterised on their anatomical localisation and drug sensitivity. These three sites have been referred to as 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} (Cortes et al., 1984). 5-HT_{1A} sites were concentrated in the hippocampus, cerebral cortex and dorsal raphe. These sites were labelled with nanomolar concentrations of [^{3}H]-5-HT, of [^{3}H]-LSD and of [^{3}H]-8-OH-DPAT (8-hydroxy-2-[di-n-propylamino]tetralin) (Hjorth et al., 1982). The 5-HT_{1B} sites were labelled by [^{3}H]-5-HT and highly concentrated in globus pallidus, subiculum and substantia nigra

and were sensitive to nanomolar concentrations of the agonist RU 24969 (5-methoxy-3-[1,2,3,6,-tetrahydropyridin-4-yl] 1H-indole) (Euvrard and Boissier, 1980) and some beta-adrenergic blockers (Middlemiss et al., 1977). The 5-HT_{1C} sites were extremely enriched in the choroid plexus of the brain ventricles and labelled with nanomolar affinities by $[^3H]$-mesulergine, $[^3H]$-5-HT and $[^3H]$-LSD. However, RU 24969 and 8-OH-DPAT, the beta-adrenergic blockers and 5-HT_2 receptor blockers such as ketanserin and pirenperone had very low affinities at these sites (Cortes et al., 1984).

3.2 5-HT autoreceptor

The 5-HT_1 sites have been suggested to control the release of 5-HT and catecholamines (Martin and Sanders-Bush, 1982a; 1982b; Engel et al., 1983; Ennis et al., 1981). The data to support the autoreceptor function of 5-HT_1 sites was provided by the observed correlation between the binding affinities of 5-HT agonists for 5-HT_1 sites and their potencies to inhibit potassium stimulated release of $[^3H]$-5-HT from hypothalamic or cortical tissues (Martin and Sanders-Bush, 1982a; 1982b; Engel et al., 1983). However, there are some discrepancies, for example, the relative binding affinity of 5-HT antagonists did not correspond to their potency in the 5-HT release test and furthermore $[^3H]$-5-HT binding is not altered by neuronal lesions of 5-HT or catecholamine neurones. It is assumed that the amount of binding sites may be very small and undetectable by binding studies. A recent study using $[^3H]$-8-OH-DPAT has demonstrated that this

ligand bound to presynaptic sites in the striatum but to postsynaptic sites in hippocampus and brain stem (Gozlan et al., 1983). The presynaptic location and pharmacological characteristics of striatal [^{3}H]-8-OH-DPAT binding led Gozlan et al., (1983) to suggest that this site represents the 5-HT autoreceptor in this brain region. However, this was questioned by Middlemiss (1985), on the grounds that 5-methoxytryptamine blocked the release of preloaded [^{3}H]-5-HT in rat brain slices prepared from striatum but it failed to displace [^{3}H]-8-OH-DPAT binding in this brain region. The 5-HT_{1b} agonist RU 24969 decreases 5-HT release in frontal cortex both *in vitro* (Middlemiss, 1984) and *in vivo* (Brazell et al., 1985), probably via 5-HT_1 receptors located on the nerve terminals in the suprachiasmatic nucleus rather than on the cell bodies located in the dorsal raphe (Marsden and Martin, 1985). It is possible that different types of 5-HT autoreceptors exist in the different regions of CNS.

3.3 Electrophysiologically-characterised 5-HT receptors

Based on the neuronal resonses to 5-HT, determined by unit recording, Aghajanian (1981) proposed the existence of three distinct 5-HT receptors, which he designated S_1, S_2 and S_3.

3.4 S_1

This receptor was characterised from unit recording of facial motoneurones (McCall and Aghajanian, 1979; 1980) and of spinal motoneurones (White and Neuman, 1980). Its activation by 5-HT

and 5-HT agonists facilitates the depolarising action of excitatory amino acids. This appears to be the physiological role of this receptor, since the activation of facial motoneurones produced by stimulation of the motor cortex is potentiated by microiontophoretic application of 5-HT (McCall and Aghajanian, 1979). The facilitatory actions of 5-HT are blocked by small doses of classical 5-HT antagonists, such as, cyproheptadine, methysergide and metergoline.

3.5 S_2.

The firing activity of 5-HT neurones is influenced by local availability of 5-HT (Aghajanian, 1978). This appears to be mediated by the autoreceptor denoted S_2 (Aghajanian, 1981). The action of these autoreceptors results in a reduction of the firing activity of 5-HT neurones. This receptor is sensitive to the action of LSD.

3.6 S_3.

In the rat forebrain the firing activity of most neurones is reduced by microiontophoretic application of 5-HT or 5-HT agonist. The receptor mediating these inhibitory responses is distinct from S_1 and S_2 receptors as indicated by the lack of effect of classical 5-HT antagonists and the weak activity of LSD (Haigler and Aghajanian, 1974a; de Montigny and Aghajanian, 1977; Aghajanian and Wang, 1978).

3.7 Discrepencies between radioligand binding and

electrophysiologically identified 5-HT receptors

The classical 5-HT antagonists have a high affinity for 5-HT_1 and 5-HT_2 sites but fail to antagonise S_3-mediated responses, the S_3 receptor appears to be a distinct from these two sites. Methiothepin has a high affinity for both the 5-HT_1 and 5-HT_2 binding sites (Leysen et al., 1981) but fails to block S_1-mediated responses in the facial motoneurones (McCall and Aghajanian, 1980), this would suggest that neither binding site corresponds to the S_1-receptor.

From the above account of 5-HT receptors, it is apparent that, as yet, there is no correlation between electrophysiological characterisation of postsynaptic 5-HT receptors and their identification by the radioligand binding method. The differences between the two types of studies may be due to several factors. For instance, the 5-HT receptor nature of the [^{3}H]-spiperone binding sites has been questioned on the grounds that the neurotransmitter has a thousand times less affinity for the site than the radioligand and concentrations of 5-HT in the range of 10^{-6} to 10^{-5} might have non-specific receptor effects (Middlemiss et al., 1980). Conversely, the nature of 5-HT_1 binding sites to be a receptor has been questioned because of its few functional correlates and due to very limited number of chemical congeners of 5-HT which bind with high affinity to this site (see Leysen, 1984). It is possible that further characterisation of 5-HT_1 and 5-HT_2 sub-types will correlate biochemical data with electrophysiological characteristics of

postsynaptic 5-HT receptors.

4. 5-HT binding sites and their second messengers

4.1 5-HT_1 sites

These 5-HT sites are linked to adenylate cyclase in the brain, which is stimulated by nanomolar concentrations of 5-HT (Fillion et al., 1979a; 1979b). This stimulatory effect of 5-HT was not blocked by 5-HT_2 site antagonists, such as mianserin or ketanserin (Barbaccia et al., 1983; Shenker et al., 1983).

4.2 5-HT_2 sites

These sites are linked to phosphatidyl inositol (PI) metabolism. The 5-HT induced PI metabolism was blocked by drugs such as pizotifen and ketanserin in the rat cerebral cortex (Berridge et al., 1982; Conn and Sanders-Bush, 1984). The hydrolysis of PI has been proposed to be a multifunctional transducing mechanism for generating a number of important intracellular signals, including calcium fluxes, prostaglandin synthesis, production of cyclic guanosine monophosphate and control of protein kinase activity (Downes, 1983).

The elucidation of second messengers for both 5-HT binding sites and their involvement in the mediation of behavioural changes due to stimulation of these sites (see section 8.) suggests that both 5-HT_1 and 5-HT_2 binding sites are true receptors.

5. Distribution of 5-HT_1 and 5-HT_2 receptors in the CNS

5-HT_1 receptors are predominant in hippocampus and striatum. The 5-HT_2 sites show a similar distribution in brain of various mammalian species including man (Leysen et al., 1983; Schotte et al., 1983). The highest density of 5-HT_2 receptors is found in the frontal parts of the cortex and it graduly decreases towards the occipital parts. In subcortical areas, the nucleus accumbens, tuberculum olfactorium and striatum contain about half the density of the prefrontal cortex and less than 10% is found in other brain areas and the spinal cord.

5.1 Cellular localisation of 5-HT_1 and 5-HT_2 receptors

Lesion studies have provided information regarding the cellular localisation of 5-HT receptors in the rat brain. The data published on 5-HT_1 receptors has depended on the $[^3H]$-ligand used (Gozlan et al., 1983). Following the destruction of noradrenaline and dopamine neurones by local injections of 6-OHDA, the $[^3H]$-5-HT binding in forebrain was unchanged. This suggests that these sites do not occur on the presynaptic catecholamine neurones or do so in very small amounts which are undetectable by means of binding studies. The destruction of cell bodies in the striatum with local injection of kainic acid resulted in a reduction of $[^3H]$-5-HT binding, but did not affect the striatal $[^3H]$-8-OH-DPAT binding. However, $[^3H]$-8-OH-DPAT binding was reduced in the hippocampus following local application of kainic acid. Destruction of 5-HT neurones following 5,6- or 5,7-DHT (dihydroxytryptamine), neither the forebrain $[^3H]$-5-HT binding

nor the hippocampal [^{3}H]-8-OH-DPAT was altered, but [^{3}H]-8-OH-DPAT binding in the striatum was decreased. This lesion data indicates that some of the 5-HT_1 receptors are located postsynaptically (those affected by kainic acid lesions), whereas the striatal [^{3}H]-8-OH-DPAT sites were localised on presynaptic 5-HT neurones. Blackburn et al.(1984) reported an increase of [^{3}H]-5-HT binding in the substantia nigra following 5,7-DHT lesions of the dorsal raphe nucleus, suggesting a postsynaptic location of 5-HT_1 sites in this region of the brain.

Following lesion of catecholamine and 5-HT neurones, the 5-HT_2 receptors, as labelled by [^{3}H]-ketanserin in frontal cortex and striatum were unaffected (Leysen et al., 1983). However, local injections of high doses of kainic acid into the frontal cortex caused a reduction of 5-HT_2 receptors; this reduction was accompanied by a decrease in glutamic acid decarboxylase activity, suggesting the presence of 5-HT_2 receptors on the GABA neurones in frontal cortex (Leysen et al., 1983). Interestingly, electrophysiological data indicated that 5-HT_2 receptors are not present on dorsal raphe nucleus (Lakoski and Aghajanian, 1985).

6. GABA receptors

Structure activity studies performed in a variety of systems indicated the existence of more than one site for GABA. These sites were designated as $GABA_A$ and $GABA_B$ (Hill and Bowery, 1981). The $GABA_A$ sites represent all "classical" bicuculline-sensitive sites whereas $GABA_B$ sites are not affected by bicuculline and do

not recognise many of the accepted GABA mimetics such as isoguvacine, 3-aminopropanesulphonic acid, and muscimol (Hill and Bowery, 1981). Furthermore, the $GABA_A$ receptor is linked to the picrotoxin and benzodiazepine sites (see below).

6.1 $GABA_A$ and related receptors

It is now widely accepted that the GABA synapse is a site of action for a variety of structurally unrelated classes of drugs, including GABA agonists, bicuculline, picrotoxin, cage convulsant bicyclophosphate esters, pentylenetetrazol, benzodiazepines (agonists, inverse agonists and antagonists), and barbiturates. These drugs either block or facilitate GABA induced responses in the CNS. On examining the chemical structures of these drugs, it becomes obvious that they can not be expected to mediate their effects by binding to the GABA recognition site because of the strict structural requirements, required at the GABA recognition site (see Ticku and Maksay, 1983).

The postsynaptic apparatus with which these drugs interact is an oligomeric receptor complex and consists of GABA recognition sites, picrotoxin sites, benzodiazepine binding sites and chloride ionophores (see Bowery et al., 1984). The three binding sites of this receptor complex have been well characterised in vitro by means of radioligand binding assays, using various ligands (see Olsen, 1981).

The stimulation of $GABA_A$ receptors increases the conductance of

chloride ions across the neuronal membrane and the direction of the ion flow depends on its electrochemical gradient. In the majority of cases an inward movement occurs (Dreifuss et al., 1969) but an outward movement is also believed to occur, for example, in sympathetic and dorsal root ganglia, and at primary afferent terminals (Adams and Brown, 1975; Deschenes et al., 1976). The GABA mimetics such as muscimol, 3-aminopropanesulphonic acid and imidazoleacetic acid also increase chloride ion conductance, whereas the receptor antagonist bicuculline prevents this change in a competitive manner (Simmonds, 1980; see Bowery et al., 1984).

6.2 Picrotoxin binding site

The binding of picrotoxin (PTX) to its site prevents the GABA-induced increase in chloride ion conductance in a non-competitive nature (Simmonds, 1980). This inhibitory effect of PTX may be dependent on the external concentration of chloride ions (see Supavilai et al., 1982). Barbiturates enhance GABA-mediated inhibition (e.g. Nicoll et al., 1975) and this may be reflected in binding studies by an enhancement of GABA binding (Willow and Johnston, 1980) and appears to be chloride ion dependent (Olsen and Snowman, 1982). Electrophysiological studies indicate that the hypnotic barbiturates increase the lifetime of the chloride channel opening (Huang and Barker, 1980; Barker et al., 1983). Barbiturates and their stereoisomers inhibit the binding of [^{3}H]-alpha-dihydropicrotoxin (DHP) and [^{35}S]-butylbicyclophosphorothionate (TBPT) to rat brain membranes (e.g.

Ticku and Olsen, 1978; Squires et al., 1983). Pentobarbital inhibition of DHP binding was competitive whilst phenobarbital inhibition was weak and occurred in micromolar range (Ticku and Olsen, 1978; Ticku, 1980). The ability of depressant and convulsant barbiturates to inhibit DHP binding led to the suggestion that the PTX site may represent a receptor site for barbiturates (Ticku and Olsen, 1978; Ticku, 1980).

Convulsant drugs, such as pentylenetetrazol, inhibit DHP and TBPT binding with potencies that correlate well with their concentrations to produce convulsions (see Ticku and Maksay, 1983) and inhibit GABA responses (Simmonds, 1978; 1982).

6.3 Effect of GABA and pentobarbitone on noradrenaline release

GABA induces calcium ion dependent release of [^{3}H]-noradrenaline from rat hippocampal synaptosomes and this effect is antagonised by picrotoxinin (Fung and Fillenz, 1983). Lower doses of pentobarbitone potentiated this GABA induced release of [^{3}H]-noradrenaline but higher doses decreased this GABA effect as did all effective concentrations of phenobarbitone (Fung and Fillenz, 1983; 1984) This latter concentration of pentobarbitone also depressed the potassium evoked release of [^{3}H]-noradrenaline by a calcium dependent but PTX insensitive mechanism (Fung and Fillenz, 1984).

6.4 Benzodiazepine receptors

Binding of [^{3}H]-diazepam indicated that specific binding could be obtained not only in the CNS tissue, but also membranes obtained from a variety of peripheral tissues (Braestrup and Squires, 1977; Gallager et al., 1981). The binding sites on kidney membranes, although possessing a high affinity for [^{3}H]-diazepam, showed fundamentally different pharmacological specificity from the brain. The differences are most marked for two benzodiazepine derivatives, clonazepam and Ro-5-4864 (4'-chloroderivative of diazepam). Clonazepam is pharmacologically active and inhibits brain [^{3}H]-diazepam binding at low concentrations but does not inhibit kidney binding even at high concentrations (Braestrup and Squires, 1977). On the other hand, Ro-5-4864 is pharmacologically inactive agent and does not inhibit [^{3}H]-diazepam binding to the brain membranes at low concentrations, but it inhibits kidney [^{3}H]-diazepam binding at nanomolar concentrations (Braestup and Squires, 1977).

The [^{3}H]-diazepam binding to brain membranes which was displaced by clonazepam was termed central (brain) benzodiazepine site; [^{3}H]-diazepam binding to peripheral tissues and inhibited by Ro-5-4864 was termed peripheral benzodiazepine site (Braestrup and Squires, 1977).

6.5 Central benzodiazepine receptor

Benzodiazepines are another group of sedatives capable of enhancing the binding of [^{3}H]-GABA to the $GABA_A$ recognition site (Skerritt et al., 1982). Electrophysiological

manifestation of this allosteric interaction apppears to be an increase in the frequency with which the GABA-operated chloride channels open (Study and Barker, 1981). The biochemically identified recognition site for benzodiazepines on brain membrane probably represents the pharmacologically relevent receptor (see Braestrup and Nielsen, 1983). The effects of benzodiazepines on this receptor are blocked by antagonists such as Ro-15-1788 (Haefely et al., 1983). A series of esters or amides of beta-carboline-3-carboxylate exert the opposite pharmacological effects to those of benzodiazepines and are proconvulsants and/or convulsant and induce panic anxiety (Haefely, 1983; Braestrup and Nielsen, 1983). The effects of these inverse agonists are blocked by Ro-15-1788 (Nutt et al., 1981; Haefely, 1983).

6.7 Peripheral benzodiazepine receptor

These sites, which also occur in the CNS, are mainly localised to glia (Schoemaker et al., 1983; Marangos et al ., 1982). Distribution of 'peripheral' sites in the CNS is different compared with central benzodiazepine receptors (Mohler and Richards, 1983). No receptor function for the glia-located peripheral sites in the CNS has been found to date, therefore they are considered at present as acceptor sites rather than true receptors (Richards et al., 1982). Central benzodiazepine receptor antagonists, such as, Ro-15-1788 have no affinity for the peripheral site (see Richards and Mohler, 1984).

6.8 $GABA_B$ receptors

Bowery and co-workers defined $GABA_B$ sites as bicuculline-insensitive GABA receptors which are activated in a stereospecific manner by (-)-baclofen (beta-P-chlorophenyl GABA), not activated by isoguvacine, 3-aminopropanesulphonic acid or muscimol, as well as many other GABA mimetics, not influenced by barbiturates or benzodiazepines and not associated with chloride ionophores, (Hill and Bowery, 1981; Bowery et al., 1983a; 1983b). $GABA_B$ site is associated with a species of calcium channel (Dunlap, 1981; Desarmenian et al., 1982; McBurney, 1984). Furthermore, evidence indicates that activation of this site decreases the inward flux of calcium ions which leads to a reduction in evoked transmitter release (e.g. Bowery et al., 1980; Fillenz and Fung, 1983; Schlicker et al., 1984).

7. Central adrenergic receptors

Binding studies have shown that receptors, which resemble pharmacologically the peripheral $alpha_1$- and $alpha_2$-adrenoceptors, are also present in the brain (U'Prichard et al., 1978; Tanka and Starke, 1980). The presence of central beta-adrenoceptors has been demonstrated by means of various techniques, including radioligand binding (see Maguire et al., 1977; Minneman et al., 1979b), electrophysiological methods (Bloom et al., 1975) and studies of cyclic adenosine monophosphate (cAMP) generating systems (see Daly, 1977; Iversen, 1977). Only beta-adrenoceptors will be discussed here in detail, because the effect of drugs acting at these receptors on the

head-twitch behaviour was examined.

As in peripheral tissues, central beta-adrenoceptors are coupled to adenyl cyclase in a stimulatory manner (Mobley and Sulser, 1979). This adenyl cyclase system consists of at least three separate protein components in the phospholipid membrane; the receptor as an integral protein with a recognition site for the neurohormone, the catalytic component of adenylate cyclase as the effector system and the guanine nucleotide regulatory protein (G-protein) that couples in a complex way the activated receptor to the enzyme leading to activation of adenylate cyclase and the formation of second messenger cAMP (see Harden, 1983). Synthesis of cAMP continues until guanosine triphosphate (GTP) is hydrolysed by a GTPase which resides on the guanine nucleotide regulatory protein (Cassel et al., 1977).

7.1 Up-regulation of central beta-adrenoceptors

Several studies have indicated an enhanced beta-adrenoceptor reactivity to intraventricular noradrenaline following depletion of the stores by reserpine or chemical sympathectomy with 6-hydroxydopamine (6-OHDA) (Geyer and Segal, 1973; Mandell, 1974). An increased responsiveness of the beta-adrenoceptor coupled adenylate cyclase system has been reported following 6-OHDA in brain tissue (Palmer, 1972; Vetulani et al., 1976a). This up-regulation of adenylate cyclase is characterised by supersensitivity to noradrenaline and iosoprenaline but not to adenosine (Vetulani et al., 1976a), thus indicating "homologous"

sensitivity changes (changes exclusive to beta-adrenoceptor stimulation). Lack of agonist can induce supersensitivity by enhancing beta-adrenoceptor coupling to the regulatory sub-unit of cAMP in a short period of time (see Harden, 1983).

7.2 Subtypes of beta-adrenoceptors in CNS

Physiological evidence suggests that two types of beta-adrenoceptors exist (Lands et al., 1967). Radioligand binding and autoradiographic techniques have identified these subtypes of beta-adrenoceptors in the CNS. In the cerebral cortex, limbic forebrain and striatum of the rat, $beta_1$-adrenoceptors are predominant while the cerebellum contains exclusively $beta_2$-subtypes (Minneman et al., 1979a; Nahorski, 1981).

Evidence suggests that $beta_1$- and $beta_2$-adrenoceptors in the CNS are independently regulated. Chronic administration of desmethylimipramine (DMI) to adult rats caused a substantial reduction in the density of $beta_1$-adrenoceptors in the cortex but had no effect on the $beta_2$-adrenoceptors; conversely, destruction of noradrenaline neurones by administration of 6-OHDA to neonatal rats which selectively destroys noradrenergic neurones originating from the locus coeruleus (Clark et al., 1972), caused a significant increase in the density of $beta_1$-adrenoceptors in the adult cerebral cortex with no change in the density of $beta_2$-adrenoceptors (Minneman et al., 1979a).These results indicate that $beta_1$-adrenoceptors are located postsynaptically. Conversely, receptors of $beta_2$-subtype are present on

noradrenergic terminals and appear to be involved in the facilitation of noradrenaline release (Westfall, 1977; see Misu and Kubo, 1983). Furthermore, the firing activity of noradrenergic neurones originating from the locus coeruleus is reduced by beta$_2$-adrenoceptor blockade (Dhalof et al., 1981).

7.3 beta-Adrenoceptors and antidepressant therapy

Chronic administration of all clinically active antidepressant treatments, including monoamine oxidase (MAO) inhibitors and electroconvulsive therapy decrease activity of the beta-adrenoceptor linked adenylate cyclase system (Vetulani and Sulser, 1975; Vetulani et al., 1976a; 1976b). For the majority of these treatments, this reduction in the sensitivity of adenylate cyclase appears to be a consequence of reduction of cortical beta-adrenoceptor density (see Sulser and Mobley, 1981). However, receptor subsensitivity does not necessarily imply functional subsensitivity (Peroutka and Snyder, 1980a; see Green and Nutt, 1983), since reduced beta-adrenoceptor density would only be expected to produce functional effects in the absence of a substantial pool of "spare receptors" (Stephenson, 1956; Moran, 1969). However, it has not been possible to investigate this question directly in vivo, because beta-adrenoceptor agonists do not produce obvious quantifiable behavioural changes when given alone.

8. Animal behavioural models for studying central 5-HT synapses

Mainly, two behavioural models in rodents are used for studying 5-HT synapses and their interaction with other neurotransmitters in the CNS. These are the hyperactivity model in the rat (5-HT hyperactivity syndrome) and head-shaking behaviour which occurs in various species.

8.1 5-HT mediated head-twitch in mice and wet dog shake behaviour in rats

Corne, Pickering and Warner (1963) reported a novel model for assesing central 5-HT function. Injection of L-5-hydroxytryptophan (L-5-HTP) in mice produced a characteristic head-twitch response, consisting of rapid intermittent side to side movements of the head. In the mouse, only the head is involved but in the rat the upper trunk may move too, resulting in the so called "wet dog shakes" (WDS) (Bedard and Pycock, 1977). These behaviours are part of the animals normal repertoire, occasional twitches being observed in untreated animals, and the similarity of the head-twitch to the pinna reflex has often been remarked (Corne and Pickering, 1967; Boulton and Handley, 1973; Bednarczyk and Vetulani, 1978). The latter is a brief head-shake induced by touching the pinna (Witkin et al., 1959).

Both the spontaneous and L-5-HTP induced head-twitching are highly dependent on sensory input to the pinna region, both being increased by locally applied irritants and inhibited by local

anaesthesia of the pinna (Boulton and Handley, 1973). Head-twitching is also reduced by novel sounds and isolation of the animals (Boulton and Handley, 1973).

Head-twitching in mice and WDS in rats can be induced by increasing brain 5-HT function in a variety of ways, including administration of either large doses of L-5-HTP alone or lower doses in combination with a decarboxylase inhibitor such as alpha-methyldopahydrazine (carbidopa), preteatment with an MAO inhibitor followed by tryptophan (Bedard and Pycock, 1977), lysergic acid diethylamide (LSD) and other hallucinogens (Corne and Pickering 1967; Bedard and Pycock, 1977; Vetulani et al., 198Ø), quipazine (Bedard and Pycock, 1977; Malick et al., 1977) and 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Bedard and Pycock, 1977; Friedman and Dallob, 1979).

8.2 Anatomical site of initiation of head-shaking behaviour

In 1963, Corne et al. examined whole brain and brain stem 5-HT concentrations at various times following L-5-HTP administration to mice. They found that head-twitches are related to brain stem concentrations of 5-HT. Bedard and Pycock (1977) from lesioning and sectioning of rat brain concluded that the WDS originate in the brain stem but can be facilitated by the presence of the diencephalic structures. Electrolytic lesions of the dorsal raphe nucleus in the rat depressed the frquency of WDS produced by L-5-HTP, LSD and quipazine, suggesting a presynaptic mechanism of action of these drugs (Vetulani et al., 1979). If head-shaking

is a model for detection of hallucinogenic activity (Corne and Pickering, 1967) then the site of initiation of this behaviour and the site of action of hallucinogens should show some similarities. The presynaptic initiation of head-shaking is supported by electrophysiological studies, which showed that administration of hallucinogens, such as, LSD, dimethyltryptamine (DMT), mescaline, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and 5-MeODMT to anesthetised rats caused a suppresion of activity of midbrain dorsal and median raphe cells, mediated by a direct action upon 5-HT neurones (See Jacobs, 1983). Much of 5-HT actions on target neurones in the forebrain is inhibitory (Haigler and Aghajanian, 1974) and it was hypothesised that the ultimate action of LSD's preferential depressant action on 5-HT neurones is to produce a disinhibition of target neurones. This disinhibition would occur most significantly in areas with densest aggregation of 5-HT axon terminals, such as, visual and limbic systems, an obvious neuronal model to account for visual hallucinations (See Jacobs, 1983).

However several studies have indicated that head-shaking behaviour is mediated by post-synaptic 5-HT receptors. Destruction of central presynaptic 5-HT neurones in the rat by administration of either 5,7-DHT or the less specific 5,6-DHT enhanced WDS induced by 5-HT (Drust and Connor, 1983) L-5-HTP (Barbeau and Bedard, 1981), and 5-Methoxytryptamine (Bednarczyk and Vetulani, 1978). Intracerebral injection of 5,7-DHT or 5,6-DHT to mice potentiated head-twitching induced by L- 5-HTP, 5-HT,

mescaline (Nakamura and Fukushima, 1978) and 5-MeODMT (Heal et al., 1985). These potentiations may be due to an increase in the density of 5-HT_2 receptors due to lesioning (Heal et al., 1985). Recent electrophysiological studies indicate a post-synaptic 5-HT mechanism of action of hallucinogens. In brain stem (the region of brain thought to be involved in the induction of head-shaking and '5-HT syndrome') and spinal cord, areas receiving direct 5-HT input, 5-HT exerts a facilitatory effect on excitatory inputs to these target cells. This facilitation is potentiated by the hallucinogens and blocked by 5-HT antagonists (see Jacobs, 1983). It could be this action of 5-HT that is responsible for the induction of head-twitching and '5-HT syndrome' behaviours. Consistent with this is the observation that hallucinogens produce these behavioural changes and 5-HT antagonists block it (Corne and Pickering, 1967). Studies measuring behavioural and electrophysiological changes simultaneously in cats support the post-synaptic mode of action of hallucinogenic drugs. Administration ofLSD, DOM, mescaline and psilocin produced depression of 5-HT unit activity lasting, on the average 3 to 4 h, while the behavioural effects lasted for at least 6 to 8 h; secondly, when the same dose of LSD was administered 24h later, it produced no behavioural changes (tolerance), but the effect on 5-HT unit activity was unaltered (Trulson and Jacobs, 1979). Furthermore, pretreatment of cats with a 5-HT receptor antagonist mianserin or ketanserin prior to administration of LSD or DOM, blocked their behavioural effects in a dose dependent manner, but did not alter the depression of 5-HT unit activity that LSD

produced in neurones of the dorsal raphe nucleus (see Jacobs 1983).

8.3 5-HT_2 receptors and mediation of head-shaking behaviour

In 1981, Peroutka, Lebovitz and Snyder reported that there was a good correlation between the ability of various drugs to inhibit spiperone binding to 5-HT_2 receptors and inhibit head-twitch behaviour. These observations were strengthened by the findings of Ortmann et al. (1982). The highly specific 5-HT_2 receptor antagonists pirenperone and ketanserin blocked WDS behaviour in rats (Colpaert and Janssen, 1983; Yap and Taylor, 1983) and pirenperone also reduced head-twitching in mice (Green et al., 1983c). It should be noted that ketanserin and pipenperone also have antagonistic properties on $alpha_1$-adrenoceptor and dopamine receptors respectively (Fozard, 1983; Green et al., 1983c). The 5-HT_{1a} receptor antagonist propranolol does not block the 5-HT mediated head-twitch in mice (Goodwin and Green, 1985) but its effects on 5-HT mediated WDS in the rat is controversial. Thus, while Matthews and Smith (1980) reported inhibition, Bedard and Pycock (1977) found no effect. The 5-HT_1 receptor agonists, 8-OH-DPAT and RU 24969, do not induce head-twitching in mice (Goodwin and Green, 1985). However, 8-OH-DPAT blocked the L-5-HTP but not 5-MeODMT induced head-twitch response in mice probably by inhibiting the release of 5-HT (Goodwin and Green, 1985).

Overall, evidence indicates that head-twitching in mice and probably WDS behaviour in the rat are 5-HT_2 receptor mediated.

9. Modulation of shaking behaviour in mice and and rats by brain neurotransmitters

9.1 Noradrenaline

The 5-HT neurones in the midbrain raphe nuclei are innervated by noradrenergic nerve terminals (Fuxe, 1965; Swanson and Hartman, 1975) originating from the locus coeruleus (Dahltstrom and Fuxe, 1964; Fuxe, 1965; Kostowski et al., 1974). Electrophysiological studies have demonstrated that the firing activity of dorsal raphe nuclei is maintained by tonic activity of noradrenergic neurones (Svensson et al., 1975; Baraban and Aghajanian, 1980). However recent experiments involving conscious animals suggested thatthis noradrenergic tone is not necessary for maintenance of raphe firing (Heym et al., 1982; Trulson and Trulson,1983; Trulson and Crisp, 1984). Biochemical studies have also shown that stimulation of alpha$_2$-adrenoceptors inhibits release of [^{3}H]-5-HT from brain slices (Frankhuyzen and Mulder, 1980; Gothert and Huth, 1980; Maura et al., 1982), and clonidine administration decreases cortical 5-hydroxyindoleacetic acid (5-HIAA) concentrations (Reinhard and Roth, 1982). Inhibition of dopamine beta-hydroxylase with alpha-methyl-P-tyrosine or lesions of the locus coeruleus increase brain 5-HIAA concentration (Johnson et al., 1972; Kostowski et al., 1974). In contrast to the action of clonidine, beta-adrenoceptor agonists such as salbutamol and clenbuterol increase 5-HT turnover (Waldmeier, 1981; Nimgaonkar et al., 1983), by increasing the tryptophan availability from the periphery to the brain (Nimgaonkar et al.,

1983).

The administration of alpha$_2$-adrenoceptor agonists, e.g. clonidine and guanabenz, inhibited the head-twitch response in mice induced by 5-HT (i.c.v.), whereas the alpha$_2$-adrenoceptor antagonists, yohimbine and piperoxan, potentiated the response (Handley and Brown, 1982). Clonidine also reduced the L-5-HTP, 5-methoxytryptamine, LSD and quipazine induced WDS in the rat (Bednarczyk and Vetulani, 1978; Vetulani et al., 1980). In the above studies, clonidine was administered before L-5-HTP, 5-HT or the 5-HT agonist. However, clonidine appeared to have no effect on L-5-HTP induced WDS, when administered 90 min after L-5-HTP (Bedard and Pycock, 1977). It is interesting to note that the beta-adrenoceptor agonists salbutamol and clenbuterol potentated the 5-HT hyperactivity syndrome in rats, when administered before L-5-HTP or quipazine but were ineffective if administered after the inducing agent (Ortmann et al., 1981; Nimgaonkar et al., 1983). A similar situation may also be true for alpha$_2$-adrenoceptor agonists and head-shaking behaviour in rats. The mechanism of action of clonidine was investigated by Bednarczyk and Vetulani (1978). These authors destroyed noradrenaline and 5-HT neurones by administering 6-OHDA and 5,6-DHT respectively. Since the effect of clonidine was not altered by these lesions, they concluded that the action of this alpha$_2$-adrenoceptor agonist was not dependent on pre- or post-synaptic noradrenaline, or presynatic 5-HT neurones. However, the data of Bednarczyk and Vetulani (1978) is difficult to interpret, because

they did not measure the extent of noradrenaline and 5-HT depletion following the chemical lesions i.e. some presynaptic noradrenaline and 5-HT neurones may still be intact following administration of neurotoxins.

The $alpha_1$-adrenoceptor agonists phenylephrine and methoxamine potentiate, whilst the corresponding antagonists prazosin and thymoxamine antagonise, the 5-HT induced head-twitch response in mice (Handley and Brown, 1982). However, Matthews and Smith (1980) reported that WB 4101 showed no effect on L-5-HTP induced WDS behaviour in rats. This discrepancy may be due to lower *in vivo* selectivity of WB 4101 compared with the drugs used by Handley and Brown (1982) (Massingham et al., 1981).

The beta-adrenoceptor antagonist propranolol failed to antagonise the 5-HT mediated head-twitch response in mice (Goodwin and Green, 1985). This result is surprising since propranolol inhibits 5-HT_2 receptor binding (Green et al., 1983b; Nahorski and Willcocks, 1983). However, when attempting to correlate binding data with 5-HT mediated behaviours, it should be remembered that most binding studies employ tissue prepared from the frontal cortex, whereas the head-twitch response in mice and WDS in rats are thought to involve the brain stem; it is possible that different subtypes of 5-HT receptors are present in these regions of the brain.

The $beta_2$-adrenoceptor agonists potentiate the L-5-HTP induced head-twitch response in mice (Delini-Stula et al., 1979; Ortmann et al., 1981; Nimgaonkar et al., 1983). The effect of $beta_1$-adrenoceptor agonists on head-twitch in mice and the effect of $beta_1$- and $beta_2$-adrenoceptor agonists on WDS behaviour in rats have not been investigated. Furthermore, it is not clear if the potentiating effect of beta-adrenoceptor agonists is dependent on presynaptic noradrenaline and 5-HT mechanisms, since in all studies, the behaviour was induced by precursor loading (Delini-Stula et al., 1979; Ortmann et al., 1981; Nimgaonkar et al., 1983), in the presence of intact presynaptic noradrenaline neurones.

9.2 Acetylcholine

Drugs acting as agonists and antagonists on acetylcholine receptors had no effect on WDS behaviour in rats induced by L-5-HTP (Bedard and Pycock, 1977).

9.3 Drugs acting on GABA and related receptors

Biochemical studies indicated that benzodiazepines reduce turnover and release of 5-HT (Jenner et al., 1975; Saner and Pletscher, 1979). Benzodiazepines such as fludiazepam and diazepam potentiate the head-twitch response in mice induced by 5-HT (i.c.v.) and mescaline in a dose dependent manner (Nakamura and Fukushima, 1977). A recent study has shown that administration of diazepam, progabide or sodium valproate for 14 days increased the $5\text{-}HT_2$ receptor density in frontal cortex and

L-5-HTP induced head-twitch response in mice (Green et al., 1985).

The acute administration of the $GABA_B$ receptor agonist baclofen reduced head-twitching induced by L-5-HTP but did not alter the response due to 5-MeODMT in mice; after 14 days of oral administration of baclofen, head-twitch responses to both L-5-HTP and 5-MeODMT were enhanced in mice, which is probably due to an increase in the density of $5\text{-}HT_2$ receptors (Metz et al., 1985). This increase in the $5\text{-}HT_2$ receptor density is probably due to a decrease in the release of 5-HT due to the stimulation of $GABA_B$ receptors by baclofen (Bowery et al., 1980; Schlicker et al., 1984).

The $GABA_A$ receptor agonist muscimol and various barbiturates failed to modulate the L-5-HTP induced head-twitch in mice and WDS in rats (Corne et al., 1963; Matthews and Smith, 1980). These results are surprising; since benzodiazepines, which exert their effects probably through the chloride channel, modulate head-twitching (see section 6.4), it is reasonable to expect other drugs, which also interact with the chloride channel, to modulate this behaviour as well.

9.4 Histamine

Some histamine antagonists reduce the L-5-HTP induced head-twitch response in mice (Corne et al., 1963). The ability of histamine to modulate head-twitching behaviour has not been studied in

detail.

9.5 Cortisol

The effect of cortisol on L-5-HTP head-twitch behaviour in mice depended upon duration of pretreatment. A single dose caused a significant decrease in the response; two daily injections had no significant effect, whilst 3-5 daily injections caused a progressive decline in responsiveness to L-5-HTP (Handley and Miskin, 1972).

9.6 Kynurenine pathway metabolites

The Kynurenine pathway metabolites, kynurenine and 3-hydroxykynurenine in low doses, caused marked potentiation of twitch response in mice, to both L-5-HTP and 5-HT. Higher doses caused antagonism of both responses but the metabolite xanthurenic acid was found to be inactive (Handley and Miskin, 1977). However, these results do not correlate with those of Lapin (1972), who found kynurenine and 3-hydroxykynurenine to be inactive over a wide range of doses against both L-5-HTP and trxptophan/phenelzine induced head-twitches in the mouse. However, Lapin (1972) found 3-hydroxykynurenine and kynurenine to reduce WDS in the rat. The discrepancy between the studies of Lapin (1972) and, Handley and Miskin (1977) is probably due to a methodological difference. Lapin (1972) used isolated mice, whereas Handley and Miskin (1977) used groups of four, and detection of head-twitch modulation is considerably less

sensitive during isolation (Boulton and Handley, 1973).

9.7 Dopamine

Drugs such as methylamphetamine, amphetamine and apomorphine which stimulate dopamine receptors directly or indirectly attenuate 5-HT mediated head-twitching in mice (Corne et al., 1963) and WDS in the rat (Bedard and Pycock, 1977). Neuroleptics also block these behaviours in both mice and rats (Corne et al., 1963; Corne and Pickering, 1967; Maj et al., 1978; Matthews and Smith, 1980). This antagonising effect of neuroleptics is probably due to their blocking of 5-HT and noradrenergic receptors as well as dopamine receptors (Maj et al., 1978). Therefore, the role of dopamine is unclear.

9.8 Antidepressants

Following chronic administration of antidepressants, studies of 5-HT_2 receptor binding have indicated a reduced receptor density in the absence of a significant changes in K_D (Peroutka and Snyder, 1980b). This effect is controversial, since increased receptor number have also been reported after chronic administration of DMI (Green et al., 1983a). As suggested by Green et al. (1983a) this discrepency could be due to the different doses, number of administrations of antidepressant drug and the withdrawal times used in various studies before examining binding. This could also be true for behavioural studies, as the effect of chronic antidepressants on head-

twitching is unclear. The effect on head-twitching caused by 5-MeODMT depends on the dose of the agonist used, the effect of large doses being reduced while low doses were potentiated when evaluated 48h after the last dose of the antidepressant (see Fuxe et al., 1983). This is in disagreement with the results of Friedman et al. (1983), who reported an enhanced response to a large dose of 5-MeODMT following a similar withdrawal time. After 72h withdrawal from chronic administration of mianserin, danitracen or amitryptyline, an increased number of L-5-HTP induced WDS in the rat were observed (Mogilnicka and Klimek, 1979). However, a recent study has demonstrated that repeated administration of various antidepressants decreased both cortical 5-HT_2 receptor density and head-twitch response, while the mice were still on treatment (Goodwin et al., 1984). The increase in 5-HT_2 receptor density reported by Green et al. (1983a) was associated with an increased sensitivity to L-5-HTP but these experiments were performed at only 18h after the last dose of antidepressant drug. Repeated electroconvulsive shock increased both head-twitching in mice and 5-HT_2 receptor number (Green et al., 1983a; Goodwin et al., 1984).

In electrophysiological experiments, the inhibitory effect of iontophoretically applied 5-HT was greatly enhanced 24h after a 14 day pretreatment with DMI and iprindole (de Montigny and Aghajanian, 1978), but it is not clear whether this effect is mediated by 5-HT_2 receptors.

9.9 Morphine

The WDS induced by i.c.v. administration of 5-HT were blocked by morphine and this inhibitory effect of morphine was reversed by naloxone (Drust et al., 1979).

9.9.1 Tryptamine

Tryptamine reduced the number of mice showing head-twitches following tranylcypromine/L-5-HTP (Jones, 1981).

9.9.2 Effect of light and dark cycle on head-shaking

The 5-MeODMT induced head-twitching in mice showed diurnal variation with peak activity occuring at midway through the light period and lowest at midway of the dark period; in contrast the 5-HT hyperactivity showed no variation over 24h (Moser and Redfern, 1984). Therefore, when using the head-twitch model it is essential to run controls at the same time.

10. Other ways of inducing head-shaking behaviour

There are several ways of elicting head-shaking behaviour which may or may not involve central 5-HT mechanisms.

10.1 Hippocampal stimulation

Electrical stimulation of dorsal hippocampus produces WDS behaviour in the rat (Rucine et al., 1977; Aihara et al., 1982). Morphine, haloperidol and chlorpromazine inhibited dose dependently the WDS and naloxone antagonised the inhibitory effect of morphine (Araki and Aihara, 1985). These results

suggest the involvement of dopamine and opioid mechanisms in the induction of WDS by hippocampal stimulation. Noradrenaline also appears to be involved in the induction of this behaviour (Yamada et al., 1983).

1Ø.2 Acetylcholine

The muscarinic cholinergic agonist carbachol administered i.c.v evoked WDS in the rat, in a dose dependent manner, which were antagonised by scopolamine, atropine, cyproheptadine, morphine, clonidine, phentolamine, haloperidol and interestingly L-5-HTP (Turski et al., 1981). Furthermore, metergoline, propranolol, bicuculline and amino-oxyacetic acid had no effect. Cyproheptadine has antihistamine, anticholinergic as well as anti-5-HT properties (Stone et al., 1961; Van Riezen, 1972) and this taken together with the results of metergoline raises the possibility that the inhibition produced by cyproheptadine may not be due to its anti-5-HT effect.Interestingly, hyoscine and atropine induced head-twitching in mice (Corne and Pickering, 1967).

1Ø.3 alpha-Melanocyte-stimulating hormone

Administration of alpha-melanocyte-stimulating hormone (i.c.v.) elicted WDS in rats , which were bloked by pretreatment with methysergide, apomorphine or fluphenazine but not by scopolamine, suggesting a possible role of 5-HT systems (Yamada and Furukawa, 1981).

10.4 Thyrotrophin-releasing hormone

The systemic and intacisternal administration of thyrotrophin-releasing hormone (TRH) to partially anaesthetised rats produced WDS amongest other behavioural changes (Prange et al., 1974). Further work involving direct injections of TRH into various regions of the brain has revealed that areas exhibiting a significant degree of shaking were located in the periaqueductal-fourth ventricular spaces, medial thalamus and hypothalamus and the medial preoptic areas (Wei et al., 1975). The brain areas involved in naloxone precipitated withdrawal shaking in morphine-dependent rats (Herz et al., 1972; Wei et al., 1975) parallels the sites of TRH-stimulated shaking (Wei et al., 1975) and the distribution of TRH (Winoker and Utiger, 1974; Brownstein et al., 1974). A recent study has indicated the involvement of dopamine and opioid systems in TRH-induced WDS but blockade of 5-HT (with methysergide), adrenergic and cholinergic receptors had no effect (Griffiths and Widdowson, 1985).

10.5 Kainic acid

Kainic acid (KA) is a structural analogue of glutamate (Shinzaki and Konishi, 1970). The i.c.v. administration of KA produces WDS (Lanthorn and Isaacson, 1978). Like endorphin-induced WDS, KA induced WDS were blocked by naloxone (Bloom et al., 1976; Lanthorn and Isaacson, 1978). The glutamate receptor blocker, glutamic acid diethylester also reduced KA-induced WDS. These results indicate the possible involvement of opioid and glutamate receptors (Lanthorn and Isaacson, 1978). Further work has

suggested the involvement of acetylcholine, catecholamines, 5-HT and GABA mechanisms (Kleinrok and Turski, 1980).

10.6 Opiate withdrawal

The occurence of WDS is one of the classical signs appearing upon abrupt termination of morphine withdrawal and after precipitation of abstinence in dependent rats with narcotic antagonists (Wei et al., 1973). WDS can also be induced by intracerebral injection of endorphins which are blocked by naloxone (Bloom et al., 1976). Endorphins, like TRH, seem to be most effective in producing WDS when injected into the medial thalamus or the periaqueductal region (Wei et al., 1973; 1975). Endorphin-induced WDS occurs after injection into the lateral ventricles but not after intracisternal injection (Bloom et al., 1976). This suggests that the site of action for endorphins is anterior to the fourth ventricle.

