STUDIES ON CHEMICAL TRANSMISSION IN THE NON-SYMPATHETIC INHIBITORY NERVOUS PATHWAY TO THE STOMACH

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September, 1980.

SUMMARY

Studies on chemical transmission in the non-sympathetic inhibitory nervous pathway to the stomach. J.S.Morris, PhD thesis, The University of Aston in Birmingham, Sept., 1980.

Vagal stimulation of the guinea-pig or rat stomach in the presence of hyoscine (0.23μM) gave a relaxation which was resistant to guanethidine (16.9μM) but reduced by hexamethonium (55.2μM) or pempidine (15.0μM). Relaxations were also produced in the presence of hexamethonium (20.0 to 55.2μM) or pempidine (2.0 to 15.0μM) alone, which were also resistant to guanethidine (16.9μM). These relaxations were reduced by low concentrations of hyoscine (0.023μM). The latency of the response in the presence of ganglion blocking drugs alone was longer than that in the presence of hyoscine alone. It was therefore argued that the ganglia of the inhibitory nerves could transmit through both nicotinic and muscarinic receptors. Muscarinic ganglionic transmission was not observed in the mouse stomach. In the presence of both hyoscine or ganglion blocking drugs alone an initial rapid relaxation to vagal stimulation was seen followed by a period of rapid recovery mediated by PGE_2 which was non-cholinergic. In the presence of hyoscine alone this was followed by a period of slow recovery. In the presence of ganglion blocking drugs alone the inhibitory response was followed by a rapid recovery and an after-contraction which was mediated by PGE_2 but also had a muscarinic component. Papaverine inhibited only the responses mediated by PGE_2 that were also muscarinic. Bradykinin (0.4 to 9.6nM) caused a relaxation of the guinea-pig stomach which was sensitive to the external calcium concentration. Relaxations to bradykinin were unaffected by tetrodotoxin (5.7μM) phentolamine (5.0 to 50.0μM) plus propranolol (5.0 to 50.0μM) or indomethacin (14.0 to 28.0μM). Desentitisation of the preparation to bradykinin produced little reduction in the size of the responses to inhibitory vagal stimulation or adrenaline (2.0 to 4.0μM) but did produce a marked reduction in the size of the response to ATP (10.0μM). In view of this it seems that bradykinin and ATP share a common step to produce gastric inhibition which is not utilised by adrenaline or the non-cholinergic, non-adrenergic inhibitory transmitter.

KEY WORDS: bradykinin, ganglia, gastric relaxation, vagus.

ACKNOWLEDGEMENTS.

This work was sponsored by the Pharmaceutical Society of Great Britain.

I thank I.C.I. for a gift of Propranolol and Geigy Pharmaceuticals for desipramine and chlorimipramine.

Considerable assistance was given by Mr. A. Richardson in the early part of this work.

Thanks to Mrs. I.R. Morris for typing this manuscript.

Above all, thanks are due to Dr. O.A. Downing for his excellent supervision of this work.

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GENERAL INTRODUCTION.

Classically the autonomic nervous system has been divided and classified according to the scheme of Langley (1921) who wrote thus:

> "I divided the autonomic system into tectal, bulbo-sacral, and sympathetic systems, and considered that each had a different developmental history.
>
> The theory that there is some fundamental differences between the sympathetic and the rest of the autonomic system was much strengthened by the discovery that the effects produced by adrenaline were apparently confined to effects caused by stimulating sympathetic nerves. Since other drugs caused effects more or less confined to those produced by stimulating tectal and bulbo-sacral nerves, it was convenient to have a common name for these nerves, and I placed them together as the parasympathetic system.....I pointed out that we should expect the cells of Auerbach's and Meissner's plexuses to be on the course of the bulbo and sacral nerves, but as there was no clear proof of their connexions, and as their obvious histological characters differed from those at any other peripheral nerve cell, I placed them in a class by themselves as the enteric nervous system".

To-day the 'enteric nervous system' is usually thought of as parasympathetic, chiefly because those 'enteric' neurones that do receive central connections, for example those of the stomach, are supplied by preganglionic nerves which run in the vagus.

The parasympathetic nerves originate from the cranial and sacral segments of the central nervous system (C.N.S.)

which run to the tissues where synapses with their post-ganglionic fibres are formed. The sympathetic nerves originate from the thoracico-lumbar segments of the spinal cord which pass through the paravertebral ganglia. The postganglionic cell bodies are generally found within these ganglia or at other ganglia (e.g. coeliac;mesenteric) remote from the innervated tissue. The organs innervated by the parasympathetic system include the salivary and lachrymal glands, the gastro-intestinal tract and abdominal digestive glands, the intrinsic eye muscles, the pulmonary airways, the heart, the urinary system, and some vascular systems (e.g. in the lungs, salivary glands and external genitalia). The sympathetic system also innervates these tissues but in addition also supplies the internal genitalia, systemic vascular beds (e.g. in the skin), sweat glands and pilo-errector muscles, and the adrenal glands. Where a tissue is under dual innervation from both systems they tend to be antagonistic in effect (e.g. the parasympathetic nerves stimulate the gut whereas those of the sympathetic system inhibit it, except for the sphincters where the reverse is true; the heart is stimulated by the sympathetic system and inhibited by the parasympathetic system; parasympathetic nerves constrict the pupils of the eye whereas sympathetic nerves dilate them). Tissues receiving only sympathetic innervation tend to be stimulated (e.g. most vascular smooth muscle).
Because of the widespread nature of sympathetic nervous responses with often an associated release of catecholamines from the adrenal medulla the sympathetic nervous system has been regarded as altering the background level of general

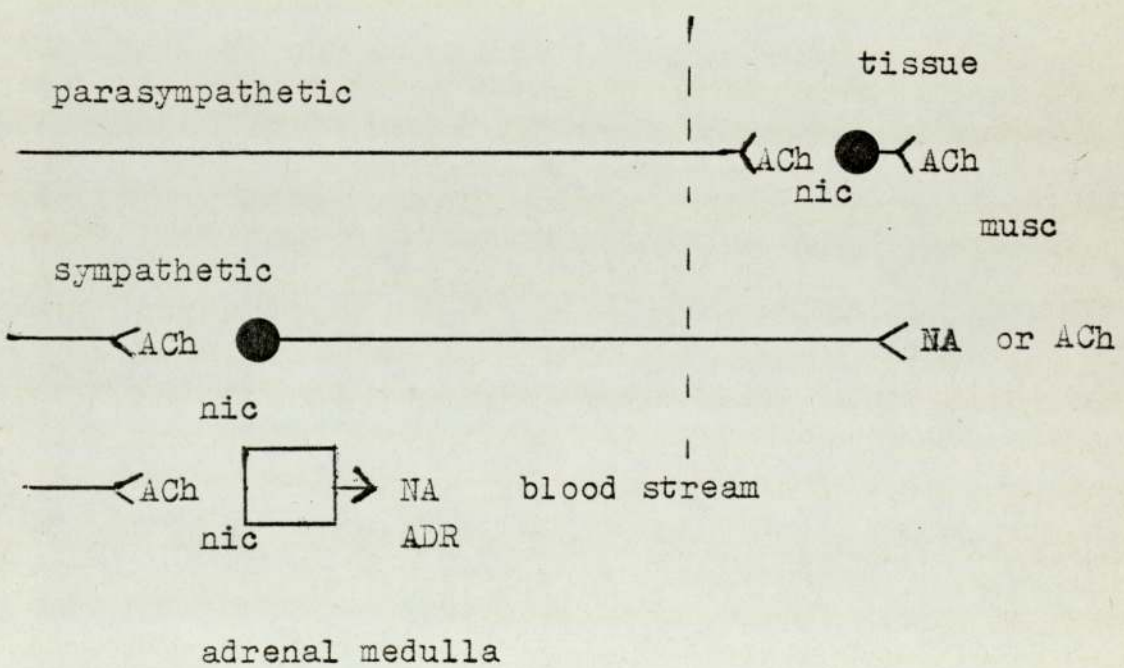
"tone" in the organism with fine control to more precise limits falling to the parasympathetic nerves.

In 1933 Dale introduced the terms adrenergic for nerves releasing adrenaline (ADR) or as it later turned out noradrenaline (NA) (West, 1950) and cholinergic for nerves releasing acetylcholine (ACh).