Clonidine and phenoxybenzamine, but not cyproheptadine, inhibited the morphine abstinence induced WDS, precipitated by nalorphine in morphine dependent rats (Bednarczyk and Vetulani, 1978). This suggests the involvement of noradrenaline but not 5-HT mechanisms. These authors suggested that the inhibitory action of clonidine is not mediated by pre- or post-synaptic alpha-receptors. Furthermore, clonidine does not require presynaptic 5-HT neurones to exert a inhibitory action on WDS induced by morphine withdrawal or by codeine and apocodeine (Bednarczyk and Vetulani, 1978; Vetulani et al., 1980).

10.7 GABA, muscimol and benzodiazepines

GABA administered systemically in large doses produced head-twitching in rats and rabbits (Smialowski et al., 1980). The $GABA_A$ receptor agonist muscimol induced WDS in the rats (Scottie de Carolis and Massotie, 1978) and some benozodiazepines produced similar changes in mice (Nakamura and Fukushima, 1976). The effects of GABA and benzodiazepines were blocked by cyproheptadine (Nakamura and Fukushima, 1976; Smialowski et al., 1980) suggesting a role of 5-HT.

10.8 Noradrenaline

The catecholamine precursor, tyramine administered i.c.v. to mice pretreated with a MAO inhibitor produced head-twitching (Orikasa et al., 1980). The $alpha_2$-adrenoceptor antagonist yohimbine also produced this response in mice (Corne and Pickering, 1967; Handley and Brown, 1982) probably by increasing the release of noradrenaline onto $alpha_1$-adrenoceptors (Handley and Brown, 1982).

It has not been investigated whether the above mentioned methods induce shaking behaviour ultimately through $5\text{-}HT_2$ receptors.

11. The 5-HT hyperactivity behaviour in the rat

Following the administration to rats of compounds which either increase synaptic 5-HT (e.g. MAO inhibitors, 5-HT releasing agents, or 5-HT precursors) or stimulate 5-HT receptors, a

stereotyped behavioural syndrome is produced (see Green and Heal, 1985). The behaviours observed consist of resting tremor, reciprocal forepaw treading, (rhythmic dorso-ventral movements of the hindlimbs), hindlimb abduction (splaying out of the hindlimbs), straub tail, lateral headweaving (slow side to side movements), salivation, head shaking, hyperreactivity and hyperactivity. A similar syndrome is also seen in other species following increases in the central 5-HT function (see Jacobs, 1976).

12. Comparison of the pharmacology of 5-HT mediated head-shaking with 5-HT hyperactivity syndrome

12.1 The 5-HT hyperactivity syndrome in rats and head-shaking response in mice and rats are produced by compounds which increase brain 5-HT function (see Green and Heal, 1985). However, higher doses of these compounds are required to produced the hyperactivity syndrome compared with head-shaking.

12.2 Head-shaking in mice and rats, and behaviours of the syndrome (including forepaw treading and hindlimb abduction) are hindbrain mediated (see section 8.2; Deakin and Green, 1978).

12.3 The 5-HT head-shaking behaviour is mediated exclusively by 5-HT_2 receptors, whereas the syndrome behaviours involves both 5-HT_1 and 5-HT_2 receptors. Thus, hyperlocomotion and hyperreactivity involve a 5-HT_1 receptor with a post-synaptic dopamine link; head weaving, forepaw treading and hindlimb abduction are

mediated by the 5-HT_2 receptor (see section 8.3; see Green and Heal, 1985).

12.4 Both types of behaviours are potentiated by $beta_2$-adrenoceptor agonists (section 9.3; Ortmann et al., 1981; Cowen et al., 1982; Nimgaonkar et al., 1983).

12.5 (-)-propranolol blocks the syndrome induced by tranylcypromine/L-tryptophan or 5-MeODMT (Green and Grahame-Smith, 1976; Costain and Green, 1978) but fails to effect the L-5-HTP induced head-twitch response in mice (Goodwin and Green, 1985).

The 5-HT mediated head-shaking behaviour was used here to assess the effect of noradrenaline and GABA systems on 5-HT systems because it is easier to quantify and may be more sensitive indicator of 5-HT receptor activation than the 5-HT hyperactivity syndrome (Drust et al., 1979). The latter occurs after higher doses of 5-HT mimetics, whereas head-shaking occurs at lower doses of these drugs. Interestingly, the appearance of hyperactivity syndrome blocks the head-shaking behaviour (Drust et al., 1979). Therefore, caution must be exercised, when using the head-shaking model, since the decrease in shakes, which coincides with the onset of the syndrome, could be interpeted wrongly as a decrease in 5-HT receptor activity.

13. Aims of the project

The initial aim of the project was to investigate thoroughly the pharmacology of head-shaking behaviour. This would include testing antagonists from each pharmacological group against causative agents from all the other groups in order to determine a 'hierarchy of antagonism'. Furthermore, it was also aimed to examine whether the 5-HT mediated head-shaking involves pre- or post-synaptic 5-HT neurones, localise the site of its initiation and the site of noradrenergic modulation by means of stereotaxic placement of cannuli and neurotoxin lesions.

EXPERIMENTAL METHODS

1. ANIMALS, ANIMAL HUSBANDRY AND LABORATORY CONDITIONS.

1.1 Mice.

Experiments reported in this thesis were carried out on Aston bred male mice of MF1 strain which weighed between 20 and 30g. Subsequent to weaning, mice were kept in groups of 25-30 (from the same birth cohort) in polypropylene cages in the animal house at an ambient temperature of 21 to 23 C under normal lighting conditions. These animals were fed a conventional 41B cube diet (Pilsbury's Ltd., Birmingham) and received tap water ad libitum. Mice were transfered to the quiet experimental room at least 5 days prior to experiment. The experimental room was maintained at $21 \pm 1^{\circ}C$, relative humidity between 50-60% and the animals were exposed to an 08.00-20.00 light-dark cycle.

1.2 Rats.

The experiments involving rats in this thesis were performed on male Hooded rats (Glaxo, Herts) weighing between 250 and 290g. These animals were fed a conventional 41B cube diet (Pilsbury's Ltd., Birmingham) and received tap water ad libitum. The rats were kept in groups of 3 in polypropylene cages in the quiet experimental room which was maintained under similar environmental conditions as those described for mice above.

All behavioural experiments were carried out between 09.00 and 19.00hrs.

2. INJECTION TECHNIQUES.

2.1 Subcutaneous (s.c.) injection.

Injection was made into the loose skin at the back of the neck of both mice and rats. Where animals received more than one s.c. injection, the second injection was made by inserting the needle under the skin in the flank of the animal. The injection volume was 10.0 ml/kg for mice and 1.0 ml/kg for rats.

2.2Intraperitoneal (i.p.) injection.

Injection was made by inserting the hypodermic needle into the abdominal wall towards the diaphragm. Care was taken not to penetrate too deeply thereby damage the internal organs. Where more than one injection was made by this route in the same animal, care was taken not to use the same injection site. The injection volume was 10.0 ml/kg for mice and 1.0 ml/kg for rats.

2.3 Intracerebroventricular (i.c.v.) injection.

The method used was that described by Brittain and Handley, (1967). A 0.25 ml tuberculin syringe was used, fitted with a 27 gauge needle which was 3 mm in length. This needle length has been found to be optimum administration of drugs into the ventricular system of mice (Handley, 1970). The injection volume was 20 µl.

The mouse was immobilised by holding the head firmly on a flat surface by holding the scruff of the skin at either side of the head. The needle was placed vertically on the mid-line of the

skull and drawn backwards until a depression was felt. This depression is the junction between the two parietal and the interparietal bones, an area which is not ossified in the mouse. The skull was penetrated and solution delivered. Any injections made outside this non-ossified area resulted in neurological damage. Mice exhibiting circling movements or having limb paralysis were immediately rejected. After i.c.v. injection the mice either remained immobile or ran around the cage. This behaviour lasted only few seconds after which time they became quiet. After 5 min the vehicle treated mice were indistiguishable from untreated controls.

After experimentation, all mice were killed by cervical dislocation and examined for the location of the hole in the skull due to needle insertion. The results from any mice which were incorrectly injected were then discarded.

3. Behavioural tests.

3.1 Induction of head-twitch behaviour in mice.

1h before experiment, mice from the same stock cage were placed in small sawdust lined polypropylene cages in groups of 3. The third mouse was included only because head-twitching is reduced when there are only 1-2 mice per cage (Boulton and Handley, 1973). This mouse formed no further part in the experiment. The remaining pair both received either carbidopa (9 mg/kg s.c.) followed 15 min later by 5-hydroxytryptophan (L-5-HTP; 200 mg/kg

i.p.) or just 5-methoxy-N,N dimethyltryptamine (5-MeODMT; 10.0 mg/kg i.p.).

3.2 Analysis of drug effects

One mouse from each pair received the test drug and the other the injection vehicle. Twitches from the two mice were counted in parallel for 5 min starting 20 min post L-5-HTP or for 12 min starting immediately after 5-meODMT. This procedure compensates for between run variability (Handley and Brown, 1982). There were at least 5 pairs per experimental group. Where it was possible to examine 3 or more doses of the test drug potency was expressed as ID_{50} (dose producing 50 % inhibition relative to control) or ED_{200} (dose producing a response frequency twice that of controls) from log dose-response regression analysis, where response = test mouse head-twitch frequency as a % of that in paired control mouse (Handley and Brown, 1982).

3.3 Induction of "wet dog shake" behaviour in rats.

Rats were maintained in groups of 3 in polypropylene cages. As in the case of mice the third rat formed no further part in the experiment. The remaining pair received quipazine (2.5 mg/kg i.p.).

3.4 The effect of Locus Coeruleus lesions on the quipazine induced "wet dog shake" behaviour.

For each run two rats were taken at random from each of the Locus Coeruleus (LC) lesioned and sham operated groups, both received

quipazine. The shakes were counted in parallel between 30 min and 60 min after quipazine.

3.4 Analysis of drug effects after LC lesions.

For each run two rats were taken at random from each of the LC lesioned and sham operated groups, one member of each pair received the test drug and the other saline by the same route. All four received quipazine. The shakes were counted in parallel for each quartet between 30 min and 60 min after quipazine. The procedure was then repeated for the remaining rats.

3.6 Statistical analysis

Drug effects relative to controls were analysed by paired t-test and 2 x 2 factorial analysis of variance blocked by run (Linton and Gallo, 1975).

Parametric and nonparametric statistics

Parametric techniques, such as the t test and analysis of variance (ANOVA), estimate population parameters. They also make a number of assumptions about the population from which the scores were drawn. Most importantly they assume that:-

i. Scores must be from a interval scale,

ii. the samples were drawn at random from the populations under consideration,

iii. the variances in the populations are homogeneous, and

iv. the scores are normally distributed in the populations.

Nonparametric techniques, such as Mann-Whitney U test, make much

weaker assumptions. The usual nonparametric assumptions are that:-

i. the samples were drawn at random from the populations under consideration and

ii. the scores underlying the ranks form a continuous distribution.

At one extreme, some statisticians argue that the assumptions underlying parametric techniques are virtually never justified for behavioural science research and as a consequence that only nonparametric techniques should be used (see Linton and Gallo, 1975). At the other extreme, many statisticians argue that violations of these assumptions do not seriously impair the usefulness of parametric techniques and that they should always be used because of their greater power (see Linton and Gallo, 1975). When there are more than one independent variable, none of the nonparametric techniques is appropriate. Therefore, results described in Chapters 3, 5 and 6 were analysed by ANOVA and paired t-test. In order to appropriately apply ANOVA to a set of data, the conditions described above for parametric techniques must be fulfilled. Head-twitch rates are an integer but interval scale measure and use of parametric statistics was therefore acceptable in the absence of obvious deviation from normality. However, many statisticians recommend ANOVA even when the data is not from interval scale (see Linton and Gallo, 1975). There is some good empirical work on the effects of violating the assumptions of normality and homogeneity of variance. In most

cases, violations of these assumptions, even fairly extreme ones, do not severly affect the outcome of ANOVA. At most, they tend to give a slightly erroneous significance level (see Linton and Gallo, 1975).

4.Ø Brain lesions, histolgy, dissection of rat brain and biocemical methods.

4.1 Lesions of the Locus Coeruleus.

Rats were anaesthetised with Sagatal (Sodium Pentobarbitone 6Ø mg/kg i.p.) and mounted in a stereotaxic instrument such that the upper incisor bar was 5.Ø mm above the inter-aural line. Two holes were drilled through the skull to allow a 3Ø gauge cannula to be lowered bilaterally to the coordinates (derived from histology and patterns of noradrenaline depletion): AP -8.2Ø mm from the bregma, ML $\pm$ Ø.8Ø mm from the midline suture, and DV - 5.5Ø mm from the dura. Five ug of 6-hydroxydopamine (6OHDA) base dissolved in 1.5 ul of Ø.9% saline containing Ø.2 mg/ml ascorbic acid were infused at the rate of lul/min and the cannula was left in place for a further minute to allow diffusion of the drug. The skin was then sutured. Sham operated rats received similar injection of the vehicle. The animals were allowed at least 21 days to recover before examining behaviour.

4.2 Histology.

This work was carried out to establish the approximate position of the LC in the strain of rats used during the work reported in this thesis.

Rats were placed in the stereotaxic instrument as described above according to the rat brain atlas of Pellegrino et al., (1979). A 30 gauge cannula (identical to the one used in actual lesioning) was lowered bilaterally and left in position for a minute. The skin was sutured and the animals were allowed to live for 24 h so that the cells through which the cannula has passed would degenerate to form a tract. After this time they were killed by cervical dislocation and the brains were removed and hardened in 10% formalin.

Sectioning was done by cutting the brain at an angle similar to that illustrated in the rat brain atlas of Pellegrino et al., (1979). The brain was blocked 3 mm on either side of the site of injection by means of a blocking guide which was made by embedding the top half of a rat's skull in a slicing block to form a mould for the brain and a guide for a razor blade.

Serial sections (50 um) were cut with a freezing microtone, stained with cresyl violet and mounted on to glass slides with the help of Miss Hilary Cross (Aston University) and the tract made by the cannula was observed under a microscope in relation to structures identified in the atlas of Pellegrino et al. (1979).

4.3 Comments.

By means of histology and from measurement of regional noaradrenaline depletion (see below), LC was found to be 1.00 mm more posterior from the bregma to that shown in the rat brain atlas of Pellegrino et al. (1979).

4.4 Dissection of rat brain.

Dissections were performed by themethod of Glowinski and Iversen (1966). Rats were killed by decapitation, their brains carefuly removed and placed on an ice-cooled glass slide. Seven regions were separated of which 5 were used for assay, the midbrain and pons-medulla being discarded.

The rhombencephalon was first separated from the rest of the brain. This region was further divided by a transverse section into the cerebellum and the pons-medulla region. A transverse section was then made at the level of the optic chiasma, which separated the cerebrum into two parts B(parietal) and C (frontal).

The hypothalamus was then disected out of part B by taking the anterior commisure as a horizontal reference and line between the posterior hypothalmus and the mammillary bodies as the caudal limit.

The striatum was then dissected out with the walls of the lateral ventricles as the internal limit and corpus collosum as the external limit. The frontal parts of the striatum were were also dissected out from part C. The midbrain was gently separated out from the remaining part of the brain and the hippocampus was carefully removed. The remainder of parts of B and C were then combined to form the corex.

4.5 Biochemical assay for monoamines.

Noradrenaline and dopamine concentrations in whole mouse brain (to

determine the extent of noradrenaline depletion after FLA-63) and in various rat brain regions (to determine the extent of LC lesion) were assayed spectrofluorometrically by the method of Chang (1964) as modified by Cox and Perhach (1973).

Brain samples were homogenised in 3 ml of cold, acidfied n-butanol. The homogenates were then shaken mechanically for 5 min and then centrifuged for 5 min at 3000 r.p.m. 2.5 ml of the supernatant was withdrawn and transferred to a centifuge tube containing 2.5 ml distilled water and 5.0 ml heptane. The tubes were then shaken for 5 min and centrifuged at 3000 r.p.m. for 5 min.

The organic layer was aspirated off and 2.5 ml of the aqueous phase was transferred to a screw-capped test tube containing 200 mg alumina; 1.0 ml of 2.0M sodium acetate containing 0.1% EDTA was added and the tubes were gently shaken for 5 min and then centrifuged at 3000 r.p.m. for 5 min. The aqueous phase was aspirated from the alumina.

The alumina was then washed by shaking gently with 2.0 ml distilled water for 2 min and centrifuged at 3000 r.p.m. for 5 min. The aqueous phase was discarded and 2.0 ml of 0.1N acetic acid were added to the alumina. The tubes were then shaken for 5 min and centrifuged at 3000 r.p.m.for 5 min; 1.0 ml of the aqueous phase was transferred to small test tube and 0.2 ml of 0.01% EDTA was added to this, after which the pH of the mixture was adjusted to 6.5 using 2N sodium hydroxide. Following this 0.1 ml of 0.1N iodine was added to the tube in order to oxidise the catecholamines. After exactly 2 min, 0.2 ml of alkaline sulphite

was added to the tube to stop the oxidation of the catecholamines. The pH of the solution was then adjusted to about 5.4 by the addition of 0.2 ml 5N acetic acid. The mixture was then heated in a boiling water bath for exactly 2 min after which the tubes were cooled over ice for 5 min. The mixture was then read at 385 nm excitation and 485 nm emission in an Aminco-Bowman spectrophotofluorimeter for noradrenaline. Following this, the solutions were heated in a boiling water bath for 3 min and fluorescence was read at320 nm excitation and 370 nm emission for dopamine.

Concentrations of noradrenaline and dopamine in whole mouse rat brain regions were calculated by running known standards and reagent blanks through the assay. Data was assessed for significance by paired t-test.

The average recoveries for the two catecholamines were:-

noradrenaline 70%

dopamine 65%

4.6 Comments.

The pH adjustment of the mixture to 6.5 after the elution of catecholamines of the alumina was found to be very important for dopamine.

4.7 General cleaning of the glassware.

Special attention was paid to the cleaning of glassware to ensure accurate results in the spectrofluorometric assay. General

glassware was soaked in Decon 90 (5% v/v solution prepaired from Decon 90 concentrate) for 24 hours. Several rinses were made with tap water and finally distilled water. Glassware was dried in a hot air oven.

5. Drug sources and vehicles used

Weights of all drugs expressed in text refer to the free base.

DRUG	SOURCE
gamma-aminobutyric acid	Sigma Ltd.
amino-oxyacetic acid	Sigma Ltd.
3-aminopropanesulphonic acid	Sigma Ltd.
*baclofen	Geigy Pharmaceuticals
bicuculline Methabromide	Sigma Ltd.
*butoxamine hydrochoride	Burroughs Wellcome
*carbidopa	Merck, Sharp and Dohme Ltd.
*7-chloro-1,3-dihydro-1-methyl-5-(4'-chlorophenyl)-2H-1,4-benzodiazepine-2-one (RO0-5-4864)	Roche Products Ltd.
diazepam (Valium)	Roche Products Ltd.
l-2,4-diaminobutyric acid	Sigma Ltd.
*Desmethylimipramine hydrochloride	Geigy Pharmaceuticals.
dobutamine hydrochoride	Lilly and Co. Ltd.
*ethyl-beta-carboline-	Glaxo Laboratories Ltd.

3-carboxylate (beta-CCE)	
*Ethyl 8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate (RO15-1788)	Roche Products Ltd.
6-hydroxydopamine hydrobromide	Sigma Ltd.
l-5-hydroxytryptophan (L-5-HTP)	Sigma Ltd.
*bis-[4-methyl-l-homopiperazinyl-thiocarbonyl]disulphide (FLA-63)	Astra Pharmacuetical Ltd.
*ICI 118,551	Imperial Chemical Industries.
imidazoleacetic acid	Sigma Ltd.
*iprindole hydrochloride	Wyeth Laboratories.
3-mercaptopropionic acid	Sigma Ltd.
5-methoxy-N,N-dimethytryptamine	Sigma Ltd.
*metoprolol tartrate	Geigy Pharmaceuticals
muscimol	Sigma Ltd.
pentobarbitone sodium (sagatal)	May and Baker Ltd.
pentylenetetrazol	Sigma Ltd.
*phenobarbitone	May and Baker Ltd.
picrotoxin	Sigma Ltd.
*practolol	Imperial Chemical Industries
*prenalterol hydrochoride	Astra Pharmaceuticals Ltd.
*procaterol hydrochoride	Warner-Lambert Co.
quipazine maleate	Miles Laboratories
*salbutamol base	Glaxo Laboratories Ltd.

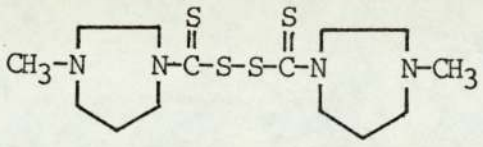
*Gift gratefully acknowledged.

Drugs were dissolved in saline (Ø.9 % w/v NaCl) except the following:-

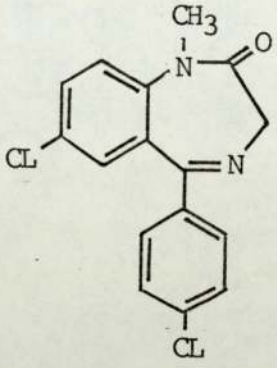
bicuculline	- dissolved in Ø.ØlN HCl.
diazepam	- commercial product "Valium" diluted in saline immediately before use.
RO15-1788	- suspended in saline with 2 drops of Tween 8Ø.
RØ5-4864	- suspended in saline with 2 drops of Tween 8Ø.
beta-CCE	- dissolved in saline by adding minimum amount of lN HCl.
pentobarbitone	- commercial product "Sagatal" diluted in saline immediately before use.
phenobarbitone	- dissolved in saline by adding minimum amount of lN NaOH.
L-5-HTP	- dissolved in saline by the addition of few drops of concentrated HCl and the pH brought to 7.Ø with NaOH.
FLA-63	- dissolved in saline by the addition of few drops of concentrated HCl and the pH brought to 7.Ø with NaOH.

6. Structure of drugs

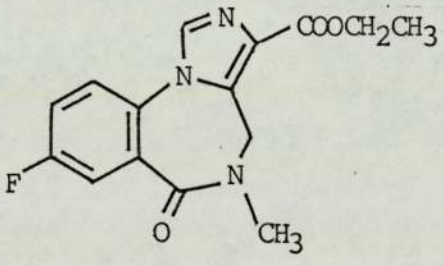
FLA-63



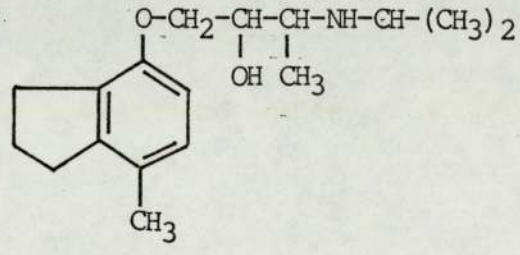
RO Ø-5-4864



RO15-1788



ICI 118,551



7.Reagents used in the biochemical assays.

Reagents used were of analar grade unless specified otherwise.

REAGENT	SOURCE
Acetic acid	BDH Chemical Ltd.
Alumina	Sigma Ltd.
n-Butanol	BDH Chemical Ltd.
Decon-90	BDH Chemical Ltd.
Ethanol (absolute)	BDH Chemical Ltd.
n-Heptane	BDH Chemical Ltd.
Hydrochloric acid (Hcl)	BDH Chemical Ltd.
Iodine	Sigma Ltd.
Sodium acetate	Sigma Ltd.
disodium-ethylene diamine tetra-acetate (EDTA)	Sigma Ltd.
Sodium sulphite (anhydrous)	Sigma Ltd.

(a) Acidified Butanol.

n-butanol containing 0.85 ml concentrated Hcl per litre.

(b) 0.01% EDTA solution.

Prepared by dissolving 37.2g of EDTA in 1M sodium acetate and made up to a volume of 1 litre. The pH was then adjusted to about 6.7-7.0 by the addition of sodium hydroxide.

(c) 0.1N iodine.

Prepared by dissolving 1.27g of iodine in 100 ml of absolute ethanol.

(d) Alkaline Sulphite.

1.0 ml of sodium sulphite solution (2.5g of anhydrous salt

dissolved in 10 ml of water) was diluted with 9.0 ml of 5N sodium hydroxide just before use.

(e) Alumina.

Approximately 200g of chromatographic grade alumina was refluxed in 1 litre of 1N Hcl for 30 min, then washed with about 20 changes of distilled water or until the the pH of the washings had risen to between 4 and 5. Finally, it was left to dry overnight at room temperature and then heated to 200 C for 2h.

CHAPTER 1.

TIME AND DOSE-EFFECT RELATIONSHIPS FOR HEAD-SHAKING BEHAVIOUR INDUCED BY L-5-HTP/CARBIDOPA AND 5-HT AGONISTS IN MICE AND RATS.

CHAPTER 1

Introduction

The experiments described in this chapter were carried out to determine the optimum protocol for studying drug effects on the head-twitch behaviour in mice and rats. In mice, head-twitch was induced by either 5-MeODMT or by preatment with carbidopa followed by L-5-HTP. In the rat it was induced with administration of quipazine.

In order to determine the optimum protocol, the following experiments were carried out:-

1. Dose response curves to quipazine (in rats), 5-MeODMT and combination of carbidopa and L-5-HTP (in mice).
2. The time course of the head-twitch induced by above drugs was also examined.

Results

1.1 L-5-HTP: time-effect relationship in mice.

The onset of the head-twitch response induced by 9.0 mg/kg (s.c.) carbidopa followed 15 min later by 180 mg/kg (i.p.) L-5-HTP occurred between the 2nd and the 5th min (Fig. 1.1a). Thereafter, the frequency of the response increased to reach a peak of about 12 responses / min between 20 and 25 min after the injection of L-5-HTP. The average response frequency fell to 1.6 responses / 10 min between 50 and 60 min after administration of L-5-HTP.

1.2 Carbidopa: dose-effect relationship in mice.

The head-twitch response induced by 180.0 mg/kg (i.p.) L-5-HTP increased as the dose of carbidopa was increased to reach a peak of 25 responses / 5 min with 12 mg/kg carbidopa (Fig. 1.1b). A further increase in the dose of carbidopa lowered the number of head-twitches observed. Head-twitches were counted for 5 min starting 20 min after L-5-HTP.

1.3 L-5-HTP: dose-effect relationship in mice.

L-5-HTP administered i.p. produced a dose-dependent increase in the number of twitches (Fig. 1.1c) reaching a maximum at 300 mg/kg in mice preteated 15 min before with 9.0 mg/kg (s.c.) carbidopa. Head-twitches were counted for 5 min starting 20 min after L-5-HTP.

1.4 5-MeODMT: time-effect relationship in mice.

The onset of the head-twitch response was within the first 2 min

after administration of 5-MeODMT (5.Ø mg/kg; i.p.). Head-twitch rates were maximal between 4 and 8 min after 5-MeODMT and occurred over a period of 15 min (Fig. 1.2a). For assessing drug effects an observation period of 12 min starting immediately after injection of 5-MeODMT was selected.

1.5 5-MeODMT: dose-effect relationship in mice.

As shown in Fig. 1.2b, 5-MeODMT produced a dose related increase in head-twitch frequency between 2.5 and 2Ø.Ø mg/kg (i.p.). Above 2Ø.Ø mg/kg signs of "5-HT hyperactivity syndrome" (Grahame-Smith, 1971) appeared and there was a decrease in the head-twitch frequency.

1.6 5-MeODMT: dose-effect relationship in rats.

Rats treated with doses ranging from Ø.25 to 1Ø.Ø mg/kg 5-MeODMT failed to show any wet dog shakes in the 45 min observation period starting immediately after administration of 5-MeODMT.

1.7 Quipazine: time-effect relationship in rats.

For these and dose: effect relationship (below 1.8) experiments the same 5 rats were used and at least a period of one week was allowed between successive runs.

The onset of the head-twitch response induced by quipazine (5.Ø mg/kg i.p.) occurred between the 3rd and 6th min and increased to reach a peak of about 8 twitches per 5 min between 45 and 55 min after injection of quipazine. The duration of the response was

over 70 min (Fig. 1.3a).

1.8 Quipazine: dose effect relationship in rats.

Quipazine produced a dose-dependent increase in the number of twitches reaching a maximum at 10 mg/kg. A further increase in the dose of quipazine produced inhibition of the head-twitch behaviour (Fig. 1.3b) and rats treated with 20 mg/kg quipazine showed behaviours of the "5-HT hyperactivity syndrome" (Grahame-Smith, 1971). The head-twitches were counted every other min between 30 and 60 min after quipazine.

Discussion

Selection of dose and time of carbidopa and L-5-HTP administration

The time-course of the L-5-HTP head-twitch was similar to that reported for the mouse by Corne et al. (1963). Carbidopa was used to inhibit peripheral L-amino acid decarboxylase, to minimise peripheral effects of 5-HT and maximise central effects (Modigh 1972). Care was needed in the choice of dose of carbidopa since, as shown in Fig. 1b, higher doses reduced the head-twitch, probably by blocking central as well as peripheral enzyme activity. Finally, the dose of 200 mg/kg L-5-HTP was chosen from its position in the middle of the linear portion of the dose-response curve.

Selection of dose and time of 5-MeODMT administration

To study drug effects a dose of 10 mg/kg (i.p.) and an observation period of 12 min starting immediately after injection of 5-MeODMT was selected.

Selection of dose and time of quipazine administration

The dose response curve obtained was similar to that described by Bedard and Pycock (1977) and Vetulani et al. (1980). The drug effects were studied in rats treated with 2.5 mg/kg (i.p.) quipazine. Head-twitches were counted every other min between 30 and 60 min after quipazine.

Head-twitching was induced with quipazine in the rat because this compound produced dose-dependent head-twitching. It was found that repeated L-5-HTP/carbidopa resulted in considerable mortality while 5-MeODMT did not produce head-twitching in the strain of rat used here. Quipazine has some undesirable properties. A large dose of quipazine (10.0 mg/kg) inhibited the spiperone induced catalepsy in rats (Frances et al., 1980). Studies demonstrated that in high doses (up to 50.0 mg/kg) it blocked MAO activity reversibly and competitively in rats brain (Rodriquez and Pardo, 1971; Green et al., 1976). Quipazine (10^{-5} M) in rat brain slices preincubated with either ^{3}H-5-HT or ^{3}H-noradrenaline caused an increase in electrically stimulated ^{3}H overflow; this effect was more predominant in brain slices preloaded with ^{3}H-5-HT. These effects on neurotransmitter release are believed to be due to antagonism of presynaptic inhibitory 5-HT and alpha-adrenoceptors on monoaminergic neurones (Schlicker et al., 1981). In mice, quipazine has been shown to induce head-twitching by two distinct mechanisms, firstly by a direct stimulation of 5-HT receptors and secondly by increasing the release of 5-HT from presynaptic neurones (Malick et al., 1977).

However, since its effects were potentiated by beta-adrenoceptor agonists and diazepam (see chapter 6) it appeared the most acceptable of the agents examined for use in this study.

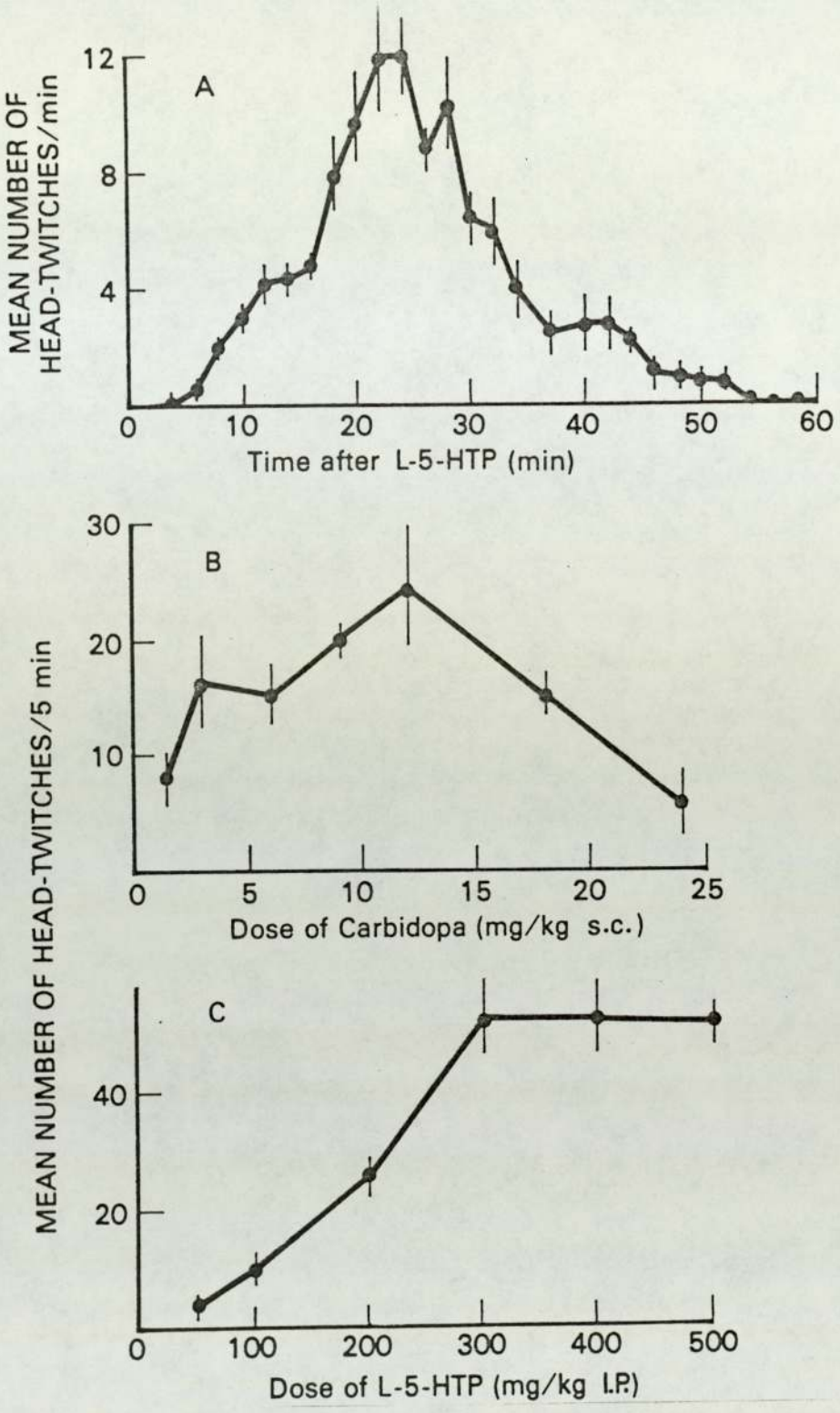


Fig. 1.1. L-5-HTP/carbidopa head-twitch response in mice.
A. L-5-HTP: time - effect relationship.
Mice (n = 5 per group) were injected with carbidopa (6 mg/kg s.c.) followed 15 min later by L-5-HTP (180 mg/kg i.p.). Head-twitches were counted every other min from 2 min to 60 min after L-5-HTP.
B. Carbidopa: dose - effect relationship.
Mice (n = 5 per group) were injected with carbidopa (1.5-24.0 mg/kg s.c.) followed 15 min later by L-5-HTP (180 mg/kg i.p.). Head-twitches were counted for 5 min starting 20 min after L-5-HTP.
C. L-5-HTP: dose - effect relationship.
Mice (n = 5 per group) were pretreated with 9 mg/kg (s.c.) carbidopa, 15 min before L-5-HTP (50-500 mg/kg i.p.) . Head-twitches were counted for 5 min starting 20 min after L-5-HTP.
Results are the means of 5 determinations and vertical bars represent s.e.m.

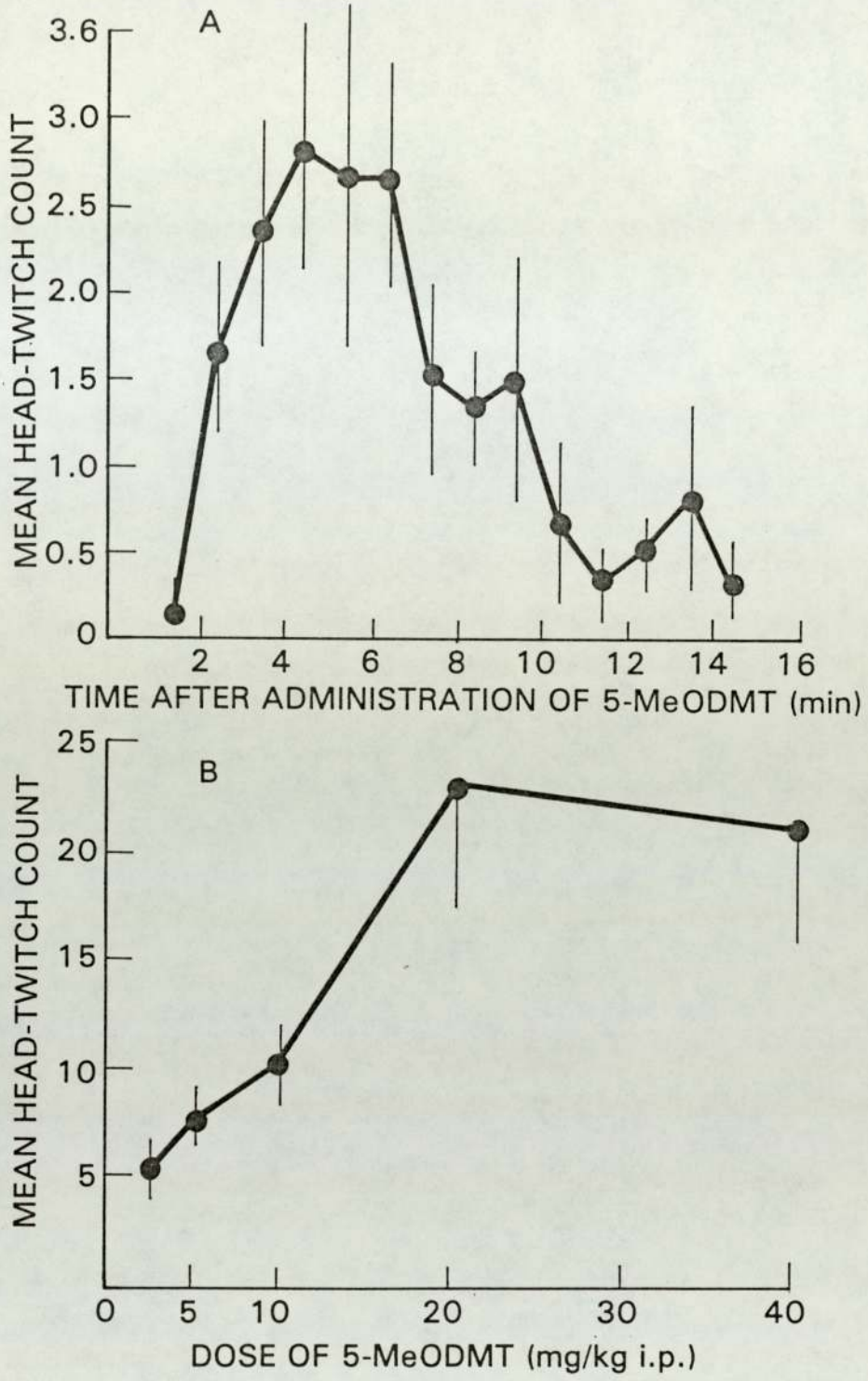


Fig. 1.2. 5-MeODMT head-twitch response in mice.
A. 5-MeODMT: time-effect relationship
Mice (n = 6 per group) were injected with 5-MeODMT (10 mg/ kg i.p.). Head-twitches were counted every other minute starting immediately after 5-MeODMT.
B. 5-MeODMT: dose-effect relationship
Mice (n = 6 per group) were injected with 5-MeODMT (2.5-40 mg/kg i.p.). Head-twitches were counted every other min between 4 min and 8 min after 5-MeODMT.

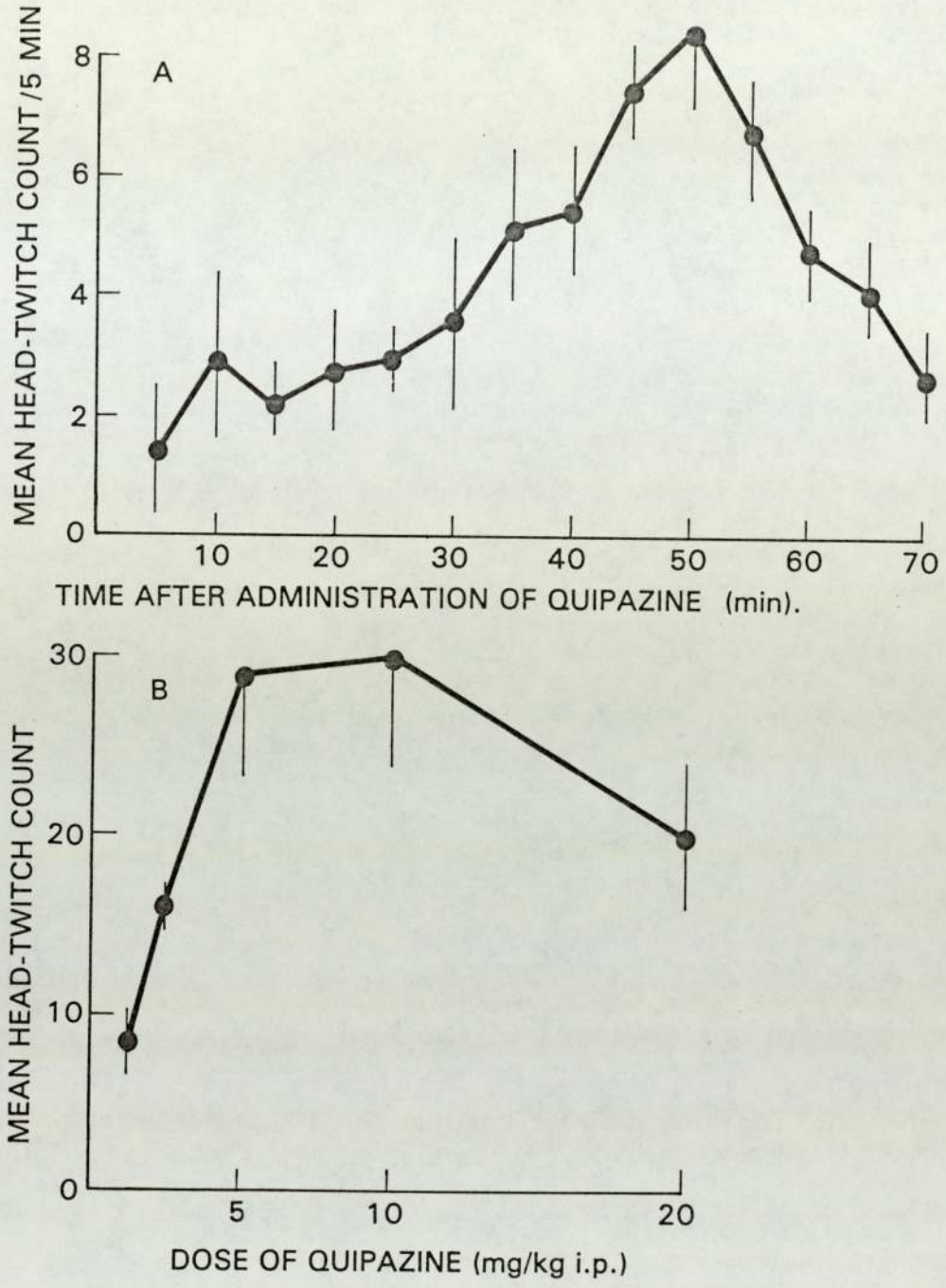


Fig. 1.3. Quipazine head-twitch response in rats.
A. Quipazine: time-effect relationship
Unoperated rats (n of runs = 5) were injected with quipazine (5.0 mg/kg i.p.). Number of head-twitches were counted for 70 min starting immediately after administration of quipazine.
B. Quipazine: Dose-effect relationship
Unoperated rats (n of runs = 5) were injected with quipazine (0.25-20 mg/kg i.p.). Head-twitches were counted every other min between 30 min and 60 min after quipazine.

CHAPTER 2.

THE EFFECT OF $BETA_1$- AND $BETA_2$-ADRENOCEPTOR AGONISTS AND ANTAGONISTS ON L-5-HTP INDUCED HEAD-TWITCH IN MICE.

CHAPTER 2

Introduction

It has been reported that selective $beta_2$-adrenoceptor agonists potentiate the 5-HT mediated head-twitch response in mice but the effect of selective $beta_1$-adrenoceptor agonists has not been examined (see Introduction for references). So it is not clear whether the potentiating effect of $beta_2$-adrenoceptor agonists is mediated through $beta_1$- or $beta_2$-adrenoceptors. Furthermore, it has not been established if both subtypes of beta-adrenoceptors are involved in modulation of 5-HT mediated head-twitch behaviour.

This work was undertaken in an attempt to characterise the beta-adrenoceptor mediated potentiation of the head-twitch response in mice, by the use of selective beta-adrenoceptor agonists and antagonists.

Results

For the results described below, mice received carbidopa(9.0 mg/kg s.c.) followed 15 min later by L-5-HTP (200.0 mg/kg i.p.). The beta-adrenoceptor agonists (dobutamine, prenalterol, procaterol and salbutamol) were injected (s.c.) simultaneously with carbidopa. The beta-adrenoceptor antagonists (butoxamine, ICI 118,551, metoprolol and practolol) were injected (s.c) immediately before the agonists. However, practolol when administered i.c.v., was injected immediately before L-5-HTP. For further details see under Experimental Methods.