Parasympathetic post-ganglionic nerves were cholinergic and sympathetic post-ganglionic nerves were adrenergic except for some exceptions (e.g. sweat gland innervation which is cholinergic despite being sympathetic in origin).

At the ganglia of both systems ACh is released.

The classical understanding of the autonomic nervous system can be illustrated, therefore, by the following diagram.



ACh = acetylcholine, NA = noradrenaline, ADR = adrenaline, musc = muscarinic receptors, nic = nicotinic receptors.

ACh is released by all the preganglionic nerves and acts chiefly at nicotinic receptors (effect mimicked by the

drug nicotine and blocked by the drugs hexamethonium or pempidine) and also by post-ganglionic parasympathetic nerves where it acts at muscarinic receptors (effects mimicked by the drug muscarine and blocked by atropine or hyoscine). Most of the sympathetic nerves release NA post-ganglionicaly except for a few exceptions (for further details and references see Keele and Neil, 1971).

For many years it was assumed that the sympathetic nerves released NA directly on to the tissue where it had its action. Later work has, however, thrown doubt on this assumption at least within the gastro-intestinal tract. Norberg (1964) has shown by fluorescence histochemical techniques that apart from vasomotor fibres most of the adrenergic nerves in the gut terminate in the enteric plexuses where they are arranged around the intramural ganglion cells in structures highly suggestive of synapses. The muscle layers proper receive very little adrenergic innervation. This same observation was later repeated by other workers (Jacobowitz, 1965; Bennett and Rogers, 1967; Read and Burnstock, 1968; van Driel and Drukker, 1973). Hollands and Vanov (1965) were of the opinion that the catecholamine containing cells observed in the musculature were greater in number than could be accounted for as being involved in vascular innervation alone and they, therefore, concluded that some direct innervation of the musculature was present. However, all the nerve cells containing catecholamines within the musculature were running parallel to the muscle fibres and not in any other direction. Åberg and Eränkö (1967) also observed these few adrenergic nerves within the musculature, running parallel to the muscle fibres. They also stained for cholinesterase and many stained nerve

fibres were seen running throughout the musculature, but in these cases nerve fibres ran accross the musculature and in all directions as one might expect to observe if the nerve cells were indeed innervating the muscle fibres. The significance of these few adrenergic nerve fibres running parallel to the muscle cells is hard to imagine. It may be argued that their density is too low for them to be involved in the effective system of muscle cell innervation but it is worth noting that in a histological study single axons were rarely observed in the musculature of the guinea-pig taenia caecum (Bennett and Rogers, 1967) but the authors produce evidence that electrical coupling between smooth muscle cells was possible and this allows impulses to propagate from muscle cell to muscle cell which could in turn make a sparse innervation effective. This type of sparse innervation but with low resistant electrical bridges between the muscle cells is now considered to be the norm for smooth muscle cells which behave as single units or 'unitary' muscles as defined by Bozler (1948). Such muscles are found in the gut, ureter and uterus (Burnstock, 1970). The occurrence of most of the adrenergic terminals within the nerve plexuses of the gut suggests that sympathetic inhibitions may be mainly effective either by reducing the release of ACh by an action on the preganglionic nerve fibres or by reducing the response to ACh on its post-ganglionic parasympathetic nerve cell bodies. In a pharmacological investigation Kosterlitz and Watt (1965) found that contractions of coaxialy stimulated guinea-pig ileum in the presence of hexamethonium were inhibited by ADR, NA and isoprenaline (ISO). This action of ADR and NA was reduced by combinations of alpha-and beta-adrenoceptor

blocking drugs and the actions of ISO was reduced by beta-adrenoceptor blocking drugs alone. Furthermore all three catecholamines reduced contractions to ACh and carbamylcholine but this action was only antagonised by beta-adrenceptor blockers. They felt that this indicated that ADR and NA were having effects at alpha-adrenoceptors located on the parasympathetic nerves and beta-adrenoceptors on the smooth muscle cells. ISO only acted at the beta-adrenoceptors of the smooth muscle cells. Paton and Vizi (1969) showed that NA and ADR reduced ACh output by guinea-pig ileum longitudinal muscle strips. In the same year Beani, Bianchi, and Crema (1969) working with the guinea-pig colon showed that sympathetic nerve stimulation in this tissue did indeed reduce the responses to pelvic nerve and transmural stimulation and also reduced the amount of ACh released by pelvic nerve and transmural (at low frequencies) stimulation. They favoured the idea that sympathetic inhibition also occurred by a direct action caused by catecholamines diffusing from their site of release at the sympathetic nerve endings on to the smooth muscle fibres. In 1970 Kosterlitz, Lydem and Watt confirmed that ACh output was reduced by alpha-adrenoceptor stimulation and also by stimulation of the sympathetic nerves in the guinea-pig ileum. The same observation was made by Gillespie and Khoyi (1974;1977) in the isolated rabbit colon, who also observed (1977) that the response to exogenous ACh was diminished by sympathetic nerve stimulation or by stimulation of beta-adrenoceptors by ISO, suggesting that as well as a reduction of ACh released from parasympathetic nerves caused by sympathetic nerve stimulation mediated via alpha-adrenoceptors, a concomitant reduction in the effectiveness

of ACh to contract the tissue also occurred in response to sympathetic nerve stimulation which was mediated by beta-adreneceptors. As stimulation of the beta-adrenoceptors inhibited the response to exogenous ACh they must be located on the smooth muscle cells themselves. Further evidence that these receptors were of the beta type was given by the fact that this response to sympathetic nerve stimulation or ISO was blocked by propranolol but not by phentolamine. These observations have provided pharmacological support for the ideas generated by histological investigations; that sympathetic inhibition is mainly effective by reducing ACh release by an action on the preganglionic parasympathetic fibres. Furthermore, the pharmacological investigations indicate that this reduction of ACh release is mediated by alpha-adrenoceptors. An action of released catecholamines is still expected as the previously mentioned investigations (Kosterlitz and Watt, 1965; Kosterlitz, Lydom and Watt, 1970: Gillespie and Khoyi, 1974:1977) distinctly showed the presence of beta-adrenoceptors on the smooth muscle, and both events occur probably simultaneously as was suggested by Beani et al (1969) and Gillespie and Khoyi (1977), in response to sympathetic nerve stimulation.

On the other hand, Gershon (1967) considered that stimulation of sympathetic nerves in the guinea-pig stomach did not reduce ACh output and argues that the effects of sympathetic nerve stimulation did not resemble those of ganglion block-ade (by ganglion blocking drugs) which should be the case if the site of action of the sympathetic nerves was indeed at the preganglionic parasympathetic fibre.

Inhibitory nerves which are non-cholinergic and non-adrenergic which release their transmitter directly onto the smooth muscle have been discovered and it is the view

of Burnstock (1979) that these fibres have a far more important role in gut inhibitions than do those of the sympathetic.

Before these fibres are considered in more detail it is worth pointing out that sympathetic reflexes do have a role in gastro-intestinal control and this has been the subject of reviews by Abrahamsson (1973) and Szurszewski and Weems (1976).

Non-cholinergic, non-adrenergic inhibitory nervous control has been found in the stomachs of fish, amphibians, birds and mammals, in the small intestine of rodents, and the cat, but not in lower vertebrates where the vagus nerves do not extend so far down the gut. They can also be found in the large intestine of a variety of mammals. Similar types of fibres have also been found in lung, heart, the urinogenital system, blood vessels and the eye, (for references see Burnstock, 1972). The non-cholinergic, non-adrenergic inhibitory nerves supplying the stomach are the subject of this study.

In the gut these fibres are thought to be involved in the reciprocal inhibition of the small intestine, where, when one muscle coat is contracted, the other is reflexly relaxed (Kottegoda, 1969). This is important in the small intestine where the contents are fluid and digestion requires mixing of the luminal contents. Alternate contractions of the two muscle coats (the longitudinal and circular) with the inhibition of the other ensures that this mixing occurs. They are also thought to be involved in the descending inhibition of the colon (Crema, 1970; Hirst, Holman and McKirdy, 1976; Hirst, 1979). When contractions occur in a segment of colon the tissue

immediately distal to it relaxes. This allows the contents, which are more solid in this part of the gut to be propelled aborally. In the stomach Cannon and Lieb (1911) noted that the stomach relaxed before the ingested mass had passed through the oesophagus in order to provide a receptacle for food. This 'receptive relaxation' was considered by Abrahamsson (1973) to involve non-cholinergic, non-adrenergic fibres. He also postulated a role for these fibres in the regulation of gastric mixing and emptying. The realisation of the existence of non-cholinergic, non-adrenergic inhibitory nerve fibres is comparatively recent. As early as 1898 Langley had observed that stimulation of the vagus could cause a relaxation of the stomach, and this was best seen when the excitatory response to vagal stimulation was blocked by atropine (May, 1904; McSwiney and Robson, 1929). Initially this was thought to be due to admixed sympathetic fibres within the vagus. In 1952 Eliasson showed that these fibres ran the whole course of the vagus because stimulation of the orbital areas of cat brain caused a gastric inhibition that was abolished by vagotomy but not by splanchnicectomy. These effects could not be reversed by a change of stimulation parameters or by gastric distension, and were independent of varying the initial tone in the experiments. Similar work in dogs showed that the inhibitory gastric fibres ran the whole course of the vagus (Semba, Fujii and Kimura, 1964) because stimulation of the medulla oblongata caused a gastric inhibition that was abolished by bilateral vagotomy. The first evidence that these fibres were non-adrenergic was given by Paton and Vane (1963) although they did not recognise it as such. They showed that the inhibitory

response of the isolated stomach to transmural or vagal stimulation, after the excitatory responses were blocked by muscarinic receptor blocking drugs, were unaffected by the adrenergic neurone blocking drug Tm 10, when at the same concentrations responses to perivascular (sympathetic) nerve stimulation were inhibited completely.

They ascribed this result as being due to stimulation of adrenergic nerve fibres at a site peripheral to the locus of action of Tm10 and did not question that the fibres may not be adrenergic.

Martinson (1965b) demonstrated that not only were the vagaly mediated inhibitory gastric responses resistant to adrenergic neurone blocking agents such as guanethidine but also to alpha-and beta-adrenoceptor blocking agents and he concluded that these fibres were not adrenergic. Campbell (1966) came to a similar conclusion regarding vagal gastric relaxation.

Similar relaxations which were resistant to adrenergic neurone blocking agents had been demonstrated in the small and large intestine of a number of mammals; in the guinea-pig taenia caecum (Burnstock, Campbell, Bennett and Holman, 1963;1964; Burnstock, Campbell and Rand, 1966) in the rabbit and human colon (Bucknell, 1964) in duodenum, ileum and colon from the rabbit, guinea-pig, rat and mouse (Holman and Hughes, 1965), in the guinea-pig ileum (Day and Warren, 1967;1968), and in the guinea-pig colon (Bianchi, Beani, Frigo and Crema, 1968). Bucknell (1964) and Day and Warren (1967;1968), also noted that their preparations were resistant to alpha-and beta-adrenoceptor blocking agents.

In electrophysiological studies on the taenia of the guinea-

pig caecum it was found that hyperpolarisation of the smooth muscle cell membrane could be induced by low frequency electrical field stimulation, and this effect was unaffected by bretylium or guanethidine (Bennett, Burnstock and Holman, 1966b). Hyperpolarisation (inhibitory junction potentials or i.j.ps.) of up to 25 mV were produced to single shocks, or if stimulated with trains of pulses a maximum hyperpolarisation was achieved of up to 35 mV within 80 ms of commencing the train of pulses firing at 10 pulses per second. Recovery was within 250 to 500 ms. On the other hand stimulating the perivasular nerve supply to the same tissue gave different characteristics of hyperpolarisation (Bennett, Burnstock and Holman, 1966a). Single shocks did not produce hyperpolarisation but hyperpolarisation produced by trains of 5 pulses per second or greater were only 16 mV in amplitude. Furthermore the latency of the response was longer varying from 150 to 300 ms. Also these hyperpolarisations were blocked by bretylium or guanethidine. Clearly then hyperpolarisation seen on field stimulation differed from that produced by sympathetic (perivascular) nerve stimulation. These hyperpolatisations were shown to be neurogenic because they were abolished by tetrodotoxin (Ttx) (Bülbring and Tomita, 1966;1967).
These intrinsic or intramural inhibitory nerves, as they were designated (Bennett et al, 1966b) were clearly similar structures to those that were responsible for mediating relaxations of the gut resistant to adrenolytic drugs. The inhibitory response of the stomach to vagal stimulation in the presence of atropine or hyoscine is sensitive to

Ganglion blocking drugs (Paton and Vane, 1963; Bülbring and Gershon, 1967; Beani, Bianchi and Crema, 1971) and this can be taken as evidence for the presence of ganglia in the nervous pathway. In most distal portions of the gut it is difficult to see where the non-cholinergic non-adrenergic nerves are supplied from. There is no inhibition of the intestines to pelvic nerve stimulation except perhaps for a limited distal portion of the guinea-pig rectum (for references see Burnstock, 1972).

However, Burnstock, Campbell and Rand (1966) have described a preparation of the guinea-pig taenia caecum where a flap of caecum wall was left attached to the longitudinal muscle band, or taenia which could then be stimulated by electrodes. In the presence of atropine, inhibitory responses were obtained which were reduced by the ganglion blocking drug pentolinium indicating that the intramural inhibitory nerves were probably post-ganglionic neurones in an autonomic pathway.

Holton (1959) when stimulating the great auricular nerve of the rabbit antidromically observed that this procedure caused the release of adenosine triphosphate (ATP) a proposed transmitter in intramural inhibitory nerves (see later), there was more ATP liberated than could be accounted for by haemolysis of erythrocytes, and this release of ATP was abolished by denervation. The liberated ATP caused vasodilatation.

This observation strongly suggests that antidromic stimulation of sensory nerves causes the release of ATP which relaxes smooth muscle. Since the vagus nerves

contain about 80% sensory fibres, it seems a genuine possibility that many of the effects seen following direct vagal stimulation could be due to this phenomenon.

Campbell (1970) and Burnstock (1972) have both pointed out that the observation of the vagal pathway being susceptible to ganglion blocking agents strongly suggests a step which involves the liberation of Ach to act at nicotinic receptors, an event that would be unlikely if the fibres involved were sensory and firing antidromically, but would be likely if the fibres were pre-ganglionic cholinergic fibres making synaptic connections with the intramural inhibitory neurones.

The nature of the chemical transmitter released from the non-cholinergic non-adrenergic nerves still remains controversial. If all of the known biologically active compounds are considered many would be discounted on the basis that they excite, or have no effect on muscle from most regions of the gastrointestinal tract (Campbell, 1970). The list of compounds that Campbell (1970) discounted in this way includes histamine, gamma aminobutyric acid, bradykinin and substance P, and the prostaglandins. He also eliminated 5-hydroxytryptamine (5-HT) on the grounds that it exerts its inhibitory action by stimulation of nerves.

Burnstock considers (1972;1979) that the transmitter is a purine nucleotide such as ATP and he makes the following points (Burnstock, 1972).

"1) ATP and the enzymes necessary for its formation are present in non-adrenergic, non-cholinergic nerves. Tritiated-adenosine is taken up by the nerves, transformed into, and stored as ATP (but not ADP) in such a way

that it is available for release during nerve stimulation.

2) ATP and its breakdown products (AMP, adenosine and inosine) are released into the perfusate during stimulation of non-cholinergic, non-adrenergic inhibitory nerves. Tritium labelled compound is released during stimulation of these nerves in tissues previously exposed to ^{3}H-adenosine.

3) The response of smooth muscle to ATP closely mimics the response to nerve stimulation. Both are characterised by rapid onset of action, and this effect is transient, being maintained for no more than 20 to 30 seconds; both produce hyperpolarisation of the smooth muscle membrane. ATP and ADP are the most active adenyl compounds, AMP and adenosine are about 100 times less active, while inosine and adenine are pharmacologically inactive.

5) When tachyphylaxis is induced by repeated administration of ATP, the inhibitory responses to non-adrenergic nerve stimulations are abolished while the inhibitory responses to periarterial adrenergic nerve stimulations persist. Quinidine blocks and dipyridamole potentiates the responses to both non-adrenergic nerve stimulation and ATP".