1.1 Effect of beta-adrenoceptor agonists and antagonists on L-5-HTP head-twitch.

Potentiation of the head-twitch behaviour was caused by both $beta_1$- (dobutamine and prenalterol) - and $beta_2$- (procaterol and salbutamol) adrenoceptor agonists (Table 1). However, none of the beta- adrenoceptor agonists, administered alone in doses of up to fifteen times their ED_{200} values (dobutamine 20 mg/kg; prenalterol 100 mg/kg; procaterol 60 mg/kg; salbutamol 20 mg/kg), induced the head-twitch response. Neither was any sign of increased locomotion, headweaving, hindlimb abduction, straub tail or forepaw treading observed in any animal pretreated with these agonists. None of the beta - adrenoceptor antagonists (metoprolol 5.0 mg/kg; practolol 10.0 ug i.c.v.; butoxamine 10.0

mg/kg; ICI 118,551 5.0 mg/kg), used caused a significant decrease in the L-5-HTP response in the absence of the agonists (Fig. 1).

1.2 Interaction of beta-adrenoceptor agonists and antagonists.

For these experiments the agonists were injected at doses close to their ED_{200} values (dobutamine 2.5 mg/kg; prenalterol 10.0 mg/kg; procaterol 5.0 mg/kg; salbutamol 0.25 mg/kg) for potentiating the head-twitch. Practolol (10.0 ug) was active against all four beta-adrenoceptor agonists when it was administered i.c.v. (Table 2). There was no indication of any selectivity in its effects; over a narrow dose range (5.0-10.0 ug i.c.v.) the response progressed from no effect to significant inhibition of all four agonists. Neither did the degree of inhibition bear any relationship to whether the agonist was $beta_1$- or $beta_2$- adrenoceptor selective. Practolol (10.0 mg/kg s.c.) was found to be inactive against all the agonists tested during its peripheral administration (Fig. 3). Metoprolol (1.25-5.0 mg/kg), like i.c.v. practolol, reduced the potentiating effects of all the four beta-adrenoceptor agonists, although this beta-adrenoceptor antagonist was investigated in less detail (Table 2). Thus the lowest dose of metoprolol (2.5 mg/kg) which inhibited the effect of prenalterol and dobutamine produced a similar degree of inhibition of procaterol and salbutamol.

Butoxamine (2.5-10.0 mg/kg), produced a dose-dependent reduction in the head-twitch potentiated by both procaterol and salbutamol (Table 3), yet the highest dose (10.0 mg/kg) was ineffective against either prenalterol or dobutamine. ICI 118,551 (1.25-5.00 mg/kg) similarly reduced procaterol and salbutamol potentiation in a dose dependent manner (Table 3) but a dose (2.5 mg/kg) producing 50% inhibition of the head-twitch when potentiated by these agonists was entirely without effect against prenalterol and dobutamine potentiation. These results are summarized in Table 3.

Discussion

All four beta-adrenoceptor agonists potentiated the head-twitch with a potency order salbutamol > dobutamine > procaterol > prenalterol. This potency order shows no obvious relationship to beta-adrenoceptor selectivity, but may be affected by relative penetration of the brain. Studies carried out *in vivo* have reported that dobutamine and prenalterol show selectivity for $beta_1$-adrenoceptors (Carlsson et al., 1977; Sonnenblick et al., 1979). However, dobutamine isomers have recently been reported to have a complex action on both alpha-, $beta_1$ and $beta_2$-adrenoceptors in vascular smooth muscle (Ruffolo and Yaden, 1983). Furthermore, salbutamol has been shown to be a selective agonist at $beta_2$-adrenoceptors both *in vivo* and in a variety of *in vitro* preparations (Brittain et al., 1968). Procaterol *in vitro* also has selectivity for $beta_2$-adrenoceptors(Yabuuchi, 1977; O'Donnell and Wanstall, 1985).

It is possible that part of the potentiation seen with dobutamine was due to stimulation of $alpha_1$-adrenoceptors. This was not investigated by using selective $alpha_1$-adrenoceptor antagonists, because blocking of these receptors prevents the occurrence of 5-HT mediated head-twitching in mice (Handley and Brown, 1982).

None of the beta-adrenoceptor agonists induced head-twitching when given alone. Furthermore, none of the antagonists reduced this response in the absence of the beta-adrenoceptor agonists. These results indicate that stimulation of beta-adrenoceptors does not lead to initiation of head-twitching and furthermore, the antagonism of these receptors fails to reduce the L-5-HTP induced behaviour. The failure of selective beta-adrenoceptor antagonists to block the L-5-HTP induced head-twitch response also indicates that as *in vitro* (Green et al., 1983b; Nahorski and Willcocks, 1983), these drugs do not bind directly to the 5-HT_2 receptor *in vivo*.

The related 5-HT receptor mediated hyperactivity syndrome in the rat is also potentiated by the $beta_2$-adrenoceptor agonists clenbuterol, salbutamol and terbutaline (Cowen et al.,1982). As with the mouse head-twitch response, the effect of $beta_2$-adrenoceptor agonists was prevented by metoprolol, however in the rat hyperactivity syndrome $beta_2$-adrenoceptor antagonist butoxamine was ineffective.

Practolol shows 9-fold selectivity, in conscious cats and dogs as well as in binding assays, for $beta_1$-adrenoceptors (Dunlap and Shanks, 1968;Leclerc et al., 1981). *In vivo* metoprolol is also selective for this receptor (Ablad et al., 1973). Conversely, *in*

vitro, in binding assays butoxamine has 5 -fold selectivity (Leclerc et al., 1981), and ICI 118,551 in isolated preparations is much more selective, for $beta_2$-adrenoceptors (O'Donnell and Wanstall, 1980). Thus the finding that dobutamine and prenalterol showed a dose-dependent inhibition after practolol or metoprolol, but were unaffected by butoxamine and ICI 118,551, demonstrates that these agonists were acting on $beta_1$- rather than $beta_2$-adrenoceptors. Since these doses of butoxamine and ICI 118,551 were not effective against $beta_1$-adrenoceptor agonists, their inhibition of salbutamol and procaterol shows that the latter agonists exerted their potentiation at least in part through $beta_2$-adrenoceptors. From this, it may be concluded that activation of both $beta_1$- and $beta_2$-adrenoceptors has a potentiating effect on the head-twitch caused by L-5-HTP.

Practolol penetrates the blood brain barrier poorly (Day et al., 1977). Its failure to block the potentiation due to any of the agonists when administered peripherally indicates that the agonists produce potentiation by a central effect.

Butoxamine and ICI 118,551 showed effects consistent with selective blockade of $beta_2$-adrenoceptors but practolol and metoprolol however showed no selectivity whatsoever. Either all effective doses of practolol and metoprolol blocked both $beta_1$-

and $beta_2$-adrenoceptors or the potentiating effect of $beta_2$ adrenoceptor activation is exerted ultimately by releasing noradrenaline onto $beta_1$-adrenoceptors. The possibility of an indirect action of $beta_2$-adrenoceptor agonists is not without support. Receptors of $beta_2$-subtype have been found on noradrenergic terminals and appear to be involved in facilitation of noradrenaline release (Westfall,1977; Dhalof et al., 1981; see Misu and Kubo, 1983). This possibilty is further investigated in chapter 6.

However, salbutamol and clenbuterol still potentiated the 5-HT hyperactivity syndrome in the rat following 6-hydroxydopamine (6-OHDA) lesions of noradrenergic pathways (Ortmann et al. 1981; Nimgaonkar et al. 1983). This 5-HT induced syndrome in the rat is mediated by both $5\text{-}HT_1$ and $5\text{-}HT_2$ receptors whereas the head-twitch response in mice involves only the $5\text{-}HT_2$ receptor (see Green and Heal 1985). Therefore, it is possible that 5-HT head-twitching in mice and 5-HT induced hyperactivity syndrome in the rat involve different neuronal pathways in the brain. The failure of 6-OHDA lesions to abolish the potentiating effects of $beta_2$-adrenoceptor agonists in the rat hyperactivity syndrome (Ortmann et al. 1981; Nimgaonkar et al. 1983) may indicate either that the doses of these agonists used in the above studies were acting non selectively on both $beta_1$- and $beta_2$-adrenoceptors or in contrast

to head-twitching in the mouse, $beta_2$-adrenoceptors involved in potentiation of behaviours in the rat are located postsynaptically.

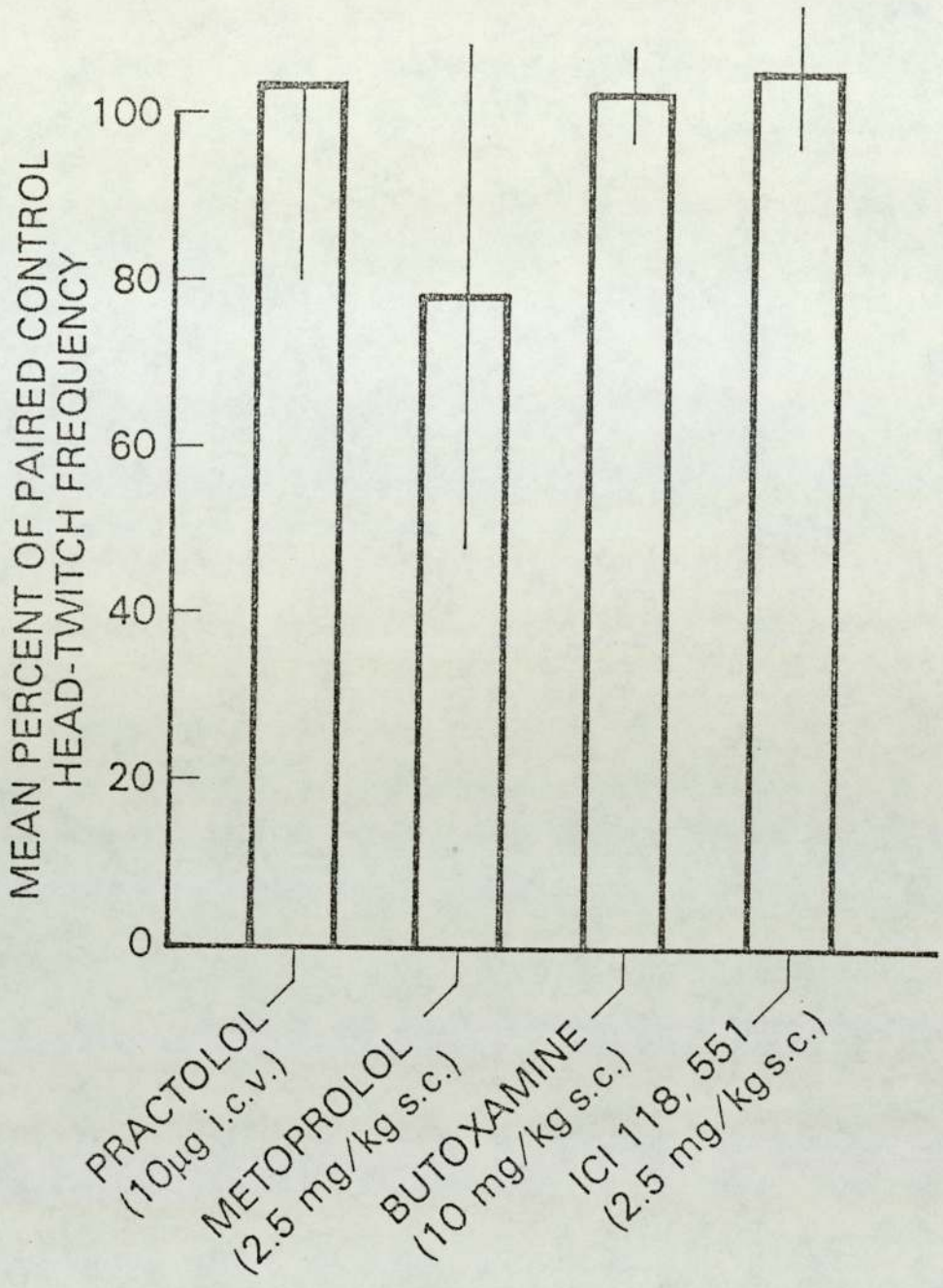


Fig. 2. 1. Effect of beta-adrenoceptor antagonists on the L-5-HTP head-twitch.
Mice received either the antagonist or saline s.c. 15 min before L-5-HTP (200 mg/kg i.p.) and carbidopa (9.0 mg/kg s.c.). Head-twitch frequency for each test mouse was recorded as percent of its paired control. Results are the means of at least 5 determinations and vertical bars represent s.e.m.
* $P < 0.05$ (test vs control values, paired t-test).

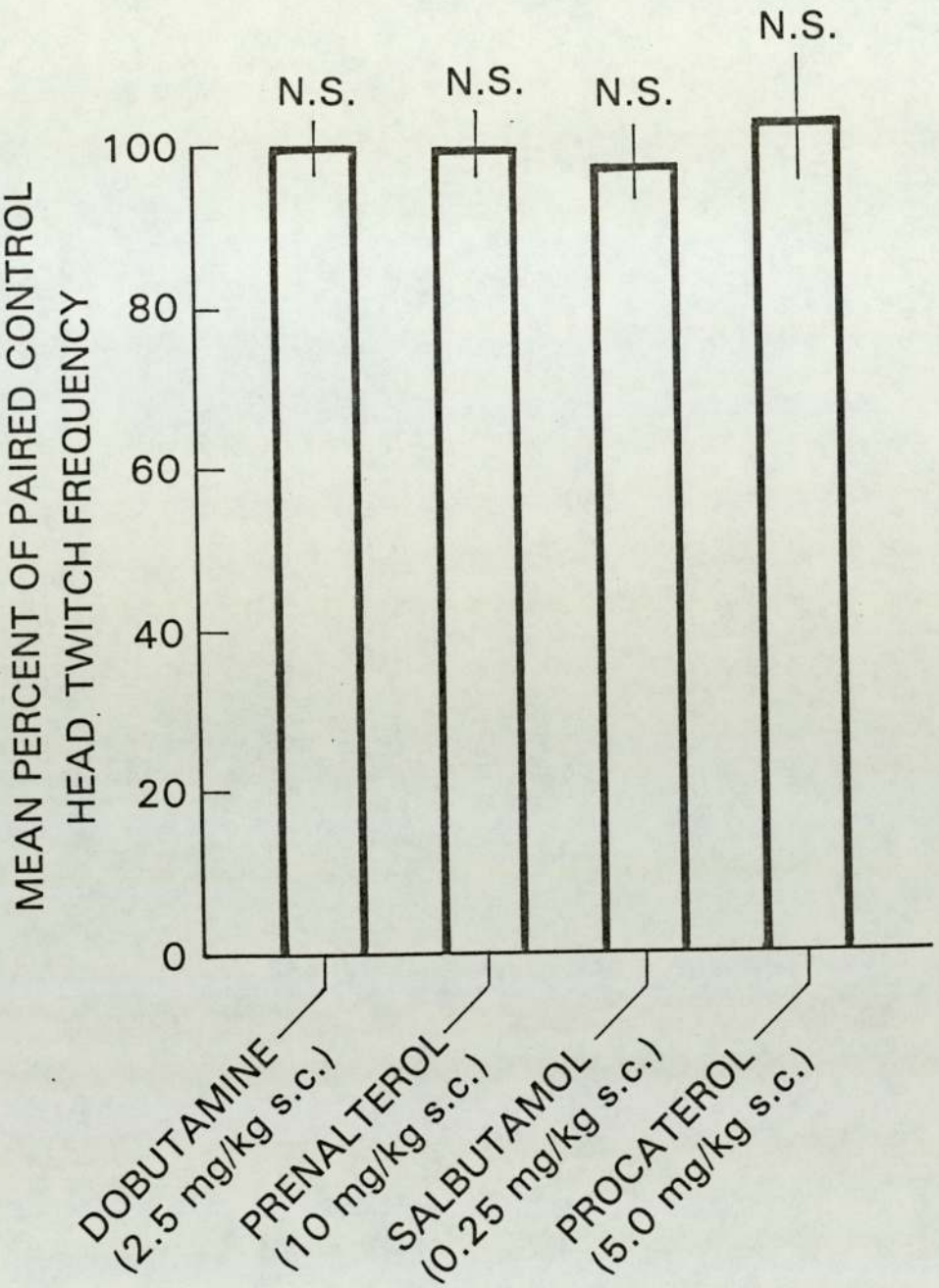


Fig. 2.2. Effect of practolol administered peripherally on the L-5-HTP head-twitch in the presence of beta-adrenoceptor agonists.
All mice received L-5-HTP (200 mg/kg i.p.) and carbidopa (9.0 mg/kg s.c.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for each test mouse was recorded as percent of its paired control. `Control' mice received beta-adrenoceptor agonist plus saline and `test' mice received beta-adrenoceptor agonist plus practolol 10 mg/ kg s.c. Results are the means of at least 5 determinations and vertical bars represent s.e.m.
N.S. not significant $P > 1.0$ (test vs control values, paired t-test).

		Linear regression (Log dose)			
Drug	Dose range tested (mg/kg)	P	r	ED_{200} (mg/kg)	95% confidence limits (mg/kg)
Dobutamine	0.5-5.0	<0.013	0.901	1.53	1.36-1.74
Prenalterol	5.0-20.0	<0.001	0.952	8.45	7.52-9.48
Procaterol	2.5-10.0	<0.001	0.925	4.50	3.90-5.19
Salbutamol	0.05-5.0	<0.002	0.900	0.12	0.06-0.24

P = Significance of least squares fit.

r = Correlation coefficient of least squares fit.

ED_{200} = dose to increase twitches to 200% of control levels.

Table 2.1. ED_{200} values of beta-adrenoceptor agonists for potentiating the L-5-HTP head-twitch response.

ANTAGONIST	prenalterol (10 mg/kg)	dobutamine (2.5 mg/kg)	procaterol (5.0 mg/kg)	salbutamol (0.25 mg/kg)
practolol (ug icv)				
5.0	92.1±1.6	97.9±4.9	93.4±3.2	93.2±33.5
7.5	82.4±1.2	78.3±5.3	91.1±2.3	75.9±7.9
10.0	64.2±3.5**	27.0±4.7*	49.6*1.6**	38.6±10.6*
metoprolol (mg/kg)				
1.25	95.2±5.9	71.6±42.2	ND	ND
2.50	53.0±3.6**	35.7±4.6*	44.4±4.6**	46.4±8.1*
5.00	40.4±4.8**	32.0±8.4*	ND	ND

Table 2.2 The effect of $beta_1$-adrenoceptor antagonists on the potentiated head-twitch.

All mice received beta-adrenoceptor agonist. Paired test and control mice received antagonist or vehicle respectively. Results are expressed as the head-twitch count for test mouse as % of paired control meaned over at least 6 runs (± s.e.m.).

ND-not determined. * $P < 0.05$; ** $P < 0.01$ (test vs control values, paired t-test).

ANTAGONIST	prenalterol (10 mg/kg)	dobutamine (2.5 mg/kg)	procaterol (5.0 mg/kg)	salbutamol (0.25 mg/kg)
butoxamine (mg/kg)				
2.5	ND	ND	91.6±4.6	90.6±32.0
5.0	ND	ND	79.4±3.8*	80.0±40.2
10.0	102.4±2.3	128.9±15.0	41.0±2.8**	18.9±4.2*
ICI 118,551 (mg/kg)				
1.25	ND	ND	71.2±2.8**	77.2±7.0*
2.50	105.2±3.3	132.5±25.5	52.2±3.8**	48.0±2.1*
5.00	ND	ND	39.2±1.7**	32.4±1.7*

Table 2.3 The effect of $beta_2$-adrenoceptor antagonists on the potentiated head-twitch.

All mice received beta-adrenoceptor agonist. Paired test and control mice received antagonist or vehicle respectively. Results are expressed as the head-twitch count for test mouse as % of paired control meaned over at least 6 runs (± s.e.m.).

ND-not determined. * $P < 0.05$; ** $P < 0.01$ (test vs control values, paired t-test).

Table 2.4. Effect of beta- adrenoceptor antagonists on the potentiation by beta- adrenoceptor agonist of the head - twitch response to L-5-HTP / carbidopa.

	AGONIST	
ANTAGONIST	$beta_1$	$beta_2$
$beta_1$	blocked	blocked
$beta_2$	no effect	blocked

CHAPTER 3

EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENT ON *IN VIVO* CENTRAL BETA-ADRENOCEPTOR SENSITIVITY

CHAPTER 3

Introduction

The common end result of long term (10 to 21 days) administration of all clinically active antidepressant treatments, including electroconvulsive therapy is a decrease in the activity of beta-adrenoceptor linked adenyl cyclase and for the majority of treatments this appears to be a consequence of "down-regulation" of cortical beta-adrenoceptor density as measured by radioligand receptor binding studies (see General Introduction for references). However, receptor subsensitivity does not necessarily imply functional subsensitivity (Peroutka and Snyder, 1980a; see Green and Nutt, 1983), since the reduced beta-adrenoceptor density would only be expected to produce functional effects in the absence of a substantial pool of "spare receptors" (Stephenson, 1956; Moran, 1969). However, it has not been possible to investigate this question directly *in vivo* because beta-adrenoceptor agonists do not produce obvious or quantifiable behavioural effects when given alone.

Although they are ineffective alone in inducing head-twitching, both $beta_1$- and $beta_2$-adrenoceptor agonists do produce a marked potentiation of L-5-HTP induced head-twitch in mice (Chapter 2). Investigation of the head-twitch and its potentiation by beta-adrenoceptor agonists should thus be capable of giving information on the *in vivo* sensitivity of both beta-adrenoceptors and $5\text{-}HT_2$ receptors to agonists after chronic administration of

antidepressants. Therefore, the experiments described in this Chapter were conducted to determine whether doses of antidepressants reported to decrease the activity of beta-adrenoceptor linked adenyl cyclase system, also leads to subsensitivity of beta-adrenoceptors *in vivo*.

Two antidepressants which are known to affect beta-adrenoceptor density (Banerjee et al., 1977) were chosen for investigation. Desmethylimipramine (DMI) is a classic monoamine uptake inhibitor (Glowinski and Axelrod, 1964) while iprindole (IPD) has little effect on monoamine systems (Ross et al., 1971). Their effects on the sensitivity of three beta-adrenoceptor agonists were investigated, prenalterol and dobutamine which have selective effects at the $beta_1$- subtype and salbutamol which has preferential effects at $beta_2$-adrenoceptors (see Chapter 2 for references).

Results

For the results described below, mice received either a single dose (administered at 09.30 hrs) or 21 days of twice-daily (administered at 09.30 hrs and 17.00 hrs) dosing with either DMI or IPD 10.0 mg/kg (i.p.). Control groups received vehicle (saline) to the same regimen. 48h after the final dose, pairs of mice were taken at random from antidepressant and vehicle groups, and placed in two cages each containing a third untreated mouse (see Experimental Methods for further details). One of each pretreated pair received one of the beta-adrenoceptor agonists (dobutamine 2.5 mg/kg; prenalterol 10.0 mg/kg; salbutamol 0.125, 0.250 and 0.500 mg/kg; all were injected s.c.) and the other saline (s.c.); simultaneously both mice were pretreated with carbidopa 9.0 mg/kg (s.c.) followed 15 min later by L-5-HTP 200 mg/kg (i.p.). The head-twitch frequency was observed in parallel for each quartet for 5 min starting 20 min after L-5-HTP.

Statistics

Results were analysed by 2 x 2 factorial analysis of variance followed by Tukey's test for unconfounded means. The significance of antidepressant efffects on beta-adrenoceptor potentiation of the head-twitch response was determined from the F value of the interaction term ($F_{[int]}$) in the analysis of variance.

1.1 Effect of single doses of DMI or IPD

Forty eight hours after a single dose of DMI or IPD, the frequency of L-5-HTP-induced head-twitching was unchanged ($P > 0.5$

in each case). The potentiating effects of prenalterol, dobutamine and salbutamol were also not significantly affected ($F_{[int]} < 3.1$, $P > 0.05$ in each case). These results are shown in Table 3.1.

1.2 Effects of chronic administration

Twice daily administration of DMI or IPD for 21 days was also without significant effect on the control head-twitch ($P > 0.1$ in each case). On the other hand, chronic dosing with both antidepressants reduced the potentiating effect of all three beta-adrenoceptor agonists ($F_{[int]} > 5.1$, $P < 0.05$ in each case). Three doses of salbutamol were examined and the percentage reduction was similar for each dose (Table 3.2).

Discussion

Acute administration of certain tricyclic antidepressants can modify the head-twitch response (Ogren et al., 1979; Friedman et al., 1983) but as reported previously for DMI and IPD (Friedman et al., 1983) such effects did not occur at 48h after dosing. This indicates that circulating drug levels had fallen below directly effective concentrations by this time. The lack of effect of chronic DMI or IPD on baseline head-twitch frequency to L-5-HTP confirms that this was also the case for chronic dosing.

For the chronic experiments, the antidepressants were administered in a regimen which has been shown to cause beta-adrenoceptor 'down-regulation' for both compounds (Banerjee et al., 1976;Minneman et al., 1979a; Peroutka and Snyder, 1980b). This regimen caused a reduction in sensitivity to beta-adrenoceptor agonists in terms of their ability to potentiate the head-twitch. It is likely that the reduced sensitivity was caused by a reduction in beta-adrenoceptor density in the absence of a large pool of 'spare' receptors (Stephenson, 1956; Moran, 1969). The reduced sensitivity extended to all three beta-adrenoceptor agonists. It has been suggested that it is only the $beta_1$-adrenoceptor which is down-regulated by antidepressants (Minneman et al.,1979a), but this is not certain; the $beta_2$-adrenoceptor agonist salbutamol has been shown to be a clinically effective antidepressant (Jouvent et al., 1977; Simon et al., 1978; Lecrubier et al., 1980) and long term administration of the more liposoluble $beta_2$-adrenoceptor agonist clenbuterol reduces beta-

adrenoceptor density (Hall et al., 1980).

The failure of chronic antidepressant treatment to modify the control head-twitch rate is also of some considerable interest. Previous studies have reported increases and decreases in both head-twitch behaviour and cortical 5-HT_2 receptor density (see Introduction for further details). As suggested by Green et al. (1983a) this discrepancy observed in 5-HT_2 receptor binding studies could be due to the different doses, number of administrations of the antidepressant drug and withdrawal times used in various studies before examining binding. This could also be true for behavioural studies.

In the present experiments, 48h after the last dose, neither chronic DMI nor IPD affected the L-5-HTP head-twitch. This could indicate that there was no change in 5-HT_2-receptor sensitivity. Before drawing this inference however, it is necessary to consider the implications of the reduced sensitivity of beta-adrenoceptors. Since the head-twitch is potentiated by beta-adrenoceptor agonists, should it not be reduced when these receptors become subsensitive? There is in fact clear evidence that this would not be the case. The influence of beta-adrenoceptors seems to be entirely facilitatory; beta-adrenoceptor antagonists have no effect whatever on head-twitching unless this has already been potentiated by a beta-adrenoceptor-dependent mechanism (see General Introduction and Chapter 2). This is precisely the situation seen here. Chronic

antidepressants were entirely without effect on the L-5-HTP head-twitch unless this was potentiated by a beta-adrenoceptor agonist.

	TREATMENTS			
BETA-AGONIST	1	2	3	4
(mg/kg)		ACUTE DMI		
dobutamine				
2.5	21.4±3.5	53.6±16.1	31.0±6.1	80.4±7.0
prenalterol				
10.0	16.5±7.4	31.0±8.4	26.2±10.9	51.0±13.4
salbutamol				
0.5	13.8±3.7	42.2±8.5	25.2±3.0	58.4±10.9
		ACUTE IPD		
dobutamine				
2.5	21.8±3.9	41.4±4.4	30.2±5.9	60.2±13.9
prenalterol				
10.0	21.0±4.3	39.3±8.3	34.2±12.8	62.8±13.6
salbutamol				
0.5	42.8±6.8	74.8±6.9	43.0±5.4	80.0±6.2

TREATMENTS

1= saline + saline; 2= saline + beta-adrenoceptor agonist

3= DMI or IPD + saline; 4= DMI or IPD + beta-adrenoceptor agonist

Results are represented as a mean of at least 5 runs ±s.e.m.

Table 3.1 The effect of acute administration of DMI and IPD on the head-twitch potentiation by beta-adrenoceptor agonists.

BETA-AGONIST	TREATMENTS			
(mg/kg)	1	2	3	4
dobutamine		CHRONIC DMI		
2.50	34.6±10.9	104.8±12.9	45.6±10.0	79.4±8.6
prenalterol				
10.0	26.2±1.9	96.7±3.2	40.0±1.8	65.6±2.6
salbutamol				
0.125	28.5±9.8	58.0±5.0	41.2±8.9	48.5±7.2
0.250	40.8±9.3	98.4±2.1	42.8±12.4	70.8±11.7
0.500	31.8±6.3	110.4±7.2	45.0±8.1	84.2±5.7
dobutamine		CHRONIC IPD		
2.50	27.5±3.9	89.7±4.8	28.8±7.0	62.3±3.0
prenalterol				
10.0	24.7±8.3	88.7±3.5	40.0±3.2	60.5±9.1
salbutamol				
0.125	24.0±8.2	93.8±11.5	23.8±8.4	60.5±11.5
0.250	33.3±7.0	98.3±2.2	25.2±5.1	76.0±5.9
0.500	32.8±3.0	118.3±10.4	25.8±3.8	87.0±6.3

TREATMENTS

1= saline + saline; 2= saline + beta-adrenoceptor agonist

3= DMI or IPD + saline; 4= DMI or IPD + beta-adrenoceptor agonist

Results are represented as a mean of at least 5 runs ±s.e.m.

Table 3.2 The effect of chronic administration of DMI and IPD on the head-twitch potentiation by beta-adrenoceptor agonists.

CHAPTER 4

MODULATION OF L-5-HTP-INDUCED HEAD-TWITCH BEHAVIOUR IN MICE BY DRUGS ACTING AT GABA AND RELATED RECEPTORS

CHAPTER 4

Introduction

As discussed in the General Introduction, GABA itself, the $GABA_A$ receptor agonist muscimol and certain benzodiazepines induce head-twitching. The GABA and benzodiazepine initiating effects were prevented by 5-HT antagonists, so appear to involve a 5-HT link. Furthermore, benzodiazepines, such as diazepam, potentiated the 5-HT induced head-twitching in mice. At the time, the effects of benzodiazepines were attributed to a direct action at 5-HT receptors (Nakamura and Fukushima, 1976). However, the close association of barbiturate and benzodiazepine receptors with the GABA receptor has since been elucidated (see General Introduction). The head-twitch inducing effects of GABA, muscimol and the benzodiazepines therefore point to a potential role for GABA receptors in the control of this behaviour. The potential involvement of GABA in modulating the 5-HT mediated head-twitch behaviour has so far been little studied.

The work described in this chapter investigated the effects of a range of drugs acting at GABA receptors and at other receptors linked to the GABA-chloride ionophore complex. These agents were tested for their ability to induce head-twitching when given alone and for their ability to modulate L-5-HTP induced head-

twitching behaviour in mice

For the results described below, drugs were injected (i.p.) simultaneously with carbidopa (9.Ø mg/kg s.c.) and followed 15 min later by L-5-HTP (2ØØ.Ø mg/kg i.p.; exceptions given in Table 4.1). Twitches were counted for 5 min starting 2Ø min post L-5-HTP. The highest dose of each drug which potentiated the L-5-HTP induced head-twitch was administered alone to determine if it induced twitching on its own. Mice were observed for 3Ø min immediately after injection.

Reminder of abbreviations

amino-oxyacetic acid (AOAA), L-2,4-diaminobutyric acid (DABA), 3-mercaptopropionic acid (3-MPA), muscimol, imidazoleacetic acid (IMAA), 3-aminopropanesulphonic acid (3-APS), pentylenetetrazol (PTZ), ethyl-beta-carboline-3-carboxylate (beta-CCE).

Results

1.1 Drugs potentiating the head-twitch response to L-5-HTP

These comprised the GABA synthesis blocker 3-MPA (2.5-10.0 mg/kg), the $GABA_A$ receptor agonists muscimol (0.12-0.50 mg/kg), 3-APS (25.0-200.0 mg/kg) and IMAA (02.5-50.0 mg/kg), and the benzodiazdpine receptor ligands diazepam (0.24,1.0 mg/kg) and RO05-4864 (5.00-10.0 mg/kg). The dose range of 3-MPA tested was limited by the occurrence of convulsions at 15 mg/kg and the doses of IMAA and diazepam which could be tested were limited by sedation, however there was no loss of righting reflex at the highest doses of these latter drugs. Table 4.2 shows ED_{200} values of these drugs, for potentiating the L-5-HTP induced head-twitch response.

ED_{200} values could not be obtained for picrotoxin because convulsions occurred at 1.0 mg/kg; there was significant potentiation at 0.5 mg/kg (fig. 4.1) and lower doses had no significant effect.

1.2 Drugs inhibiting the head-twitch response to L-5-HTP

These comprised the GABA metabolism inhibitor AOAA (6.0-24.0 mg/kg) and the GABA uptake blocker DABA (5.0-20.0 mg/kg) as well as GABA itself (12.5-100 ug i.c.v.), the $GABA_B$ receptor agonist baclofen (1.25-5.0 mg/kg), the benzodiazepine receptor ligands

beta-CCE (20.0-80.0 mg/kg) and RO15-1788 (2.5-10.0 mg/kg) and the barbiturate phenobarbitone (15.0-60.0 mg/kg). Mice given 60.0 mg/kg phenobarbitone were heavily sedated but loss of righting reflex did not occur at this dose and a lower dose (7.5 mg/kg i.p.) of this barbiturate had no significant effect on the head-twitch response. Table 4.3 shows ID_{50} values of these drugs for inhibiting the L-5-HTP induced head-twitch response.

No dose response curve could be obtained for bicuculline because of convulsions occurring at 1.0 mg/kg; 0.5 mg/kg reduced the head-twitch (fig. 4.1) but lower doses were ineffective.

1.3 Drugs with biphasic effects on the head-twitch response to L-5-HTP

Low doses of pentobarbitone (3.75 to 15 mg/kg) potentiated the head-twitch as well as causing obvious excitement, whereas doses of 25 to 60 mg/kg reduced it (fig. 4.2). These higher doses were associated with sedation but the righting reflex was still present.

Pentylenetetrazol also produced biphasic effects with potentiation appearing at 20 mg/kg and inhibition at 40 mg/kg (fig. 4.3). Convulsions occurred at 50 mg/kg.

1.4 Drug effects alone

Only muscimol induced head-twitching when administered alone; 1.Ø mg/kg produced Ø.66 twitches/min over a observation period of 3Ø min immediately after injection. Other drugs (3-MPA 1Ø mg/kg; IMAA 5Ø.Ø mg/kg; 3-APS 2ØØ.Ø mg/kg; diazepam 1.ØØ mg/kg; ROØ5-4864 1Ø.Ø mg/kg) which potentiated the L-5-HTP induced head-twitching failed to initiate this behaviour when administered alone.

Discussion

Selective agonists for $GABA_A$ and $GABA_B$ receptors had opposite effects on head-twitch frequency. Thus the $GABA_B$ agonist baclofen (Hill and Bowery, 1981; Bowery et al., 1983a; 1983b) inhibited the head-twitch, while the $GABA_A$ receptor agonists muscimol, 3-APS and IMAA (Krogsgaard-Larsen et al., 1977; Hill and Bowery, 1981) produced potentiation. Both effects were dose related. The inhibitory effect of the $GABA_A$ receptor antagonist bicuculline (Curtis et al., 1970; 1971; Hill and Bowery, 1981) was consistent with the potentiating effect of corresponding agonists. The inhibitory effect of baclofen on L-5-HTP induced head-twitching has also been reported by Metz et al., (1985), who found it to be ineffective against the direct agonist 5-methoxy N,N-dimethyltryptamine. This suggests that baclofen inhibits the head-twitch by reducing 5-HT release.

AOAA inhibits GABA-transferase (Wallach, 1961) and DABA inhibits neuronal GABA uptake (Sutton and Simmonds, 1974). These agents therefore raise endogenous GABA. Like GABA itself, they resulted in dose-related inhibition of the head-twitch. The results from the selective agonists suggest that this inhibition may be exerted through a predominant effect at the $GABA_B$ receptor. Spontaneous head-twitching after peripheral GABA administration in rat and rabbit (Smialowski et al., 1980) may be due to a

different balance of receptor activation in these species, favouring potentiating effects at the $GABA_A$ receptor. Interestingly, the GABA depleting agent (glutamic acid decarboxylase inhibitor) 3-MPA (Horton and Meldrum, 1973) induced potentiation, suggesting a tonic inhibitory role for GABA in the mouse.

As discussed in the Introduction, $GABA_A$ receptors are a part of multi-receptor complex containing benzodiazepine and barbiturate receptors. Ligands at these latter sites should therefore have predictable effects on the L-5-HTP induced head-twitch.

Benzodiazepine receptor ligands showed the expected profile. Diazepam potentiated head-twitching (as reported by Nakamura and Fukushima, 1977) and the "inverse agonist" beta-CCE (Polc et al., 1982) inhibited it. RO15-1788 is a benzodiazepine with antagonistic effects against both classic benzodiazepines and "inverse agonists" (Hunkeler et al., 1981), Ro-15-1788 inhibited the head-twitch with four times the potency of beta-CCE. This could be interpeted either as being due to blockade of the effects of ongoing release of the putative endogenous ligand, or as due to intrinsic partial inverse agonist properties (File et al., 1982).

The preconvulsant RO05-4864 shows selectivity for peripheral binding site *in vitro* (see General Introduction). RO05-4864 potentiated the head-twitch with a potency approximately one tenth that of diazepam; however it has recently been shown to bind to the picrotoxin / barbiturate site (Simmonds, 1984; Ticku and Ramanjaneyulu, 1984). The potentiating effect of RO05-4864 may therefore not be due to an effect at the so called benzodiazepine "acceptor" site.

By analogy with the effect of $GABA_A$ receptor agonists and benzodiazepine receptor ligands, the predicted effect of barbiturates would be to increase head-twitching frequency. However, the early work of Corne et al., (1963) showed a wide range of barbiturates to be inactive up to very high doses. In the present experiments both pentobarbitone and phenobarbitone had marked effects. The difference from the results of Corne et al., (1963) probably lies in their use of a quantal (number of mice/group showing head-twitch) rather than a graded (head-twitch rate) response as a measure of head-twitch intensity, combined with our use of a pretreatment (200 mg/kg L-5-HTP + 9 mg/kg carbidopa) which resulted in 100% incidence of head-twitching. Phenobarbitone binds only weakly to the GABA-linked barbiturate receptor and, unlike pentobarbitone, does not potentiate GABA or benzodiazepine binding ; pentobarbitone on the other hand is a

potent ligand for this site (see General Introduction). The potentiation shown by pentobarbitone would be the predicted effect of agonist activity at the GABA-linked barbiturate receptor. The inhibitory effect shown by both barbiturates indicates the possibility of a second site of barbiturate action, perhaps that associated with inhibition of transmitter release (Fung and Fillenz, 1984).

The effect of two further ligands for the picrotoxin/barbiturate site, picrotoxin and pentylenetetrazol, were similarly complex. The testable dose range of picrotoxin was narrow because of its steep dose response curve for convulsant activity. The highest subconvulsant dose caused potentiation while lower doses were inactive. This was unexpected since picrotoxin is also a chloride channel blocker (Simmonds, 1980) and would thus be predicted to cause inhibition, as did the GABA antagonist bicuculline. Pentylenetetrazol is also a convulsant but less potent than picrotoxin. For this compound high sub-convulsant doses did produce inhibition but potentiation was seen at lower doses. This may again indicate multiple sites of action.

Of all the drugs tested, only muscimol caused detectable head-twitching when given alone. Muscimol is the most selective agonist for $GABA_A$ receptors of those used here (Hill and Bowery,

1981), so would be the least likely to be "self-damping" through an opposing action at $GABA_B$ receptors. Spontaneous head-twitching in mice has also been noted following certain benzodiazepines but not diazepam (Nakamura and Fukushima, 1976). The head-twitch inducing effect of benzodiazepines in mice and GABA in rats and rabbits is prevented by 5-HT antagonists (Nakamura and Fukushima, 1976 ; Smialowski et al., 1980), so that these effects depend on an intact serotonergic system and probably represent a modulatory effect on the primary, 5-HT dependent, head-twitch inducing mechanism.

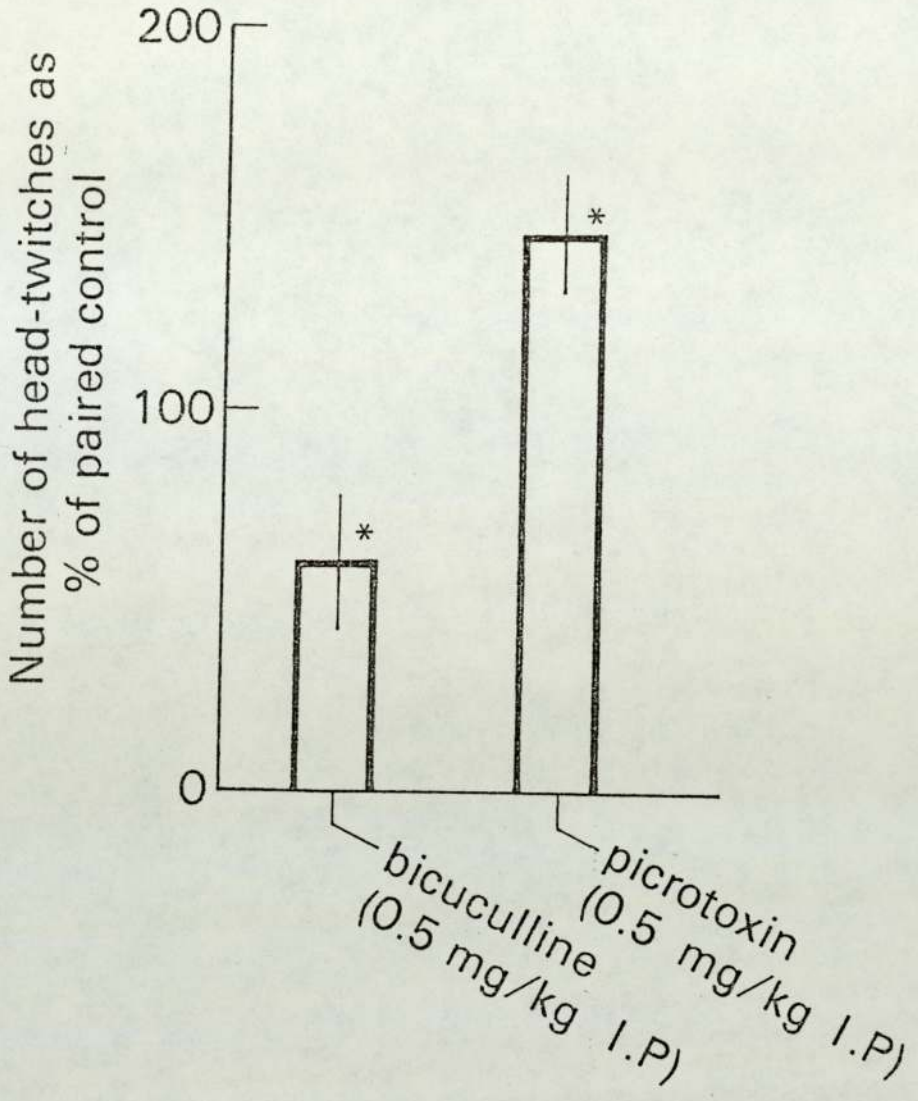


Fig. 4.1 Effect of GABA antagonists on the L-5-HTP induced head-twitch.

All mice received L-5-HTP (200 mg/kg i.p.) and carbidopa (9 mg/kg s.c.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for test mouse was recorded as percent of its paired control. Control mice received vehicle and the test mice received picrotoxin or bicuculline . Results are the means of at least 6 determinations and vertical bars represent s.e.m.

* $P < 0.05$

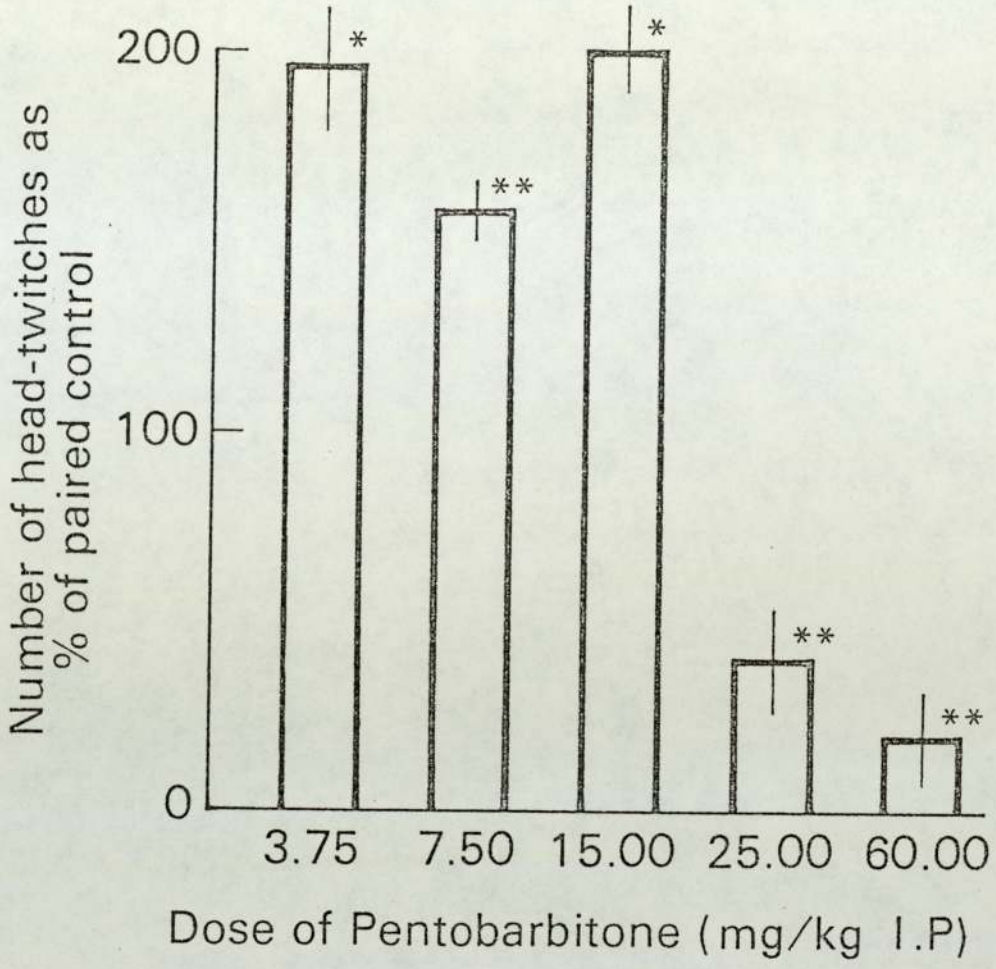


Fig. 4.2 Effect of pentobarbitone on the L-5-HTP induced head-twitch.

All mice received L-5-HTP (200 mg/kg i.p.) and carbidopa (9 mg/kg s.c.). Pairs of mice vere assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for test mouse was recorded as percent of its paired control. Control mice received vehicle and the test mice received pentobarbitone. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

* $P < 0.05$ ** $P < 0.001$

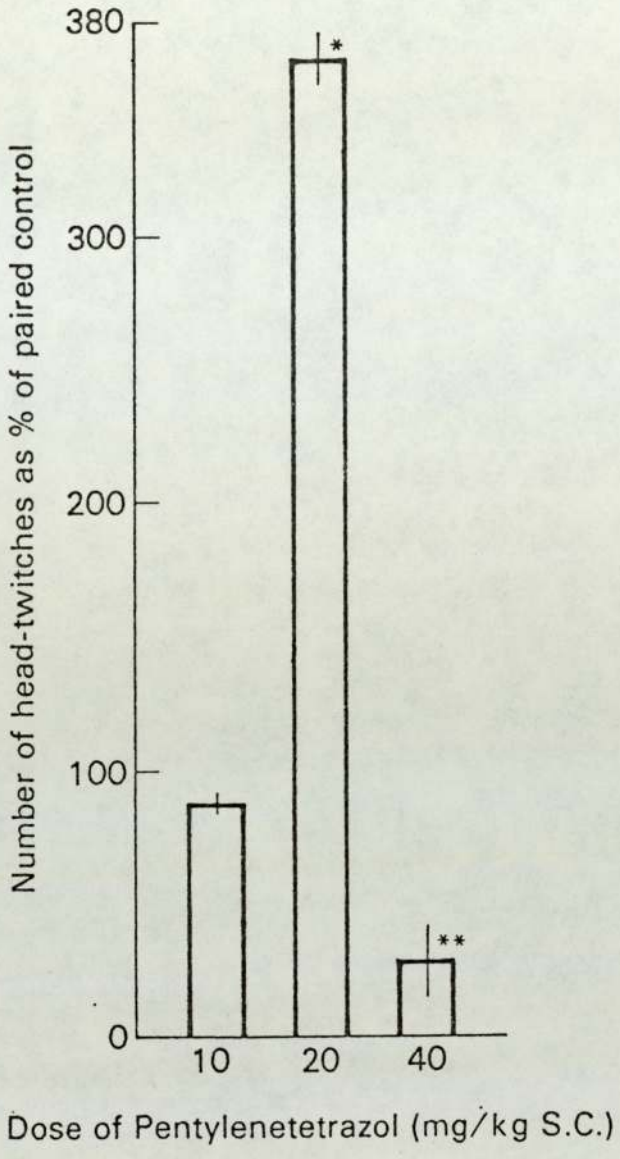


Fig. 4.3 Effect of pentylenetetrazol on the L-5-HTP induced head-twitch.

All mice received L-5-HTP (200 mg/kg i.p.) and carbidopa (9 mg/kg s.c.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for test mouse was recorded as percent of its paired control. Control mice received vehicle and the test mice received pentylenetetrazol. Results are the means of at least 6 determinations and vertical bars represent s.e.m.

* $P < 0.05$ ** $P < 0.001$

Drug	Route of administration	Time injected before L-5-HTP
GABA	i.c.v*	Ø
AOAA	s.c.	6 h
DABA	s.c.	24 h
3-MPA	i.p.	Ø
muscimol	i.p.	Ø
IMAA	i.p.	30 min
PTZ	s.c.	Ø

* by the method of Brittain and Handley, 1967.

Table 4.1 Drug administration

	Linear regression (Log dose)				
Drug	Dose range tested (mg/kg)	P	r	ED_{200} (mg/kg)	95% confidence limits (mg/kg)
3-MPA	2.50-10.00	<0.005	0.910	6.20	2.00-18.30
muscimol	0.12- 0.50	<0.001	0.983	0.26	0.19-0.36
IMAA	12.50-50.00	<0.02	0.921	16.02	9.93-25.85
3-APS	25.00-200.00	<0.037	0.945	83.45	45.54-152.90
Diazepam	0.25-1.00	<0.016	0.936	0.70	0.28-1.80
RO05-4864	5.00-10.00	<0.044	0.901	5.94	1.72-10.49

P = Significance of least squares fit.

r = Correlation coefficient of least squares fit.

ED_{200} = dose to increase twitches to 200% of control levels.

Table 4.2 Drugs potentiating the head-twitch response to L-5-HTP

Linear regression

(Log dose)

Drug	Dose range tested (mg/kg)	P	r	ID_{50} (mg/kg)	95% confidence limits (mg/kg)
GABA	12.50-100.00*	<0.002	0.906	35.50*	21.90-57.31*
AOAA	6.00-24.00	<0.001	0.956	10.90	8.45-24.23
DABA	5.00-20.00	<0.05	0.920	22.37	10.48-37.13
baclofen	1.25-5.00	<0.002	0.981	2.66	1.84-3.86
RO15-1788	2.50-10.00	<0.01	0.908	13.06	6.56-26.00
beta-CCE	20.00-80.00	<0.04	0.903	41.05	10.12-60.03
phenobarbitone	15.00-60.00	<0.001	0.974	18.05	12.16-26.76

P = Significance of least squares fit.

r = Correlation coefficient of least squares fit.

ID_{50} = dose to decrease twitches to 50% of contrnl levels.

*ug administered i.c.v. by the method of Brittain and Handley, 1967.

Table 4.3 Drugs inhibiting the head-twitch response to L-5-HTP

CHAPTER 5

INVOLVEMENT OF NORADRENALINE IN POTENTIATION OF THE HEAD-TWITCH RESPONSE BY GABA-RELATED DRUGS.

CHAPTER 5

Introduction

The results obtained in Chapter 3 show that drugs acting at GABA, benzodiazepine and barbiturate receptors have potent effects on the L-5-HTP induced head-twitch response in mice. Muscimol and 3-aminopropanesulphonic acid (3-APS) which are $GABA_A$ receptor agonists, diazepam and low doses of pentobarbitone caused dose-dependent potentiation of the L-5-HTP head-twitch response. Furthermore, higher doses of pentobarbitone, and all effective doses of phenobarbitone and baclofen reduced this behaviour. The barbiturate receptor antagonist picrotoxin (PTX) however produced potentiation.

The experiments described here represent a preliminary attempt to determine the mechanism of these effects. The effect of these agents on head-twitching induced by 5-methoxy -N,N-dimethyltryptamine (5-MeODMT) were examined in order to exclude a presynaptic effect on 5-HT neurones; 5-MeODMT is a direct 5-HT receptor agonist (Fuxe et al. 1972). Noradrenaline plays an important role in modulating the 5-HT mediated head-twitch and beta-adrenoceptor agonists exert a marked potentiating effect. The involvement of noradrenergic mechanisms in the effect of GABA-related drugs has therefore been investigated.

For the results described below, one mouse from each pair received the drug under test and the other received the vehicle. Both pretreated mice received a submaximal dose of 5-MeODMT (10 mg/kg i.p.). Twitches from each mouse were counted for alternate minutes from 0 to 12 minutes after 5-MeODMT. In the experiments involving a third pretreatment, i.e. FLA-63 or metoprolol, twitches in quartets of mice were counted in parallel over the same time period. The members of the quartet represented each of the four pretreatments (drug/drug; drug/vehicle; vehicle/drug; vehicle/vehicle). Runs with pairs or quartets were repeated until the appropriate group size was reached for each experiment.

Statistical analysis

Results were analysed by paired t-test and 2 x 2 factorial analysis of variance; both were carried out on raw data. The significance ($P_{[int]}$) of drug interactions with FLA-63 or metoprolol was determined from the F value of the interaction term in the analysis of variance.

Results

The doses of GABA-related drugs, used for the results described below, were chosen from the work carried out in Chapter 3. The doses for muscimol, 3-APS and diazepam are close to their ED_{200} values for potentiating the L-5-HTP induced head-twitch response in mice. The doses of pentobarbitone and PTX were chosen from their dose response analysis for potentiating the L-5-HTP head-twitch. The dose of baclofen used was close to its ID_{50} value for reducing the L-5-HTP response.

1.1 Drug effects on head-twitch induced by 5-MeODMT

The head twitch response produced by 5-MeODMT (10.0 mg/kg) was potentiated by pretreatment of mice with 3-APS (80.0 mg/kg), diazepam (0.7 mg/kg), PTX (0.5 mg/kg) or pentobarbitone (6.0 mg/kg) administered (i.p.) 30 min before 5-MeODMT and by muscimol (0.125 mg/kg), injected (i.p.) 20 min before 5-MeODMT (Table 5.1). In contrast, the $GABA_B$ receptor agonist baclofen (2.5 mg/kg) inhibited the 5-MeODMT induced head-twitch when administered 30 min before the 5-HT agonist (Table 5.1).

Diazepam (0.7 mg/kg i.p) and pentobarbitone (6.0 mg/kg i.p.) did not produce sedation. No convulsions were seen in any mice treated with PTX (0.5 mg/kg i.p.). None of the GABA-related drugs (muscimol, 3-APS, diazepam, PTX or pentobarbitone) induced head-twitching when administered alone at the above doses during the 30 min observation period starting immediately after administration.

1.2 Effect of FLA-63 on brain catecholamine levels

Table 5.2 shows that 40.0 mg/kg (i.p.) of FLA-63 administered 4h beforehand to mice significantly reduced brain noradrenaline to approximately 10% of control levels but had no significant effect on brain dopamine.

1.3 Effect of FLA-63 on 5-MeODMT induced head-twitch

The 5-MeODMT (10.0 mg/kg) induced-head-twitch response was significantly potentiated by pretreatment of mice with FLA-63 (40.0 mg/kg) administered 4h previously ($P < 0.05$, Tukey's test for unconfounded means; Fig. 5.1). FLA-63 (40.0 mg/kg i.p.) failed to induce head-twitching when administered alone 4h beforehand.

1.4 Effect of GABA-related drugs on 5-MeODMT induced head-twitch in the presence of FLA-63

Treatment with FLA-63 (40.0 mg/kg) 4h previously prevented any further potentiation of 5-MeODMT (10.0 mg/kg) head-twitching by muscimol (0.125 mg/kg), 3-APS (80.0 mg/kg), diazepam (0.7 mg/kg), PTX (0.5 mg/kg) or pentobarbitone (6.0 mg/kg) as assessed from the interaction terms in the analysis of variance ($F_{[int]} \geq 7.62$, $P < 0.05$ in each case; Fig. 1). Similar pretreatment with FLA-63 also prevented the inhibitory effect of baclofen ($F_{[int]} \geq 24.58$, $P < 0.01$; Fig. 1).

1.5 Effect of metoprolol on the potentiation of 5-MeODMT head-twitch induced by FLA-63, muscimol and diazepam

Potentiation of the 5-MeODMT (10.0 mg/kg) head-twitch response by FLA-63 (40.0 mg/kg), muscimol (0.125 mg/kg) or diazepam (0.7 mg/kg) (administered 4h, 20 min and 30 min before the 5-HT agonist respectively) was prevented by the beta-adrenoceptor antgonist metoprolol (2.5 mg/kg s.c.) administered 15 min before 5-MeODMT ($F_{[int]} \geq 20.16$, $P < 0.01$ in each case; Fig. 2). However, metoprolol had no significant effect on control responses ($P > 0.05$, Tukey's test for unconfounded means; Fig. 2).

Discussion

The GABA-related drugs, muscimol, 3-APS, diazepam, PTX and pentobarbitone potentiated and, baclofen reduced the head-twitch response to 5-MeODMT with a similar potency to their effects on L-5-HTP induced head-twitching seen in Chapter 3. This demonstrates that their mechanism of action does not involve presynaptic 5-HT mechanisms, since 5-MeODMT is a direct 5-HT receptor agonist (Fuxe et al., 1972).

Noradrenaline has potent modulatory actions on the 5-HT mediated head-twitch response in both mice and rats (see below and General Introduction for references). GABA-related drugs, such as pentobarbitone and baclofen, modulate the release of noradrenaline in tissue preparations of the brain (see General Introduction for further details). Therefore, it is possible that GABA-related drugs modulate the 5-HT mediated behaviour by affecting the release of noradrenaline. This possibility was investigated by blocking the synthesis of noradrenaline and by beta-adrenoceptor blockade.

Potentiation of the head-twitch by FLA-63

FLA-63 is a dopamine beta-oxidase inhibitor (Svensson and Waldeck, 1969). It depleted whole-brain noradrenaline by 90% without affecting brain dopamine. 5-HT concentrations have not been reported to be affected by this compound. The 5-HT mediated head-twitch appears to be dependent on noradrenergic transmission for its occurrence (Maj et al., 1978; Handley and Brown, 1982),

the observed potentiation by FLA-63 was therefore surprising. A similar result has been obtained by Nakamura and Fukushima (1978) with reserpine in mice, although in this case the other monoamines would also be depleted. 6-hydroxydopamine administered i.c.v. to rats failed to antagonise the head-twitch (Bednarczyk and Vetulani, 1978) but a moderate reduction in quipazine induced head-twitch following Locus Coeruleus lesions with this substance was observed (Chapter 6).

The essential nature of the noradrenergic contribution has been demonstrated by the blocking effect of a variety of $alpha_1$-adrenergic antagonists in mice (Handley and Brown, 1982). It is possible that FLA-63 did not block the head-twitch in the present experiments because the small amount of remaining noradrenaline was sufficient to activate $alpha_1$-adrenoceptors over the short time-course of the 5-MeODMT head-twitch. 5-MeODMT increases noradrenaline turnover (Fuxe et al., 1972) and this may indicate that this substance itself releases noradrenaline.

As to the occurrence of actual potentiation in the presence of FLA-63, this was sensitive to metoprolol, suggesting activation of beta-adrenoceptors. Beta-adrenoceptor antagonists do not themselves reduce head-twitching in mice (Chapter 2; Goodwin and Green, 1985) showing that any activation of these receptors under control conditions is not sufficient to maintain a tonic facilitation. FLA-63 could itself release noradrenaline but there appear to be no reports of such an effect. Alternatively, beta-

adrenoceptor supersensitivity could be responsible. Lack of agonist can induce supersensitivity by enhancing beta-adrenoceptor coupling to the regulatory sub-unit of cAMP within the required time scale used here (see Harden, 1983). Release of remaining noradrenaline by 5-MeODMT could thus become sufficient to induce metoprolol-sensitive potentiation. Interestingly cortisol, which is a potent facilitator of coupling (Davies and Lefkowitz, 1980; 1981) also induces head-twitch potentiation in low doses (Handley and Miskin,1972), although it is not known whether this is metoprolol-sensitive.

Potentiation by muscimol, 3-APS, diazepam and pentobarbitone

Of these four agents, only muscimol induces head-twitching when given alone (Chapter 4). However at the dose used here muscimol did not induce twitching. Muscimol and 3-APS are selective agonists at $GABA_A$ receptors (Hill and Bowery, 1981). Accessory receptors which govern the frequency and duration of opening of the GABA-operated chloride channel are important sites of action for benzodiazepines and barbiturates respectively (see Bowery et al., 1984). The potentiating effect of these agents were not additive with FLA-63, in fact there was complete occlusion of their effects. An alternative test of noradrenaline involvement was performed using beta-adrenoceptor blockade. Muscimol, the more selective of the two $GABA_A$ receptor agonists, and diazepam were examined in the presence of metoprolol and their potentiating effect was abolished, clearly demonstrating that its occurrence was due to a beta-adrenoceptor dependent mechanism.

Potentiating effect of PTX

PTX is a barbiturate receptor antagonist and blocks the GABA-induced change in chloride conductance noncompetitively (see Ticku and Maksay, 1983). However, at subconvulsant doses, it had the same effect as the chloride-channel activating drugs, i.e. it produced potentiation. This potentiation was not seen after FLA-63. This is suggestive of a noradrenergic link but further investigation, for instance beta-blockade, will be necessary to establish this. The underlying mechanism of the potentiating effect could be linked to the head-twitch depressant actions of phenobarbitone and high dose pentobarbitone and may involve a different receptor site. Barbiturate receptors have not yet been fully characterised, but there are indications that some may exist separate from the $GABA_A$ receptor (see Johnston and Willow, 1982).

Blocking effect of baclofen

Baclofen is a selective agonist at $GABA_B$ receptors; these are linked to the calcium ion channel (see Bowery et al., 1984). Metz and co-workers (1985) have previously reported that, while baclofen antagonised the L-5-HTP head-twitch, it was ineffective against the head-twitch induced by 5-MeODMT. This indicates that the known inhibitory effect of baclofen on 5-HT release (Schlicker et al., 1984) is likely to be involved. In the present study, baclofen was a potent inhibitor of 5-MeODMT head-twitching. The potency was similar to that previously described for inhibition of L-5-HTP (Chapter 4). The difference may lie in

the pretreatment regimen used. Metz and co-workers gave a loading dose of 10 mg/kg baclofen followed by a further 10 mg/kg in drinking water over the ensuing 24h. In the present experiments, a single dose of 2.5 mg/kg was administered 30 min before 5-MeODMT, the resulting antagonism indicates that under these conditions baclofen exerts its effects at or beyond the site of action of 5-MeODMT since the latter is a direct agonist. The antagonism of the head-twitch by baclofen was prevented by FLA-63. Put another way, the FLA-63 potentiated head-twitch was insensitive to baclofen. Baclofen inhibits noradrenaline release (Bowery et al., 1980; Hill and Bowery, 1981), but it is difficult to envisage a mechanism by which noradrenaline depletion would negate the consequences of this effect. It remains to be investigated whether the potentiating effects of other agents also confer resistance to baclofen inhibition.

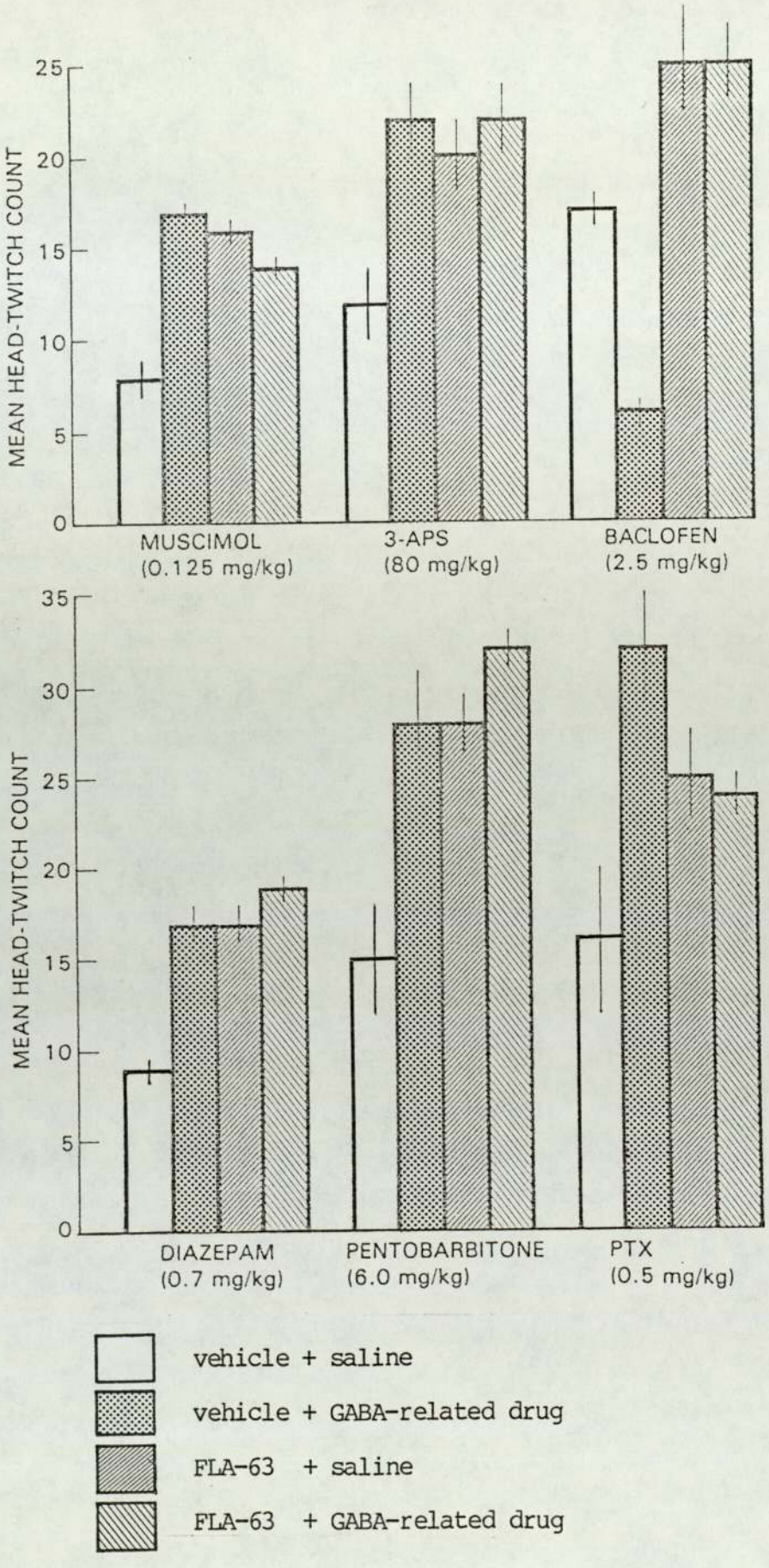


Fig. 5.1 The effect of GABA-related drugs on 5-MeODMT head-twitch in the presence and absence of FLA-63 .
Results are mean $\pm$ s.e.m. for each group of 6 mice. Head-twitch rates were counted every alternate min from 0 to 12 min after 5-MeODMT 10.0 mg/kg. All drugs were administered i.p. FLA-63 was given 4h before and other drugs 30 min before (muscimol 20 min before) 5-MeODMT.

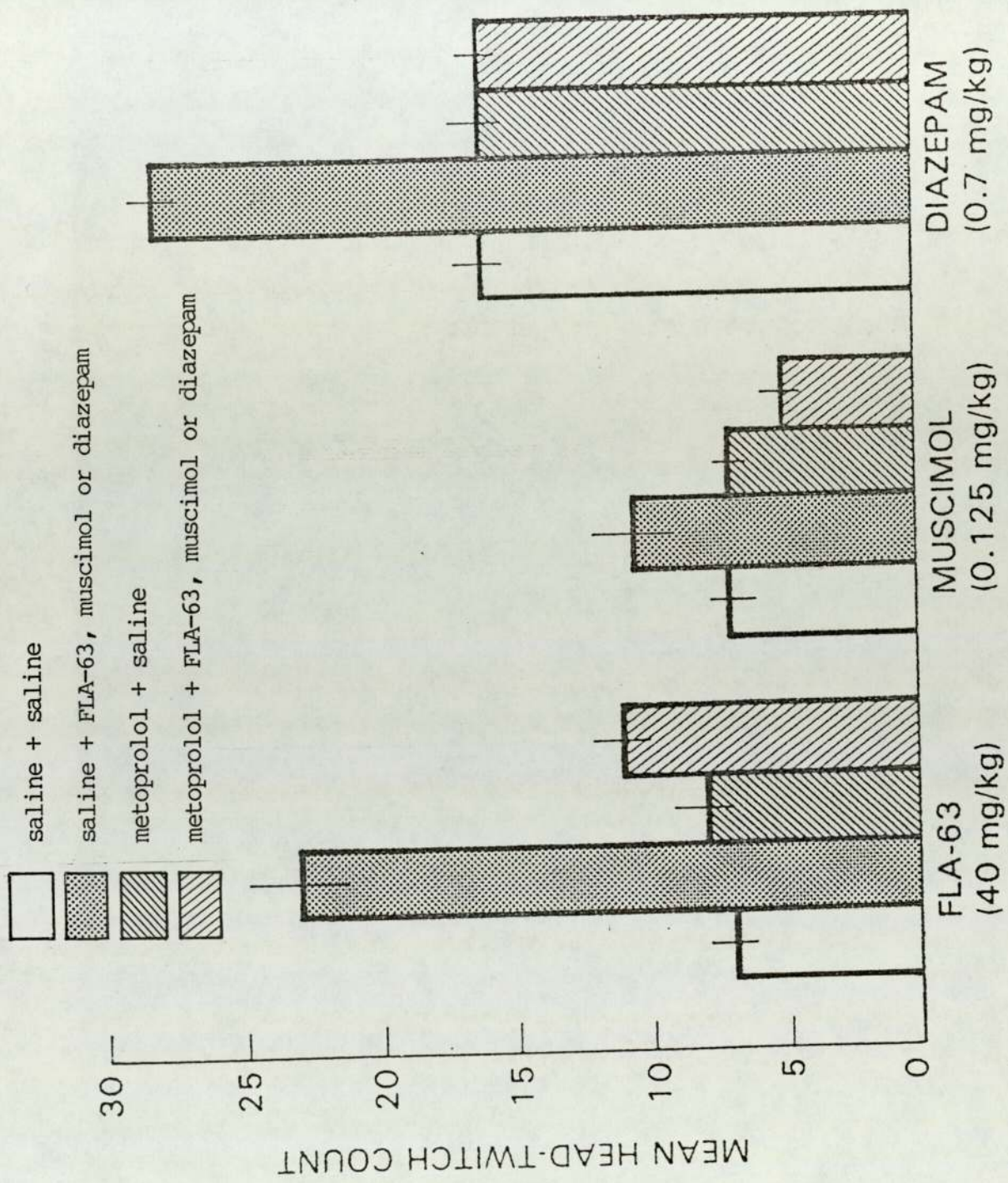


Fig. 5.2 The effect of metoprolol on head-twitch potentiation induced by FLA-63, muscimol and diazepam.
Restlts are mean ± s.e.m. for each group of 6 mice. Head-twitch rates were counted every alternate min from 0 to 12 min after 5-MeODMT 10.0 mg/kg. FLA-63, muscimol and diazepam were administered i.p. (4h, 20 min and 30 min respectively) before 5-MeODMT. Metoprolol (2.5 mg/kg s.c.), was injected 15 min before 5-MeODMT

Drug	Dose (mg/kg i.p.)	Mean % of control head-twitch response	
muscimol	0.125	234.3±43.1**	(12)
3-APS	80.0	266.1±37.9**	(14)
baclofen	2.5	34.4±4.7**	(20)
diazepam	0.7	189.3± 37.2*	(6)
PTX	0.5	245.1±33.9*	(6)
pentobarbitone	6.0	183.7±25.9*	(6)

Table 5.1. Effect of GABA-related drugs on 5-MeODMT induced head-twitch response in mice.

Effects reported as % of the twitches from the control (vehicle pretreated) animal tested at the same time, ±s.e.m. Number of observations given in brackets. The drugs were administered 30 min (except muscimol which was administered 20 min) before 5-MeODMT. Head-twitches were observed in parallel on alternate min for control and test mice from 0 to 12 min after 5-MeODMT injection (10.0 mg/kg i.p.)

*$P < 0.05$; **$P < 0.01$ (paired t-test).

	Catecholamine levels (ng/g wet weight)	
	Vehicle	FLA-63
Noradrenaline	385±25	40±20**
Dopamine	655±69	690±55

Table 5.2 Effect of FLA-63 on brain catecholamine levels 4h after FLA-63 (40.0 mg/kg i.p.).

Groups of mice were pretreated with either FLA-63 (40.0 mg/kg) or vehicle.

Results are mean values of 4 determinations each consisting of 3 pooled brains ±s.e.m.

**$P < 0.01$ (paired t-test).

CHAPTER 6

INVOLVEMENT OF LOCUS COERULEUS IN THE POTENTIATION OF THE HEAD-TWITCH RESPONSE BY DIAZEPAM AND BETA-ADRENOCEPTOR AGONISTS

CHAPTER 6

Introduction

The work described in Chapter 2 established that both $beta_1$- and $beta_2$-adrenoceptor selective agonists potentiate the L-5-HTP induced head-twitch response in mice. However, it has not been investigated whether the same is true in rats. All the studies describing the potentiation of head-twitching in mice by $beta_2$-adrenoceptor agonists induced the behaviour by means of precusor loading. It is not clear whether this beta-adrenoceptor induced potentiation involves presynaptic 5-HT mechanisms.

Furthermore, work presented in Chapter 5 established that the potentiation of L-5-HTP head-twitch behaviour in mice by GABA-related drugs (described in Chapter 4), are noradrenaline dependent. Potentiation of the 5-MeODMT head-twitch by $GABA_A$ receptor agonists, diazepam and pentobarbitone was prevented by noradrenaline depletion and by the $beta_1$-adrenoceptor antagonist metoprolol.

The present experiments were undertaken in order to further characterise the nature of the noradrenergic involvement in the head-twitch by examining drug effects in rats with bilateral 6-hydroxydopamine (6-OHDA) lesions of the Locus Coeruleus (LC).

Results

1.1 Effect of LC lesions on brain catecholamines

Bilateral LC lesions were performed in 10 rats and the same number of animals were sham-operated according to the procedure described under Experimental Methods. The animals were allowed at least 21 days to recover before examining behaviour. Seven days after final behavioural testing all the rats (10 lesioned and 10 sham-operated) were killed; their brains were removed and disected according to the method of Glowinski and Iversen (1966). The various disected regions were assayed fluorometrically for catecholamine levels by the method described by Chang (1964) as modified by Cox and Perhach (1973).

Table 6.1 shows that bilateral lesions of the LC produced a selective reduction (to 7.1$\pm$ 1.0 % of controls) in the forebrain noradrenaline (hippocampus + cerebral cortex) and cerebellum (to 10.0 $\pm$ 3.5 % of controls). The noradrenaline content of the hypothalamus (99.6$\pm$25.2 % of controls) and dopamine of the striatum remained unchanged (103.0$\pm$ 31.6 % of controls). All rats were successfully lesioned.

1.2 Effect of LC lesions on the quipazine induced head-twitch behaviour

The LC lesioned animals showed significantly fewer twitches

compared with sham operated rats at 2.5 mg/kg and 5.Ø mg/kg i.p. quipazine (Table 6.2). Head-twitches were counted in parallel between 3Ø and 6Ø min after quipazine.

1.3 Effect of LC lesions on action of drugs

For the results described below, the same rats were used for investigating each drug. At least 7 days were allowed before testing the next drug. All animals were used for each drug. Head-twitches were counted in parallel for each quartet between 3Ø and 6Ø min post quipazine (for further details see Expermental Methods).

The beta-adrenoceptor agonists prenalterol (Ø.25 mg/kg), procaterol (1.5 mg/kg) and salbutamol (Ø.25 mg/kg) injected (s.c.) simultaneously with quipazine (2.5 mg/kg i.p.) potentiated the head-twitch response in sham-operated rats ($P < Ø.Ø1$ in each case). However, dobutamine (2.Ø mg/kg s.c.) injected simultaneously with quipazine (2.5 mg/kg i.p.) failed to potentiate the head-twitch response ($P > Ø.Ø5$). In a preliminary run a dose of 3.Ø mg/kg of this beta-agonist did produce a potentiation of quipazine head-twitching in sham operated rats but decreased the response in LC lesioned animals (sham operated + saline = 27; sham operated + dobutamine = 43; LC lesions + saline = 21; LC lesions + dobutamine = 16 head-twitches between

30 and 60 min after quipazine). This inhibition was associated with appearance of 5-HT 'hyperactivity syndrome' being more intense in lesioned compared with sham operated rats. Therefore the dose of dobutamine was reduced in order to examine the effect of LC lesions on the dobutamine induced potentiation of the head-twitch by beta-adrenoceptor agonists. As before, the lesioned rats showed fewer twitches than did the sham-operated controls ($P < 0.01$ in each case). The effect of LC on action the of drugs was assessed from the significance of the interaction term in the analysis of variance. Lesions increased the potentiation caused by dobutamine 2.0 mg/kg ($F_{[int]} = 14.56$, $P < 0.01$) and prenalterol 0.25 mg/kg ($F_{[int]} = 7.64$, $P < 0.05$). It was also noted that the 5-HT syndrome appeared more intense. Conversely LC lesions significantly reduced the potentiating effect of procaterol 1.5 mg/kg ($F_{[int]} = 12.69$, $P < 0.01$) and salbutamol 0.25 mg/kg ($F_{[int]} = 13.36$, $P < 0.01$); the 5-HT syndrome also appeared reduced but no attempt was made to quantitate this.

Diazepam (0.25 mg/kg i.p.) administered 15 min before quipazine (2.5 mg/kg i.p.) potentiated the head-twitch behaviour in sham-operated rats ($F = 6.37$, $P < 0.05$). LC lesions prevented this potentiation ($F_{[int]} = 16.52$, $P < 0.01$). When the two groups of lesioned rats were compared (Tukey's test) it was found that the above dose of diazepam significantly reduced head-twitching in lesioned rats.

Discussion

All the operated rats showed a satisfactory depletion of forebrain noradrenaline (>90%), with no significant depletion of hypothalamic noradrenaline or striatal dopamine, indicating that selective lesioning of forebrain LC connections had been obtained. Lesions of LC itself, rather than the more usual dorsal bundle lesions were performed in order to obtain a more complete destruction of LC connections throughout the brain. The observed depletions in cerebellum suggest that it was likely that this aim was achieved, although spinal concentrations were not examined.

LC lesions significantly reduced the frequency of the quipazine head- twitch. It has previously been shown that the 5-HT mediated head-twitch in both mice and rats is modified by drugs affecting noradrenergic transmission. Thus it is reduced or abolished by selective antagonists of $alpha_1$-adrenoceptors and potentiated by the corresponding agonists; $Alpha_2$-adrenoceptor antagonists , on the other hand, produce potentiation, while the agonists produce a dose-dependent inhibition (see General Introduction). Although $alpha_1$-adrenoceptor activity appears to be essential for head-twitching to occur in mice (Handley and Brown, 1982), previous work with noradrenaline depletion has failed to detect any inhibitory effect of reserpine (Nakamura and Fukushima, 1978),

intraventricular 6-OHDA (Bednarczyk and Vetulani, 1978) or dopamine beta-hydroxylase inhibition with FLA-63 (see Chapter 5) on 5-HT mediated head-twitching. Possibly only minimal noradrenaline release is necessary onto $alpha_1$-adrenoceptors at critical sites. Whatever the receptors involved, the reduction in twitch-rate in lesioned rats suggests a net tonic facilitatory role for noradrenergic LC neurones under the conditions of these experiments.

As in the case of L-5-HTP response in mice (see Chapter 2), both $beta_1$- and $beta_2$-adrenoceptor agonists potentiated the head-twitch response to quipazine in rats. Dobutamine failed to potentiate the quipazine induced head-twitch in sham operated rats but higher doses of this $beta_1$-adrenoceptor agonist did potentiate the response in these animals but reduced it in LC lesioned rats. This was probably due to the 'hyperactivity syndrome' behaviours being more intense in lesioned than sham operated animals. The appearance of the syndrome behaviours appear to block head-twitching (Drust et al., 1979) and therefore the dose of dobutamine was reduced for subsequent investigation. The present results also reveal a differential effect of LC lesions on the response to these agonists. The potentiation due to dobutamine and prenalterol, which are agonists selective for $beta_1$-adrenoceptors (Carlsson et al.,

1977; Sonnenblick et al., 1979), was further increased. This effect would be typical of the up-regulation shown by post-synaptic $beta_1$-adrenoceptors when deprived of their agonist (Minneman et al., 1979a). In contrast, the potentiating effects of salbutamol and procaterol, two agonists selective for $beta_2$-adrenoceptors, (Brittain et al., 1968; Yabbuuchi, 1977; O'Donnell and Wanstall, 1985), was prevented by LC lesions. This suggests a presynaptic location for the relevant $beta_2$-adrenoceptors. There is evidence for the existence of presynaptic $beta_2$-adrenoceptors on adrenergic nerve endings, which facilitate noradrenaline release (Westfall, 1977; see Misu and Kubo, 1983). $Beta_2$-adrenoceptors have also been reported on noradrenergic cell bodies of LC, with a facilitatory effect on neuronal firing in response to sensory input (Dhalof et al., 1981). Thus the potentiating effect of $beta_2$-adrenoceptor agonists would be exerted ultimately by facilitation of noradrenaline release onto post-synaptic $beta_1$-adrenoceptors and therefore susceptible to antagonism by selective $beta_1$-adrenoceptor antagonists and LC lesions. This interpretation also explains the pattern of inhibition shown by the beta-adrenoceptor antagonists described in Chapter 2, i.e. the head-twitch potentiation by agonists selective for $beta_1$-adrenoceptors is selectively blocked by $beta_1$-adrenoceptor antagonists only, while the potentiating effect of $beta_2$-adrenoceptor agonists is prevented by antagonists

of both subtypes of beta-adrenoceptor.

Diazepam, together with other agonists of the GABA-chloride ionophore complex, increased the head-twitching induced by 5-methoxy -N,N-dimethyltryptamine and this potentiation was noradrenaline dependent, since it was prevented by noradrenaline depletion and by beta-adrenoceptor blockade (see Chapter 5). Diazepam was chosen for investigation here because of its low toxicity. In rat, as in mouse, it further increased head-twitch rates. In lesioned rats, this potentiation was reversed to inhibition, showing that LC neurones provide the necessary noradrenergic component and also revealed an LC-independent depressant effect.

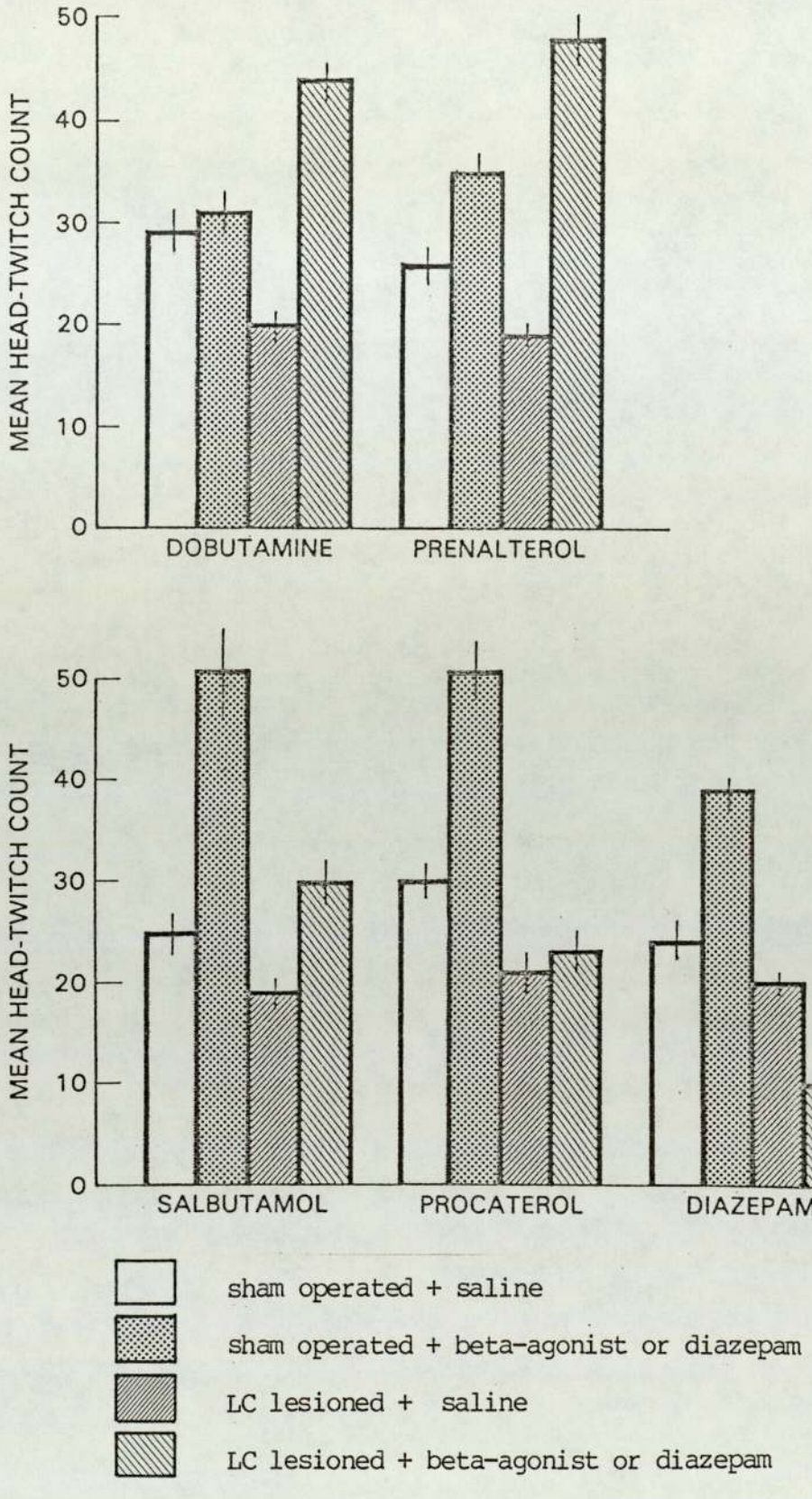


Fig. 6.1 Effect of LC lesions on potentiation produced by beta-adrenoceptor agonists and diazepam.

Results are reprsented as means of 5 observations and vertical bars represent s.e.m. The head-twitches were counted in parallel for each quartet of rats.

	Number of head-twitches (±s.e.m.)	
Dose of quipazine (mg/kg i.p.)	sham-operated	LC lesioned
2.5	40.4±1.3	26.4±1.4**
5.0	57.7±2.0	40.6±2.7**

Table 6.1 The effect of LC lesions on quipazine induced head-twitch response.

**$P < 0.01$ different from control group run at the same time (paired t-test).

	sham operated	LC lesioned	lesion % of control
NA			
hippocampus + cerebral cortex	293.6±29.8	19.8±8.1**	7.1±1.0
cerebellum	470.4±35.6	56.6±10.3**	10.0±3.5
hypothalamus	1438.3±69.8	1421.9±28.9	99.6±25.2
DA			
striatum	3210.9±25.9	3300.1±30.6	103.0±31.6

Table 6.2 Regional noradrenaline (NA) and dopamine (DA) concentrations in control and 6-OHDA lesioned rats.

Values are mean (± s.e.m.) in ng/g wet weight of tissue (n per group =10).

**$P < 0.01$ different from appropriate control group (paired t-test)

General Discussion

The head-twitch behaviour in mice and rats induced by L-5-HTP and 5-HT agonists is simple and easier to quantitate than the 'hyperactivity syndrome' in rats. Therefore head-shaking behaviour was used here to investigate the effect of drugs acting at beta-adrenoceptors and GABA related receptors on 5-HT neurotransmission in the CNS.

The effect of drugs on the head-twitch response in both mice and rats was examined at peak time of the response and the dose of the inducing agent was selected which produced approximately 50% of the maximal response, to enable detection of potentiating and inhibitory drug effects. Test drugs were administered such that they were exerting maximum effect at the time of counting the head-twitches.

The results obtained in Chapter 2 demonstrate that $beta_1$- (dobutamine and prenalterol) and $beta_2$- adrenoceptor (procaterol and salbutamol) selective agonists potentiated the L-5-HTP induced head-twitch response in mice. Like propranolol (Goodwin and Green, 1985) selective $beta_1$- (metoprolol and practolol) or $beta_2$- (butoxamine and ICI 188,551) adrenoceptor antagonists did not reduce the L-5-HTP head-twitch response. In the case of $alpha_1$-adrenoceptors it has been proposed that a tonic input onto these receptors is necessary (Handley and Brown, 1982), but the results obtained here demonstrate that a noradrenergic tone feeding onto beta-adrenoceptors is not necessary for the

occurrence of 5-HT mediated head-twitching. Furthermore, unlike $alpha_1$-adrenoceptor stimulation and $alpha_2$-adrenoceptor antagonism (Corne and Pickering, 1967; Handley and Brown, 1982), both $beta_1$- and $beta_2$-adrenoceptor agonists failed to induce head-twitching in mice.

The selective $beta_1$-adrenoceptor antagonists, metoprolol and practolol blocked the potentiation produced by dobutamine and prenalterol of the L-5-HTP-induced head-twitch response. The potentiation due to these agonists was unaltered by the $beta_2$-adrenoceptor antagonists, butoxamine and ICI 118,551. This indicates that the potentiating effects of these agonists were $beta_1$-selective. However, the potentiating effects of the selective $beta_2$-adrenoceptor agonists were prevented by both $beta_1$- and $beta_2$-adrenoceptor antagonists. From these results it appears that both subtypes of beta-adrenoceptors are involved in potentiation of L-5-HTP head-twitch behaviour in mice. The above results obtained with butoxamine and ICI 118,551 show selective antagonism of $beta_2$-adrenoceptors, whereas metoprolol and practolol appeared to show no selectivity. These results can be explained as follows:-

i. Either all the effective doses of metoprolol and practolol blocked both $beta_1$- and $beta_2$-adrenoceptors, or

ii. the potentiating effects of $beta_2$-adrenoceptor agonists are exerted by releasing noradrenaline onto $beta_1$-adrenoceptors.