Despite this strong evidence in favour of ATP as the transmitter not all workers have supported this view. Using tritium labelled adenine nucleotide and noradrenaline (Kuchii, Miyahara and Shibata, 1973a) it was found that electrical field stimulation of guinea-pig taenia caecum at high intensity (60v) caused the release of (^{3}H) adenine nucleotide. However, the inhibitory action of electrical stimulation was proportional to (^{3}H) noradrenaline release and not

associated with the release of (^{3}H) nucleotide Furthermore, these workers also showed that cold storage for more than 8 days, cooling to 19 degrees centrigade, or Ttx treatment abolished the inhibitory responses to electrical stimulation and to nicotine. After these treatments, nicotine and electrical stimulation elicited only contractions; the release of labelled NA was inhibited but that of labelled adenosine nucleotide was not. In another study (Kuchii, Miyahara and Shibata, 1973b) it was shown that cold storage progressively reduced NA uptake by the taenia caecum of the guinea-pig, but not that of (^{3}H) adenosine. Using thin layer chromatography these workers estimated that 68% of the radioactive adenosine was converted to ATP, while 18% remained (^{3}H) adenosine in fresh preparation, and this was unaffected by 8 days cold storage. As it had been previously proved that cold storage progressively caused degeneration of nerves in the taenia (Hattori, Kurahashi, Mori and Shibata, 1972) they concluded that as cold storage did not affect uptake or distribution of metabolites of tritiated adenosine, these processes must be occuring in non-neural tissue. But it must be pointed out that Hattori et al (1972) had only investigated degeneration of sympathetic nerves and Kuchii et al (1973b) have assumed that intramural inhibitory nerves follow a similar degeneration pattern which may well not be the case, indeed as it has been proved that the intramural inhibitory nerves of the taenia have ganglionic connections (Burnstock et al 1966) these nerves will have their cell bodies intact and still connected to the neural processes, therefore, will be more resistant to degenerative processes than the cut sympathetic fibres.

Ohga and Taneike (1977) considered ATP or a related compound

was unlikely to be the inhibitory transmitter in porcine stomach strips because ATP had only excitatory effects on this tissue. Furthermore, after desensitisation of the strips to ATP, inhibitory responses could be evoked by electrical stimulation without any modifications. Weston (1973) made the same observation regarding ATP desensitisation in the rabbit duodenum.

Another observation that does not support the 'purinergic nerve' hypothesis was made by Spedding, Sweetman, and Weetman (1974). They found that the relaxation of the taenia from the caecum of the guinea-pig to ATP was blocked by 2-2'-pyridylisatogen tosylate (PIT) but the response of this preparation to field stimulation, in the presence of hyoscine, was not blocked.

Fasth, Hultén, Jahnberg and Martinson (1973) have suggested that the transmitter may be a peptide on the basis that the response to injected bradykinin (Bk) in the cat <u>in vivo</u> closely resembles the response to gastric relaxation elicited by vagal stimulation. Gillespie and McKnight (1978) gave a similar discussion regarding a role for peptides as the inhibitory neurotransmitter in the annococcygeus muscle of rats, rabbits and cats.

The other candidate suggested to be the non-adrenergic inhibitory neurotransmitter is Vasoactive Intestinal Polypeptide (VIP), (Fahrenkrug 1979). Bloom and Polak (1978) and Humphrey and Fisher (1973) suggest that VIP is the neurotransmitter based on the fact that it is released into the venous effluent when physiological mechanisms known to be mediated via non-cholinergic non-adrenergic

nerve fibres are evoked namely (1) the gastric relaxation mediated by high threshold vagal fibres; (2) the intestinal vasodilatation elicited by mechanical stimulation of the intestinal mucosa (3) the colonic vasodilatation mediated via the pelvic nerves and (4) the effects of local intra-arterial infusion of VIP mimicked the studied mechanism in the respective organs. Cocks and Burnstock (1979) found that the response to VIP does not resemble the responses to non-cholinergic non-adrenergic nerve stimulation in the guinea-pig taenia caecum, in that the response to VIP was very slow to develop, taking about 4 minutes to reach maximum effect, whereas, that to nerve stimulation was immediate. ATP in contrast produced a rapid response.

Fahrenkrug, Galbo, Holst and Schaffalitzky de Muckadel (1978) showed that VIP was released in the presence of atropine but release was blocked by hexamethonium. They also showed that the release was significantly reduced by stimulation of the splanchnic nerves and this process was abolished by the alpha-adrenoceptor blocking agent phenoxybenzamine. It seems unlikely that sympathetic nerves which exert inhibitory action on the tissue, would inhibit the release of transmitter from other inhibitory nerves. As sympathetic nerves are now thought to exert their control by reducing Ach release from parasympathetic fibres the possibility is raised that VIP is released from cholinergic nerves along with Ach. If this were the case VIP release may, therefore, be reduced by sympathetic nerve stimulation. Injected Ach can release VIP but this effect is blocked by atropine

(Fahrenkrug et al, 1978), therefore, the VIP detected was not due to release from other nerve fibres caused by liberated Ach, and therefore, liberation from parasympathetic nerves along with Ach seems the most likely explanation; the slow inhibition caused by VIP (Cocks and Burnstock, 1979) would act as a self limiting component to the nerve action.

Burnstock (1978) discusses the possibility of (sympathetic) nerves releasing more than one transmitter and it may well be that other nerves have a similar process.

METHODS.

Whole stomach preparation.

Whole stomachs with vagi attached were removed from albino guinea-pigs of either sex (wt range 200 - 500g), from male wistar rats (wt range 100 - 200g), or from mature albino mice also of either sex. An incision was made in the fundus and a glass cannula of 0.5 cm internal diameter was inserted and tied in. Any stomach contents were carefully removed by washing with physiological saline solution.

The pyloric sphincter and oesophagus were then tied off. The preparation was then lowered into a 100 ml organ bath containing physiological saline in the case of guinea-pigs and rats, or into a 50 ml organ bath containing physiological saline in the case of mice. The stomach was then filled with physiological saline from a small (200 ml) reservoir connected to the glass cannula with rubber tubing. The resevoir was raised until the solution/air interface in the resevoir was 2.0 cm above the point of entry of the cannula into the stomach. The resevoir was then sealed from the atmosphere and the airspace connected to a low pressure air transducer (Devices UPI). Recordings of intraluminal pressure changes were made with a Devices pen recorder. Pressure changes in this thesis are given in units of kilo Pascals (kPa). Apart from the recording apparatus this method is similar to that described by Paton and Vane (1963).

The vagi were stimulated by means of bipolar platinum electrodes with stimulation parameters of: 20 - 40 v; 0.2 - 1.0 ms; 1 - 800 Hz.

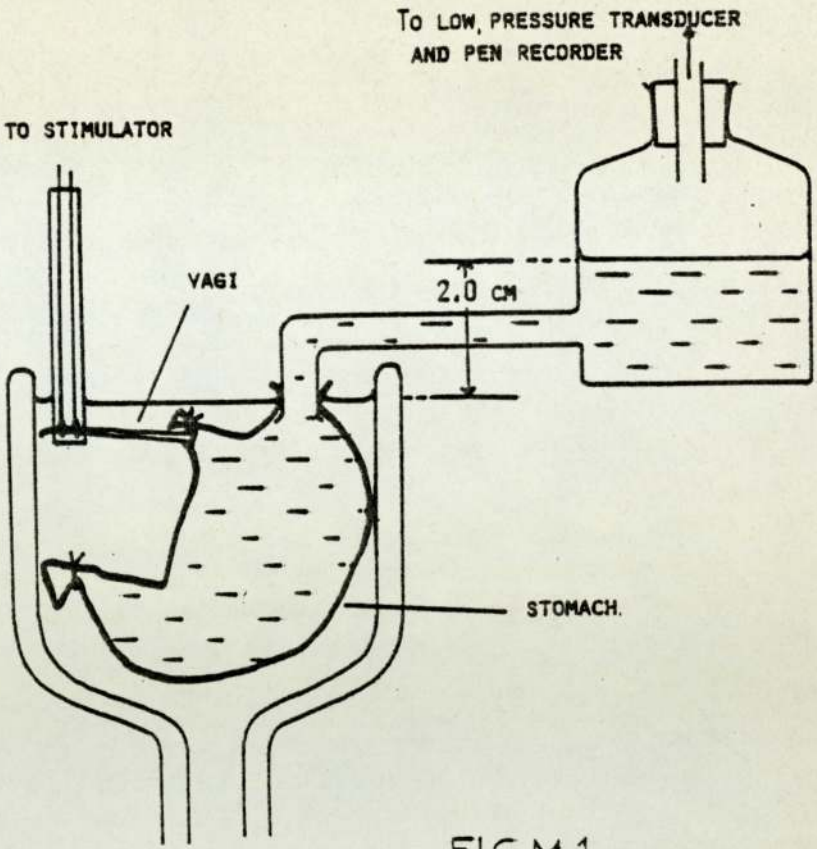


FIG M.1

FIG M.1. The apparatus for the whole isolated stomach preparations.