These two possibilities were further investigated as described in

Chapter 6 by lesioning noradrenergic pathways originating from the LC. The lesions of LC were carried out rather than the more usual dorsal bundle, because the location of the beta-adrenoceptors involved is unknown. This work was carried out in the rat so that stereotaxic lesions could be performed. Bilateral lesions of LC were performed with 6-OHDA and the control animals received similar injections of the vehicle. Quipazine was used to induced head-twitching in rats, because 5-MeODMT used previously for the mouse work proved to be unsuitable for use in rats due to the appearance of the hyperactivity syndrome at all doses (see Chapter 1). Quipazine is a direct 5-HT agonist but it has been suggested that it induces head-twitching by a combination of pre- and post-synaptic mechanisms (Malick et al., 1977). The effects of beta-adrenoceptor agonists on the head-twitching in the rat have not been investigated previously. The results obtained in Chapter 6, show that beta-adrenoceptor agonists potentiate the quipazine induced behaviour in the rat as they do the L-5-HTP response in the mouse (Chapter 2). Since only one dose of each beta-adrenoceptor agonists was tested, it is not possible to comment on the potency of the agonists used but, in agreement with the results obtained in the mouse (Chapter 2), both $beta_1$-selective (dobutamine and prealterol) and $beta_2$-selective (procaterol and salbutamol) agonists potentiated the quipazine induced head-twitch response in the rat.

Bilateral lesions of the LC significantly reduced quipazine head-twitch rates compared with sham operated controls. This contrasts

with the finding of Bednarczyk and Vetulani (1978) that i.c.v. 6-OHDA had no significant effect on L-5-HTP-induced head-twitching in rats, although these authors did show a downward trend. In this latter work however, brain noradrenaline levels were not measured. The reduced head-twitch rates in the lesioned rats may indicate that a proportion of the receptors on which quipazine acts are located on the LC neurones. Quipazine and LSD may induce head-twitching by a common mechanism (Vetulani et al., 1981) and the latter sensitises LC neurones to the excitatory effects of sensory input (Aghajanian, 1980). In this context it may be relevant that head-twitching itself depends on sensory input from the pinna (Boulton and Handley, 1973). A further possibility is that LC is responsible for the noradrenergic tone believed necessary for the maintenance of raphe firing (Dahlstrom and Fuxe, 1964; Baraban and Aghajanian, 1980), since quipazine is partly dependent on 5-HT neurones for its effect (Vetulani et al., 1979), but recent studies involving conscious animals suggested that this tone is not necessary for raphe firing (Heym et al., 1982; Trulson and Trulson, 1983; Trulson and Crisp, 1984).

LC lesions differentially affected the potency of beta-adrenoceptor agonists. There was a significant increase in the potentiating effect of $beta_1$-adrenoceptor agonists, dobutamine and prenalterol. This suggests that these receptors are postsynaptic to LC neurones; $beta_1$-adrenoceptor density has been reported to increase after LC lesions (Minneman et al., 1979a).

In contrast , the potentiating effects of salbutamol and procaterol were drastically reduced. This suggests that the $beta_2$-adrenoceptors activated by these latter agonists are located on the LC neurones leading to increased noradrenaline release (Dahlof et al., 1981). These findings correlate with studies in the mouse in which the effects of $beta_1$-adrenoceptor agonists were prevented by $beta_1$-adrenoceptor antagonists, whilst the effects of $beta_2$-adrenoceptor agonists was prevented by antagonists selective for both subtypes of beta-adrenoceptors (Chapter 2). Therefore, LC appears to innervate the $beta_1$-adrenoceptors concerned with potentiation of 5-HT mediated head-twitch behaviour.

The mode of action of the tricyclic and related antidepressants is not yet known. Although the majority block the neuronal reuptake of noradrenaline and/or 5-hydroxytryptamine (5-HT) (Glowinski and Axelrod, 1964; Carlsson, 1976), it is now thought unlikely that this is the mechanism of their clinical effects. Reuptake blockade occurs with the first dose, while the antidepressant effect is delayed for 7 to 21 days (Klein and Davies, 1969; Oswald et al., 1972); in addition, the atypical or second generation antidepressants such as iprindole (IPD) do not consistently affect monoamine reuptake (Ross et al., 1971). The delay in onset of the therapeutic effect has led to considerable interest in the chronic pharmacology of these drugs. As a result it has been demonstrated that all clinically active antidepressant treatments, including monoamine oxidase inhibitors

and electroconvulsive therapy, lead to decreased activity of beta-adrenoceptor linked adenyl cyclase (Vetulani et al., 1976b). For the majority of treatments (exceptions are nisoxetine, zimelidine and mianserin; see Sulser and Mobley, 1981) this appears to be a consequence of "down-regulation" of cortical beta-adrenoceptor density as measured by radioligand receptor binding studies (Banerjee et al., 1977; Wolfe et al., 1978; Bergstrom and Kellar, 1979). These effects occur on chronic (10 to 21 day) dosing only (Vetulani et al., 1976b; Peroutka and Snyder, 1980a). However, receptor subsensitivity does not necessarily imply functional subsensitivity (Peroutka and Snyder, 1980a; see Green and Nutt, 1983), since the reduced beta-adrenoceptor density would only be expected to produce functional effects in the absence of a substantial pool of "spare receptors" (Stephenson, 1956; Moran, 1969). However, it has not been possible to investigate this question directly in vivo because beta-adrenoceptor agonists do not produce any behavioural effects when given alone (Chapter 2).

Although they are ineffective alone, beta-adrenoceptor agonists do produce a marked potentiation of the 5-HT mediated head-twitch behaviour in mice and rats (Chapters 2 and 6). Therefore, investigation of the head-twitch potentiation in mice by beta-adrenoceptor agonists was used as a behavioural model for examining the in vivo sensitivity of beta-adrenoceptors following chronic pretreatment with antidepressants.

Two antidepressants which are known to affect beta-adrenoceptor density (Banerjee et al., 1977) were chosen for investigation in Chapter 3. DMI and IPD were chosen for study here, because they "down regulate" beta-adrenoceptors by diferent mechanisms (Banerjee et al., 1977). Thus, DMI is a classic monoamine uptake inhibitor (Glowinski and Axelrod, 1964) while IPD has little effect on monoamine systems (Ross et al., 1971). Their effects on the sensitivity of three beta-adrenoceptor agonists were investigated, prenalterol, dobutamine and salbutamol. The results obtained in Chapter 3 show that chronic administration (21 days) of either DMI or IPD reduced the potency of all three beta-adrenoceptor agonists for potentiating the L-5-HTP head-twitch response in mice. Therefore, these results demonstrate that a decrease in the sensitivity of beta-adrenoceptor linked adenyl cyclase system, following pretreatment with antidepressants, reduces the function of beta-adrenoceptors *in vivo*. Since all active antidepressant treatments tested to date decrease the sensitivity of the beta-adrenoceptor linked adenyl cyclase system and the demonstration that this change is functional *in vivo*, suggests that it may be one of the ways in which this class of drugs exert their clinical effects.

It has been suggested that only the $beta_1$-adrenoceptor is down-regulated by the chronic administration of antidepressants (Minneman et al.,1979a), but this is not certain; the $beta_2$-adrenoceptor agonist salbutamol has been shown to be a clinically effective antidepressant (Jouvent et al., 1977; Simon et al.,

1978; Lecrubier et al., 1980) and long term administration of the more liposoluble $beta_2$-adrenoceptor agonist clenbuterol reduces beta-adrenoceptor density (Hall et al., 1980). However the results obtained in Chapter 2 indicate that, at least as far as the head-twitch is concerned, the relevant $beta_2$-adrenoceptors are presynaptic and exert their effects by facilitating the release of noradrenaline onto $beta_1$-adrenoceptors. The results obtained in Chapter 6 support this conclusion. Thus 6-OHDA lesions of LC potentiate the effect of $beta_1$-adrenoceptor agonists but abolishes that of $beta_2$-adrenoceptor agonists. The findings of Chapter 3, that chronic DMI and IPD administration produces subsensitivity to both $beta_1$- and $beta_2$-adrenoceptor agonists, could therefore be accounted for by a reduction in the functional sensitivity of both adrenoceptor subtypes, or of $beta_1$-adrenoceptors alone, but not by a selective reduction in $beta_2$-adrenoceptor sensitivity.

It has been speculated that beta-adrenoceptor density may be increased in depression (see Pandey and Davis, 1979). The influence of beta-adrenoceptors on 5-HT mediated head-twitching is entirely facilitatory; beta-adrenoceptor antagonists have no effect on head-twitching unless this has been already potentiated by beta-agonists (Chapter 2; Goodwin and Green, 1985). This was also seen in chapter 3. Chronic antidepressant pretreatment was without effect on L-5-HTP head-twitching unless it was potentiated by a beta-adrenoceptor agonist. In terms of the behavioural model used here, antidepressants would be able to

reduce the effect of such 'up-regulation' without inducing net underactivity in the underlying system. Antidepressants are also characterised by their ability to relieve depression without elevating normal mood. Interactions of antidepressants with head-twich behaviour thus show some interesting parallels with their effects in man.

In Chapter 4 the effect of drugs acting at GABA receptors and other chloride ionophore-related sites was studied for their ability to modulate the head-twitch induced by L-5-HTP in the mouse. The results obtained show that the $GABA_A$ receptor agonists, muscimol, IMAA and 3-APS, produced a dose related potentiation, while bicuculline inhibited the head-twitch. The $GABA_B$ receptor agonist, baclofen, produced dose related inhibition. Diazepam potentiated, whilst the 'inverse' benzodiazepine receptor agonist beta-CCE inhibited the L-5-HTP head-twich response. The benzodiazepine receptor antagonist RO-15-1788 also produced inhibition. RO-Ø5-4864, a ligand for the benzodiazepine 'acceptor' site, potentiated the head-twitch. Pentobarbitone and PTZ potentiated the L-5-HTP-induced head-twitch at low doses, changing to inhibition as the dose was increased. All effective doses of phenobarbitone produced inhibition of this response. Picrotoxin in subconvulsant doses, produced only potentiation. GABA itself (administered i.c.v.), AOAA (GABA transferase inhibitor) and DABA (GABA neuronal reuptake blocker) inhibited the L-5-HTP head-twitch, while 3-MPA (GABA depleter) potentiated it. These results demonstrate that

both $GABA_A$ and $GABA_B$ receptors modulate the L-5-HTP response in mice. Since head-twitching was induced by precurser loading, it is possible that the actions of GABA-related drugs were exerted through presynaptic 5-HT mechanisms, such as on release and reuptake of 5-HT. This possibility was investigated in Chapter 5. The effect of muscimol, 3-APS, diazepam, PTX, pentobarbitone and baclofen on 5-MeODMT induced head-twitch response in mice was tested. It was found that these GABA-related drugs exerted similar effects on 5-MeODMT as on L-5-HTP induced head-twitch response in mice. These results demonstrate that the actions of GABA-related drugs do not involve presynaptic 5-HT mechanisms. However, recently Metz et al. (1985) reported that the $GABA_B$ receptor agonist baclofen blocked the L-5-HTP and not 5-MeODMT induced head-twitch response in mice. These authors suggested that the ability of baclofen to block the L-5-HTP induced head-twitch is due to inhibition of 5-HT release. In support of this baclofen does block 5-HT release *in vitro* from brain tissue preloaded with [^{3}H]-5-HT (Schlicker et al., 1984). This action of baclofen is unlikely to occur *in vivo* following large doses of L-5-HTP, since the 5-HT mediated behaviours resulting from precursor loading are due to 'spill over' of 5-HT into the synaptic cleft (Grahame-Smith, 1971; Green and Heal, 1985). This 'spill over' process is unlikely to be under physiological control requiring calcium ions. Therefore, the reason for discrepancy between the present results and those reported by Metz et al. (1985) is unknown.

From the above results it appears that GABA-related drugs modulate 5-HT head-twitch behaviour by:-

i. either exerting their effects directly on the '5-HT head-twitch initiating mechanism' or

ii. indirectly through another neurotransmitter capable of modulating this behaviour.

The exact site of 5-HT head-twitch initiation is unknown and therefore investigation of the first possibility is very difficult. The second possibility was examined in detail with respect to noradrenaline and beta-adrenoceptors.

$GABA_A$ receptors are capable of modulating noradrenaline release; GABA triggers Calcium ion dependent noradrenaline release from hippocampal synaptosomes (Fung and Fillenz, 1983). This effect is mimicked by low concentrations of pentobarbitone (Fung and Fillenz, 1984). Higher concentrations of pentobarbitone inhibited release as did all effective concentrations of phenobarbitone (Fung and Fillenz, 1983; 1984). This pattern is very similar to the effects of phenobarbitone and pentobarbitone on the L-5-HTP head-twitch response described in Chapter 4. Thus, low doses of pentobarbitone increased head-twitch response, whereas higher doses of this barbiturate and all effective doses of phenobarbitone reduced this behaviour. However GABA enhancement of noradrenaline release has been reported to be PTX sensitive, while PTX in subconvulsant doses induced potentiation of the L-5-HTP and 5-MeODMT head-twitch, rather

than the inhibition predicted by this mechanism (Chapters 4 and 5). In vitro work has demonstrated that barbiturates, PTZ and PTX bind to the same site (See Ticku and Maksay, 1983). This PTX binding site has not yet been fully characterised and it has been suggested that subtypes may exist and some of these sites may not be associated with $GABA_A$-receptor (Johnston and Willow, 1982). It is possible that the potentiating action of PTX on L-5-HTP and and 5-MeODMT head-twitch responses, is associated with antagonism of the site which higher doses of pentobarbitone and all effective doses of phenobarbitone stimulate to exert their effects on head-twitching. The $GABA_B$ receptor agonist baclofen, blocks 5-HT mediated head-twitch behaviour and has been shown to decrease potassium evoked release of noradrenaline from cortical slices (Bowery et al., 1980).

The possible involvement of noradrenaline in mediating the actions of GABA-related drugs was investigated in Chapters 5 and 6. The pretreatment of mice with FLA-63 (dopamine beta-hydroxylase inhibitor) to block noradrenaline synthesis prevented the modulating actions of muscimol, 3-APS, diazepam, pentobarbitone, PTX and baclofen on the 5-MeODMT induced head-twitch. Furthermore, in mice the potentiating effects of muscimol and diazepam were blocked by the $beta_1$-adrenoceptor antagonist, metoprolol. These results suggests that GABA-related drugs require the presence of noradrenaline and beta-adrenoceptors to modulate the 5-HT mediated head-twitch response. Noradrenaline neurones of the LC are inhibited by iontophoretic GABA and

diazepam (Cedarbaum and Aghajanian, 1977; Grant et al., 1980), but this result was obtained under anaesthesia which is capable of reversing drug effects on this nucleus (see Foote etal., 1983). The possible involvement of neurones originating from the LC in mediating the actions of GABA-related drugs was examined in Chapter 6. Rats with bilateral 6-OHDA lesions of LC which were also used for examination of beta-adrenoceptor agonists, were used to investigate the effect of diazepam on the quipazine induced head-twitch response. Since the same lesioned and sham operated rats were used several times, only the effect of diazepam was examined here, because it is probably the least toxic of GABA-related drugs investigated in mice (Chapters 4 and 5). The potentiating effect of diazepam was not only abolished by LC lesions but was converted into inhibition, suggesting an underlying inhibitory effect of this drug which is independent of LC. The LC neurones were shown to be essential for the production of potentiation by diazepam and in the mouse at least, the potentiating effects of diazepam and muscimol were prevented by the $beta_1$-adrenoceptor antagonist, metoprolol (Chapter 5). Therefore, LC appears to innervate beta-adrenoceptors involved in potentiation of head-twitch by GABA-related drugs. The actual site of action of GABA drugs is not clear from the present results but two possibilities exist:-

i. modulation of noradrenaline release (Fung and Fillenz, 1984; Bowery et al., 1980). This is likely to involve neurones originating from the LC which innervate postsynaptic $beta_1$-adrenoceptors, since LC lesions and metoprolol abolished the

potentiating effect of diazepam or

ii. direct action on the postsynaptic GABA-receptors which are dependent on activation of $beta_1$-adrenoceptors to exert their inhibitory effect; these $beta_1$-adrenoceptors are innervated by neurones originating from the LC. This aspect of the pharmacology of head-twitching shows strong parallels with certain electrophysiological findings. Postsynaptic interactions between noradrenaline and GABA have been established in cortex and cerebellum (Woodward et al., 1979), where the pathways originating from the LC increase GABA-mediated inhibition by a beta-adrenoceptor dependent mechanism (Moises et al., 1981; Waterhouse et al., 1982); the iontophoretic application of the beta-adrenoceptor antagonist sotalol to purkinje neurones reduced baseline responsiveness to GABA as well as abolishing beta-agonist induced increases in GABA potency (Waterhouse et al., 1982). Further work has demonstrated that this beta-adrenoceptor is of $beta_1$-subtype, since the potentiating effects of noradrenaline were blocked by practolol ($beta_1$-adrenoceptor antagonist) and not zinterol ($beta_2$-adrenoceptor antagonist) (Yeh, 1981). This is consistent with the finding that $beta_1$-adrenoceptor blockade abolished the potentiation due to muscimol and diazepam of 5-HT mediated head-twitch response.

The beta-adrenoceptor mediated enhancement of GABA inhibition discussed above seems to be a component of the mechanism whereby noradrenaline pathways originating from the LC are proposed to

enhance the effectiveness of sensory input in a variety of brain areas by increasing the signal/noise ratio (see Foote et al., 1983).

These effects of noradrenergic transmission from LC, on sensory processing and GABA mediated responses, could provide an explanation for noradrenaline- and GABA-interactions with the 5-HT mediated head-twitch behaviour. Head-twitching due to 5-HT agonists is dependent on sensory input from the pinna region since it is abolished by local anaesthesia of the pinna (Boulton and Handley, 1973). In appearance, it is identical with the pinna reflex (e.g. Corne et al., 1963). It may represent a higher-level modulation of this reflex, since both vertical transection at the posterior commisure (Bedard and Pycock, 1977) and 5-HT receptor antagonism (Corne et al., 1963) abolish the head-twitch but not the pinna reflex. The vast majority of head-twitch inducing agents are hallucinogens (Corne and Pickering, 1967; Bednarzcyk and Vetulani, 1978) and it has been suggested that the head-twitch represents a lowering of the threshold for detection of sensory input from the pinna region (Boulton and Handley, 1973). The resulting signal, may be further modulated by noradrenaline neurones originating in LC.

From the present results, it is not clear whether the GABA-related drugs exert their effects by modulating noradrenaline release or by acting postsynaptically on the site where noradrenaline potentiates the inhibitory effect of GABA. Further

work involving the local application of GABA-related drugs to pre- and post-synaptic noradrenergic neurones originating from the LC is necessary to establish the exact site of action of these drugs.

The results in this thesis indicate that interactions between GABA and noradrenaline neurotransmissions result in major changes in sensitivity to the effects of a 5-HT receptor agonist. The exact site of these interactions is not clear. These three transmitters are implicated in the mode of action of a very wide variety of centrally acting drugs and in the causation of CNS disease. For example, both noradrenaline and 5-HT are involved in the aetiology of depression (see Van Praag, 1978). In addition the "down-regulation" of beta-adrenoceptors by antidepressants requires the presence of 5-HT neurones, if not of 5-HT itself (Nimgaonkar et al., 1985), the GABA-agonist progabide, has been claimed to be an effective antidepressant (Lloyd et al., 1983) and chronic administration of DMI reduces the beta-adrenoceptor enhancement of GABA inhibition (Yeh and Woodward, 1983; Waterhouse et al., 1984). Further investigation of this complex three-way interaction between neurotransmitters may indicate the mechanism by which such effects occur. Head-shaking behaviour in mice and rats appears to be a useful model for studying these interactions in vivo.

References

Ablad, B., Carlsson, D. and Ek, L. (1973). Pharmacological studies of two new cardioselective adrenergic beta-receptor antagonists. Life Sci., 12, 107-119.

Adams, P.R. and Brown, D.A. (1975). Actions of gamma-aminobutyric acid on sympathetic ganglion cells. J. Physiol. London, 250, 85-120.

Aghajanian, G.K. (1978). Feedback regulation of central monoaminergic neurons: evidence from single cell recording studies. In: Essays in Neurochemistry and Neuropharmacology, Vol. 3 (Youdim, M.B.H. and Lovenberg, W. Eds.), p1-32. Wiley, London.

Aghajanian, G.K. (1980). Mescaline and LSD facilitate the activation of locus coeruleus neurons by peripheral stimuli. Brain Res., 186, 492-498.

Aghajanian, G.K. (1981). The modulatory role of serotonin of multiple receptors in brain. In: Serotonin Neurotransmission and Behaviour (Jacobs, B.L. and Gelperin, A. Eds.), p156-185. MIT Press, Cambridge, Massachusetts.

Aghajanian, G.K. and Wang, R.Y. (1978). Physiology and Pharmacology of central serotonerigic neurons. In: Psychopharmacology: A generation of progress (Lipton, M.A., Dimascio,A. and Killam, K.F., Eds.) p171-183. Raven Press, New York.

Aihara, H., Araki, H. and Ohzeki, M. (1982). Hippocampal kindling and effects of antiepileptic drugs. Jpn. J. Pharmac., 32, 37-45.

Amin, A.H., Crawford, T.B.B. and Gaddum, J.H. (1954). The distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. J. Physiol., 126, 596-618.

Araki, H. and Aihara, H. (1985). Effects of morphine and neuroleptics on wet-dog shaking behaviour elicited by hippocampal stimulation in rats. Psychopharmacology, 85, 301-305.

Banerjee, S.P., Kung, L.S., Riggin, S.J. and Chanda, S.K. (1977). Development of beta-adrenergic receptor subsensitivity by antidepressants. Nature, 268, 455-456.

Baraban, J.M. and Aghajanian, G.K. (1980). Suppression of firing activity of 5-HT neurons in the dorsal raphe by alpha adrenoceptor antagonists. Neuropharmacology, 19, 355-365.

Barbaccia, M.L., Brunello, N. Chang, D.M. and Costa, E., (1983). Serotonin-elicited amplification of adenylate cyclare activity in hippocampal membranes from adult rat. J. Neurochem., 40, 1671-1679.

Barbeau, H. and Bedard, P. (1881). Denervation supersensitivity to 5-hydroxytryptophan to rats following spinal transection and 5,7-dihydroxytryptamine injection. Neuropharmacology, 20, 611-616.

Barker, J.L., Gratz, E., Owen, D.G. and Study, R.E. (1983). Pharmacological effects of clinically important drugs on the excitability of cultured mouse spinal neurons. In Actions and Interactions of GABA and Benzodiazepines (Bowery, N.G. Ed.). Raven Press, New York.

Bedard, P. and Pycock, C.J. (1977). "Wet-dog shake" behaviour in

the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology, 16, 663-670.
Bednarczyk, B. and Vetulani, J. (1978). Antagonism of clonidine to shaking behaviour in morphine abstinence syndrome and to head-twhtches produced by serotonergic agents in the rat. Pol. J. Pharmac. Pharm., 30, 307-322.
Bergstrom, D.A., and Kellar, K.J. (1979). Adrenergic and serotonergic receptor binding in rat brain after chronic desmethylimipramine treatment. J. Pharmac. Exp. Ther., 209, 256-261.
Berridge, M.J., Downes, P.C. and Handley, M.R. (1982). Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. Biochem. J., 206, 587-595.
Bertler, A. and Rosengren, E. (1959). Occurrence and distribution of catecholamines in brain. Acta. Physiol. Scand., 47, 350-361.
Blackburn, T.P., Bowery, N.G., Cox, B., Hudson, A.L., Martin, D. and Price, G.W. (1984). Lesion of the dorsal raphe nucleus increases the nigral concentration of 5-HT_1 receptors. Br. J. Pharmac., 82, 203P.
Bloom, F., Segal, D., Ling, N. and Guillemin, R. (1976). Endorphins: profound behavioural effects in rats suggest new etiological factors in mental illness. Science, 194, 630-632.
Bloom, F.E., Siggins, B., Hoffer, B.J., Segal, M. and Oliver, A.P. (1975). Cyclic nucleotides in the central synaptic actions of catecholamines. In: Advances in cyclic nucleotide research (Drummond, G.I., Greengard, P. and Robinson, G.A. Eds.), Raven Press, New York.
Boulton, C.S. and Handley, S.L. (1973). Factors modifying the head- twitch response to 5-hydroxytryptophan. Psychopharmacologia 20, 204-214.
Bowery, N.G., Doble, A., Hill, D.R., Hudson, A.L., Middlemiss, D.N., Shaw, J.S. and Turnbull, M.J. (1980). (-)-Baclofen decreases transmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature, 283, 92-84.
Bowery, N.G., Hill, D.R. and Hudson, A.L. (1983a). Characteristics of $GABA_b$ receptor binding sites on rat whole brain membranes. Br. J. Pharmac., 78, 181-206.
Bowery, N.G., Hill, D.R., Hudson, A.L., Price, G.W., Turnbull, M.J.and Wilkin, G.P. (1983b). Heterogeneity of mammalian GABA receptors. In: Actions of GABA-receptors and Benzodiazepines (Bowery, N.G. Ed.). Raven Press, New York.
Bowery, N.G., Price, G.W., Hudson, A.L., Hill, D.R., Wilkin, G.P. and Turnbull, M.J.(1984). GABA receptor multiplicity. Visualization of different receptor types in the mammalian CNS. Neuropharmacology, 23, 219-221.
Braestrup, C. and Nielsen, M. (1978). Benzodiazepine receptors. In: Handbook of Psychopharmacology (Iversen, L., Iversen, S.D. and Snyder, S.H. Eds.), Vol. 17, p285-384. Plenum Press, New York.
Braestrup, C. and Nielsen, M. (1982). Neurotransmitters and CNS Disease. Anxiety. The Lancet, II, 1031-1034.
Braestrup, C. and Squires, R.F. (1977). Specific benzodiazepine receptors in rat brain characterized by high affinity ^3H-diazepam binding. Proc. Natn. Acad. Sci. U.S.A. 74, 3805-3809.
Brazell, M.P., Marsden, C.A., Nisbet, A.P. and Routledge, C.

(1985). The 5-HT_1 receptor agonist RU-24969 decreases 5-hydroxytryptamine (5-HT) release and metabolism in the rat frontal cortex in vitro and in vivo. Br. J. Pharmac., 86, 209-216.

Brittain, R.T., Farmer, J.B., Jack, D., Martin, L.E. and Simpson, N.T. (1968). Alpha-(t-butylamino(methyl]-4-hydroxy-m-xylene-alpha^1, alpha^3-diol (AH 3365): a selective beta-adrenergic stimulant. Nature 219, 862-863.

Brittain, R.T. and Handley, S.L. (1967). Temperature changes produced by injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. J. Physiol., 192, 805-813.

Brownstein, M.J., Palkovits, M., Saavedra, J.M., Bassiri, R.M. and Utiger, R.D. (1974). Thyrotropin-relesing hormone in specific nuclei of rat brain. Science, 185, 267-269.

Carlsson, A. (1976). The contribution of drug research to investigating the nature of endogenous depression. Pharmakopsychiatrie Neuropsychopharmakologie, 9, 2-10.

Carlsson, A., Dahlof, C.-G. and Hedberg, A. (1977). Differentiation of cardiac chronotropic and inotropic effects of beta-adrenoceptor agonists. Naunyn Schmiedebergs Arch. Pharmac., 300, 101-104.

Cassel., D., Lefkowitz, H. and Sellinger, Z. (1977). The regulatory GTPase cycle of turkey erythrocyte adenylate cyclase. J. Cyclic Nucleic Res., 3, 893-406.

Cedarbaum, J.M. and Aghajanian, G.K. (1977). Catecholamine receptors on locus coeruleus neurons: pharmacological characterization. Eur. J. Pharmac., 44, 375-385.

Chang, C.C. (1964). A sensitive method for spectrophotofluorometric assay of catecholamines. Int. J. Neuropharmacology, 3, 643-649.

Clark, D.W.J., Laverty, R. and Phelam, E.L. (1972). Long-lasting peripheral and central effects of 6-hydroxydopamine in rats. Br. J. Pharmac., 44, 233-243.

Colpaert, F.C. and Janssen, P.A. (1983). The head-twitch response to intraperitoneal injection of 5-hydroxytryptophan in the rat: antagonist effects of purported 5-hydroxytryptamine antagonists and pirenperone an LSD antagonist. Neuropharmacology, 22, 993-1000.

Conn, P.J. and Sanders-Bush, E. (1984). Selective 5-HT-2 antagonists inhibit serotonin stimulated phosphatidylinositol metabolism in cerebral cortex. Neuropharmacology, 23, 993-996.

Corne, S.J., Pickering, R.W. and Warner, B.T. (1963). A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. Br. J. Pharmac., 20, 106-120.

Corne, S.J. and Pickering, R.W. (1967). A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. Psychopharmacologia, 11, 65-78.

Cortes, R., Palacious, J.M. and Pazos, A. (1984). Visualisation of multiple serotonin receptors in the rat brain by autradiography. Br. J. Pharmac., 82, 202P.

Costain, D.W. and Green, A.R. (1978). beta-Adrenoceptor antagonists inhibit the behavioural responses of rats to increased brain 5-hydroxytryptamine. Br. J. Pharmac., 64, 193-200.

Cowen, P.J., Grahame-Smith, D.G., Green, A.R. and Heal, D.J. (1982). beta- Adrenoceptor agonists enhance 5-hydroxytryptamine-mediated behavioural responses. Br. J. Pharmac., 76, 265-270.

Cox, R.M. and Perhach, J.C. (1973). A sensitive rapid and simple method form simultaneous spectrofluorometric determination of norepinepherine, dopamine, 5-hydroxytryptamine and 5-hydroxyindole acetic acid in discrete areas of the brain. J. Neurochem., 20, 1777-1780.

Curtis, D.R., Duggan, A.W., Felix, A. and Johnston, G.A.R. (1970). GABA, bicuculline and central inhibition. Nature, 226, 1222-1224.

Curtis, D.R., Duggan, A.W., Felix, A. and Johnston, G.A.R. (1971). Bicuculline, an antagonist of GABA and synaptic inhibition in the spinal cord of the cat. Brain Res., 32, 69-96.

Dahlstrom, A. and Fuxe, K. (1964). Evidence for the existence of monoamine-containing neurons in the central nervous system. I. demonstration of monoamines in the cell bodies of brainstem neurons. Acta. Physiol. Scand., 62, Suppl. 232, 1-55.

Daly J.W. (1977). The formation, degradation and function of cyclic nucleotides in the nervous system. Int. Rev. Neurobiol., 20, 105-168.

Davies, A.O. and Lefkowitz, J.R. (1980). Corticosteroid-induced differential regulation of beta-adrenergic receptors in circulating human polymorphonuclear leukocytes and mononuclear leukocytes. J. Clin. Endocrinol. Metab., 51, 599-605.

Davies, A.O. and Lefkowitz, J.R. (1981). Agonist-promoted high affinity state of the beta-adrenergic receptor in human neutrophils: modulation by corticosteroids. J. Clin. Endocrinol. Metab., 53, 703-708.

Day, M.D., Hemsworth, R.A. and Street, J.A. (1977). The central uptake of beta-adrenoceptor antagonists. J. Pharm. Pharmac., 29: 52P.

Deakin, J.F.W. and Green, A.R. (1978). The effects of putative 5-hydoxytryptamine antagonists on the behaviour produced by administration of tranylcypromine and L-dopa to rats. Br. J. Pharmac., 64, 201-209.

Delini-Stula, A., Vassout, A., Radeke, E. and Ortmann, R. (1979). Psychopharmacological profile of beta$_2$-receptor stimulants. Naunyn Schmiedebergs Arch. Pharmac., 307, R65.

De Montigny, C. and Aghajanian, G.K. (1977). Preferential action of 5-methoxydimethytryptamine and 5-methoxytryptamine on presynaptic serotonin receptors: a comparative iontophoretic study with LSD and serotonin. Neuropharmacology, 16, 811-818.

De Montigny, C. and Aghajanian, G.K. (1978). Tricyclic antidepressants: Long-term treatment increases responsitivity of rat forebrain neurons to serotonin. Science, 202, 1303-1306.

Desarmenian, M., Feltz, P., Loeffler, J.P., Occhipinti, G. and Santangelo, F. (1982). Multiple GABA-receptors on A sigma and C primary afferent neurones in the adult rat. Br. J. Pharmac., 76, 289P.

Desarmenian, M., Feltz, P., Occhipinti, G., Santangelo, F. and Schlichter, R. (1984). Co-existence of $GABA_a$ and $GABA_b$ receptors on A and C primary afferents. Br. J. Pharmac., 81,

327-334.
Deschenes, M., Feltz, P. and Lamour, Y. (1976). A model for an estimate in vivo of the ionic basis of presynaptic inhibition: an intracellular analysis of the GABA-induced depolarisation in rat dorsal root ganglia. Brain Res., 118, 486-493.
Dhalof, C., Englberg, G. and Svensson, T.H. (1981). Effect of beta-adrenoceptor antagonists on the firing rate of noradrenaline neurons in the Locus Coeruleus of the rat. Naunyn Schmiedebergs Arch. Pharmac., 317, 26-30.
Downes, C.P. (1983). Inositol phospholipids and neurotransmitter-receptor signalling mechanisms. Trends Neurosci., 6, 313-316.
Dreifuss, J.J., Kelly, J.S. and Krnjevic, K. (1969). cortical inhibition and gamma-aminobutyric acid. Expl. Brain Res., 9, 137-154.
Drust, E.G. and Connor, J.D.(1983).Pharmacological analysis of shaking behaviour induced by enkephalins, thyrotropin-releasing hormone or serotonin in rats: evidence for different mechanisms. J. Pharmac. Exp. Ther., 224, 148-154.
Drust, E.G., Sloviter, R.S. and Connor, J.D. (1979). Effect of morphine on 'wet-dog' shakes caused by cerebroventricular injection of serotonin. Pharmacology, 18, 299-305.
Dunlap, D. and Shanks, R.G. (1968). Selective blockade of adrenoceptive beta-receptors in the heart. Br. J. Pharmac., 32, 201-218.
Dunlap, K. (1981). Two types of gamma-aminobutyric acid receptor on embryonic sensory neorones. Br. J. Pharmac., 74, 579-586.
Euvrard, C. and Boisser, J.R. (1980). Biochemical assessment of the central 5-HT agonist activity of RU 24969 (a piperidinylindole). Eur. J. Pharmac., 63, 65-72.
Engel, G., Gothert, M., Muller-Schweinitzer, E., Schliker, E., Sistonen, L. and Stadler, P.A. (1983). Evidence for common pharmacological properties of [^{3}H]-5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. Naunyn Schmiedebergs Arch. Pharmac., 324, 116-124.
Ennis, C., Kemp, J.D. and Cox, B. (1981). Characterisation of inhibitory 5-hydroxytryptamine receptors that modulate dopamine release in the striatum. J. Neurochem., 36, 1515-1520.
Falck, B., Hillarp, N.A., Thiema, G. and Torp, A. (1962). Fluorescence of catecholamines and related compounds condensed with formaldehyde. J. Histochem. Cytoche., 10, 348-354.
File, S.E., Lister, R.G. and Nutt, D.J. (1982). The anxiogenic action of benzodiazepine antagonists. Neuropharmacology, 21, 1033-1037.
Fillion, G., Beaudoin, D., Rousselle, J.C., Fillion, M.P., Dray, F., and Jacobs, J. (1979a). Decrease of [^{3}H]-5-HT high affinity binding and 5-HT adenylate cyclase activation after kainic acid lesion in rat brain striatum. J. Neurochem., 33, 567-570.
Fillion, G., Rousselle, J.C., Beaudoin, D., Pradelles, P., Goiny, M., Dray, F., and Jacobs, J. (1979b). Serotonin sensitive adenylate cyclase in horse brain synptosomal membranes. Life Sci., 24, 1813-1822.
Foote, S.L., Bloom, F.E. and Aston-Jones, G. (1983). Nucleus Locus coeruleus: new evidence of anatomical and

physiological specificity. Physiol. Rev., 63, 844-914.
Fozard, J.R. (1983). Mechanism of the hypotensive effect of ketanserin. J. Cardiovasc. Pharmac., 4, 829-838.
Frances, H., Lecrubier, Y., Puech, A.J. and Simon, P. (1980). Evidence for the role of noradrenaline in some effects of quipazine. Psychopharmacology, 67, 307-310.
Frankhuyzen, A.L. and Mulder, A.H. (1980). Noradrenaline inhibits depolarization-induced [^{3}H]-serotonin release from slices of rat hippocampus. Eur. J. Pharmac., 63, 179-182.
Friedman, E., Cooper, T.B. and Dallob, A. (1983). Effects of chronic antidepressant treatment on serotonin receptor activity in mice. Eur. J. Pharmac., 89, 69-76.
Friedman, E. and Dallob, A. (1979). Enhanced serotonin receptor activity after chronic treatment with imipramine or amitriptytline. Commun. Psychopharmac., 3, 89-92.
Fung, S.-C. and Fillenz, M. (1983). The role of pre-synaptic GABA and benzodiazepine receptors in the control of noradrenaline release in rat hippocampus. Neurosci. Letts., 42, 61-66.
Fung, S.-C. and Fillenz, M. (1984). The actions of barbiturates on release of noradrenaline from rat hippocampal synaptosomes. Neuropharmacology, 23, 1113-1116.
Fuxe, K. (1965). Evidence for the existence of monoamine neurons in the central nervous system. IV. Distribution of monoamine nerve terminals in the central nervous system. Acta. Physiol. Scand., 64, Suppl. 247, 37-85.
Fuxe, K., Holmsdtedt, B. and Jonsson, G. (1972). Effects of 5-methoxy N-N dimethyltryptamine on central monoamine neurones. Eur. J. Pharmac., 19, 25-34.
Fuxe, K., Ogren,S.-O., Agnati, L.F., Benfenati, F., Fredholm, B., Andersson, K., Zini, I. and Eneroth, P. (1983). Chronic antidepressant treatment and central 5-HT synapses. Neuropharmacology, 22, 389-400.
Gallager, D.W., Mallorga, P., Oertel, W., Henneberry, R. and Tallman, J. (1981). [^{3}H]-Diazepam binding in mammalian central nervous system: pharmacological characterisation. J. Neurosci., 1, 218-228.
Geyer, M.A. and Segal, D.S. (1973). Differental effects of reserpine and alpha-methyl-p-tyrosine on norepinepherine and dopamine induced behavioural activity. Psychopharmacologia, 29, 131-138.
Glowinski, J. and Axelrod, J. (1964). Inhibition of uptake tritiated-noradrenaline in the intact rat brain by imipramine and structurally related compounds. Nature, 204, 1318-1319.
Glowinski, J. and Iversen, L.L. (1966). Regional studies of catecholamine in the rat brain. I. The disposition of [^{3}H]-norepinepherine, [^{3}H]-dopamine and [^{3}H]-dopa in various regions of the rat brain. J. Neurochem., 13, 655-669.
Gold, M.R. and Martin, A.R. (1984). gamma-Aminobutyric acid and glycine activate Cl^- channels having different characteristics in CNS neurones. Nature, 308, 639-641.
Goodwin, G.M., Green, A.R. and Johnson, P. (1984). 5-HT_2 receptor characteristics in frontal cortex and 5-HT_2 receptor-mediated head-twitch behaviour following antidepressant treatment to mice. Br. J. Pharmac., 83, 235-.
Goodwin, G.M. and Green, A.R. (1985). A behavioural and

biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT_1 and 5-HT_2 receptors. Br. J. Pharmac., 84, 743-753.

Gothert, M. and Huth, H. (1980). alpha-Adrenoceptor-mediated modulation of 5-hydroxytryptamine release from rat brain cortex slices. Naunyn Schmiedebergs Arch. Pharmac., 313, 21-26.

Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski, J. and Hamon, M. (1983). Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature, 305, 140-142.

Grabowska, M., Antkiewicz, L. and Michaluk, J. (1974). A possible interaction of quipazine with central dopamine structures. J. Pharm. Pharmac., 26, 74-76.

Grahame-Smith, D.G. (1971). Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. J. Neurochem., 18, 1053-1066.

Grant, S.J., Huang, Y.H. and Redmond Jr, D.E. (1980). Benzodiazepines attenuate single unit activity in the locus coeruleus. Life Sci., 27, 2231-2236.

Green, A.R. (1984). 5-HT mediated behaviour. Animal studies. Neuropharmacology, 23, 1521-1528.

Green, A.R. and Grahame-Smith, D.G. (1976). (-)-Propranolol inhibits the behavioural responses of rats to increased 5-hydroxytryptamine in the central nervous system. Nature, 262, 594-596.

Green, A.R. and Heal, D.J. (1985). The effects of drugs on serotonin-mediated behavioural models. In: Neuropharmacology of serotonin. (Green, A.R. Ed.), p326-265. Oxford University Press, Oxford.

Green, A.R., Heal, D.J., Johnson, P., Laurence, B.E. and Nimgaonkar, V.L. (1983a). Antidepressant treatments: effects in rodents on dose-response curves of 5-hydroxytryptamine- and dopamine-mediated behaviours and 5-HT_2 receptor number in frontal cortex. Br. J. Pharmac., 80, 377-385.

Green, A.R., Johnson, P., Mountford, J.A. and Nimgaonkar, V.L. (1985). Some anticonvulsant drugs alter monoamine-mediated behaviour in mice in ways similar to electoconvulsive shock; implications for antidepresaant theraphy. Br. J. Pharmac., 84, 337-346.

Green, A.R., Johnson, P. and Nimgaonkar, V.L. (1983b). Interactions of beta-adrenoceptor agonists and antagonists with the 5-hydroxytryptamine$_2$ (5-HT_2) receptor. Neuropharmacology, 22, 657-660.

Green, A.R. and D.J. Nutt (1983). Antidepressants. In: Preclinical Psychopharmacology, Part 1, (Grahame-Smith, D.G.), p1-37. Excerpta Medica, Oxford.

Green, A.R., O'Shaughnessy, K., Hammond, M., Schachter, M., and Grahame-Smith, D.G. (1983c). Inhibition of 5-hydroxytryptamine-mediated behaviour by the putative 5-HT_2 antagonist pirenperone. Neuropharmacology, 22, 573-578.

Green, A.R., Youdim, M.B.H. and Grahame-Smith, D.G. (1976). Quipazine: its effects on rat brain 5-HT metabolism, monoamine oxidase activity and behaviour. Neuropharmacology, 15, 173-179.

Griffiths, E.C. and Widdowson, P.S. (1985). Involvement of other neurotransmitters in TRH-induced wet-dog shaking. Br. J.

Pharmac., 86, 458P.
Haefely, W. (1983). The biological basis of benzodiazepine actions. The benzodiazepines today-two decades of research and clinical experience. J. Psychoact. Drugs, 15, 19-39.
Haefely, W., Bonetti, E.P., Burkard, W.P., Cumin, R., Laurent, J.P., Mohler, H., Pieri, L., Polc, P., Richards, J.G., Schaffner, R. and Scherschlicht, R., (1983). Benzodiazepine antagonists. In: The Benzodiazepines-From Molecular Biology to Clinical Practice (Costa, E. Ed.), p137-146. Raven Press, New York.
Haefely, W., Pieri, L., Polc, P., and Schaffner, R. (1981). General Pharmacology and neuropharmacology of benzodiazepine derivatives. In: Handbook of experimental Pharmacology. Vol. 55 Pt II (Hoffmeister F. and Stille G., Eds), p13-262. Springer-Verlag, Berlin.
Haigler, H.J. and Aghajanian, G.K. (1974). Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic neurons and neurons receiving serotonergic input. J. Pharmac. Exp. Ther., 188, 688-699.
Hall, H., Salldmark, M. and Ross, S.B. (1980). Clenbuterol, a central beta-adrenoceptor agonist. Acta Pharmacol. Toxicol. 47, 159-160.
Handley, S.L. (1970). Study of some of the pharmacological actions of noradrenaline and related compounds injected into the brains of mice. Ph.d. Thesis, Aston University, Birmingham.
Handley, S.L. and Brown, J. (1982). Effects on the 5-hydroxytryptamine induced head-twitch of drugs with selective actions on alpha$_1$- and alpha$_2$-adrenoceptors. Neuropharmacology, 21, 507-510.
Handley, S.L. and Miskin, R.C. (1972). Effect of cortisol on a central response to 5-hydroxytryptophan. Br. J. Pharmac., 45, 179-180.
Handley, S.L. and Miskin, R.C. (1977). The interaction of some kynurenine pathway metabolites with 5-hydroxytryptophan and 5-hydroxytryptamine. Psychopharmacologia (Berl.), 51, 305-309.
Handley, S.L., Mithani, S. and Singh, L. (1985). Locus Coeruleus lesions do not affect diazepam- or alpha-adrenergic modulation of operant conflict. Br. J. Pharmac., 84, 87P.
Harden, T.K. (1983). Agonist-induced desensitization of the beta-adrenergic receptor-linked adenylate cyclase. Pharmac. Rev., 35, 5-32.
Heal, D.J., Metz, A. and Philpot, J. (1985). 5,7-dihydroxytryptamine lesioning increases both 5-HT$_2$-mediated behaviour and cortical 5-HT$_2$ receptor number in mice. Br. J. Pharmac., 85, 311P.
Herz, A., Teschemacher, H., Albus, K. and Ziegelgansberger, S. (1972). Morphine abstinence syndrome in rabbits precipitated by injection of morphine antagonists into ventricular system and restricted parts of it. Psychopharmacologia, 26, 219-235.
Heym, J., Trulson, M.E. and Jacobs, B.L. (1982). Raphe unit activity in freely moving cats: effects of phasic auditory and visual stimuli. Brain Res., 232, 29-39.
Hill, D.R. and Bowery, N.G. (1981). ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline insensitive GABA$_b$ sites in rat brain. Nature, 290, 149-152.

Hjorth, S., Carlsson, A., Lindberg, P. Sanchez, D., Wikstrom, H., Arvidsson, L.-E., Hacksell, U and Nilsson,J.L.G. (1982). (-Hydroxy-2-(di-n-propylamino) tetralin, 8-OH-DPAT, a potent and selective simplified ergot congener with central 5-HT-receptor stimulating activity. J. Neurol. Trans., 55, 169-188.

Horton, R.W. and Meldrum, B.S. (1973). Seizures induced by allylglycine, 3-mercaptopropionic acid and 4-deoxypyridoxine in mice and photosensitive baboons, and different modes of inhibition of cerebral glutamic acid decarboxylase. Br. J. Pharmac., 49, 52-63.

Huang, L.M. and Barker, J.L. (1980). Pentobarbital: Stereospecific actions of (+) and (-) isomers revealed on cultured mammalian neurones. Science, 207, 195-197.

Hunkeler, W., Mohler, H., Pieri, L., Polc, P., Bonetti, E.P., Cumin R., Schaffner, R. and Haefely, W. (1981). Selective antagonists of benzodiazepines. Nature, 290, 514-516.

Iversen, L.L. (1977). Catecholamine-sensitive adenylate cyclase in nervous tissue. J. Neurochem., 29, 5-12.

Jacobs, B.L. (1976). An animal behavioural model for studing centrtal serotonergic synapses. Life Sci., 19, 777-786.

Jacobs, B.L. (1983). Mechanism of action of hallucinogenic drugs: focus upon postsynaptic serotonergic receptors. In: Part 1 Preclinical Psychopharmacology. (Grahame-Smith, D.G. Ed.), p344-376. Excerpta Medica.

Jenner, P., Chadwick, P., Reynolds, E.H. and Marsden, C.D. (1975). Altered 5-HT metabolism with clonazepam, diazepam and diphenyl hydantoin. J. Pharm. Pharmac., 27, 707-710.

Johnson, G.A., Kim, E.G. and Boukma, S.J. (1972). 5-Hydroxy-indole levels in rat brain after inhibition of dopamine-beta-hydroxylase. J. Pharmac. Exp. Ther., 180, 539-546.

Johnston, G.A.R. and Willow, M. (1982). GABA and barbiturate receptors. Trends Pharmac. Sci., 3, 328-330.

Jones, R.S.G. (1981). In vivo pharmacological studies on the interaction between tryptamine and 5-hydroxytyptamine. Br. J. Pharmac., 73, 485-493.

Jouvent, R., Lecrubier, Y., Peuch, A.J., Simon, P. and Widlocher, D. (1977). De l'etude experimentale d'un stimulant beta-adrenergique a la mise en evidence de son activite antidepressive chez l'homme. Encephale 3, 285-293.

Kellar, K.J., Cascio, C.S., Butler, J.A. and Kurtzke, R.N. (1981). Differential effects of electroconvulsive shock and antidepressant drugs on serotonin-2 receptors in rat brain. Eur. J. Pharmac., 69, 515-518.

Kendall, D.A. and Nahorski, S.R. (1983). Temperature-dependent 5-hydroxytryptamine (5-HT)-sensitive [^{3}H]spiperone binding to rat cortical membranes: regulation by quanine nucleotide and antidepressant treatment. J. Pharmac. Exp. Ther., 227, 429-434.

Klein, D.F. and Davis, J.M. (1969) In: Diagnosis and Drug Treatment of Psychiatric Disorders, (Williams and Wilkins, Baltimore), p187-298.

Kleinrok, Z. and Turski, L. (1980). Kainic acid-induced wet dog shakes in rats. The relation to central neutransmitters. Naunyn Schmiedibergs Arch. Pharmac., 314, 37-46.

Kostowski, W., Samanin, R., Bareggi, S.R., More, V., Garattini, S. and Valzelli, L. (1974). Biochemical aspects of the

interaction between midbrain raphe and locus coeruleus in the rat. Brain Res., 82, 178-182.
Krogsgaard-Larsen, P., Johnston, G.A.R., Lodge, D. and Curtis, D.R. (1977). A new class of GABA agonist. Nature, 268, 53-55.
Lakoski, J.M. and Aghajanian, G.K. (1985). Effects of ketanserin on neuronal responses to serotonin in the preferontal cortex, lateral geniculate and dorsal raphe nucleus. Neuropharmacology, 24, 265-273.
Lands, A.M., Lirdena, F.P. and Buzzo, H.J. (1967). Differentiation of receptors responsive to isoproternol. Life Sci., 6, 2241-2249.
Lanthorn, T. and Isaacson, R.L. (1978). Studies of kainate-induced wet-dog shakes in the rat. Liofe Sci., 22, 171-178.
Lapin, I.P. (1972). Interaction of kynurenine and its metabolites with tryptamine, serotonin and its precursors and oxotremorine. Psychopharmacologia (Berl.), 26, 237-247.
Lecrubier, Y., Peuch, A.J., Frances, H., Jouvent, R., Simon, P. and Widlocher, D. (1981). Beta-adrenergic stimulation and antidepressant activity. Acta. Psychiat. Scand., 63, 173-178.
Leclerc, G., Rouot, B., Velly, J. and Schvartz, J. (1981) Beta-adrenergic subtypes. Trends Pharmac. Sci., 17, 18-20.
Leysen, J.E. (1984). Problems in <u>in vivo</u> receptor binding stuidies and identification and role of serotonin receptor sites. Neuropharmacology, 23, 247-254.
Leysen, J.E., Awouters, F., Kennis, L., Laudron, P.M., Vanderberk, J. and Janssen, P.A.J. (1981). Receptor binding profile of R41468, a novel antagonist at 5-HT_2 receptors. Life Sci., 28, 1015-1022.
Leysen, J.E., Van Gompel, P., Verwimp, M. and Niemegeers, C.J.E. (1983). Role and localization of serotonin$_2$ (S_2)-receptor binding sites: effects of neuronal lesions. In: CNS receptors. From molecular pharmacology to behaviour (Mandel, P. and DeFeudis, F.V., Eds.), p373. Raven Press, New York.
Linton, M. and Gallo Jr, P.S. (1975). The practical statistician: simplified handbook of statistics. Brookside/Cole Publishing company, Montery, California.
Lloyd, K.G., Morselli, P.L., Depoortere, H., Fournier, V., Zivkobvic, B., Scatton, B., Brodkkamp, C., Worms, P. and Bartholini, G. (1983). The potential use of GABA agonists in psychiatric disorders: Evidence from studies with progabide in animals and clinical trials. Pharmac. Biochem. Behav., 18, 957-966.
Lloyd, K.G. and Pilc, A. (1984). Chronic antidepressants and GABA synapses. Neuropharmacology, 23, 841-842.
Maguire, M.E., Ross, E.M. and Gilman, A.G. (1977). beta-Adrenergic receptor: ligand binding properties and the interaction with adenyl cyclase. Adv. Cyclic Nucleotide Res., 8, 1-83.
Maj, J., Baran, L., Bigajska, K., Rogoz, A. and Skuza, G. (1978). The influence of neuroleptics on the behavioural effect of 5-hydroxytryptophan. Pol. J. Pharmac. Pharm., 20, 431-440.
Malick, J.B., Dosen, E. and Barnett, A. (1977). Quipazine-induced head-twitch in mice. Pharmac. Biochem. Behav., 6, 325-329.
Mandell, A.J. (1974). The role of adaptive regulation in the

pathophysiology of psychiatric disease. J. Psychiat. Res., 11, 173-179.

Marangos, P.J., Patel, J., Boulenger, J.P. and Clark-Rosenberg, R. (1982). Characterization of peripheral type benzodiazepine binding sites in brain using [^{3}H]-RO5-4864. Mol. Pharmac., 22, 26-33.

Marsden, C.A. and Martin, K.F. (1985). In vivo voltametric evidence that the 5-HT autoreceptor is not of the 5-HT_{1A} subtype. Br. J. Pharmac., 86, 445P.

Martin, L.L. and Sanders-Bush, E. (1982a). The serotonin autoreceptor: antagonism by quipazine. Neuropharmacology, 21, 445-450.

Martin, L.L. and Sanders-Bush, E. (1982b). Comparison of the pharmacological characteristics of 5-HT_1 and 5-HT_2 binding sites with those of serotonin autoreceptors which modulate serotonin release. Naunyn Schmiedibergs Arch. Pharmac., 321, 165-170.

Massingham, R., Dubocovich, M.L., Shepperson, N.B. and Langer, S.Z. (1981). In vivo selectivity of prazosin but not WB 4101 for postsynaptic alpha-1-adrenoceptors. J. Pharmac. Exp. Ther., 217, 467-474.

Matthews, W.D. and Smith, C.D. (1980). Pharmacological profile of a model for central serotonin receptor activation. Life Sci., 26, 1397-1402.

Maura, G., Gemignani, A. and Raiteri, M. (1982). Noradrenaline inhibits serotonin release through $alpha_2$-adrenoceptors located on serotonergic nerve terminals. Naunyn Schmiedibergs Arch. Pharmac., 320, 272-274.

McBurney, R.N. (1984). Membrane actions of GABA in cultured central neurones. In: Actions and Interactions of GABA and Benzodiazepines. (Bowery, N.G. Ed.) p43-58. Raven Press, New York.

McCall, R.B. and Aghajanian, G.K. (1979). Serotonergic facilitation of facial motoneurone excitation. Brain Res., 169, 11-27.

McCall, R.B. and Aghajanian, G.K. (1980). Pharmacological characterisation of serotonin receptors in the facial motor nucleus: a microiontophoretic study. Eur. J. Pharmac., 65, 175-183.

Metz, A., Goodwin, G.M. and Green, A.R. (1985). The administration of baclofen to mice increases 5-HT_2-mediated head-twitch behaviour and 5-HT_2 receptor number in frontal cortex. Neuropharmacology, 24, 357-360.

Middlemiss, D.N. (1984). 8-Hydroxy-2- (di-n-propylamino)tetralin is devoid of activity at the 5-hydroxytryptamine autoreceptor in rat brain. Implications for the proposed link between the autoreceptor and the [^{3}H]-5-HT recognition site. Naunyn Schmiedibergs Arch. Pharmac., 327, 18-22.

Middlemiss, D.N. (1985). The putative 5-HT_1 receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. J. Pharm. Pharmac., 37, 434-437.

Middlemiss, D.N., Blakeborough, L. and Leather, S.R. (1977). Direct evidence for an interaction of adrenergic blockers with the 5-HT receptor. Nature, 267, 289-290.

Middlemiss, D.N., Carroll, J.A., Fisher, R.W. and Mounsey, I.J. (1980). Does [^{3}H]spiroperidol labels a 5-HT receptor in the frontal c ortex of the rat? Eur. J. Pharmac., 66, 253-254.

Minneman, K.P., Dibner, M.D., Wolfe, B.B. and Molinoff, P.B. (1979a). Beta$_1$- and beta$_2$-adrenergic receptors in rat cerebral cortex are independently regulated. Science, 204, 866-868.

Minneman, K.P., Hegstrand, L.R. and Molinoff, P.B. (1979b). Simultaneous determination of beta-1 and beta-2 adrenergic receptors in tissues containing both receptor subtypes. Mol. Pharmac., 16, 34-46.

Misu, Y. and Kubo, T. (1983) Presynaptic beta-adrenoceptors. Trends Pharmac. Sci., 4, 506-508.

Mobley, P.L. and Sulser, F. (1979). Norepinepherine stimulated cyclic AMP accumulation in rat limbic forebrain slices: Partial mediation by a subpopulation of receptor with neither alpha or beta characteristics. Eur. J. Pharmac., 60, 221-227.

Modigh, K. (1972). Central and peripheral effects of 5-hydroxy-tryptophan on motor activity in mice. Psychopharmacologia, 22, 48-54.

Mogilnicka, E. and Klimek, V. (1979). Mianserin, danitracen and amitriptyline withdrawal increases the behavioural responses of rats to L-5-HTP. J. Pharm. Pharmac., 31, 704-705.

Mohler, H. and Richards, J.G. (1983). Autoradiographic visualization of ^{3}H-R0-5-4864 binding in the CNS. Br. J. Pharmac., 79, 280P.

Moises, H.C., Waterhouse, B.D. and Woodward, D.J. (1981). Locus Coeruleus stimulation potentiates purkinje cell responses to afferent input: the climbing fiber system. Brain Res., 222, 43-64.

Monroe, P.J. and Smith, C.D. (1983). Characteristics of multiple (^{3}H)5-hydroxytryptamine binding sites in rat spinal cord tissue. J. Neurochem., 41, 349-355.

Moran, J. F., Triggle, C.R. and Triggle, D.J. (1969). The mechanism of action of 2-halogenoethylamines at the adrenergic alpha-adrenoceptor and a further investigation of the 'spare receptor' hypothesis, J. Pharm. Pharmac., 21, 38-46.

Moser, P.C. and Redfern, P.H. (1984). Diurnal variation in behavioural responses to 5-HT receptor stimulation. Br. J. Pharmac., 82, 269P.

Nahorski, S.R. (1981). Identification and significance of beta-adrenoceptor subtypes. Trends Pharmac. Sci., 2, 95-98.

Nahorski, S.R. and Willcocks, A.L. (1983). Interactions of beta-adrenoceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex. Br.J. Pharmac., 78: 107P.

Nakamura, M. and Fukushima, H. (1976). Head-twitches induced by benzodiazepines and the role of biogenic amines. Psychopharmacology, 49, 259-261.

Nakamura, M. and Fukushima, H. (1977). Head-twitches induced by benzodiazepines and the role of biogenic amines. Psychopharmacology, 49, 259-261.

Nakamura, M. and Fukushima, H. (1978). Effects of reserpine, parachloropheylalaninie, 5,6 dihydroxytryptamine and fludiazepam on the head twitches induced by 5-hydroxytryptamine and 5-methoxytryptamine in mice. J. Pharm. Pharmac., 30, 254-256.

Nicoll, R.A., Eccles, J.C., Oshima, T. and Rubia, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. Nature, 258, 625-627.

Nimgaonker, V.L., Green, A.R., Cowen, P.J., Heal, D.J., Grahame-Smith, D.G. and Deakin, J.F.W. (1983). Studies on the mechanisms by which clenbuterol, a beta-adrenoceptor agonist, enhances 5-HT mediated behaviours and increases brain 5-HT metabolism in the rat. Neuropharmacology, 22, 739-749.

Nimgaonkar, V.L., Goodwin, G.M., Davies, C.L. and Green, A.R. (1985). Down-regulation of beta-adrenoceptors in rat cortex by repeated administration of desipramine, electroconvulsive shock and clenbuterol requires 5-HT neurones but not 5-HT. Neuropharmacology, 24, 279-283.

Nutt, D.J., Cowen, P.J. and Little, H.J. (1981). Unusual interactions of benzodiazepine receptor antagonists. Nature, 295, 436-438.

O'Donnell, S.R. and Wanstall, J.C. (1980). Evidence that ICI 118,551 is a potent highly $beta_2$-selective adrenoceptor antagonist and can be used to characterise beta-adrenoceptor populations in tissues. Life Sci., 27, 671-677.

O'Donnell, S.R. and Wanstall, J.C. (1985). Responses to the $beta_2$- selective agonist procaterol of vascular and aterial preparations with different functional beta-adrenoceptor populations. Br. J. Pharmac., 84, 227-235.

Ogren, S.-O., Fuxe, K., Agnati, L.F., Gustafsson, J.A., Jonsson G. and Holm, A.L. (1979). Re-evaluation of the indoleamine hypothesis of depression. Evidence for a reduction of functional activity of central 5-HT systems by anti-depressant drugs, J. Neurol. transm. 46, 85-103.

Olsen, R.W. (1981). GABA - benzodiazepine - barbiturate receptor interactions. J. Neurochem., 37, 1-13.

Olsen, R.W. and Snowman, A. (1982). Chloride dependent enhancement by barbiturates of gamma-aminobutyric acid receptor binding. J. Neurosci., 2, 1812-1823.

Orikasa, S., Sukurada, S. and Kisara, K. (1980). Head-twitch response induced by tyramine. Psychopharmacology, 67, 53-59.

Ortmann, R., Martin, S., Radeke, E. and Delini-Stula, A. (1981). Interaction of beta-adrenoceptor agonists with the serotonergic system in rat brain. A behavioural study using the L-5-HTP syndrome. Naunyn Schmiedebergs Arch. Pharmac., 316, 225-230.

Ortmann, R., Bischoff, S., Radeke, E., Buech, O. and Delini-Stula, A. (1982). Correlations between different measures of antiserotonin activity of drugs. Study with neuroleptics and serotonin receptor blockers. Naunyn Schmiedebergs Arch. Pharmac., 321, 265-270.

Oswald, I., Brezinova, V. and Dunleavy, D.L.F. (1972). On the slowness of action of tricyclic antidepressant drugs, Br. J. Psychiat. 120, 673-677.

Palmer, G.C. (1972). Increased cyclic AMP response in norepinepherine in the rat brain following 6-hydroxydopamine. Neuropharmacology, 11, 145-149.

Pandey, G.N. and Davis, J.M. (1979). Treatment with antidepressants, sensitivity of beta-adrenergic receptors and effective illness. In: Neuroreceptors basic and clinical aspects. (Usdin, E., Bunney, Jr. W.E. and Davis J.M. Eds.), p99-121. J. Wiley and Sons.

Pedigo, N.W., Yamamura, H.I. and Nelson, D.L. (1981). Discrimination of multiple [^{3}H]-5-hydroxytryptamine binding sites by neuroleptic spiperone in rat brain. J. Neurochem., 36, 220-226.

Pellegrino, L.J., Pellegrino, A.S. and Cushman, A.J. (1979). A stereotaxic atalas of the rat brain. Plenum Press. New York and London.

Peroutka, S.J., Lebovitz, R.M. and Snyder, S.H. (1981). Two distinct central serotonin receptors with different physiological funtions. Science, 212, 827-829.

Peroutka, S.J. and Snyder, S.H. (1979). Multiple serotonin receptors: differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol. Mol. Pharmac., 16, 687-699.

Peroutka, S.J. and Snyder, S.H (1980a). Regulation of serotonin$_2$ (5-HT$_2$) receptors labelled with ^{3}H-spiroperidol by chronic treatment with the antidepressant amitriptyline. J. Pharmac., Exp. Ther., 215, 582-587.

Peroutka, S.J. and Snyder, S.H. (1980b). Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. Science, 210, 88-90.

Petty, F. and Coffman, J.A. (1984). Plasma GABA: A possible indicator of altered GABA function in psychiatric illness. Neuropharmacology, 23, 859-860.

Pilc, A. and Lloyd, K.G. (1984). Chronic antidepressants and GABA "B" receptors: A GABA hypothesis of antidepressant drug action. Life Sci., 35, 2149-2154.

Polc, P., Bonetti, E.P., Schaffener, R. and Haefely, W. (1982). A three state of the benzodiazepine receptor explains the interactions between the benzodiazepine antagonist RO 15-1788, benzodiazepine tranquilizers, beta-carbolines and phenobarbitone. Naunyn Schmiedebergs Arch. Pharmac., 321, 260-264.

Prange, Jr, A.J., Breese, G.R., Cott, J.M., Martin, B.R., Cooper, B.R., Wilson, I.C. and Plotnikoff, N.P. (1974). Thyrotropin releasing hormone: antagonism of pentobarbital in rodents. Life Sci., 14, 447-455.

Reinhard, J.F. and Roth, R.H. (1982). Noradrenergic modulation of serotonin synthesis and metabolism, I. inhibition by clonidine in vivo. J. Pharmac. Exp. Ther., 221, 541-546.

Richards, J.G., Mohler, H. and Haefley, W. (1982). Benzodiazepine binding sites: receptors or acceptors ? Trends Pharmac. Sci., 3, 233-235.

Richards, J.G. and Mohler, H. (1984). Benzodiazepine receptors. Neuropharmacology, 23, 233-242.

Rodriguez, R. and Pardo, E.G. (1971). Quipazine, a new type of antidepressant agent. Psychopharmacologia, 21, 89-100.

Ross, S.B., Renyi, A.L. and Ogren, S.-O. (1971). A comparison of the inhibitory activities of iprindole and imipramine on the uptake of 5-hydroxytryptamine and noradrenaline in brain

slices. Life Sci., 10, 1267-1277.
Rucine, R., Rose, P.R. and Burnham, W.M. (1977). After discharge threshold and kindling rates in dorsal and ventral hippocampus and dentate gyrus. Can. J. Neurol. Sci., 4, 273-278.
Ruffolo Jr, R.R. and Yaden, E.L. (1983). Vascular effects of the stereoisomers of dobutamine. J. Pharmac. Exp. Ther., 224, 46-50.
Saner, A. and Pletscher, A. (1979). Effects of diazepam on central 5-hydroxytryptamine synthesis. Eur. J. Pharmac., 55, 315-318.
Schlicker, E. and Gothert, M. (1981). Antagonistic properties of quipazine at presynaptic receptors and alpha-adrenoceptors in rat brain cortex slices. Naunyn Schmiedibergs Arch. Pharmac., 317, 204-208.
Schlicker, E., Classen, K. and Gothert, M. (1984). $GABA_b$ receptor- mediated inhibition of serotonergic release in the rat. Naunyn Schmiedebergs Arch. Pharmac., 326, 99-105.
Schoemaker, H., Boles, R.G., Horst, W.D. and Yamamura, H.I. (1983). Specific high affinity binding for [^{3}H]-RO5-4864 in rat brain and kidney. J. Pharmac. Exp. Ther., 225, 61-69.
Schotte, A., Maloteatx, J.M. and Laduron, P.M. (1983). Characterization and regional distribution of serotonin S_2-receptors in human brain. Brain Res., 276, 231-235.
Scotti De Carolis, A. and Massoti, M. (1978). Electroencephalgraphic and behavioural investigations on "GABAergic" drugs: Muscimol, baclofen and sodium-hydroxybutyrate. Prog. Neuropsychopharmacology, 2, 431-442.
Shenker, A., Maayani, S., Weinstock, H. and Green, J.P. (1983). Characterization of a serotonin receptor coupled to adenylate cyclase in adult guinea pig hippocampus. Soc. Neurosci. Abs., 9, 1152.
Shinozaki H. and Konishi, S. (1970). Actions of several anthelminetics and insecticides on rat cortical neurones. Brain Res., 24, 368-371.
Simmonds, M.A. (1978). Classification of some GABA antagonists with regard to site of action and potency in slices of rat cuneate nucleus. Eur. J. Pharmac., 80, 347-358.
Simmonds, M.A. (1980). Evidence that bicuculline and picrotoxin act at seperate sites to antagonise gamma-aminobutyric acid in rat cuneate nucleus. Neuropharmacology, 19, 39-45.
Simmonds, M.A. (1982). Presynaptic actions of gamma-aminobutyric acid and some antagonists in a slice preparation of cuneate nucleus. Br. J. Pharmac., 63, 495-502.
Simmonds, M.A. (1984). Physiological and pharmacological characterisation of the actions of GABA. In: Actions and Interactions of GABA and Benzodiazepine. (Bowery, N.G. Ed.), p27-42. Raven Press, New York.
Simon, P., Lecrubier, Y., Jouvent, R., Peuch, A.J., Allilaire J.F. and Widlocher, D. (1978). Experimental and clinical evidence of the antidepressant effect of an beta-adrenergic agonist. Psychol. Med., 8, 335.
Skerritt, J.H., Willow, M. and Johnston, G.A.R. (1982). Diazepam enhancement of low affinity GABA binding to rat brain membranes. Neurosci. Lett., 29, 63-66.
Smialowski, A., Smialowska, M., Reichenberg, K., Byrska, B.

and Vetulani, J. (1980). Motor depression and head-twitches induced by i.p. GABA. Psychopharmacology, 69, 295-298.
Sonnenblick, E.H., Frishman, W.H. and Lejemtel, T.H. (1979). Dobutamine: a new synthetic cardioactive symathetic amine. N. Engl. J. Med., 300, 17-22.
Squires, R.F., Casida, J.E., Richardson, M. and Salderup, E. (1983). ^{35}S-t-butylbicyclophosphorthionate binds with high affinity to brain specific sites coupled to $GABA_A$ ion recognition sites. Mol. Pharmac., 23, 326-336.
Steinbusch, H.W.M. (1981). Distribution of serntonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. Neuroscience, 6, 557-618.
Steinbusch, H.W.M. and Niewenhuys, R. (1983). The raphe nuclei of the brainstem: a cytoarchitectonic and immunohistochemical study. In: Chemical Neuroanatomy (Emson, P., Ed.), pl31-207. Raven Press, New York.
Stephenson, R.P. (1956). Modification of receptor theory. Br. J. Pharmac. Chemother., 11, 379-393.
Stone, C.A., Wenger, H.C., Ludden, C.T., Stavorski, J.M. and Rose, C.A. (1961). Antiserotonin and antihistaminic properties of cyproheptadine. J. Pharmac. Exp. Ther., 131, 73-84.
Suchowsky, G.K., Pegrassi, L., Moretti, A. and Bonsignori, A. (1969). The effect of 4H-3 methylcarboxamide 1, 3-benzoxazine-2-one (FI 6654) on monoaminoxidase and cerebral 5-HT. Archs. Int. Pharmacodyn. Ther., 182, 332-340.
Study, R.E. and Barker, J.L. (1981). Diazepam enhancement and (-) pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responsesin cultured central neurons. Proc. Natn. Acad. Sci., 78, 7180-7184.
Sulser, F. and Mobley, P.L. (1981). Regulation of central noradrenergic receptor function: New vistas on the mode of action of antidepressant treatments. In: Neuroreceptors-Basic and Clinical aspects. (Usdin, E., Bunney, W.E. and Davis, J.M. Eds.), p55-83. John Wiley and Sons Ltd.
Supavilai, P., Mannonen, A., Collins, J.F. and Karobath, M. (1982). Anion-dependent modulation of ^{3}H-muscimol binding and of GABA-stimulated ^{3}H-flunitrazepam binding by picrotoxin and related CNS convulsants. Eur. J. Pharmac., 81, 687-691.
Sutton, I. and Simmonds, M.A. (1974). The selective blockade by diaminobutyric acid of neuronal uptake of [^{3}H]-GABA in rat brain <u>in vivo</u>. J. Neurochem., 23, 273-274.
Svensson, T.H., Bunney, B.S. and Aghajanian, G.K. (1975). Inhibition of both noradrenergic and serotonergic neurons in brain by the alpha-adrenoceptor agonist clonidine. Brain Res., 92, 291-306.
Svensson, T.H. and Waldeck, B. (1969). On the significance of central noradrenaline for motor activity: experiments with a new dopamine beta-hydroxylase inhibitor. Eur. J. Pharmac., 7, 278-282.
Swanson, L.W. and Hartman, B.K. (1975). The central adrenergic system. An immunofluorescence study of the location of cell bodies and the efferent connections in the rat utilizing dopamine-beta-hydroxylase as a marker. J. Comp. Neurol., 163, 467-505.
Tanka, T. and Starke, K. (1980). Antagonist/agonists-preferring

alpha$_1$/alpha$_2$ receptors ? Eur. J. Pharmac., 63, 191-194.
Theobold, W., Buch, H., Kupp, P., Stenger, E. and Heimann, H. (1968). Pharmakologische and experimental psychologische untersuchungen mit 2 Inhaltstoffen des Fliegenpilzes (Amanita Muscaria). Arzneim. Forsch., 18, 311-315.
Thyagarajan, R., Ramanlaneyulu, R. and Ticku, M.K. (1983). Enhancement of diazepam and gamma-aminobutyric acid binding by (+) etomidate and pentobarbital. J. Neurochem., 41, 575-585.
Ticku, M.K. (1980). Is picrotoxinin site at the GABA synapse a site of action for barbiturates. Brain Res., Bull, 5, (Suppl. 2), 919-923.
Ticku, M.K. and Maksay, G. (1983). Convulsant / depressant site of action at the allosteric benzodiazepine-GABA receptor-ionophore complex. Life Sci., 33, 2363-2375.
Ticku, M.K. and Olsen, R.W. (1978). Interactions of barbiturates with dihydropicrotoxinin binding sites related to the GABA-ionophore system. Life Sci., 22, 1643-1651.
Ticku, M.K., Thyagarajan, R. and Ramanjaneyulu, R. (1983). In: Neurotrnsmitters, Seizures and Epilepsies. (Fariello, F., Engel, J.R., marselli, P.L. and Quensey, R.F. Eds). Raven Press, New York.
Ticku, M.K. and Ramanjaneyulu, R. (1984). RO05-4864 inhibits the binding of [^{35}S]t-butylbicyclophosphorthionate to rat brain membranes. Life Sci., 34, 631-638.
Trulson, M.E. and Crisp, T. (1984). Role of norepinepherine in regulating the activity of serotonin-containing dorsal raphe neurons. Life sci., 35, 511-515.
Trulson, M.E. and Jacobs, B.L. (1979). Dissociations between the effects of LSD on behaviour and raphe unit activity in freely moving cats. Science, 205, 515-518.
Trulson, M.E. and Trulson, V.M. (1983). Chloral hydrate anesthesia alters the responsiveness of dorsal raphe neurons to psychoactive drugs. Life Sci., 32, 949-956.
Turski, L., Cruczwar, S.J., Turski, W. and Kleinrok, Z. (1981). Studies of carbachol-induced wet-dog shake behaviour in rats. Psychopharmacology, 73, 81-83.
Twarog, B.M. and Page, I.H. (1953). Serotonin content of some mammalian tissues and urine, and a method of determination. Am. J. Physiol., 175, 157-161.
U'Pritchard, D.C., Greenberg, D.A. and Snyder, S.H. (1978). The labelling of two alpha-adrenergic receptors in the CNS by catecholamines and other ligands: ion ansd nucleotide interactions. In: Recent advances in the Pharmacology of adrenoceptors. Elsevier, North Holland.
Van Praag, H.M. (1978). Amine hypotheses of affective disorders. In: Handbook of Psychopharmacology. (Iversen, L.L., Iversen, S.D. and Snyder, S.H. Eds.), pl3. Plenum Press, New York.
Van Reizen, H. (1972). Differential central effects of the 5-HT antagonists mianserine and cyproheptadine. Naunyn Schmiedibergs Arch. Pharmac., 198, 256-269.
Vetulani, J., Bednarczyk, B., Reichenberg, K. and Rokosz, A. (1980). Head-twitches induced by LSD and quipazine: Similarities and differences. Neuropharmacology, 19, 155-158.
Vetulani, J.F., Stawarz, R.J., Dingel, J.V. and Sulser, F. (1976a). A possible common mechanism of action of

antidepressant treatments. Naunyn Schmiedebergs Arch. Pharmac., 293, 109-114.
Vetulani, J., Byrska, B. and Reichenberg, K. (1979). Head twitches produced by serotonergic drugs and opiates after lesion of the mesostriatal serotonergic system of the rat. Pol. J. Pharmac. Pharm., 31, 413-423.
Vetulani, J., Stawarz, R.J. and Sulser, F. (1976b). Adaptive mechanisms of the noradrenergic cyclic AMP generating system in the limbic forebrain of the rat: adaptation to persistent changes in the avilability of norepinepherine(NE). J. Neurochem., 27, 661-666.
Vetulani, J. and Sulser, F. (1975). Actions of various antidepressant treatments reduces reactivity of noradrenergic cyclic AMP-generating system in limbic forebrain. Nature, 257, 495-496.
Waldmeier, P.C. (1981). Stimulation of central serotonin turnover by beta-adrenoceptor agonists. Naunyn Schmiedibergs Arch. Pharmac., 317, 115-119.
Wallach, D.P. (1961). Studies on the GABA pathway-I The inhibition of gamma-aminobutyric acid-alpha-ketoglutaric acid transaminase in vitro and in vivo by U-7524 (amino-oxyacetic acid). Biochem. Pharmac., 5, 323-331.
Waterhouse, B.D., Moises, H.C. Yeh, H.H., Geller, H.M. and Woodward, D.J. (1984). Comparison of norepinepherine-and benzodiazepine-induced augmentation of purkinje cell responses to gamma-aminobutyric acid (GABA). J. Pharmac. Exp. Ther., 228, 257-267.
Waterhouse, B.D., Moises, H.C., Yeh, H.H. and Woodward, D.J. (1982). Norepinephrine enhancement of inhibitory synaptic mechanisms in cerebellum and cerebral cortex: mediation by beta-adrenergic receptors. J. Pharmac. Exp. Ther., 21, 495-506.
Wei, E., Loh, H. and Way, E.L. (1973). Brain sites of precipitated abstinence in morphine dependent rats. J. Pharmac. Exp. Ther., 185, 108-115.
Wei, E., Sigel, S., Loh, H., and way, E.L. (1975). Thyrotrophin-releasing hormone and shaking behaviour in rat. Nature, 253, 739-740.
Westfall, T.C. (1977). Local regulation of adrenergic neurotransmission. Physiol. Rev., 57, 659-728.
White, S.R. and Newman, R.S. (1980). Facilitation of spinal motoneurone excitability by 5-hydroxytryptamine and noradrenaline. Brain Res., 188, 119-127.
Willow, M. and Johnston, G.A.R. (1980). Enhancement of GABA binding by pentobarbitone. Neurosci. Lett., 18, 323-327.
Winoker, A. and Utiger, R.D. (1974). Thyrotropin-releasing hormone: regional distribution in rat brain. Science, 185, 265-267.
Witkin, L.B., Spitaletta, P. and Plummer, A.J. (1959). Effects of some central depressants on two simple reflexes in the mouse. J. Pharmac. Exp. Ther., 126, 330-333.
Woodward, D.J., Moises, H.C., Waterhouse, B.D., Hoffer, B.J. and Freedman, R. (1979). Modulatory action of norepinepherine in the central nervous system. Federation Proceedings, 38, 2109-2116.
Wolfe, B.B., Harden, T.K., Sporn, J.R. and Molinoff, P.B.

(1978). Presynaptic modulation of beta adrenergic receptors in rat cerebral cortex after treatment with antidepressants. J. Pharmac. Exp. Ther., 207, 446-457.

Yabbuuchi, Y. (1977). The beta-adrenoceptor stimulant properties of OPC-2009 on guinea-pig isolated tracheal, right atrial and left atrial prepartions. Br. J. Pharmac., 61, 513-521.

Yamada, K. and Furukawa, T. (1981). The yawning elicted by alpha-melanocyte-stimulating hormone involves serotonergic-dopaminergic-cholinergic neuron link in rats. Naunyn Schmiedibergs Arch. Pharmac., 316, 155-160.

Yamada, K., Matsuki, J. and Furukawa, T. (1983). Wet-dog body shaking induced by electrical stimulation of hippocampus in the intact and reserpinized rat. Res. Commun. chem. Pathol. Pharmac., 39, 29-37.

Yap, C.Y. and Taylor, D.A. (1983). Involvement of 5-HT_2 receptors in the wet-dog shake behaviour induced by 5-hydroxytryptophan in the rat. Neuropharmacology, 22, 801-804.

Yeh, H.H. (1981). The noradrenergic system and the devolping cerebellum. Doctoral Disseration. The University of Texas Health Sciences Center at Dallas.

Yeh, H.H. and Woodward, D.J. (1983). Alterations in beta adrenergic physiological response characteristics after long-term treatment with desmethylimipramine: Interaction between norepinepherine and gamma-aminobutyric acid in rat cerebellum. J. Pharmac. Exp. Ther., 226, 126-134.

Br. J. Pharmac. (1985), **86**, 297–303

Modulation of 5-hydroxytryptamine-induced head-twitch response by drugs acting at GABA and related receptors

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1 The effects of drugs acting at the γ-aminobutyric acid (GABA) receptors and other chloride ionophore-related sites have been studied for their ability to modulate the head-twitch induced by 1-5-hydroxytryptophan (5-HTP) in the mouse.

2 The $GABA_a$ receptor agonists, muscimol, imidazoleacetic acid and 3-aminopropanesulphonic acid, produced a dose-related potentiation, while bicuculline inhibited the head-twitch. The $GABA_b$ receptor agonist, baclofen, produced dose-related inhibition.

3 Diazepam potentiated the head-twitch while the 'inverse' benzodiazepine receptor agonist ethyl-β-carboline-3-carboxylate inhibited the head-twitch. The antagonist Ro15-1788 also produced inhibition. Ro05-4864, a ligand for the benzodiazepine 'acceptor' site, potentiated the head-twitch.

4 Pentobarbitone and pentylenetetrazol potentiated the 5-HTP-induced head-twitch at low doses, changing to inhibition as the dose was increased. Picrotoxin in subconvulsant doses, produced only potentiation. More than one site may be involved in the action of these substances.

5 GABA, amino-oxyacetic acid and 1-2-4-diaminobutyric acid inhibited the head-twitch, while the GABA-depletor, 3-mercaptopropionic acid potentiated it.

6 Of all the agents tested, only muscimol produced head-twitching when given alone.

7 It was concluded that both $GABA_a$ and $GABA_b$ receptors modulate the head-twitch response to 5-HTP.

Introduction

5-Hydroxytryptamine (5-HT) receptor agonists induce a characteristic head-twitch response, the frequency of which is dose-dependent (Corne *et al.*, 1963). This effect provides a model for the study of central 5-HT receptor activation and appears to be due to activation of $5\text{-}HT_2$ receptors (Peroutka *et al.*, 1981; Ortmann *et al.*, 1982; Green *et al.*, 1983). Because it is simple to quantitate, it has proved an attractive model for the study of transmitter interactions with 5-HTergic mechanisms. Such studies have demonstrated a role for dopaminergic (Maj *et al.*, 1978) and noradrenergic (Ortmann *et al.*, 1981; Handley & Brown, 1982; Handley & Singh, 1984a) mechanisms in the modulation of the 5-HTergic head-twitch response.

The potential involvement of γ-aminobutyric acid (GABA) has so far been little studied. GABA itself induces spontaneous head-twitching following peripheral administration to the rat and rabbit, but is ineffective in the mouse (Smialowski *et al.*, 1980). The $GABA_a$ receptor agonist, muscimol, has also been reported to induce the head-twitch (Scottie de Carolis & Massotti, 1978), as have certain benzodiazepines (Nakamura & Fukushima, 1976). The GABA and benzodiazepine effects at least were prevented by 5-HT antagonists (Nakamura & Fukushima, 1976; Smialowski *et al.*, 1980). Although the effects of benzodiazepines were formerly attributed to a direct action at 5-HT receptors (Nakamura & Fukushima, 1976), the close association between subpopulations of benzodiazepine and GABA receptors has since been elucidated (Study & Barker 1981; Skerritt *et al.*, 1982). The head-twitch inducing effects of GABA, muscimol and the benzodiazepines therefore point to a potential role for GABA receptors in the control of this response.

We have therefore investigated the effects of a range of drugs acting at GABA receptors and at other receptors on the GABA-chloride ionophore complex. These agents were tested for their ability to induce head-twitching when given alone and for their ability

to modulate l-5-hydroxytryptophan (5-HTP) induced head-twitching behaviour. Some of these results have appeared in preliminary form (Handley & Singh, 1984b; 1985).

Methods

Animals

Male albino mice (MF1 strain, bred in our laboratories) weighing between 20 and 30 g were kept in the experimental room in groups of 25 (from the same birth cohort) on an 08 h 00 min to 20 h 00 min light-dark cycle at constant temperature (21 ± 1°C) for at least 5 days before the experiment. All behavioural studies were performed between 09 h 00 min and 18 h 00 min.

Induction of a head-twitch behaviour

One hour before experiment, mice from the same stock cage were placed in small sawdust lined polythene cages in groups of three. The third mouse was included only because head-twitching is reduced when there are only 1–2 mice per cage (Boulton & Handley, 1973). This mouse performed no further part in the experiment. The remaining pair received carbidopa (9 mg kg^{-1}, s.c.) followed 15 min later by 5-HTP (200 mg kg^{-1}, i.p.).

Analysis of drug effects

Drugs were injected i.p. 15 min before 5-HTP (exceptions given in Table 1). One mouse from each pair received the test drug and the other the injection vehicle. Twitches from the two mice were counted in parallel for 5 min starting 20 min post 5-HTP. This procedure compensates for between run variability (Handley & Brown, 1982). There were at least 6 pairs per experimental group. Drug effects relative to controls were assessed by paired t test carried out on the raw data. Potency was expressed as ID_{50} (dose producing a 50% inhibition relative to controls) or ED_{200} (dose producing a response frequency twice that of the controls) from log dose-respose regression analysis, where response = test mouse head-twitch frequency expressed as a % of that in paired control mouse (Handley & Brown, 1982). A minimum of four dose levels was investigated for each drug.

The highest dose of each drug which potentiated the 5-HTP-induced head-twitch was administered alone to determine whether it induced twitching on its own. Mice were observed for 30 min immediately after injection.

Solutions and drugs

Drugs were dissolved in saline (0.9% w/v NaCl) except the following: bicuculline (dissolved in 0.01N HCl); diazepam (commercial product Valium diluted in saline immediately before use); Ro15-1788 and Ro05-4864 (suspended in saline with 2 drops of Tween 80); ethyl-β-carboline-3-carboxylate (dissolved in saline by adding minimum amount of 1N HCl); pentobarbitone (commercial product, Sagatal, diluted in saline immediately before use); phenobarbitone (dissolved in saline by adding minimum amount of 1N NaOH).

The drugs were obtained from the following sources: GABA, amino-oxyacetic acid (AOAA), bicuculline methobromide, 1-2,4-diaminobutyric acid (DABA), 3-mercaptopropionic acid (3-MPA), muscimol, imidazoleacetic acid (IMAA), 3-aminopropanesulphonic acid (3-APS), pentylenetetrazol, picrotoxin and 1-5-hydroxytryptophan (5-HTP) were all obtained from Sigma, Poole, Dorset; Ro15-1788 ethyl 8-fluoro-5, 6-dihydro-5-methyl-6-oxo-4 H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate, Ro05-4864 7-chloro-1,3-dihydro-1-methyl-5-(4′-chlorophenyl)-2H-1,4-benzodiazepine-2-one and diazepam (Valium; Roche products, Welwyn Garden City, Herts); baclofen (Geigy pharmaceuticals, Macclesfield, Cheshire); car-

Table 1 Drug administration

Drug	*Route of administration*	*Time injected before 5-HTP*
GABA	i.c.v*	0
AOAA	s.c.	6 h
DABA	s.c.	24 h
3-MPA	i.p.	0
Muscimol	i.p.	0
IMAA	i.p.	30 min
Pentylenetetrazol	s.c.	0

* by the method of Brittain & Handley (1967).
GABA, γ-aminobutyric acid; AOAA, amino-oxyacetic acid; DABA 1-2,4-diaminobutyric acid; 3-MPA; 3-mercaptopropionic acid; IMAA; imidazoleacetic acid.

Table 2 Drugs potentiating the head-twitch response to 5-hydroxytryptophan (5-HTP) linear regression (log dose)

Drug	*Dose-range tested (mg kg^{-1})*	P	r	*ED_{200} (mg kg^{-1})*	*95% confidence limits (mg kg^{-1})*
3-MPA	2.50– 10.00	<0.005	0.910	6.20	2.10– 18.30
Muscimol	0.12– 0.50	<0.001	0.983	0.26	0.19– 0.36
IMAA	12.50– 50.00	<0.02	0.921	16.02	9.33– 25.85
3-APs	25.00–200.00	<0.037	0.945	83.45	45.54–152.90
Diazepam	0.25– 1.00	<0.016	0.936	0.70	0.28– 1.80
Ro05-4864	5.00– 10.00	<0.044	0.901	5.94	1.72– 10.49

For abbreviations, see Table 1; 3-APS, 3-aminopropanesulphonic acid.
P = Significance of least squares fit.
r = Correlation coefficient of least squares fit.
ED_{200} = dose to increase twitches to 200% of control levels.

bidopa (Merck, Sharp and Dohme, Hoddesdon, Herts); ethyl-β-carboline-3-carboxylate (βCCE, Glaxo, Greenford, Middx); pentobarbitone (Sagatal) and phenobarbitone (May and Baker, Dagenham, Essex). Drug doses in text refer to the weight of free base.

Results

Drugs potentiating the head-twitch response to 5-hydroxytryptophan

Table 2 shows ED_{200} values for drugs which potentiated the 5-HTP head-twitch. These comprised the GABA synthesis blocker 3-MPA, the $GABA_a$ receptor agonists muscimol, 3-APS and IMAA, and the benzodiazepine receptor ligands diazepam and Ro05-4864. The dose-range of 3-MPA tested was limited by the occurrence of convulsions at 15 mg kg^{-1} and the doses of IMAA and diazepam which could be tested were limited by sedation; however there was no loss of righting reflex at the highest doses of these latter drugs.

ED_{200} values could not be obtained for picrotoxin because convulsions occurred at 1.0 mg kg^{-1}; there was significant potentiation at 0.5 mg kg^{-1} (122.2 ± 8.0% of paired control; $P<0.05$) and lower doses had no significant effect.

Drugs inhibiting the head-twitch response to 5-hydroxytryptophan

Table 3 shows ID_{50} values for drugs which inhibited the head-twitch response. These comprised the GABA metabolism inhibitor AOAA and the GABA uptake blocker DABA as well as GABA itself, the $GABA_b$ receptor agonist baclofen, the benzodiazepine receptor ligands βCCE and Ro15-1788 and the barbiturate, phenobarbitone. Mice given 60.0 mg kg^{-1} phenobarbitone were heavily sedated but loss of righting reflex

Table 3 Drugs inhibiting the head-twitch response to 5-hydroxytryptophan: linear regression (log dose)

Drug	*Dose-range tested (mg kg^{-1})*	P	r	*ID_{50} (mg kg^{-1})*	*95% confidence limits (mg kg^{-1})*
GABA	12.50–100.00*	<0.002	0.906	35.50*	21.90–57.31*
AOAA	6.00– 24.00	<0.001	0.956	10.90	8.45–24.23
DABA	5.00– 20.00	<0.05	0.920	22.37	10.48–37.13
Baclofen	1.25– 5.00	<0.002	0.981	2.66	1.84– 3.86
Ro15-1788	2.50– 10.00	<0.01	0.908	13.06	6.56–26.00
βCCE	20.00– 80.00	<0.04	0.903	41.05	10.12–60.03
Phenobarbitone	15.00– 60.00	<0.001	0.974	18.05	12.16–26.76

For abbreviations, see Table 1; βCCE, ethyl-β-carboline-3-carboxylate.
P = Significance of least squares fit.
r = Correlation coefficient of least squares fit.
ID_{50} = dose to decrease twitches to 50% of control levels.
*μg administered i.c.v. by the method of Brittain & Handley (1967).