In some experiments the stomach was stimulated by field stimulation, or stimulated transmurally using platinum electrodes with stimulation parameters of 100 v; 1 ms; 1 - 800 H_3. In other experiments the blood vessels supplying the stomach were also dissected out and stimulated by bipolar platinum electrodes with stimulation parameters of 20 - 40 v; 0.5 - 1 ms; 20 - 40 H_3. This is effectively sympathetic stimulation as the sympathetic fibres run with the blood vessels to the stomach. A diagram of the apparatus is shown in FIG M1.

Longitudinal muscle strip preparation.

A strip of tissue, about 1 cm long was removed from the lesser curvature of the guinea-pig stomach with vagi attached. This strip was then pinned out, mucosal side uppermost onto a cork disc immersed in physiological saline. The mucosal and circular muscle were then removed by scraping carefully with a scapel blade. The preparation was then lowered into a 50 ml organ bath containing physiological saline and connected to a Devices isotonic recorder. Changes in length of the tissue were recorded on a Devices pen recorder. The vagi were stimulated using bipolar platinum electrodes with stimulation parameters of: 20 - 40 v; 0.2 - 1 ms; 20 - 40 H_3.

Taenia Caecum preparation with flap of caecal wall attached.

This preparation was originally described by Burnstock et al (1966). The taenia were removed from the caecum with a flap of the circular muscle caecal wall still attached, and the tissue was carefully washed with physiological

saline. The tissue was then lowered into a 50 ml organ bath and attached to a Devices isotonic transducer. Changes in length were recorded on a Devices pen recorder. The flap of caecal wall was drawn through bipolar platinum ring electrodes and the caecal wall was stimulated using stimulation parameters of 20 - 40 H_3; 1 ms; 80 - 100 v.

Physiological Saline Solutions.

One of three physiological salines were used, either McEwen's (1956) solution, or one of two Krebs' solutions, one solution having twice the concentration of calcium present than the other. The Krebs' solutions had the following compositions: 118.4 mM Na Cl, 3.7 mM KCl, 2.6 or 5.2 mM Ca Cl_2, 1.2 mM Mg SO_4, 2.2 mM KH_2 PO_4, 24.9 mM Na HCO_3 and 10.0 mM glucose. All three solutions were gassed both before and during use by 5% CO_2 in O_2 and the fluid in the organ bath was maintained at 37 degrees centigrade.

Experiments were performed in McEwen's (1956) solution except for some experiments in Chapter 6, when Krebs' solution with either 2.6 or 5.2 mM Ca Cl_2 was used, and this is indicated in the relevant text.

Drugs used.

All drugs were added directly to the bath.

Drugs used were: Adenosine 5-triphosphate, Grade 11 disodium salt (Sigma); Adrenaline hydrogen tartrate (BDH); Bradykinin triacetate (Sigma); Carbachol chloride (BDH); Chlorimipramine hydrochloride (Geigy); Desipramine hydrochloride (Geigy); Guanethidine sulphate (Ciba); Hemicholinium-3 (Aldrich); Hexamethonium Bromide (Sigma); 5-Hydroxytryptamine (Sigma); Indomethacin (Sigma);

McN-A-343 (McNeil); Papaverine (BDH); Pempidine bitartrate (May & Baker); Phentolamine mesylate (Ciba); Physostigmine Sulphate (BDH); Propranolol hydrochloride (I.C.I.); Prostaglandin E_2 (Upjohn); Prostaglandin $F_{2\alpha}$ (Upjohn); Sodium nitroprusside (BDH); Tetrodotoxin (Sigma).

Hyoscine hydrobromide (BDH).

For Krebs' solution see Pharmacological experiments on isolated preparations.

The staff of the Department of Pharmacology, University of Edinburgh.

Churchill Livingstone (1970) London.

CHAPTER 1.

Non-cholinergic non- adrenergic relaxation of the isolated whole guinea-pig stomach in the presence of hyoscine.

INTRODUCTION.

Non-cholinergic, non-adrenergic inhibitory fibres innervating the stomach have been shown to occur in the vagi of guinea-pigs, (Campbell, 1966; Beani et al, 1971) in rats, (Heazell, 1974;1975) and in cats, (Martinson, 1965b). The non-adrenergic nature of these responses is demonstrated by their resistance to adrenergic neurone blocking drugs (Campbell, 1966; Beani et al, 1971; Heazell 1974;1975) and resistance to alpha-and beta-adrenoceptor blockers (Martinson, 1965b; Beani et al, 1971). Beani et al (1971) showed that the drug of choice for producing the best block of periarterial (sympathetic) stimulation was guanethidine (16.9 μM) and it is, therefore, the drug chosen in this part of the study.

The response to stimulation of these non-cholinergic, non-adrenergic inhibitory nerves was abolished by ganglion blocking drugs (Paton & Vane, 1963). Careful inspection of FIG 12 trace 6 given in the paper by Paton and Vane (1963) which shows the response to vagal stimulation in the presence of hyoscine and hexamethonium does reveal a residual relaxation.

Later workers (Bülbring and Gershon, 1967; Beani et al, 1971) claimed only a reduction of the non-cholinergic, non-adrenergic relaxation by ganglion blocking drugs. This section of the work is designed to confirm these findings in the isolated whole guinea-pig stomach.

RESULTS.

Effect of hyoscine on responses to vagal stimulation.

FIG 1.1 shows the effect on supramaximal vagal stimulation of increasing concentrations of hyoscine added to the organ bath in a cumulative manner. It can be seen that as the concentration of hyoscine is increased the response which was initially a contraction is slowly changed into a relaxation. This is shown quantitatively in FIG 1.2 which is a concentration/effect curve for the action of hyoscine on the response of the stomach to supramaximal vagal stimulation. The response measured was the response at cessation of stimulation, contractions being designated positive and relaxations being designated negative. Values for both contractions and relaxations were expressed as a percentage of the maximum relaxation. This method was chosen because the initial contractions varied greatly in size, as is indicated by the large standard error for the first point in FIG 1.2. The maximum relaxations were less variable. Maximum relaxation occurred in the presence of 0.23μM hyoscine, the size of the response at this concentration being 0.83$\pm$0.07 kPa (mean$\pm$S.E. of the mean, n=20).

Effect of guanethidine on responses to vagal stimulation in the presence of hyoscine.

In 7 out of 7 experiments hyoscine resistant vagal relaxations were unaffected by guanethidine (16.9μM) when responses to periarterial (sympathetic) stimulation were greatly reduced by 8μM guanethidine (FIG I.3).

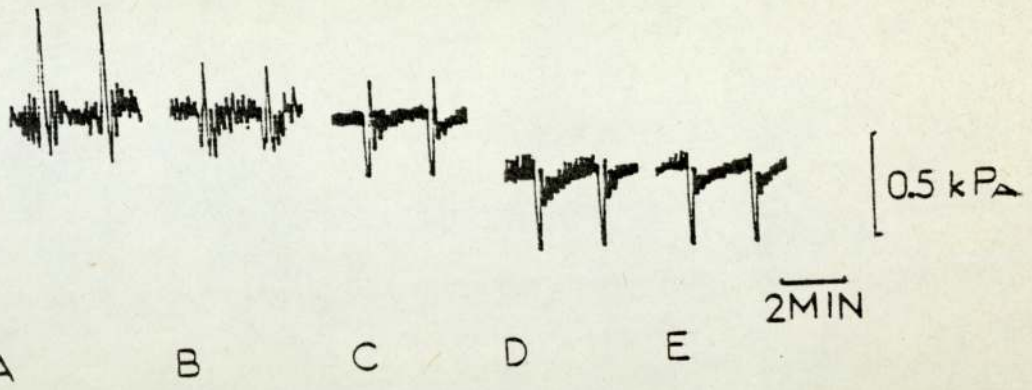


FIG 1.1

FIG 1.1. The effect of hyoscine on responses to supramaximal vagal stimulation of the isolated whole guinea-pig stomach.