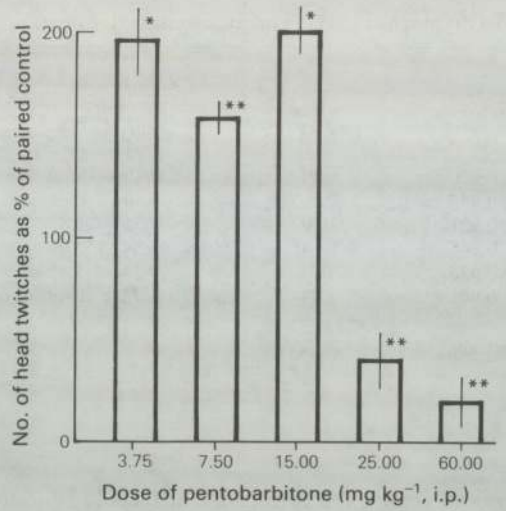

Figure 1 Effect of pentobarbitone on the 5-hydroxytryptophan (5-HTP)-induced head-twitch. All mice received 5-HTP (200 mg kg^{-1}, i.p.) and carbidopa (9 mg kg^{-1}, s.c.). Pairs of mice were assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for test mouse was recorded as % of its paired control. Control mice received vehicle and the test mice received pentobarbitone. Results are the means of at least 6 determinations and vertical lines represent s.e.mean: * $P<0.05$; ** $P<0.001$.

did not occur at this dose and a lower dose (7.5 mg kg^{-1} i.p.) of this barbiturate had no significant effect on the head-twitch response.

No dose-response curve could be obtained for bicuculline because of convulsions occurring at 1.0 mg kg^{-1}; 0.5 mg kg^{-1} (30.1 ± 9.0% of paired control; $P<0.05$) significantly reduced the head-twitch but lower doses were ineffective.

Drugs with biphasic effects on the head-twitch response to 5-hydroxytryptophan

Low doses of pentobarbitone (3.75 to 15 mg kg^{-1}) potentiated the head-twitch as well as causing obvious excitement, whereas doses of 25 to 60 mg kg^{-1} reduced it (Figure 1). These higher doses were associated with sedation but the righting reflex was still present.

Pentylenetetrazol also produced biphasic effects with potentiation appearing at 20 mg kg^{-1} and inhibition at 40 mg kg^{-1} (Figure 2). Convulsions occurred at 50 mg kg^{-1}.

Drug effects alone

Only muscimol induced head-twitching when administered alone; 1.0 mg kg^{-1} produced 0.66 twitches per min over an observation period of 30 min immediately after injection.

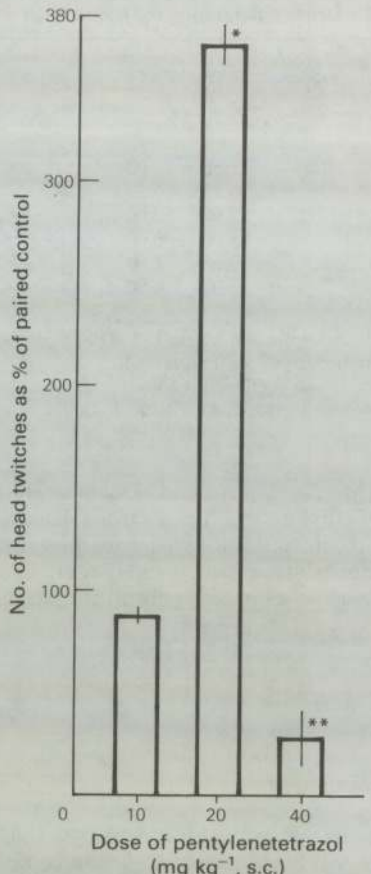

Figure 2 Effect of pentylenetetrazol on the 5-hydroxytryptophan (5-HTP)-induced head-twitch. All mice received 5-HTP (200 mg kg^{-1}, i.p.) and carbidopa (9 mg kg^{-1}, s.c.), pairs of mice were assigned at random to control or test conditions and observed in parallel. Head-twitch frequency for test mouse was recorded as % of its paired control. Control mice received vehicle and the test mice received pentylenetetrazol. Results are the means of at least 6 determinations and vertical lines represent s.e.mean: * $P<0.05$; ** $P<0.001$.

Discussion

There is evidence for two types of GABA receptor; $GABA_a$ linked to the chloride ionophore (see McBurney, 1984) and $GABA_b$ which is chloride ionophore-independent and is associated with inhibition of monoamine release (Bowery *et al.*, 1980; Hill & Bowery, 1981). Selective agonists for these two receptors had opposite effects on head-twitch frequency. Thus the $GABA_b$ agonist baclofen (Hill & Bowery, 1981; Bowery *et al.*, 1983) inhibited the head-twitch, while the $GABA_a$ receptor agonists muscimol, 3-APS

and IMAA (Hill & Bowery, 1981) produced potentiation. Both effects were dose-related. The inhibitory effect of the $GABA_a$ receptor antagonist bicuculline (Hill & Bowery, 1981) was consistent with the potentiating effect of corresponding agonists. The inhibitory effect of baclofen on 5-HTP-induced head-twitching has also been reported by Metz *et al.*, (1985), who found it to be ineffective against the direct agonist 5-methoxy *N,N*-dimethyltryptamine. This suggests that baclofen inhibits the head-twitch by reducing 5-HT release.

AOAA inhibits GABA-transferase (Wallach, 1961) and DABA inhibits neuronal GABA uptake (Sutton & Simmonds, 1974). These agents therefore raise endogenous GABA. Like GABA itself, they resulted in dose-related inhibition of the head-twitch. The results from the selective agonists suggest that this inhibition may be exerted through a predominant effect at the $GABA_b$ receptor. Spontaneous head-twitching after peripheral GABA administration in rat and rabbit (Smialowski *et al.*, 1980) may be due to a different balance of receptor activation in these species, favouring potentiating effects at the $GABA_a$ receptor. Interestingly, the GABA depleting agent (glutamic acid decarboxylase inhibitor) 3-MPA (Horton & Meldrum, 1973) induced potentiation, suggesting a tonic inhibitory role for GABA in the mouse.

Since $GABA_a$ receptors are part of a multi-receptor complex containing benzodiazepine and barbiturate receptors (see Olsen, 1981), ligands at these latter sites should have predictable effects on the 5-HTP induced head-twitch. Benzodiazepine receptor ligands showed the expected profile. Diazepam potentiated head-twitching (as reported by Nakamura & Fukushima, 1977) and the 'inverse agonist' βCCE (Polc *et al.*, 1982) inhibited it. Ro15-1788 is a benzodiazepine with antagonistic effects against both classic benzodiazepines and 'inverse agonists' (Hunkeler *et al.*, 1981). Ro15-1788 inhibited the head-twitch with four times the potency of βCCE. This could be interpreted either as being due to blockade of the effects of ongoing release of the putative endogenous ligand, or as due to intrinsic partial inverse agonist properties (File *et al.*, 1982).

A second benzodiazepine binding site has recently been described which appears to be non-neuronal, and has been suggested to be a silent 'acceptor' rather than a receptor (Richards *et al.*, 1982). The preconvulsant Ro05-4864 shows selectivity for this site *in vitro*. Ro05-4864 potentiated the head-twitch with a potency approximately one tenth that of diazepam; however it has recently been shown to bind to the picrotoxin/barbiturate site (Simmonds, 1984). The potentiating effect of Ro05-4864 may therefore not be due to an effect at the so called benzodiazepine 'acceptor' site.

The third component of the chloride ionophore receptor complex binds picrotoxin and barbiturates (Ticku & Olsen, 1978). Barbiturates prolong the duration of chloride channel opening (Huang & Barker, 1980) and enhance GABA-mediated responses (Nicoll *et al.*, 1975). By analogy with the effect of $GABA_a$ receptor agonists and benzodiazepine receptor ligands, the predicted effect of barbiturates would be to increase head-twitching frequency. However, the early work of Corne *et al.*, (1963) showed a wide range of barbiturates to be inactive up to very high doses. In the present experiments both pentobarbitone and phenobarbitone had marked effects. The difference from the results of Corne *et al.* (1963) probably lies in their use of a quantal (number of mice/group showing head-twitch) rather than a graded (head-twitch rate) response as a measure of head-twitch intensity, combined with our use of a pretreatment ($200\,mg\,kg^{-1}$ 5-HTP + $9\,mg\,kg^{-1}$ carbidopa) which resulted in head-twitching in all mice. Phenobarbitone binds only weakly to the GABA-linked barbiturate receptor and, unlike pentobarbitone, does not potentiate GABA or benzodiazepine binding; pentobarbitone on the other hand is a potent ligand for this site (Thyagarajan *et al.*, 1983). The potentiation shown by pentobarbitone would be the predicted effect of agonist activity at the GABA-linked barbiturate receptor. The inhibitory effect shown by both barbiturates indicates the possibility of a second site of barbiturate action, perhaps that associated with inhibition of transmitter release (Fung & Fillenz, 1984).

The effect of two further ligands for the picrotoxin/barbiturate site, picrotoxin and pentylenetetrazol (see Ticku & Maksay, 1983) were similarly complex. The testable dose-range of picrotoxin was narrow because of its steep dose-response curve for convulsant activity. The highest subconvulsant dose caused potentiation while lower doses were inactive. This was unexpected since picrotoxin is also a chloride channel blocker (Simmonds, 1980) and would thus be predicted to cause inhibition, as did the GABA antagonist bicuculline. Pentylenetetrazol is also a convulsant but less potent than picrotoxin. For this compound high sub-convulsant doses did produce inhibition but potentiation was seen at lower doses. This may again indicate multiple sites of action.

Of all the drugs tested only muscimol caused detectable head-twitching when given alone. Muscimol is the most selective agonist for $GABA_a$ receptors of those used here (Hill & Rowery, 1981), so would be the least likely to antagonize its own effects through an opposing action at $GABA_6$ receptors.

In conclusion, GABAergic mechanisms appear to play an important role in the modulation of this 5-HT-induced behaviour. This could have clinical significance. Head-twitching is induced by hallucinogens (Corne & Pickering, 1987) and hallucinatory episodes have been reported after baclofen withdrawal (e.g. Stein, 1977), pentylenetetrazol (see Lal & Emett-

Oglesby, 1983), and muscimol (Theobald *et al.*, 1968) administration.

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References

BOULTON, C.S. & HANDLEY, S.L. (1973). Factors modifying the head-twitch response to 5-hydroxytryptophan. *Psychopharmac.*, **31,** 205–214.

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., MIDDLEMISS, D.N., SHAW, J.S. & TURNBALL, M.J. (1980). (-)-Baclofen decreases transmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature*, **283,** 92–94.

BOWERY, N.G., HILL, D.R. & HUDSON, A.L. (1983). Characteristics of $GABA_b$ receptor binding sites on rat whole brain membranes. *Br. J. Pharmac.*, **78,** 181–206.

BRITTAIN, R.T. & HANDLEY, S.L. (1967). Temperature changes produced by injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. *J. Physiol.*, **192,** 805–813.

CORNE, S.L., PICKERING, R.W. & WARNER, B.T. (1963). A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **20,** 106–120.

CORNE, S.J. & PICKERING, R.W. (1967). A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. *Psychopharmac.*, **11,** 65–78.

FILE, S.E., LISTER, R.G. & NUTT, D.J. (1982). The anxiogenic action of benzodiazepine antagonists. *Neuropharmac.*, **21,** 1033–1037.

FUNG, S-C & FILLENZ, M. (1984). The actions of barbiturates on release of noradrenaline from rat hippocampal synaptosomes. *Neuropharmac.*, **23,** 1113–1116.

GREEN, A.R., O'SHAUGHNESSY, K., HAMMOND, M., SCHACHTER, M. & GRAHAME-SMITH, D.G. (1983). Inhibition of 5-hydroxytryptamine- mediated behaviour by the putative $5\text{-}HT_2$ antagonist pirenperone. *Neuropharmac.*, **22,** 573–578.

HANDLEY, S.L. & BROWN, J. (1982). Effects on the 5-hydroxytryptamine induced head-twitch of drugs with selective actions on $alpha_1$- and $alpha_2$- adrenoceptors. *Neuropharmac.*, **21,** 507–510.

HANDLEY, S.L. & SINGH, L. (1984a). The effect of beta-adrenoceptor agonists and antagonists on head-twitch in male mice. *Br. J. Pharmac.*, **81,** 127P.

HANDLEY, S.L. & SINGH, L. (1984b). GABA modulates the head-twitch induced by 5-HTP. *Br. J. Pharmac.*, **82,** 340P.

HANDLEY, S.L. & SINGH, L. (1985). $GABA_a$ agonists potentiate and baclofen antagonises the L-5-HTP head-twitch. *Br. J. Pharmac.*, **84,** 86P.

HILL, D.R. & BOWERY, N.G. (1981). ^{3}H-baclofen and ^{3}H-GABA bind to bicuculline insensitive $GABA_b$ sites in rat brain. *Nature*, **290,** 149–152.

HORTON, R.W. & MELDRUM, B.S. (1973). Seizures induced by allylglycine, 3-mercaptopropionic acid and 4-deoxypyridoxine in mice and photosensitive baboons, and different modes of inhibition of cerbral glutamic acid decarboxylase. *Br. J. Pharmac.*, **49,** 52–63.

HUANG, L.M. & BARKER, J.L. (1980). Pentobarbital: Stereospecific actions of (+) and (−) isomers revealed on cultured mammalian neurones. *Science*, **207,** 195–197.

HUNKELER, W., MOHLER, H., PIERI, L., P., BONETTI, E.P., CUMIN, R., SCHAFFNER, R. & HAEFELY, W. (1981). Selective antagonists of benzodiazepines. *Nature*, **290,** 514–516.

LAL, H. & EMETT-OGLESBY, M.W. (1983). Behavioural analoges of anxiety. Animal models. *Neuropharmac.*, **22,** 1423–1441.

MAJ, J., BARAN, L., BIGAJSKE, K., ROGOZ, A. & SKUZA, G. (1978). The influence of neuroleptics on the behavioural effect of 5-hydroxytryptophan. *Pol. J. Pharmac. Pharm.*, **30,** 431–440.

McBURNEY, R.N. (1984). Membrane actions of GABA in cultured central neurones. In *Actions and Interactions of GABA and Benzodiazepines*. ed. Bowery, N.G. pp. 43–58. New York: Raven Press.

METZ, A., GOODWIN, G.M. & GREEN, A.R. (1985). The administration of baclofen to mice increases $5\text{-}HT_2$-mediated head-twitch behaviour and $5\text{-}HT_2$ receptor number in frontal cortex. *Neuropharmac.*, **24,** 357–360.

NAKAMURA, M. & FUKUSHIMA, H. (1976). Head-twitches induced by benzodiazipines and the role of biogenic amines. *Psychopharmac.*, **49,** 259–261.

NAKAMURA, M. & FUKUSHIMA, H. (1977). Effect of benzodiazepines on central serotonergic neurone systems. *Psychopharmac.*, **53,** 121–126.

NICOLL, R.A., ECCLES, J.C., OSHIMA, T. & RUBIA, F. (1975). Prolongation of hippocampal inhibitory postsynaptic potentials by barbiturates. *Nature*, **258,** 625–627.

OLSEN, R.W. (1981). GABA-benzodiazepine – barbiturate receptor interactions. *J. Neurochem.*, **37,** 1–13.

ORTMANN, R., BISCHOFF, S., RADEKE, E., BUECH, O. & DELINI-STULA, H. (1982). Correlations between different measures of antiserotonin activity of drugs. Study with neuroleptics and serotonin receptor blockers. *Naunyn-Schmiedebergs Arch. Pharmac.*, **321,** 265–270.

ORTMANN, R., MARTIN, S., RADEKE, E. & DELINI-STULA, H. (1981). Interactions of beta-adrenoceptor agonists with the serotonergic system in rat brain. A behavioural study using L-5-HTP syndrome. *Naunyn-Schmiedebergs Arch. Pharmac.*, **316,** 225–230.

PEROUTKA, S.J., LEBOVITZ, R.M. & SNYDER, S.H. (1981). Two distinct central serotonin receptors with different physiological functions. *Science*, **212,** 827–829.

POLC, P., BONETTI, E.P., SCHAFFENER, R. & HAFELY, W. (1982). A three state of the benzodiazepine receptor explains the interactions between the benzodiazepine antagonist RO 15-1788, benzodiazepine tranquillizers, beta-carbolines and phenobarbitone. *Naunyn-Schmiedebergs Arch. Pharmac.*, **321,** 260–264.

RICHARDS, J.G., MOHLER, H. & HAEFLEY, W. (1982). Benzodiazepine binding sites: receptors or acceptors? *Trends Pharmac. Sci.*, **3,** 233–235.

SCOTTIE DE CAROIS, A. & MASSOTTI, M. (1978). Electroencephalgraphic and behavioural investigations on 'GABAergic' drugs: Muscimol baclofen and sodium-hydroxybutyrate. *Prog. Neuropsychopharmac.*, **2,** 431–442.

SIMMONDS, M.A. (1980). Evidence that bicuculline and picrotoxin act at separate sites to antagonise gamma-aminobutyric acid in rat cuneate nucleus. *Neuropharmac.*, **19,** 39–45.

SIMMONDS, M.A. (1984). Physiological and pharmacological characteristics of the actions of GABA. In *Actions and Interactions of GABA and Benzodiazepine.* ed. Bowery, N.G. pp. 27–42. New York: Raven Press.

SKERRITT, J.H., WILLOW, M. & JOHNSTON, G.A.R. (1982). Diazepam enhancement of low affinity GABA binding to rat brain membranes. *Neurosci. Lett.*, **29,** 63–66.

SMIALOWSKI, A., SMIALOWSKA, M., REICHENBERG, K., BYRSKA, B. & VETULANI, J. (1980). Motor depression and head-twitches induced by i.p. GABA. *Psychopharmac.*, **69,** 295–298.

STEIN, R. (1977). Hallucinations after sudden withdrawal of baclofen. *Lancet*, **ii,** 44–45.

STUDY, R.E. & BARKER, J.L. (1981). Diazepam enhancement and (−) pentobarbital: Fluctuation analysis reveals different mechanisms for potentiation of gamma-aminobutyric acid responses in cultured central neurons. *Proc. natn. Acad. Sci., U.S.A.*, **78,** 7180–7184.

SUTTON, I. & SIMMONDS, M.A. (1974). The selective blockade by diaminobutyric acid of neuronal uptake of [^{3}H]-GABA in rat brain in vivo. *J. Neurochem.*, **23,** 273–274.

THEOBALD, W., BUCH, H., KUPP, P., STENGER, E. & HEIMANN, H. (1968). Pharmakologische and experimental psychologische untersuchungen mit 2 Inhaltstoffen des Fliegenpilzes (Amanita muscaria). *Arzneim. Forsch.*, **18,** 311–315.

THYAGARAJAN, R., RAMANLANEYULU, R. & TICKU, M.K. (1983). Enhancement of diazepam and gamma-aminobutyric acid binding by (+) etomidate and pentobarbital. *J. Neurochem.*, **41,** 475–585.

TICKU, M.K. & MAKSAY, G. (1983). Convulsant/depressant site of action at the allosteric benzodiazepine-GABA receptor-ionophore complex. *Life Sci.*, **33,** 2363–2375.

TICKU, M.K. & OLSEN, R.W. (1978). Interactions of barbiturates with dihydropicrotoxinin binding sites related to the GABA-ionophore system. *Life Sci.*, **22,** 1643–1651.

WALLACH, D.P. (1961). Studies on the GABA pathway-I. The inhibition of gamma-aminobutyric acid-alpha-ketoglutaric acid transaminase in vitro and in vivo by U-7524 (amino-oxyacetic acid). *Biochem. Pharmac.*, **5,** 323–331.

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THE EFFECT OF β-ADRENOCEPTOR AGONISTS AND ANTAGONISTS ON HEAD-TWITCH IN MALE MICE

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Previous studies have shown that β_2-adrenoceptor agonists potentiate the quipazine hyperactivity syndrome mediated by 5-hydroxytryptamine (5-HT) receptors. The effect of antagonists was however not clearcut (Cowan et al, 1982). Since β_2-adrenoceptor agonists also potentiate the L-5-hydroxytryptophan (L-5-HTP) head-twitch (Ortmann et al, 1981), we have investigated the interactions of β_1- and β_2-adrenoceptor selective agonists and antagonists on this model.

Male TO mice (18-30g) received the agonists salbutamol or dobutamine s.c. simultaneously with carbidopa (9mg/kg s.c.) 15 min before L-5-HTP (200mg/kg i.p.) Head twitches were counted for 5 minutes starting 20 minutes after L-5-HTP. Each mouse was observed in parallel with a control animal (saline/L-5-HTP/carbidopa).

Potentiation of the head-twitch syndrome was caused by both the β_1- and β_2- adrenoceptor agonists (ED200 i.e. dose to double the control response:- dobutamine 1.53 (1.36-1.74) mg/kg;salbutamol 0.12(0.06-0.24) mg/kg). The legitimacy of the β_1/β_2-adrenoceptor subclassification has recently been questioned (Leclerc et al, 1981); however the effect of antagonists did provide some evidence that separate receptors may be involved (Table 1). The antagonists were injected s.c. immediately before salbutamol or dobutamine (except for practolol which was administered icv [Brittain and Handley, 1967]); parallel control groups received agonist only (control groups for practolol also received saline icv.). None of the selective antagonists affected the L-5-HTP response when given alone. The ability of butoxamine to prevent the effect of salbutamol while leaving dobutamine potentiation unaffected suggests that the former effect was caused by an action at β_2-adrenoceptors while the latter effect was not. However both the β_1-adrenoceptor antagonists metoprolol and practolol, prevented the respose to both agonists. This raises questions about the selectivity of the antagonists at the doses used; although practolol does have a relatively high selectivity it is still only 9 times more potent at β_1-adrenoceptors (Leclerc et al,1981).

Table 1 Interaction of β_1- and β_2-adrenoceptor ligands on head-twitch counts following L-5-HTP/carbidopa (mean ± s.e.mean).

	AGONIST					
ANTAGONIST	dobutamine (2.5mg/kg) alone	+antagonist	salbutamol (0.25mg/kg) alone	+antagonist	saline alone	+antagonist
metoprolol (2.5 mg/kg)	43.0±7.3	13.9±1.9**	38.1±6.4	17.9±5.2**	28.0±12.2	14.0±3.8
practolol (10 ug icv)	34.0±7.0	10.9±2.9**	84.9±7.3	34.9±12.2***	13.6±3.1	12.6±3.9
butoxamine (10 mg/kg)	32.7±7.7	39.4±7.0	44.4±2.5	9.0±2.6***	17.0±0.7	17.4±1.4

(n/group=7)

* p<0.05 ** p<0.01, *** p<0.001 paired t-test

These results suggest the possibility that both β_1- and β_2-adrenoceptors may be involved in modulation of the 5-HT mediated head-twitch response. The complex pattern of antagonist action may indicate problems of selectivity or alternatively may reflect the neuronal organisation of the receptors involved.

Brittain, R.T. & Handley, S.L. (1967) J. Physiol. 192, 805-813.
Cowan, P.J. et al (1982) Br. J. Pharmac. 76, 265-270.
Leclerc, G. et al (1981) Trends Pharmac. Sci. 17, 18-20.
Ortmann, R. et al (1981) Naunyn-Schmiedebergs Arch. Pharmac. 316, 225-230.

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GABA MODULATES THE HEAD-TWITCH INDUCED BY L-5-HTP

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A recent study has shown that systemic administration of GABA caused head-twitching and other behavioural changes in rats and rabbits but that similar doses in mice failed to induce the head-twitch response (Smialowski et al, 1980). We have investigated the effect of centrally administered GABA and of drugs which affect brain GABA concentrations on head-twitch behaviour produced by L-5-hydroxytryptophan (L-5-HTP).

Male MF1 mice (20-30g) received carbidopa (9 mg/kg s.c.) 15 min before L-5-HTP (200 mg/kg i.p.). Head-twitches were counted for 5 min starting 20 min after L-5-HTP. Each mouse was observed in parallel with a control animal (saline / carbidopa / L-5-HTP). GABA was administered i.c.v. (Brittain and Handley,1967); control groups for GABA received saline i.c.v. L-2,4-diamino-n-butyric acid (DABA) and amino-oxyacetic acid (AOAA) were administered 24 and 6 hours before L-5-HTP respectively. 3-Mercaptopropionic acid (3MPA) was given simultaneously with L-5-HTP. The two antagonists, picrotoxin and bicuculline were given (i.p.) 15 min before L-5-HTP; control groups for bicuculline received 0.01N HCL vehicle (1ml/100g).

Table 1. Head-twitch counts following L-5-HTP/carbidopa (mean ± s.e.mean).

Test Treatment	Head-twitches	Control Treatment	Head-twitches
picrotoxin (0.5 mg/kg)	35.5 ± 6.3*	saline	24.8 ± 4.7
picrotoxin (0.5 mg/kg) + GABA (36.0 ug i.c.v.)	10.1 ± 2.3*	GABA (36.0 ug i.c.v.)	4.5 ± 1.5
bicuculline (1.0 mg/kg)	16.8 ± 5.6*	vehicle	31.0 ± 12.7

n/group = 5. * $P < 0.05$ Wilcoxon matched-pair signed-ranks test.

GABA itself, DABA (Sutton and Simmonds, 1974) and AOAA (Wallach, 1961) which increase brain GABA concentrations inhibited the L-5-HTP head-twitch (ID_{50} i.e. dose to halve the control response :- GABA 35.5 [57.3-21.9] ug i.c.v.; DABA 22.3 [37.1-10.4] mg/kg i.p.; AOAA 10.9 [24.2-8.4] mg/kg s.c.) while 3MPA which inhibits GABA synthesis (Horton and Meldrum, 1973) increased it (ED_{200} i.e. dose to double the control response :- 6.2 [18.3-2.1] mg/kg i.p.). In line with this picrotoxin which probably acts at a site related to but not identical with the GABA sites (Simmonds, 1980), also increased head-twitching and prevented the GABA-induced reduction (Table 1). However bicuculline, a selective antagonist at $GABA_a$ sites (Hill and Bowery, 1981), antagonised the L-5-HTP head-twitch (Table 1). This suggests the possibility that the inhibitory effect of GABA in the mouse might be related to an effect at $GABA_b$ receptors.

Brittain, R.T. and Handley, S.L (1967) J. Physiol. 192, 805-813.
Hill, D.R. and Bowery, N.G. (1981) Nature. 290, 149-152.
Horton, R.W. and Meldrum, B.S. (1973) Br. J. Pharmacol. 49, 52-63.
Simmonds, M.A. (1980) Neuropharmacol. 19, 39-45.
Smialowski, A. et al, (1980) Psychopharmacol. 69, 295-298.
Sutton, I. and Simmonds, M.A. (1974) J. Neurochem. 23, 273-274.
Wallach, D.P. (1961) Biochem. Pharmacol. 5, 323-331.

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86P

$GABA_A$ AGONISTS POTENTIATE AND BACLOFEN ANTAGONISES THE L-5-HTP HEAD-TWITCH

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We have previously reported the effect of drugs which modify central GABA transmission on the head-twitch induced by L-5-hydroxytryptophan (L-5-HTP) (Handley and Singh, 1984). We now examine the effect of GABA agonists on this behaviour.

Male MF1 mice (20-30g) received carbidopa (9.0 mg/kg; s.c.) 15 min before L-5-HTP (200 mg/kg; i.p.) Head-twitches were counted for 5-min starting 20 min after L-5-HTP. Each mouse was observed in parallel with a control animal (saline /carbidopa/L-5-HTP).

The GABA receptor agonists muscimol, imidazoleacetic acid (IMAA) and 3-aminopropanesulphonic acid (3-APS) (Hill and Bowery, 1981) were administered before L-5-HTP and the ED_{200} (the dose to double the control response) was calculated for each drug (Table 1). IMAA and 3-APS did not cause head-twitching when given alone at the ED_{200} for potentiating L-5-HTP. Muscimol caused a low incidence of head twitching which was insufficient to account for its potentiation of L-5-HTP.

Table 1. ED_{200} Values for $GABA_a$ receptor agonists. (95% confidence limits in brackets).

Drug	Time of Administration	ED_{200} mg/kg (i.p.)
muscimol	Simultaneously with L-5-HTP	0.26 (0.19 - 0.36)
IMAA	30 minutes before L-5-HTP	16.02 (9.93 - 25.85)
3-APS	15 minutes before L-5-HTP	83.45 (45.54 - 152.9)

The $GABA_b$ receptor agonist baclofen (Hill and Bowery, 1981) was administered (i.p.) simultaneously with carbidopa and ID_{50} (dose to halve the control response) was calculated to be 2.66 mg/kg (95% confidence limits: 3.86 - 1.84).

These results suggest that the stimulation of $GABA_a$ receptors potentiate the L-5-HTP head-twitch, which is in line with the action of $GABA_a$ receptor antagonist bicuculline, shown to reduce this behaviour (Handley and Singh, 1984). The $GABA_b$ agonist baclofen reduced this syndrome, supporting our previous suggestion that the inhibitory effect of GABA on the L-5-HTP head-twitch in the mouse may be related to an effect at these receptors (Handley and Singh, 1984).

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Hill, D.R. and Bowery, N.G. (1981) Nature 290, 149-152.

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LOCUS COERULEUS LESIONS DO NOT AFFECT DIAZEPAM- OR α-ADRENERGIC-MODULATION OF OPERANT CONFLICT

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Agonists and antagonists of α-adrenoceptors have well defined effects in animal models of fear and anxiety (Handley & Mithani, 1983; 1984 a,b,c) however the neuronal basis of these effects is as yet unknown. The noradrenergic Locus Coeruleus-dorsal bundle system (LC-db) has been implicated in the expression of fear-motivated behaviours (Redmond & Huang, 1979), although not all workers agree on this involvement (Mason & Fibiger, 1979). We have therefore investigated the effect of LC lesions on the effects of α-adrenoceptor ligands in an operant conflict model of anxiety.

Male Lister Hooded rats received 6-OHDA or vehicle bilaterally into LC. 6 rats which were subsequently shown to have sustained at least 80% depletion of hippocampo/cortical noradrenaline (hypothalamic noradrenaline and striatal dopamine concentrations were unaffected) and 8 shamoperated controls were trained on a VI_{30}sec schedule of reinforcement for condensed milk reward. The schedule was divided into four 5 min periods, the second being accompanied by reward-contingent footshock and the fourth being time-out. Rats were reinforced daily and drugs injected at weekly intervals commencing 21 days post-lesion.All rats successfully aquired the reinforcement schedule. Footshock-induced suppression of lever pressing was titrated to produce <6 lever presses/5 min for diazepam, clonidine and prazosin and later adjusted to 6-10 lever presses/5 min for yohimbine and phenylephrine. There were no significant differences in performance between lesioned and shamoperated rats during unpunished or time-out periods, or in the shock intensity required to produce the specified response suppression level in the punished period.

Table 1. Lever presses/rat/5 min during punished period (mean +/-sem).

drug/dose		lesioned	sham operated	
diazepam	2.5	31.0+/-5.1 (3.5 +/-1.2)	42.5+/-9.3(3.5 +/- 0.9)	ns
clonidine	0.025	8.5+/-2.3 (2.8 +/-0.7)	13.5+/-4.4(5.4 +/- 2.2)	ns
prazosin	0.05	8.3+/-1.9 (3.5 +/-1.7)	7.4+/-0.4(2.6 +/- 1.4)	ns
phenyl-ephrine	0.5	0.3+/-0.4 (6.6 +/-0.5)	1.1+/-0.36(6.9+/-1.0)	ns
Yohimbine	2.5	0.2+/-0.7 (6.3 +/-1.0)	1.0+/-0.9(5.9+/-1.2)	ns

All drugs were $p<0.05$ vs previous day's vehicle control (shown in brackets). ns:- drug effects on lesioned vs sham, $p>0.05$.

Table 1 shows that the antipunishment effects of clonidine and prazosin were not modified by the presence of LC lesions,neither were the punishment-enhancing effects of yohimbine or phenylephrine. These results suggest that an intact LC-db system is not essential either for normal performance, or for the expression of the effects of the above drugs, in this model of anxiety.

Handley, S.L. & Mithani, S. (1983) Br. J. Pharmac. 78 110P.
Handley, S.L. & Mithani, S. (1984a) Br. J. Pharmac. 82 341P.
Handley, S.L. & Mithani, S. (1984b) Br. J. Pharmac. 81 128P.
Handley, S.L. & Mithani, S. (1984c) Archs. Pharmac. in press.
Mason, S.T. & Fibiger, H.C. (1979) Life Sci. 25 2141-2147.
Redmond, D.E. & Huang, Y.H. (1979) Life Sci. 25 2149-2162.

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Psychopharmacology

Involvement of noradrenaline in potentiation of the head-twitch response by GABA-related drugs

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Abstract. The involvement of noradrenaline in the potentiation of head-twitching by drugs acting at GABA and related receptors has been examined in mice. The direct 5-HT agonist 5-methoxy-N,N-dimethyltryptamine was used to induce the head-twitch. The dopamine beta-oxidase inhibitor FLA-63 depleted whole brain noradrenaline by 90% and potentiated head-twitching when this was measured 4 h after injection. The $GABA_A$-receptor agonists muscimol and 3-aminopropanesulphonic acid (3-APS), a low dose of pentobarbitone, diazepam and picrotoxin (PTX) potentiated control head-twitch rates but had no further effect when head-twitching had been potentiated by FLA-63. The potentiating action of FLA-63 was prevented by the beta-adrenoceptor antagonist metroprolol; the latter having no effect on control head-twitch rates. Muscimol and diazepam potentiation was examined and found to be blocked by metoprolol. The $GABA_B$-receptor agonist baclofen reduced head-twitching. This was also prevented by FLA-63. The role of beta-adrenoceptors in modulating these actions of GABA-related drugs is discussed.

Key words: GABA and related receptors – Noradrenaline – Beta-adrenoceptors – 5-HT head-twitch

Drugs acting at GABA, benzodiazepine, and barbiturate receptors have potent effects on 5-HT-mediated behaviours such as the head-twitch. Muscimol and 3-aminopropanesulphonic acid (3-APS), which are $GABA_A$ receptor agonists (Hill and Bowery 1981), diazepam and low doses of pentobarbitone cause dose-dependent potentiation of the L-5-hydroxytryptophan (L-5-HTP) head-twitch response (Handley and Singh 1984a, 1985a, b). GABA, muscimol and certain benzodiazepines can also initiate head-twitching (Nakamura and Fukushima 1976; Scottie de Carolis and Massoti 1978; Smialowski et al. 1980). Higher doses of pentobarbitone, and all effective doses of phenobarbitone and baclofen reduce the L-5-HTP head-twitch (Handley and Singh 1985a, b; Metz et al. 1985). The barbiturate receptor antagonist picrotoxin (PTX) (see Ticku and Macksay 1983), however, produced potentiation (Handley and Singh 1984a, 1985b).

The experiments described here represent a preliminary attempt to determine the mechanism of these effects. The effect of these agents on head-twitching induced by 5-methoxy-N, N-dimethyltryptamine (5-MeODMT) were examined in order to exclude a presynaptic effect on 5-HT release following L-5-HTP; 5-MeODMT is a direct 5-HT receptor agonist (Fuxe et al. 1972). Noradrenaline plays an important role in modulating the serotonergic head-twitch (Bednarczyk and Vetulani 1978; Maj et al. 1978; Vetulani et al. 1980; Handley and Brown 1982) and beta-adrenoceptor agonists exert a marked potentiating effect (Delini-Stula et al. 1979; Ortmann et al. 1981; Nimgaonkar et al. 1983; Handley and Singh 1984b). The effect of noradrenaline depletion and beta-adrenoceptor blockade on the effect of GABA-related drugs has therefore been investigated.

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Materials and methods

Animals. Male albino mice (MF1 strain, bred in our laboratories) weighing between 20 and 30 g were kept in groups of 25 (from the same birth cohort) on an 08.00–20.00 hour light-dark cycle at constant temperature ($21 \pm 1°$ C) for at least 5 days prior to experiment. All behavioural studies were performed between 09.30 and 18.00 hours.

One hour before experiment, mice from the same stock cage were placed in small sawdust-lined polythene observation cages in groups of three. The third mouse was included only because head-twitching is reduced when there are only one to two mice per cage (Boulton and Handley 1973). This mouse formed no further part in the experiment.

Effect of drugs on 5-MeODMT-induced head-twitch behaviour. One mouse from each pair received the drug under test and the other received the vehicle. Both pretreated mice received a submaximal dose of 5-MeODMT (10 mg/kg IP). Twitches from each mouse were counted for alternate minutes from 0 to 12 min after 5-MeODMT. In the experiments involving a third pretreatment, i.e. FLA-63 or metoprolol, twitches in quartets of mice were counted in parallel over the same time period. The members of the quartet represented each of the four pretreatments (drug/drug; drug/vehicle; vehicle/drug; vehicle/vehicle). Runs with pairs or quartets were repeated until the appropriate group size was reached for each experiment. This design compensated for between run variation and, for the quartets, allowed factorial analysis of variance to be performed.

Determination of brain noradrenaline and dopamine levels. Groups of mice received FLA-63 or vehicle. Four hours later, the mice were killed and brains removed. For mea-

Table 1. Effect of GABA-related drugs on 5-MeODMT induced head-twitch response in mice

Drug	Dose (mg/kg IP)	Mean % of control head-twitch response
Muscimol	0.125	234.3 ± 43.[illegible] (12)
3-APS	80.0	266.1 ± 37.9[b] (14)
Baclofen	2.5	34.4 ± 4.7[b] (20)
Diazepam	0.7	189.3 ± 37.2[a] (6)
PTX	0.5	245.1 ± 33.9[a] (6)
Pentobarbitone	6.0	183.7 ± 25.9[a] (6)

Effects reported as % of the twitches from the control (vehicle pretreated) animal tested at the same time, ±SEM. Number of observations given in brackets. The drugs were administered 30 min (except muscimol which was administered 20 min) before 5-MeODMT. Head-twitches were observed in parallel on alternate minutes for control and test mice from 0 to 12 min after 5-MeODMT injection (10.0 mg/kg IP).
[a] $P < 0.05$; [b] $P < 0.01$ (paired t-test)

surement of noradrenaline and dopamine, the brains of three mice were pooled. Noradrenaline and dopamine levels were determined fluorometrically as described by Chang (1964).

Statistical analysis. Results were analysed by paired t-test and 2 × 2 factorial analysis of variance; both were carried out on raw data. The significance [$P_{(int)}$] of drug interactions with FLA-63 or metoprolol was determined from the F value of the interaction term in the analysis of variance. This method allows the effects of an active drug to be evaluated in the presence and absence of a second drug which is also active (Linton and Gallo 1975).

Drugs. Drugs were dissolved in saline (0.9% w/v Nacl solution) except the following: diazepam (commercial product "Valium" diluted in saline immediately before use); pentobarbitone (commercial product "Sagatal" diluted in saline immediately before use); FLA-63 and 5-methoxy-N,N-dimethyltryptamine (dissolved in saline by addition of concentrated HCl and the pH brought to 7.0 with NaOH). Doses of drugs refer to the weight of the free base.

Drugs were obtained from the following sources: muscimol, 3-aminopropanesulphonic acid (3-APS), picrotoxin (PTX), 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) were all obtained from Sigma. Diazepam (Roche Products), pentobarbitone (May and Baker), *bis*-(4-methyl-1-homopiperazinylthiocarbonyl)disulphide (FLA-63; Astra Pharmaceuticals) and baclofen (Geigy Pharmaceuticals).

Results

5-MeODMT produced a dose-related increase in head-twitch frequency between 2.5 and 20 mg/kg (IP data not shown). A maximum of 50.0 ± 3.8 (mean of five observations) head-twitches were obtained with 20.0 mg/kg (IP) 5-MeODMT; above this dose signs of "5-HT syndrome" appeared and there was a decrease in the head-twitch frequency.

Drug effects on head-twitch induced by 5-MeODMT. The head twitch response produced by 5-MeODMT (10.0 mg/kg) was potentiated by pretreatment of mice with 3-APS (80,0 mg/kg), diazepam (0.7 mg/kg), PTX (0.5 mg/kg) or pentobarbitone (6.0 mg/kg) administered 30 min before 5-MeODMT and by muscimol (0.125 mg/kg), 20 min before 5-MeODMT (Table 1). In contrast, the $GABA_B$ agonist baclofen (2.5 mg/kg) inhibited the 5-MeODMT-induced

Table 2. Effect of FLA-63 on brain catecholamine levels 4 h after FLA-63 (40.0 mg/kg IP)

	Catecholamine levels (ng/g wet weight)	
	Vehicle	FLA-63
Noradrenaline	385 ± 25	40 ± 20[a]
Dopamine	655 ± 69	690 ± 55

Groups of mice were pretreated with either FLA-63 (40.0 mg/kg) or vehicle.
Results are mean values of four determinations each consisting of three pooled brains ±SEM.
[a] $P < 0.01$ (paired t-test)

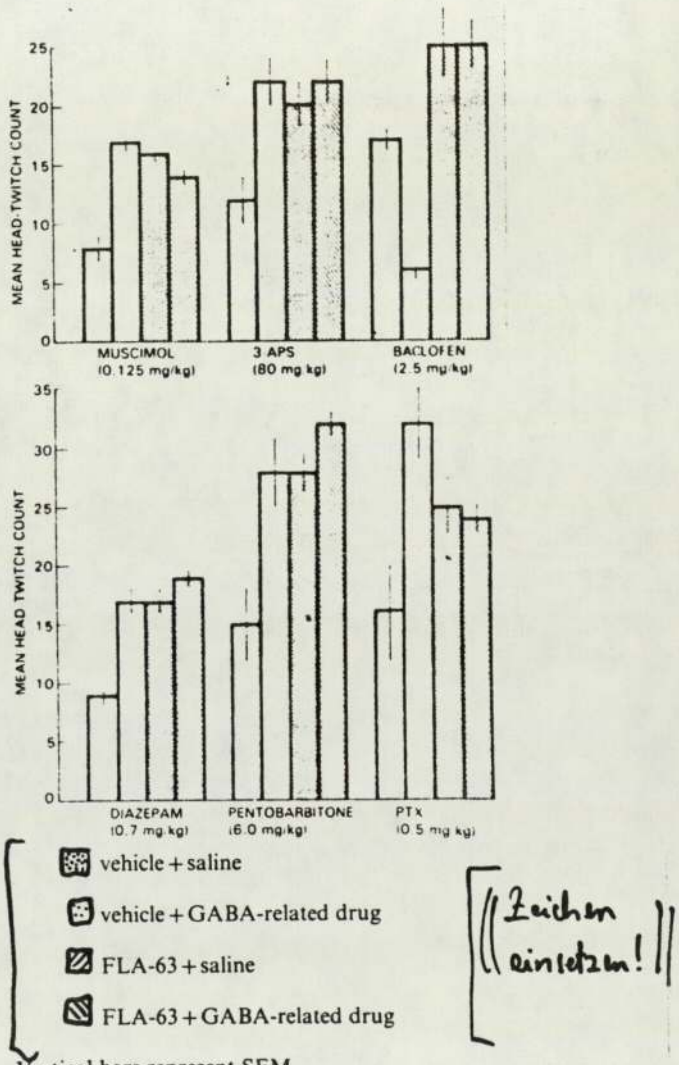

Fig. 1. The effect of GABA-related drugs in the presence of FLA-63 on 5-MeODMT head-twitch. The head-twitch response was observed in parallel for each quartet of mice

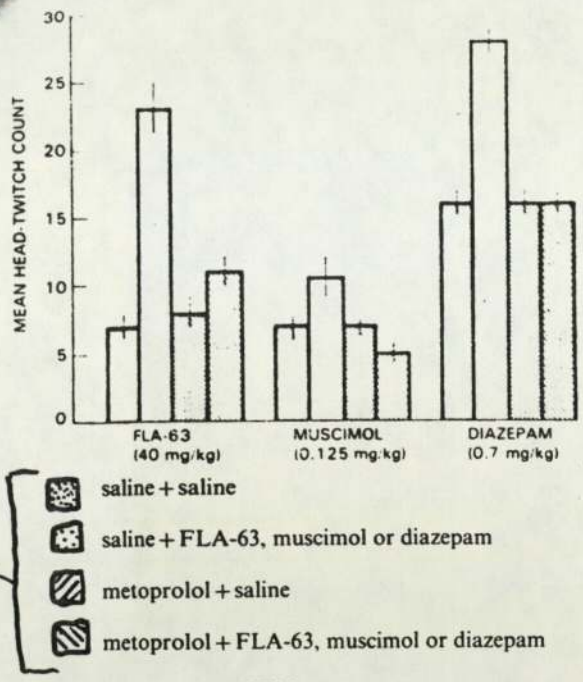

Fig. 2. The effect of metoprolol on head-twitch potentiation induced by FLA-63, muscimol and diazepam. The head-twitch response was observed in parallel for each quarted of mice

head-twitch when administered 30 min before the 5-HT agonist (Table 1).