A = initial contractions

B = response in the presence of 0.023μM hyoscine

C = response in the presence of 0.046μM hyoscine

D = response in the presence of 0.23μM hyoscine

E = response in the presence of 0.46μM hyoscine

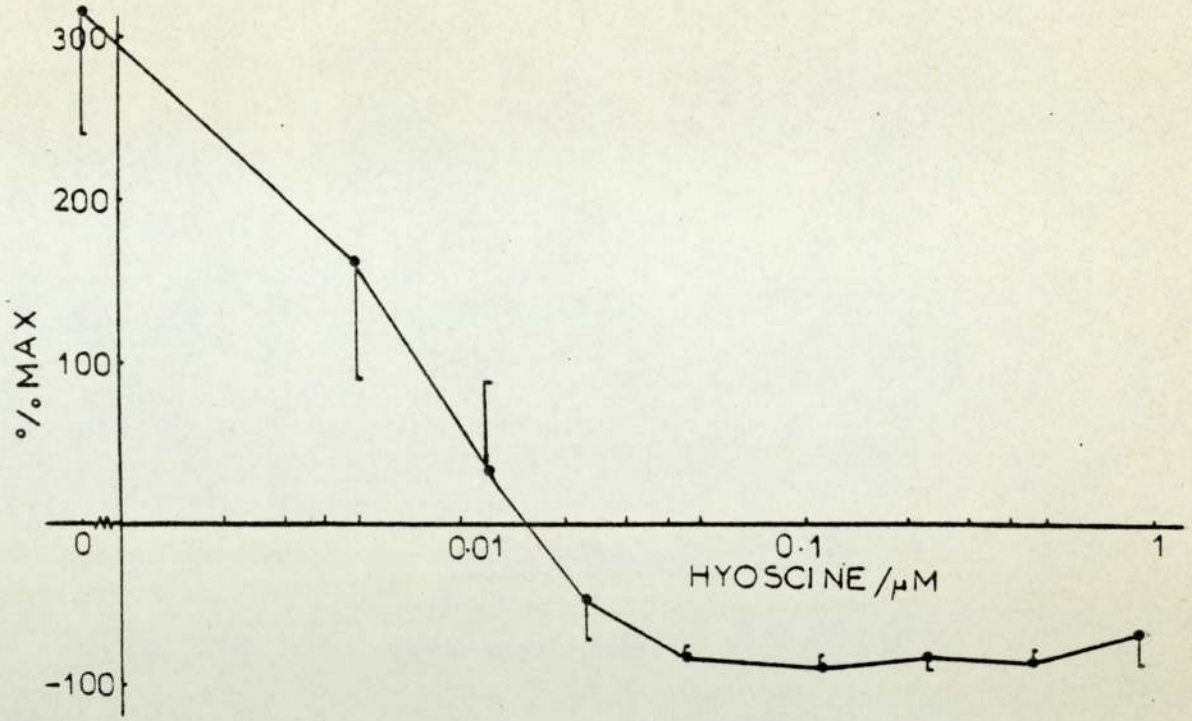


FIG 1.2

FIG I.2. Concentration/effect curve for hyoscine on responses to supramaximal vagal stimulation of the isolated whole guinea-pig stomach.

Positive values indicate contractions negative values indicate relaxations.

The vertical bars indicate standard errors of the means for each point.

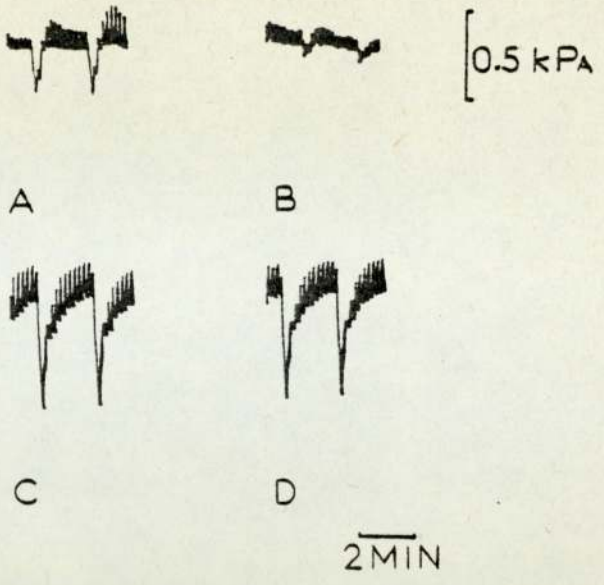


FIG 1.3

FIG 1.3. The effect of guanethidine on relaxations to supramaximal periarterial and vagal stimulation of the whole isolated guinea-pig stomach.

A Initial relaxations to supramaximal periarterial stimulation.

B Same preparation as A. Relaxation to supramaximal periarterial stimulation in the presence of 8.0 μM guanethidine.

C Different preparation to A and B. Relaxation to supramaximal vagal stimulation in the presence of 0.46 μM hyoscine.

D Same preparation as C. Relaxation to supramaximal vagal stimulation in the presence of 0.46 μM hyoscine and 16.9 μM guanethidine.

Effect of ganglion blocking drugs after hyoscine.

The vagal inhibitory responses in the presence of hyoscine were reduced by ganglion blocking drugs as for example FIG 1.4. Hexamethonium was maximally effective at 55.2 μM and pempidine at 15 μM, concentrations. The relaxations could not be completely abolished by ganglion blocking drugs plus hyoscine.

The effect of drugs on tone.

The fall in baseline of the trace in FIG 1.1 is indicative of the fall in tone of the preparation caused by the addition of hyoscine. This fall in tone was not significantly different ($P > 0.05$) in the presence of Ttx to that produced without Ttx in the bathing fluid. The effect of two other drugs on tone is shown in FIG 1.5. 13.3 μM Papaverine greatly lowered the tone, which was followed by 0.062 and 0.62 μM physostigmine which greatly increased the spontaneous activity, with little effect on tone, but any slight changes would be masked by the spontaneous activity produced.

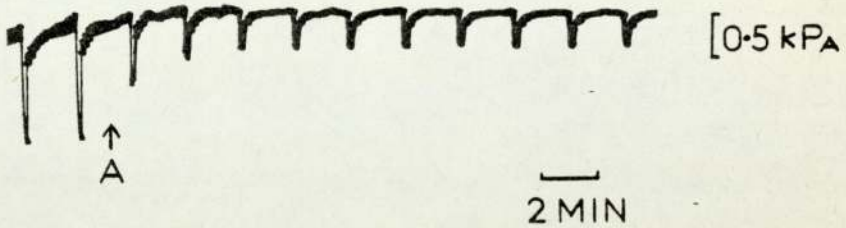


FIG 1.4

FIG 1.4. The effect of ganglion blockade on relaxations to supramaximal vagal stimulation, of the whole isolated guinea-pig stomach in the presence of hyoscine.

0.46μM hyoscine present throughout

15.0μM pempidine added at A.

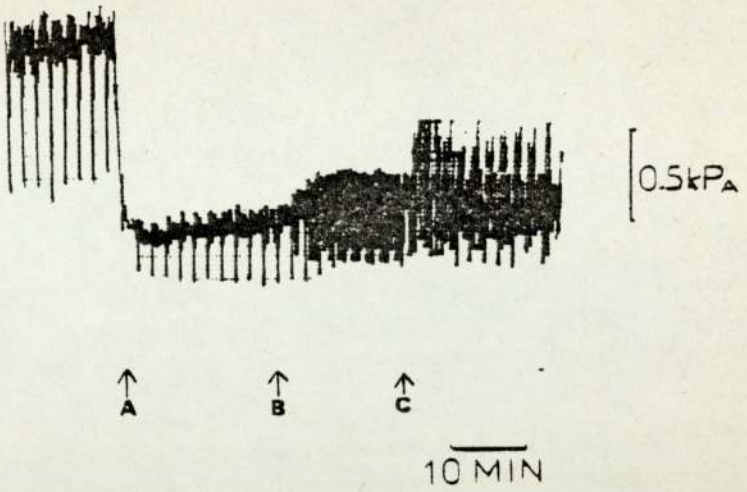


FIG 1.5

FIG 1.5. The effect of papaverine and physostigmine on the tone of the isolated whole guinea-pig stomach

15.0 μM pempidine present throughout.

relaxations shown are to supramaximal vagal stimulation

13.3μM papaverine added at A, 0.062μM physostigmine added at B, increased to 0.62μM physostigmine at C.

DISCUSSION.