Diazepam (0.7 mg/kg IP) and pentobarbitone (6.0 mg/kg IP) did not produce sedation. No convulsions were seen in any mice treated with PTX (0.5 mg/kg IP). None of the GABA-related drugs or FLA-63 induced head-twitching when administered alone at these doses.

Effect of FLA-63 on brain catecholamine levels. Table 2 shows that 40.0 mg/kg (IP) of FLA-63 administered 4 h beforehand to mice significantly reduced brain noradrenaline to approximately 10% of control levels but had no significant effect on brain dopamine.

Effect of FLA-63 on 5-MeODMT head-twitch. The 5-MeODMT (10.0 mg/kg)-induced head-twitch response was significantly potentiated by pretreatment of mice with FLA-63 (40.0 mg/kg) administered 4 h previously ($P<0.05$, Tukey's test for unconfounded means; Fig. 1).

Effect of GABA-related drugs in the presence of FLA-63. Treatment with FLA-63 (40.0 mg/kg) 4 h previously prevented any further potentiation of 5-MeODMT (10.0 mg/kg) head-twitching by muscimol (0.125 mg/kg), 3-APS (80.0 mg/kg), diazepam (0.7 mg/kg), PTX (0.5 mg/kg) or pentobarbitone (6.0 mg/kg) as assessed from the interaction terms in the analysis of variance [$F_{(int)} \geqq 7.62$, $P<0.05$ in each case; Fig. 1]. Similar pretreatment with FLA-63 also prevented the inhibitory effect of baclofen [$F_{(int)} \geqq 24.58$, $P<0.01$; Fig. 1].

Effect of metoprolol on the potentiation induced by FLA-63, muscimol and diazepam. Potentiation of the 5-MeODMT (10.0 mg/kg) head-twitch response by FLA-63 (40.0 mg/kg), muscimol (0.125 mg/kg) or diazepam (0.7 mg/kg) (administered 4 h, 20 min and 30 min before the 5-HT agonist, respectively) was prevented by the beta-adrenoceptor antagonist metoprolol (2.5 mg/kg SC) administered 15 min before 5-MeODMT [$F_{(int)} \geqq 20.16$, $P<0.01$ in each case; Fig. 2]. However, metoprolol had no significant effect on control responses ($P>0.05$, Tukey's test for unconfounded means; Fig. 2).

Discussion

The dopamine beta-oxidase inhibitor FLA-63 was chosen for investigation of the possible involvement of noradrenaline in the head-twitch potentiation induced by GABA-related drugs because of its high selectivity in depleting only noradrenaline (Svensson and Waldeck 1969). It was predicted that this drug would cause a degree of reduction in the control rate of head-twitching because the serotonergic head-twitch appears to need intact alpha$_1$-adrenoceptors in order to occur, since it is prevented by alpha$_1$-adrenoceptor antagonists (Handley and Brown 1982). It was therefore surprising that, instead, potentiation was consistently observed, despite a 90% reduction in brain noradrenaline. The absence of any inhibition suggests that very little noradrenaline is needed to activate the alpha$_1$-adrenoceptors involved. Preliminary experiments with the tyrosine hydroxylase inhibitor alpha-methyl *p*-tyrosine indicated complete abolition of head twitching; in this case, dopamine would have been depleted as well (results not given). Noradrenaline depletion with 6-hydroxy dopamine ICV failed to affect the L-5-HTP head-twitch (Bednarczyk and Vetulani 1978), but the degree of depletion was not reported. The occurrence of actual potentiation after FLA-63 is more puzzling. A similar potentiation was reported for reserpine by Nakamura and Fukushima (1978), but in this case the other monoamines would also be depleted. Possible explanations would include a reduction in alpha$_2$-adrenoceptor occupation, since these are inhibitory to head-twitching (Handley and Brown 1982) or the occurrence of a supersensitivity response in either alpha$_1$- or beta-adrenoceptors, both of which facilitate head-twitching. We have so far investigated only this last possibility. Metoprolol, in a dose which prevents the potentiating effect of beta-adrenoceptor agonists, has no effect on the control head-twich rate (present results and Handley and Singh 1984 b); it did, however, prevent the FLA-63 potentiation, indicating the involvement of beta-adrenoceptors. Although changes in actual beta-adrenoceptor density need several days to occur, altered agonist availability can cause marked effects on beta-adrenoceptor sensitivity, at least in vitro within the required time scale. This effect is thought to be due to changes in the degree of coupling of the beta-adrenoceptor to the regulatory sub-unit of cyclic adenosine monophosphate (see Harden 1983).

The potentiating effect of FLA-63 complicates the interpretation of its effects on GABA-related drugs. Head-twitch rates, in the presence of FLA-63 or of each of these drugs alone, were below the maximum obtainable. The experimental design was chosen to allow the statistical evaluation of the effect of one active drug in the presence of another which is also active (Linton and Gallo 1975). The results therefore demonstrate that muscimol, 3-APS, diazepam, PTX and pentobarbitone did not produce any further

potentiation in the presence of FLA-63. Since beta-adrenoceptors had already been implicated in the potentiating effect of FLA-63, the effect of metoprolol was examined against two of these agents, muscimol, the more specific of the two $GABA_A$ receptor agonists, and diazepam. Their potentiating effect was abolished, indicating that this potentiation involved the participation of beta-adrenoceptors.

What could be the mechanism of this noradrenergic involvement? GABA is capable of triggering noradrenaline release from synaptosomes by a chloride-channel-dependent mechanism (Fung and Fillenz 1984). This effect has been observed in hippocampal synaptosomes and is mimicked by low doses of pentobarbitone (Fung and Fillenz 1983, 1984). However, it is blocked by picrotoxin, while this drug not only fails to block the head-twitch but actually potentiates it (Handley and Singh 1984b, 1985b). A further possibility is the post-synaptic interaction which has been shown to occur in cortex and cerebellum between $GABA_A$ receptors and beta-adrenoceptors. Sensory stimulation can inhibit purkinje neurones. This inhibition depends on GABA and is markedly potentiated by beta-adrenoceptor agonists and stimulation of locus coeruleus (Waterhouse et al. 1982; see also Foote et al. 1983). This has been proposed to constitute the noise-reducing element of the mechanism by which the locus coeruleus improves signal/noise ratio and so enhances the effectiveness of sensory input (Foote et al. 1983). Preliminary experiments have established that potentiation of head-twitching by diazepam is prevented by locus coeruleus lesions (Handley and Singh submitted). In addition, the head-twitch is modulated by forebrain mechanisms (Bedard and Pycock 1977) and depends for its occurrence on sensory input from the pinna (Boulton and Handley 1973). This possibility therefore appears to be worthy of further investigation.

Barbiturate effects on head-twitching are complex; while low doses of pentobarbitone potentiate, higher doses cause inhibition as do all doses of phenobarbitone (Handley and Singh 1985b). The potentiating effect of pentylenetetrazole is consistent with the latter, and could indicate that its site of action is different from that of low dose pentobarbitone (Handley and Singh 1985b). The finding that pentobarbitone produced no further potentiation in the presence of FLA-63 may also indicate a noradrenergic involvement at such an alternative site.

Baclofen, a selective agonist at $GABA_B$ receptors (see Bowery et al. 1984) antagonises head-twitching induced by the 5-HT precursor L-5-HTP (Handley and Singh 1985a, b; Metz et al. 1985). Metz and co-workers (1985) have reported that baclofen does not antagonise head-twitching induced by the direct agonist 5-MeODMT. This indicates that the inhibitory effect of baclofen on L-5-HTP head-twitching is likely to be due to inhibition of 5-HT release (Schlicker et al. 1984). In contrast, the present experiments demonstrate a potent antagonism of 5-MeODMT-induced head-twitching, indicating a post-synaptic mechanism. The difference may lie in the pretreatment regimen used. Metz and co-workers (1985) gave a loading dose of 10 mg/kg baclofen followed by a further 10 mg/kg in drinking water over the ensuing 24 h. In the present study, a single dose of 2.5 mg/kg was administered 30 min before 5-MeODMT. The antagonistic effect of baclofen on the 5-MeODMT head-twitch did not occur in the presence of FLA-63. Baclofen inhibits noradrenaline release (Bowery et al. 1980; Hill and Bowery 1981), but it is difficult to envisage a mechanism by which noradrenaline depletion would negate the consequences of this effect. Further investigation will be necessary to establish whether other agents which potentiate the head-twitch also confer resistance to baclofen inhibition.

The present results indicate that interactions between GABAergic and noradrenergic mechanisms result in major changes in sensitivity to the effects of a 5-HT receptor agonist. These three transmitters are implicated in the mode of action of a very wide variety of centrally acting drugs and in the causation of CNS disease. For example, both noradrenaline and 5-HT are involved in the aetiology of depression (see Van Praag 1978). In addition the "down-regulation" of beta-adrenoceptors by antidepressants requires the presence of 5-HT neurones, if not of 5-HT itself (Nimgaonkar et al. 1985) and GABA-agonists have been claimed to be effective antidepressants (Lloyd et al. 1983). Further investigation of this complex three-way interaction between neurotransmitters may indicate the mechanism by which such effects occur.

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References

Bedard P, Pycock CJ (1977) "Wet-dog shake" behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology 16:663–670

Bednarczyk B, Vetulani J (1978) Antagonism of clonidine to shaking behaviour in morphine abstinence syndrome and to head-twitches produced by serotonergic agents in the rat. Pol J Pharmacol Pharm 30:307–322

Boulton CS, Handley SL (1973) Factors modifying the head-twitch response to 5-hydroxytryptophan. Psychopharmacologia 20:204–214

Bowery NG, Doble A, Hill DR, Hudson AL, Middlemiss DN, Shaw JS, Turnbull MJ (1980) (-)-Baclofen decreases transmitter release in the mammalian CNS by an action at a novel GABA receptor. Nature 283:92–94

Bowery NG, Price GW, Hudson AL, Hill DR, Wilkin GP, Turnbull MJ (1984) GABA receptor multiplicity. Visualization of different receptor types in the mammalian CNS. Neuropharmacology 23:219–231

Chang CC (1964) A sensitive method for spectrophotofluorometric assay of catecholamines. Int J Neuropharmacol 3:643–649

Delini-Stula A, Vassout A, Radeke E, Ortmann R (1979) Psychopharmacological profile of $beta_2$-rceptor stimulants. Naunyn-Schmiedeberg's Arch Pharmacol 307:R258

Foote SL, Bloom FE, Aston-Jones G (1983) Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. Physiol Rev 63:844–914

Fung S-C, Fillenz M (1983) The role of pre-synaptic GABA and benzodiazepine receptors in the control of noradrenaline release in rat hippocampus. Neurosci Lett 42:61–66

Fung S-C, Fillenz M (1984) The actions of barbiturates on release of noradrenaline from rat hippocampal synaptosomes. Neuropharmacology 23:1113–1116

Fuxe K, Holmstedt B, Jonsson G (1972) Effects of 5-methoxy N-N dimethyltryptamine on central monoamine neurones. Eur J Pharmacol 19:25–34

Handley SL, Brown J (1982) Effects on the 5-hydroxytryptamine induced head-twitch of drugs with selective actions on $alpha_1$- and $alpha_2$-adrenoceptors. Neuropharmacology 21:507–510

Handley SL, Singh L (1984a) GABA modulates the head-twitch induced by 5-HTP. Br J Pharmacol 82:340P

Handley SL, Singh L (1984b) The effect of beta-adrenoceptor ago-

nists and antagonists on head-twitch in male mice. Br J Pharmacol 81:127P

Handley SL, Singh L (1985a) $GABA_A$ agonists potentiate and baclofen antagonises the L-5-HTP head-twitch. Br J Pharmacol 84:86P

Handley SL, Singh L (1985b) Modulation of serotonergic head-twitch response by drugs acting at GABA and related receptors. Br J Pharmacol 86:297–303

Harden TK (1983) Agonist-induced desensitization of the beta-adrenergic receptor-linked adenylate cyclase. Pharmacol Rev 35:5–32

Hill DR, Bowery NG (1981) ^{3}H-Baclofen and ^{3}H-GABA bind to bicuculline insensitive $GABA_b$ sites in rat brain. Nature 290:149–152

Linton M, Gallo PS Jr (1975) The practical statistician: simplified handbook of statistics. Brookside/Cole Publishing Company, Montery, California

Lloyd KG, Morselli PL, Depoortere H, Fournier V, Zivkobvic B, Scatton B, Brodkkamp C, Worms P, Bartholini G (1983) The potential use of GABA agonists in psychiatric disorders. Evidence from studies with progabide in animals and clinical trials. Pharmacol Biochem Behav 18:957–966

Maj J, Baran L, Bigajska K, Rogoz A, Skuza G (1978) The influence of neuroleptics on the behavioural effect of 5-hydroxytryptophan. Pol J Pharmacol Pharm 30:431–440

Metz A, Goodwin GM, Green AR (1985) The administration of baclofen to mice increases 5-HT_2-mediated head-twitch behaviour and 5-HT_2 receptor number in frontal cortex. Neuropharmacology 24:357–360

Nakamura M, Fukushima H (1976) Head-twitches induced by benzodiazepines and the role of biogenic amines. Psychopharmacology 49:259–261

Nakamura M, Fukushima H (1978) Effects of reserpine, parachloropheylalaninie, 5,6 dihydroxytryptamine and fludiazepam on the head twitches induced by 5-hydroxytryptamine and 5-methoxytryptamine in mice. J Pharm Pharmacol 30:254–256

Nimgaonkar VL, Green AR, Cowen PJ, Heal DJ, Grahame-Smith DG, Deakin JFW (1983) Studies on the mechanisms by which clenbuterol, a beta-adrenoceptor agonist, enhances 5-HT mediated behaviours and increases brain 5-HT metabolism in the rat. Neuropharmacology 22:739–749

Nimgaonkar VL, Goodwin GM, Davies CL, Green AR (1985) Down-regulation of beta-adrenoceptors in rat cortex by repeated administration of desipramine, electroconvulsive shock and clenbuterol requires 5-HT neurones but not 5-HT. Neuropharmacology 24:279–283

Ortmann R, Martin S, Radeke E, Delini-Stula A (1981) Interaction of beta-adrenoceptor agonists with the serotonergic system in rat brain. A behavioural study using the L-5-HTP syndrome. Naunyn-Schmiedeberg's Arch Pharmacol 316:225–230

Schlicker E, Classen K, Gothert M (1984) $GABA_b$ receptor-mediated inhibition of serotonergic release in the rat. Naunyn-Schmiedeberg's Arch Pharmacol 326:99–105

Scottie de Carolis A, Massotti M (1978) Electroencephalgraphic and behavioural investigations on "GABAergic" drugs: Muscimol, baclofen and sodium-hydroxybutyrate. Prog Neuropsychopharmacol 2:431–442

Smialowski A, Smialowska M, Reichenberg K, Byrska B, Vetulani J (1980) Motor depression and head-twitches induced by i.p. GABA. Psychopharmacology 69:295–298

Svensson TH, Waldeck B (1969) On the significance of central noradrenaline for motor activity: experiments with a new dopamine beta-hydroxylase inhibitor. Eur J Pharmacol 7:278–282

Ticku MK, Maksay G (1983) Convulsant/depressant site of action at the allosteric benzodiazepine-GABA receptor-ionophore complex. Life Sci 33:2363–2375

Van Praag HM (1978) Amine hypotheses of affective disorders. In: Iversen LL, Iversen SD, Snyder SH (eds) Handbook of Psychopharmacology. Plenum Press, New York, p 13

Vetulani J, Bednarczyk B, Reichenberg K, Rokosz A (1980) Head-twitches induced by LSD and quipazine: Similarities and differences. Neuropharmacology 19:155–158

Waterhouse BD, Moises HC, Yeh HH, Woodward DJ (1982) Norepinephrine enhancement of inhibitory synaptic mechanisms in cerebellum and cerebral cortex: mediation by beta-adrenergic receptors. J Pharmacol Exp Ther 221:495–506

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The modulation of head-twitch behaviour by drugs acting on beta-adrenoceptors: evidence for the involvement of both $beta_1$- and $beta_2$-adrenoceptors

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Abstract. Drugs selective for either the $beta_1$- or $beta_2$-adrenoceptor have been investigated for their effects on the head-twitch behaviour induced by L-5-hydroxytryptophan (L-5-HTP) in mice. All four agonists, dobutamine and prenalterol ($beta_1$-), and salbutamol and procaterol ($beta_2$-), potentiated the effect of L-5-HTP although they were ineffective in inducing the head-twitch when administered alone. The corresponding antagonists, practolol and metoprolol ($beta_1$-) and butoxamine and ICI 118,551 ($beta_2$-), were without effect on the L-5-HTP head-twitch. The antagonists each significantly reduced the effect of the corresponding agonists but, while butoxamine and ICI 118,551 were inactive against dobutamine and prenalterol potentiation, both practolol and metoprolol reduced the effect of salbutamol and procaterol. Thus it is argued that dobutamine and prenalterol potentiation is due to an action at $beta_1$-adrenoceptors, while at least a component of the potentiating effect of salbutamol and procaterol is exerted through $beta_2$-adrenoceptors. The lack of effect of the antagonists alone is discussed.

Key words: Head-twitch – L-5-hydroxytryptophan – $Beta_1$-adrenoceptors – $Beta_2$-adrenoceptors

Large doses of L-5-hydroxytryptophan (L-5-HTP) have been reported to produce a head-twitching response in mice (Corne et al. 1963). L-5-HTP is the immediate precursor of 5-hydroxytryptamine (5-HT). The response can also be produced by intracerebral administration of 5-HT itself (Suchowsky et al. 1969; Handley and Miskin 1976). Considerable evidence has been accumulated to suggest that the head-twitch response to L-5-HTP in mice and rats results from the formation of 5-HT in the brain (Corne et al. 1963; Corne and Pickering 1967; Matthews and Smith 1980) and is mediated by $5\text{-}HT_2$ receptors (Peroutka et al. 1981; Ortmann et al. 1982; Green et al. 1983b).

Interactions of selective alpha-adrenoceptor agonists and antagonists with 5-HT-mediated behaviours in rats and mice have been widely investigated (Bedard and Pycock 1977; Bednarczyk and Vetulani 1978; Maj et al. 1978; Vetulani et al. 1980; Ortmann et al. 1981; Handley and Brown 1982), but the effect of beta-adrenoceptor ligands has been less studied. Agonists selective for $beta_2$-adrenoceptors, such as salbutamol (Delini-Stula et al. 1979), clenbuterol (Nimgaonkar et al. 1983), fenoterol and terbutaline (Ortmann et al. 1981) potentiate the L-5-HTP-induced head-twitch response in mice, but the effects of $beta_1$-selective agonists have not been examined. Non-selective beta-adrenoceptor antagonists, such as propranolol, reduce 5-HT mediated hyperactivity syndrome in the rat (Green and Grahame-Smith 1976; Costain and Green 1978) but this is believed to be due to a direct effect on 5-HT receptors (Middlemiss et al. 1977; Green et al. 1983a; Nahorski and Willcocks 1983). However, propranolol does not block the 5-HT head-twitch in mice (Goodwin and Green 1985) or wet dog shake behaviour in rats (Bedard and Pycock 1977) and all the selective antagonists for either $beta_1$- or $beta_2$-adrenoceptors so far tested, such as metoprolol and ICI 118,551, are essentially without effect on the $5\text{-}HT_2$ receptor and do not modulate 5-HT induced hyperactivity syndrome in the rat (Costain and Green 1978; Green et al. 1983a; Nahorski and Willcocks 1983).

It has been reported that both subtypes of beta-adrenoceptors exist in the brain (Minneman et al. 1979). The present work was undertaken in an attempt to characterise the beta-adrenoceptor mediated potentiation of the head-twitch, by the use of selective agonists and antagonists.

Some of the results presented here have been communicated to the British Pharmacological Society (Handley and Singh 1984).

Materials and methods

Male albino mice (TO strain, bred in our laboratories) weighing between 18 and 30 g were kept in a quiet room in groups of 25 on an 08.00–20.00 hour light – dark cycle at constant temperature ($20 \pm 1°$ C) for 5 days prior to experiment. One hour prior to experimentation, groups of mice were placed in small opaque polythene cages lined with sawdust. All behavioural studies were performed between 09.30 and 18.00 hours.

Effect of beta-adrenoceptor agonists on L-5-HTP head-twitch. For these experiments, pairs of mice were housed together 1 h prior to experiment; one was the "test" animal and received the beta agonist simultaneously with the carbidopa, while the other "control" mouse received saline. Both mice were pretreated with carbidopa/L-5-HTP as

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above. A third mouse was included in the cage to maximise the twitch rate of the experimental pair (Boulton and Handley 1973) but took no further part in the experiment. Each mouse was observed in parallel with its paired control (saline/carbidopa/L-5-HTP), to compensate for inter-run variability (Handley and Brown 1982); head-twitches were counted for 5 min, starting 20 min after L-5-HTP. Log dose-response4 regression analysis was performed using response = head-twitch count for the test mouse expressed as a percentage of its paired control mouse from the same run. For potentiation of the head-twitch the ED_{200}, the dose producing a response twice that of the control, was calculated from the regression analysis with 95% confidence limits.

Agonist-antagonist interactions. The mice were paired as described for agonists. All mice received carbidopa/L-5-HTP as before and the agonists were injected simultaneously with carbidopa. One mouse of each pair received antagonist and the other received saline immediately before agonist injection. Antagonists were injected SC, except for practolol which was also administered ICV (Brittain and Handley 1967). Results were analysed by paired *t*-test on raw data. For the figures and tables, responses were expressed as count for each test mouse as a percentage of its paired control.

Drugs. Drugs were obtained from the following sources: dobutamine hydrochloride (Lilly Research), metoprolol tartrate (Geigy Pharmaceuticals), practolol and ICI 118,551 (ICI Pharmaceuticals), prenalterol hydrochloride (Astra Pharmaceuticals), procaterol hydrochloride (Warner-Lambert Co.), salbutamol base (Glaxo Laboratories), butoxamine hydrochloride (Burroughs Wellcome), L-5-HTP (Sigma Ltd.) and carbidopa (Merck, Sharp and Dohme Ltd.). Drugs were dissolved in 0.9% w/v NaCl solution (saline), except L-5-HTP wich was dissolved in saline by the addition of few drops of concentrated HCl, and the pH brought to 7.0 with NaOH. Doses of drugs refer to the weight of the free base.

Results

L-5-HTP: time-effect relationship. The onset of the head-twitch response induced by 9.0 mg/kg carbidopa and 180 mg/kg L-5-HTP occurred between the 2nd and the 5th min (Fig. 1a). Thereafter, the frequency of the response increased to reach a peak of about 12 responses/min between 20 and 25 min after the injection of L-5-HTP. The average response frequency fell to 1.6 responses/10 min between 50 and 60 min after administration of L-5-HTP.

Carbidopa: dose-effect relationship. The head-twitch response increased as the dose of carbidopa was increased to reach a peak of 25 responses/5 min with 12 mg/kg carbidopa (Fig. 1b). A further increase in the dose of carbidopa lowered the number of head-twitches observed.

L-5-HTP: dose-effect relationship. L-5-HTP produced a dose-dependent increase in the number of twitches (Fig. 1c), reaching a maximum at 300 mg/kg.

On the basis of this data (Fig. 1), the effect of beta-adrenoceptor ligands was examined in mice which were pre-

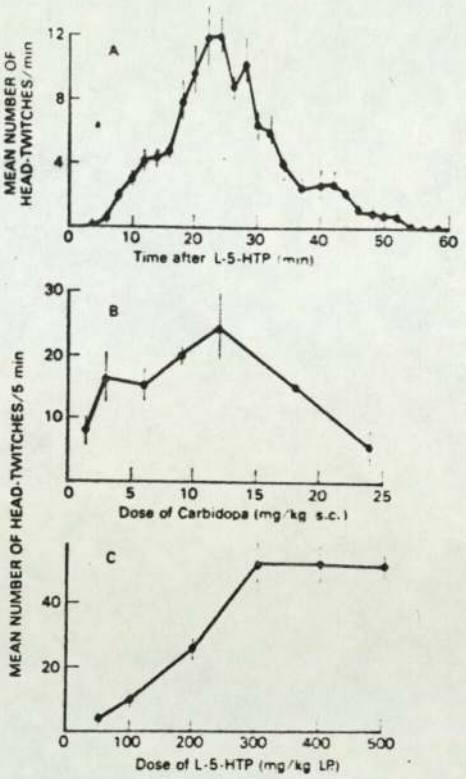

Fig. 1. A L-5-HTP: time – effect relationship. Mice ($n = 5$ per group) were injected with carbidopa (6 mg/kg SC) followed 15 min later by L-5-HTP (180 mg/kg IP). Head-twitches were counted every other min from 2 min to 60 min after L-5-HTP. **B** Carbidopa: dose – effect relationship. Mice ($n = 5$ per group) were injected with carbidopa (1.5–24.0 mg/kg SC) followed 15 min later by L-5-HTP (180 mg/kg IP). Head-twitches were counted for 5 min starting 20 min after L-5-HTP. **C** L-5-HTP: dose – effect relationship. Mice ($n = 5$ per group) were pretreated with 9 mg/kg (SC) carbidopa, 15 min before L-5-HTP (50–500 mg/kg IP). Head-twitches were counted for 5 min starting 20 min after L-5-HTP. Results are the means of five determinations and *vertical bars* represent SEM

treated with 9 mg/kg (SC) carbidopa, followed 15 min later by 200 mg/kg (IP) L-5-HTP. Head-twitches were counted for 5 min starting 20 min after L-5-HTP.

Effect of beta-adrenoceptor agonists and antogonists on L-5-HTP head-twitch. Potentiation of the head-twitch syndrome was caused by both $beta_1$- and $beta_2$-adrenoceptor agonists (Table 1). However, none of the beta-adrenoceptor agonists, administered alone in doses of up to 15 times their ED_{200} values, induced the head-twitch response. Neither was any sign of increased locomotion, headweaving, hindlimb abduction, straub tail or forepaw treading observed in any animal pretreated with these agonists. None of the beta-adrenoceptor antagonists used caused a significant decrease in the L-5-HTP response in the absence of the agonists (Fig. 2).

Interaction of beta-adrenoceptor agonists and antagonists. For these experiments the agonists were injected at doses close to the ED_{200} for potentiating the head-twitch. Practolol was active against all four agonists when it was administered ICV (Table 2). There was no indication of any selectivity in its effects; over a narrow dose range (5.00–10.00 µg ICV) the response progressed from no effect to significant

Table 1. ED_{200} values of beta-adrenoceptor agonists for potentiating the L-5-HTP head-twitch response (200 mg/kg L-5-HTP/9.0 mg/kg carbidopa).

Linear regression (Log dose)

Drug	Dose range tested (mg/kg)	*P*	*r*	ED_{200} (mg/kg)	95% confidence limits (mg/kg)
Dobutamine	0.5–5.0	<0.013	0.901	1.53	1.36–1.74
Prenalterol	5.0–20.0	<0.001	0.952	8.45	7.52–9.48
Procaterol	2.5–10.0	<0.001	0.925	4.50	3.90–5.19
Salbutamol	0.05–5.0	<0.002	0.900	0.12	0.06–0.24

P = Significance of least squares fit
r = Correlation coefficient of least squares fit
ED_{200} = dose to increase twitches to 200% of control levels

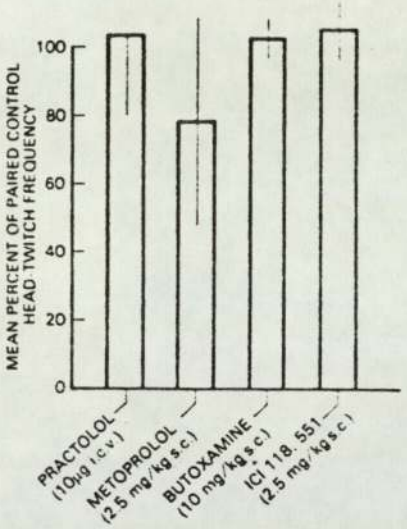

Fig. 2. Effect of beta-adrenoceptor antagonists on the L-5-HTP head-twitch. Mice received either the antagonist or saline SC 15 min before L-5-HTP (200 mg/kg IP) and carbidopa (9.0 mg/kg SC). Head-twitch frequency for each test mouse was recorded as percent of its paired controls. Results are the means of at least five determinations and *vertical bars* represent SEM. $^{*}P<0.05$ (test vs control values, paired *t*-test

Table 2. The effect of $beta_1$-adrenoceptor antagonists on the potentiation of the head-twitch response by beta-adrenoceptor agonists

Antagonist	Prenalterol (10 mg/kg)	Dobutamine (2.5 mg/kg)	Procaterol (5.0 mg/kg)	Salbutamol (0.25 mg/kg)
Practolol (μg ICV)				
5.0	92.1±1.6	97.9±4.9	93.4±3.2	93.2±33.5
7.5	82.4±1.2	78.3±5.3	91.1±2.3	75.9±7.9
10.0	64.2±3.5[b]	27.0±4.7[a]	49.6±1.6[b]	38.6±10.6[a]
Metoprolol (mg/kg)				
1.25	95.2±5.9	71.6±42.2	ND	ND
2.50	53.0±3.6[b]	35.7±4.6[a]	44.4±4.6[b]	46.4±8.1[a]
5.00	40.4±4.8[b]	32.0±8.4[a]	ND	ND

All mice received beta-adrenoceptor agonist, L-5-HTP (200 mg/kg) and carbidopa (9.0 mg/kg). Paired test and control mice received antagonist or vehicle respectively. Results are expressed as the head-twitch count for antagonist-treated mouse as % of paired control meaned over at least six runs (±SEM)

ND not determined. [a] $P<0.05$; [b] $P<0.01$ (test vs control values, paired *t*-test)

Table 3. The effect of $beta_2$-adrenoceptor antagonists on the potentiation of the head-twitch response by beta-adrenoceptor agonists

Antagonist	Prtenalterol (10 mg/kg)	Dobutamine (2.5 mg/kg)	Procaterol (5.0 mg/kg)	Salbutamol (0.25 mg/kg)
Butoxamine (mg/kg)				
2.5	ND	ND	91.6±4.6	90.6±32.0
5.0	ND	ND	79.4±3.8[a]	80.0±40.2
10.0	102.4±2.3	128.9±15.0	41.0±2.8[b]	18.9±4.2[a]
ICI 118,551 (mg/kg)				
1.25	ND	ND	71.2±2.8[b]	77.2±7.0[a]
2.50	105.2±3.3	132.5±25.5	52.2±3.8[b]	48.0±2.1[a]
5.00	ND	ND	39.2±1.7[b]	32.4±1.7[a]

All mice received beta-adrenoceptor agonist, L-5-HTP (200 mg/kg) and carbidopa (9.0 mg/kg). Paired test and control mice received antagonist or vehicle respectively. Results are expressed as the head-twitch count for antagonist-pretreated mouse as % of paired control meaned over at least six runs (±SEM)

ND mot determined. [a] $P<0.05$; [b] $P<0.01$ (test vs control values, paired *t*-test)

inhibition of all four agonists. Neither did the degree of inhibition bear any relationship to whether the agonist was $beta_1$- or $beta_2$-adrenoceptor selective. Practolol was found to be inactive against all the agonists tested during peripheral administration [dobutamine (2.5 mg/kg) 99.5±2.0%; prenalterol (10.0 mg/kg) 99.0±2.5%; salbutamol (0.25 mg/kg) 96.0±3.5%; procaterol (5.0 mg/kg) 103.5±7.0% of appropriate paired control; $P>1.0$ in each case]. Results with metoprolol were similar to ICV practolol, although this compound was investigated in less detail (Table 2). Thus the lowest dose of metoprolol which inhibited the effect of prenalterol and dobutamine produced a similar degree of inhibition of procaterol and salbutamol.

Butoxamine, a $beta_2$-antagonist, produced a dose-dependent reduction in the head-twitch potentiated by both procaterol and salbutamol (Table 3), yet the highest dose was ineffective against either prenalterol or dobutamine. ICI 118,551 similarly reduced procaterol and salbutamol potentiation in a dose dependent manner (Table 3), but a dose producing 50% inhibition of the head-twitch when potentiated by these agonists was entirely without effect against prenalterol and dobutamine potentiation.

Discussion

The initial experiments were necessary to establish the optimum protocol for investigation of drug effects on the head-twitch. The time-course of the L-5-HTP head-twitch was similar to that reported for the mouse by Corne et al. (1963). Carbidopa was used to inhibit the peripheral L-amino acid decarboxylase, to minimise peripheral effects of 5-HT and maximise central effects (Modigh 1972). Care was needed in the choice of dose of carbidopa since, as

shown in Fig. 1b, higher doses reduced the head-twitch, probably by blocking central as well as peripheral enzyme activity. Finally, the dose of 200 mg/kg L-5-HTP was chosen from its position in the middle of the linear portion of the dose-response curve.

All four beta-adrenoceptor agonists potentiated the head-twitch, with a potency order salbutamol > dobutamine > procaterol > prenalterol. This potency order shows no obvious relationship to beta-adrenoceptor selectivity, but may be affected by relative penetration of the brain. Studies carried out in vivo have reported that dobutamine and prenalterol show selectivity for $beta_1$-adrenoceptors (Carlsson et al. 1977; Sonnenblick et al. 1979). However, dobutamine isomers have recently been reported to have a complex action on alpha-, $beta_1$- and $beta_2$-adrenoceptors in vascular smooth muscle (Ruffolo and Yaden 1983). Furthermore, salbutamol has been shown to be a selective agonist at $beta_2$-adrenoceptors both in vivo and in a variety of in vitro preparations (Brittain et al. 1968). Procaterol in vitro also has selectivity for $beta_2$-adrenoceptors (Yabuuchi 1977; O'Donnell and Wanstall 1985).

No agonist induced the head-twitch when given alone. The ability to enhance, but not to induce, head-twitching may reflect the known ability of noradrenergic systems, notably locus coeruleus, to increase signal to noise ratio of sensory input and to facilitate responses to other transmitters, without themselves inducing firing (Foote et al. 1983). This effect appears to involve beta- as well as alpha-adrenergic mechanisms (Rogawski and Aghajanian 1980; Waterhouse et al. 1981, 1982; Madison and Nicoll 1982; see also Foote et al. 1983).

The related 5-HT receptor-mediated hyperactivity syndrome in the rat is also potentiated by the $beta_2$-adrenoceptor agonists clenbuterol, salbutamol and terbutaline (Cowen et al. 1982). As with the mouse head-twitch response, the effect of $beta_2$-adrenoceptor agonists was prevented by metoprolol; however in the rat hyperactivity syndrome $beta_2$-adrenoceptor antagonist butoxamine was ineffective.

Practolol shows 9-fold selectivity in conscious cats and dogs as well as in binding assays for $beta_1$-adrenoceptors (Dunlap and Shanks 1968; Leclerc et al. 1981), and in vivo metroprolol is also selective for this receptor (Ablad et al. 1973). Conversely, in vitro in binding assays butoxamine has 5-fold selectivity (Leclerc et al. 1981), and ICI 118,551 in isolated preparations is much more selective, for $beta_2$-adrenoceptors (O'Donnell and Wanstall 1980). Thus our finding that dobutamine and prenalterol showed a dose-dependent inhibition after practolol or metoprolol, but were unaffected by butoxamine and ICI 118,551, demonstrates that these agonists were acting on $beta_1$- rather than $beta_2$-adrenoceptors. Since these doses of butoxamine and ICI 118,551 were not effective against $beta_1$-adrenoceptor agonists, their inhibition of salbutamol and procaterol shows that the latter agonists exerted their potentiation at least in part through $beta_2$-adrenoceptors. From this, it may be concluded that activation of both $beta_1$- and $beta_2$-adrenoceptors has a potentiating effect on the head-twitch caused by L-5-HTP.

Practolol penetrates the blood brain barrier poorly (Day et al. 1977). Its failure to block the potentiation due to any of the agonists when administered peripherally indicates that the agonists produce potentiation by a central effect.

Butoxamine and ICI 118,551 showed effects consistent with selective blockade of $beta_2$-adrenoceptors, but practolol and metoprolol showed no selectivity whatsoever. Either all effective doses of practolol and metoprolol blocked both $beta_1$- and $beta_2$-adrenoceptors or the potentiating effect of $beta_2$-adrenoceptor activation is exerted ultimately by releasing noradrenaline onto $beta_1$-adrenoceptors. The possibility of an indirect action of $beta_2$-adrenoceptor agonists is not without support. Receptors of $beta_2$-subtype have been found on noradrenergic terminals and appear to be involved in facilitation of noradrenaline release (Westfall 1977; Dhalof et al. 1981; see Misu and Kubo 1983). However, 6-hydroxydopamine lesions of locus coeruleus, dorsal and ventral bundles did not prevent the potentiating effect of salbutamol or clenbuterol on the related hyperactivity syndrome in the rat (Ortmann et al. 1981; Nimgaonkar et al. 1983). Effects of such lesions on head-twitching are currently under investigation.

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References

Ablad B, Carlsson D, Ek L (1973) Pharmacological studies of two new cardioselective adrenergic beta-receptor antagonists. Life Sci 12:107–119

Bedard P, Pycock CJ (1977) "Wet-dog shake" behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. Neuropharmacology 16:663–670

Bednarczyk B, Vetulani J (1978) Antagonism of clonidine to shaking behaviour in morphine abstinence syndrome and to head-twitches produced by serotonergic agents in the rat. Pol J Pharmacol Pharm 30:307–322

Boulton CS, Handley SL (1973) Factors modifying the head-twitch response to 5-hydroxytryptophan. Psychopharmacologia 20:204–214

Brittain RT, Handley SL (1967) Temperature changes produced by the injection of catecholamines and 5-hydroxytryptamine into the cerebral ventricles of the conscious mouse. J Physiol 192:805–813

Brittain RT, Farmer JB, Jack D, Martin LE, Simpson NT (1968) – [(*t*-butylamino)methyl]-4-hydroxy-m-xylene-1,3-diol (AH 3365): a selective beta-adrenergic stimulant. Nature 219:862–863

Carlsson E, Dahlof C-G, Hedberg A, Persson H, Tangstraud B (1977) Differentiation of cardiac chronotropic and inotropic effects of beta-adrenoceptor agonists. Naunyn-Schmiedebergs Arch Pharmacol 300:101–105

Corne SJ, Pickering RW (1967) A possible correlation between drug-induced hallucinations in man and a behavioural response in mice. Psychopharmacologia 11:65–78

Corne SJ, Pickering RW, Warner BT (1963) A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. Br J Pharmacol 20:106–120

Costain DW, Green AR (1978) Beta-adrenoceptor antagonists inhibit the behavioural responses of rats to increased brain 5-hydroxytryptamine. Br J Pharmacol 64:193–200

Cowen PJ, Grahame-Smith DG, Green AR, Heal DJ (1982) Beta-adrenoceptor agonists enhance 5-hydroxytryptamine-mediated behavioural responses. Br J Pharmacol 76:265–270

Day MD, Hemsworth RA, Street JA (1977) The central uptake of beta-adrenoceptor antagonists. J Pharm Pharmacol 29:52P

Delini-Stula A, Vassout A, Radeke E, Ortmann H (1979) Psychopharmacological profile of $beta_4$-receptor stimulants. Naunyn-Schmiedeberg's Arch Pharmacol 307:R65

Dhalof C, Englberg G, Svensson TH (1981) Effect of beta-adreno-

ceptor antagonists on the firing rate of noradrenaline neurons in the locus coeruleus of the rat. Naunyn-Schmiedeberg's Arch Pharmacol 317:26–30

Dunlap D, Shanks RG (1968) Selective blockade of adrenoceptive beta-receptors in the heart. Br J Pharmacol 32:201–218

Foote SL, Bloom FE, Aston-Jones G (1983) Nucleus locus coeruleus: new evidence of anatomical and physiological specificity. Physiol Rev 63:844–914

Goodwin GM, Green AR (1985) A behavioural and biochemical study in mice and rats of putative selective agonists and antagonists for 5-HT_1 and 5-HT_2 receptors. Br J Pharmacol 84:743–753

Green AR, Grahame-Smith DG (1976) (-)-Propranolol inhibits the behavioural responses of rats to increased 5-hydroxytryptamine in the central nervous system. Nature 262:594–596

Green AR, Heal DJ (1985) The effects of drugs on serotonin-mediated behavioural models. In: Green AR (ed) Neuropharmacology of serotonin. Oxford: Oxford University Press

Green AR, Johnson P, Nimgaonkar VL (1983a) Interactions of beta-adrenoceptor agonists and antagonists with the 5-hydroxytryptamine$_2$ (5-HT_2) receptor. Neuropharmacology 22:657–660

Green AR, O'Shaughnessy K, Hammond M, Schachter M, Grahame-Smith DG (1983b) Inhibition of 5-hydroxytryptamine-mediated behaviour by the putative 5-HT_2 antagonist pirenperone. Neuropharmacology 22:573–578

Handley SL, Brown J (1982) Effects on the 5-hydroxytryptamine induced head-twitch of drugs with selective actions on alpha$_1$- and alpha$_2$-adrenoceptors. Neuropharmacology 21:507–510

Handley SL, Miskin RC (1876) The interaction of some kynurenine pathway metabolites with 5-hydroxytryptophan and 5-hydroxytryptamine. Psychopharmacologia 51:305–309

Handley SL, Singh L (1984) The effect of beta-adrenoceptor agonists and antagonists on the head-twitch in male mice. Br J Pharmacol 81:127P

Leclerc G, Rouot B, Velly J, Schwartz J (1981) Beta-adrenergic subtypes. TIPS 17:18–20

Madison DV, Nicoll RA (1982) Noradrenaline blocks accomodation of pyramidal cell discharge in hippocampus. Nature 299:636–638

Maj J, Baran L, Bigajska K, Rogoz A, Skuza G (1978) The influence of neuroleptics on the behavioural effect of 5-hydroxytryptophan. Pol J Pharmacol Pharm 30:431–440

Matthews WD, Smith CD (1980) Pharmacological profile of a model for central serotonin receptor activation. Life Sci 26:1397–1403

Middlemiss DN, Blakeborough L, Leather SR (1977) Direct evidence for an interaction of adrenergic blockers with the 5-HT receptor. Nature 267:289–290

Minneman KP, Dibner MD, Wolfe BB, Molinoff PB (1979) Beta$_1$- and beta$_2$-adrenergic receptors in rat cerebral cortex are independently regulated. Science 204:866–868

Misu Y, Kubo T (1983) Presynaptic beta-adrenoceptors. TIPS 4:506–508

Modigh K (1972) Central and peripheral effects of 5-hydroxytryptophan on motor activity in mice. Psychopharmacologia 23:48–54

Nahorski SR, Willcocks AL (1983) Interactions of beta-adrenoceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex. Br J Pharmacol 78:107P

Nimgaonkar VL, Green AR, Cowen PJ, Heal DJ, Grahame-Smith DG, Deakin JFW (1983) Studies on the mechanisms by which clenbuterol, a beta-adrenoceptor agonist, enhances 5-HT-mediated behaviours and increases brain 5-HT metabolism in the rat. Neuropharmacology 22:739–749

O'Donnell SR, Wanstall JC (1980) Evidence that ICI 118,551 is a potent highly beta$_2$-selective adrenoceptor antagonist and can be used to characterise beta-adrenoceptor populations in tissues. Life Sci 27:671–677

O'Donnell SR, Wanstall JC (1985) Responses to the beta$_2$-selective agonist procaterol of vascular and aterial preparations with different functional beta-adrenoceptor populations. Br J Pharmacol 84:227–235

Ortmann R, Martin S, Radeke E, Delini-Stula E (1981) Interaction of beta-adrenoceptor agonists with the serotonergic system in rat brain. A behavioural study using the L-5-HTP syndrome. Naunyn-Schmiedeberg's Arch Pharmacol 316:225–230

Ortmann R, Bischoff S, Radeke E, Buech O, Delini-Stula H (1982) Correlations between different measures of antiserotonin activity of drugs. Study with neuroleptics and serotonin receptor blockers. Naunyn-Schmiedeberg's Arch Pharmacol 321:265–270

Peroutka SJ, Lebovitz RM, Snyder SH (1981) Two distinct central serotonin receptors with different physiological functions. Science 212:827–829

Ruffolo JR, RR, Yaden EL (1983) Vascular effects of the stereoisomers of dobutamine. J Pharmacol Exp Ther 224:46–50

Rogawski MA, Aghajanian GK (1980) Modulation of lateral geniculate neurone exciteability by noradrenaline microiontophoresis or locus coeruleus stimulation. Nature 287:731–734

Sonnenblick EH, Frishman WH, Lejemtel TH (1979) Dobutamine: a new synthetic cardioactive symathetic amine. N Engl J Med 300:17–22

Suchowsky GK, Pegrassi L, Moretti A, Bonsignori A (1969) The effect of 4H-3 methylcarboxamide 1,3-benzoxazine-2-one (FI 6654) on monoaminoxidase and cerebral 5-HT. Arch Int Pharmacodyn Ther 182:332–340

Vetulani J, Bednarczyk B, Reichenberg K, Rokosz A (1980) Head-twitches induced by LSD and quipazine: Similarities and differences. Neuropharmacology 19:155–158

Waterhouse BD, Moises HC, Woodward DJ (1981) Alpha-receptor mediated facilitation of somatosensory cortical neuronal responses to excitatory synaptic inputs and iontophoretically applied acetylcholine. Neuropharmacology 20:907–920

Waterhouse BD, Moises HC, Yeh HH, Woodward DJ (1982) Norepinephrine enhancement of inhibitory synaptic mechanisms in cerebellum and cerebral cortex: mediation by beta-adrenergic receptors. J Pharmacol Exp Ther 221:495–506

Westfall TC (1977) Local regulation of adrenergic neurotransmission. Physiol Rev 57:659–728

Yabbuuchi Y (1977) The beta-adrenoceptor stimulant properties of OPC-2009 on guinea-pig isolated tracheal, right atrial and left atrial preparations. Br J Pharmacol 61:513–521

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