A relaxation by the isolated guinea-pig stomach in response to vagal stimulation has been demonstrated. That the fibres mediating this response are non-adrenergic is confirmed by the resistance of the response to guanethidine (16.9μM)at a concentration sufficient to abolish responses to periarterial (sympathetic) stimulation. This is in agreement with other workers (Campbell, 1966; Beani et al, 1971). As has been previously shown (Bülbring and Gershon, 1967; Beani et al, 1971) the relaxations were reduced but not abolished by ganglion blocking drugs. The fact that responses were not eliminated by a combination of hyoscine and ganglion blocking drugs suggests that transmission is possible by a way which does not require a cholinergic step. Bülbring and Gershon (1967) suggested that ganglia utilising 5-HT transmission occured in the pathway to the stomach of guinea-pigs and mice on the basis that the vagal response was reduced in the presence of desensitisation to 5-HT during the early phase (Paton and Perry, 1953) of block produced by nicotine. Both responses to vagal stimulation and 5-HT were abolished but both responses recovered in the second (competitive) phase of nicotine block. Stimulation of the mouse stomach caused release of 5-HT, and this effect was abolished by Ttx. The response to vagal stimulation resembled that of 5-HT both in the presence and absence of hyoscine, and the vagal inhibitory response was completely abolished when desensitisation to 5-HT was combined with competitive block of nicotinic Ach receptors.

In view of this finding Beani et al (1971) tried to abolish the vagal relaxation of atropine pretreated strips of

guinea-pig stomach by submitting them to 5-HT desensitisation and hexamethonium. Although vagal responses were reduced this could be completely accounted for by a fall in tone of the preparation caused by 5-HT. In other experiments in which the order of drug treatments were reversed they observed desensitisation to 5-HT left the hexamethonium - resistant vagal inhibition unchanged. Further they were unable to reduce vagal relaxations with methysergide which antagonised the response to 5-HT. The role of 5-HT in non-adrenergic relaxation of the guinea-pig stomach is re-investigated in chapter 3.

Hyoscine caused a fall in tone of the preparation probably being due to an action directly on the smooth muscle of the stomach because after abolition of nervously mediated responses by Ttx hyoscine still caused a fall in tone that did not significantly differ from that seen in the untreated preparation.

The reduction in tone causes great difficulty in interpretation of results as a smaller sized response, if accompanied by a fall in tone as for example seen with papaverine in FIG 1.5 may be entirely due to a reduction in the capacity of the preparation to relax further. McSwiney and Wadge (1928) showed that relaxations due to vagal stimulation were greatest when tone was highest showing that tone does have an effect on the apparent size of the response observed. In order to make a correction for changes in tone, responses could be measured relative to the original baseline. FIG 1.5 illustrates a drawback of this method of measurement. Measuring relative to the original baseline might lead one to conclude in the case of FIG 1.4 that papaverine was enhancing the relaxation

due to vagal stimulation and that physostigmine may have slightly reduced the responses, a conclusion that seems extremely unlikely to be a true reflection of the actual pharmacological events. Therefore, whenever possible procedures that do not involve large tone changes have been adopted in the rest of this work.

SUMMARY.

1) Non-adrenergic relaxations resistant to hyoscine in response to vagal stimulation have been demonstrated in the isolated guinea-pig stomach.

2) The responses in the presence of hyoscine were reduced but not abolished by ganglion blocking drugs.

3) Hyoscine reduced the tone of the preparation by a direct relaxant action on the stomach smooth muscle cells.

4) The relevance of tone changes in the preparation to the measurements of the results is discussed.

CHAPTER 2.

Non-cholinergic, non-adrenergic, gastric inhibition in the presence of ganglion blocking drugs alone.

INTRODUCTION.

The reduction by ganglion blocking drugs of the responses to non-cholinergic, non-adrenergic nerve stimulation in the presence of hyoscine or atropine is well known (see Chapter 1). However, few workers have looked at the effect of stimulating the vagi in the presence of ganglion blocking drugs alone. Paton and Vane (1963) showed that when hexamethonium alone was added to the bathing fluid, responses to vagal stimulation were blocked. Stimulus parameters used by Paton and Vane were large pulse widths (1 ms) and low frequency (1-5 Hz). This section of the work re-examines the effect of vagal stimulation in the presence of ganglion blocking drugs alone with a narrower pulse width (200μs) and a larger range of frequencies (1-200 Hz).

If the fibres could transfer an impulse when nicotinic receptors of the ganglia are blocked, there must be an alternative pathway, either utilising a different transmitter or receptor, or involving fibres which run straight through from the vagus to the tissue without a ganglionic step.

Transmission at sympathetic ganglia can also take place via muscarinic ACh receptors (Jones, 1963). The possibility that the vagal inhibitory pathway also contains ganglionic muscarinic receptors was, therefore, investigated.

RESULTS.

Effect of ganglion blocking drugs alone in response to vagal stimulation of the whole isolated guinea-pig stomach.

When ganglion blocking drugs were added in increasing concentration to previously untreated preparations, responses to supramaximal stimulation of the vagus nerves were converted from contractions to relaxations in a manner similar to that seen with hyoscine alone as shown in CHAPTER 1. This is illustrated in FIG 2.1. Concentration/effect curves are shown in FIG 2.2 for the action of hexamethonium on responses to supramaximal vagal stimulation and FIG 2.3 for the action of pempidine on responses to supramaximal vagal stimulation. As was the case in FIG 1.2 for the action of hyoscine on response to supramaximal vagal stimulation, responses were expressed as a percentage of the maximum relaxation produced, because of the large variation in the amplitude of contractions in untreated preparations.

Maximum relaxations were achieved in the presence of 20μM hexamethonium or 2μM pempidine. It is possible to exceed the maximally effective concentrations mentioned above with very little reduction in the size of the response; e.g. the response in the presence of 441.9μM hexamethonium was not significantly smaller ($P>0.05$) than with 20μM. Similarly in the presence of 75.0μM pempidine the response was not significantly smaller ($P>0.01$) than with 2μM.

In the presence of hyoscine, maximum sized relaxations were 0.83 ± 0.07 kPa (mean $\pm$ s.e. mean; $n=20$) in the presence of hexamethonium, maximum sized relaxations of 0.64 ± 0.10 kPa

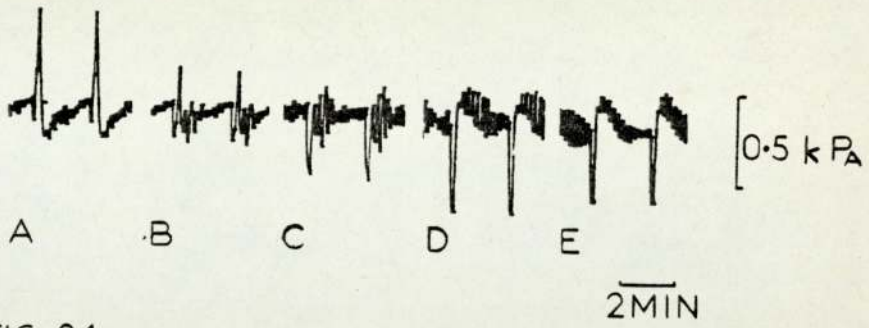


FIG 2.1

FIG 2.1. The effect of hexamethonium on responses to supramaximal vagal stimulation of the isolated whole guinea-pig stomach

A = initial contractions
B = 0.55 μM hexamethonium
C = 2.21 μM hexamethonium
D = 55.2 μM hexamethonium
E = 441.8 μM hexamethonium

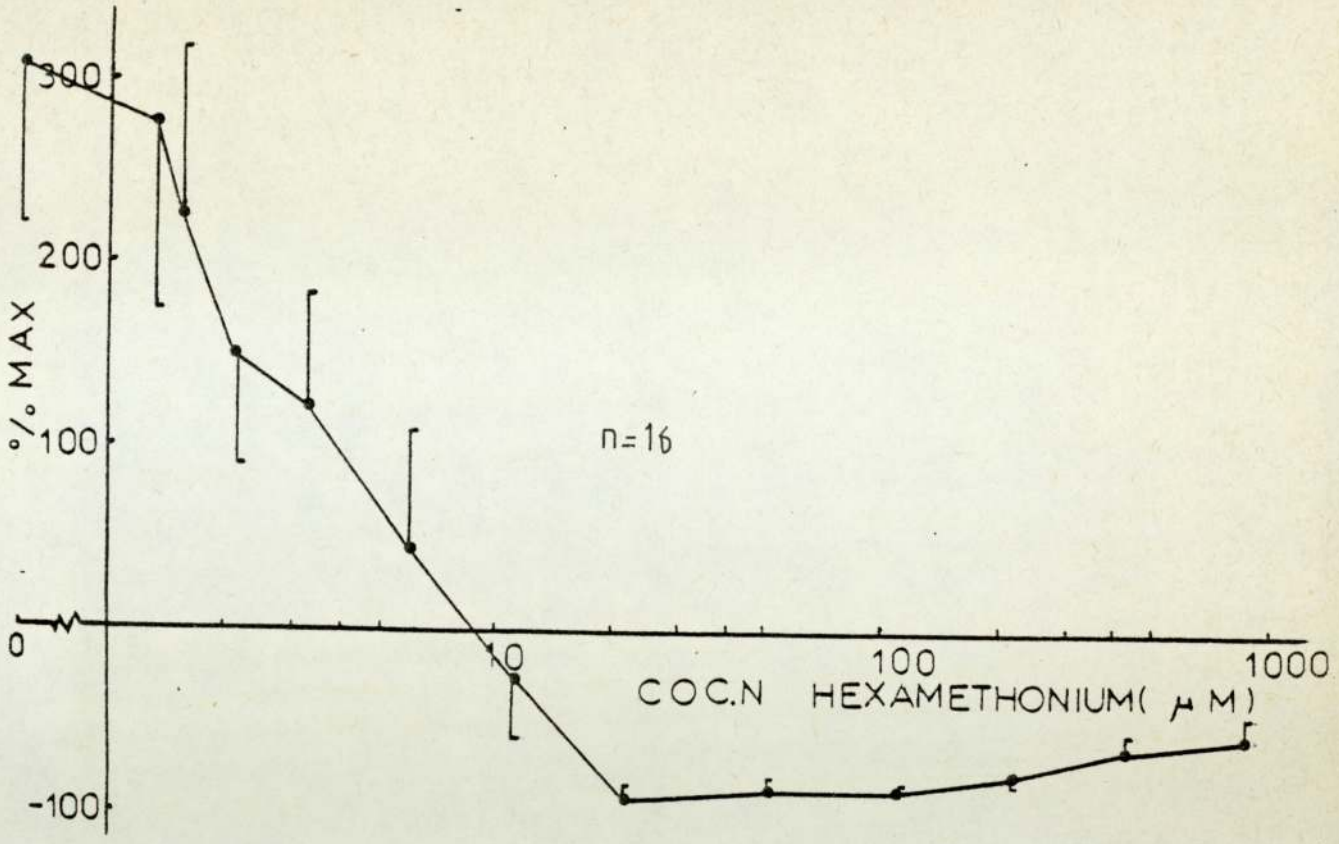


FIG 2.2

FIG 2.2 Concentration/effect curve for the action of hexamethonium on responses to supramaximal vagal stimulation of the isolated whole guinea-pig stomach.

Positive values indicate contractions, negative values relaxations.

The vertical bars indicate standard errors of the means for each point.

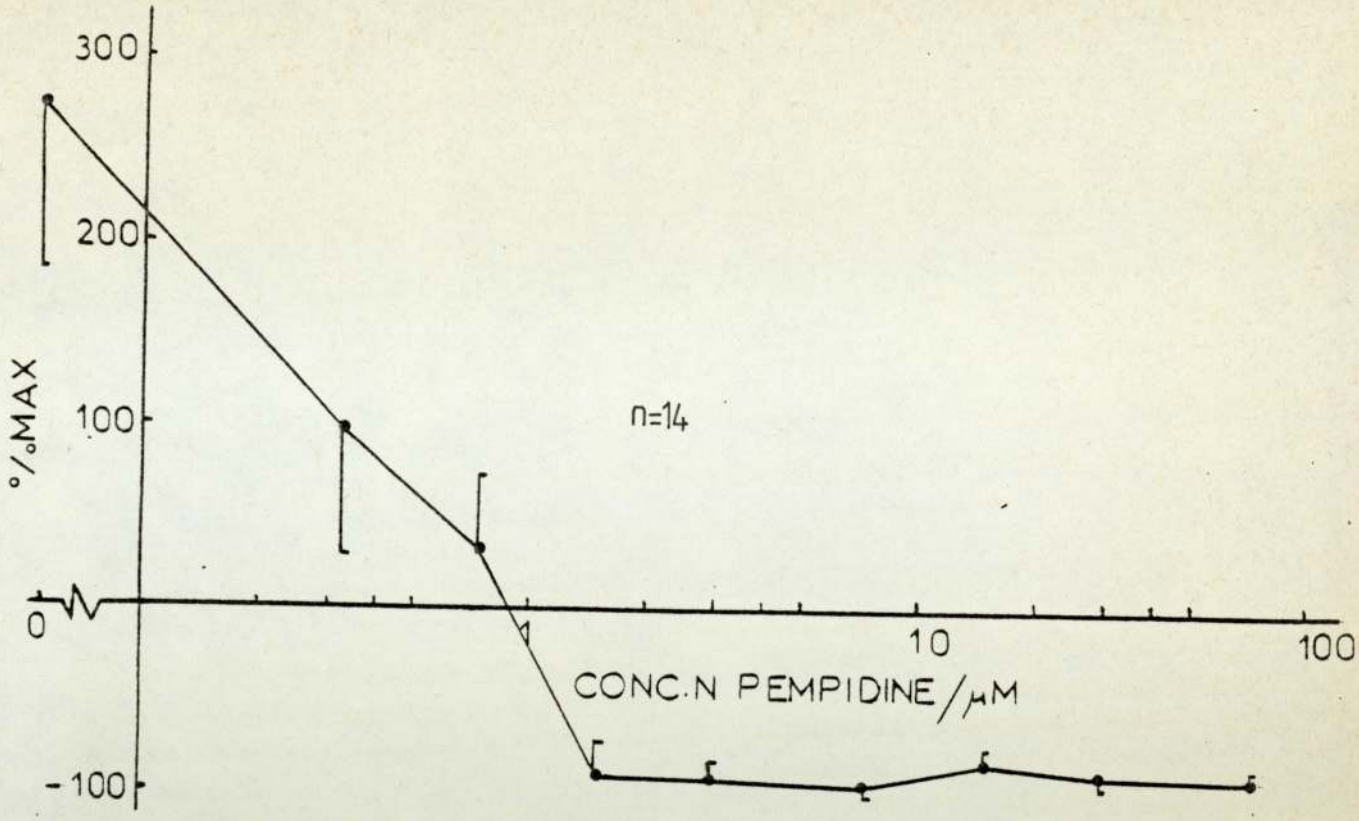


FIG 2.3

FIG 2.3. Concentration/effect curve for the action of pempidine on responses to supramaximal vagal stimulation of the isolated whole guinea-pig stomach.

the vertical bars indicate standard errors of the means for each point

positive values indicate contractions negative values indicate relaxations

(mean ± s.e. mean;n =16) were produced and in the presence of pempidine maximum sized relaxations were 0.61±0.05 kPa (mean ± s.e. mean;n=14). The size of these relaxations in the presence of each antagonist did not significantly differ (P>0.01.)

Effect of frequency of stimulation of the vagus nerves in the presence of pempidine or hyoscine alone.

Two frequency/effect curves showing the effect of varying frequency of stimulation of the vagi with a small pulse width (200μs) at supramaximal voltage, in the presence of hyoscine or pempidine alone are shown in FIG 2.4. When points of the same frequency of stimulation were compared for each drug they did not differ significantly (P>0.01). When these experiments were repeated using 1.0ms pulse width instead of 200μs, relaxations to vagal nerve stimulations were generally not seen with frequencies of stimulation below 5 Hz.

Effect of guanethidine on responses to supramaximal vagal stimulation in the presence of ganglion blocking drugs.

Responses to supramaximal vagal stimulation in the presence of pempidine (15.0μM) or hexamethonium (55.2μM) were unaffected by guanethidine (16.9μM) whereas responses to supramaximal periarterial (sympathetic) stimulation were abolished (FIG 2.5).

The form of responses to vagal stimulation in the presence of ganglion blocking drugs alone compared to those in the presence of hyoscine alone.

Ganglion blocking drugs did not cause a large tone change as had been seen with hyoscine (FIG 2.1 C.f. FIG 1.1). The form of the response also differed from that in the presence of hyoscine alone (FIG 2.6).

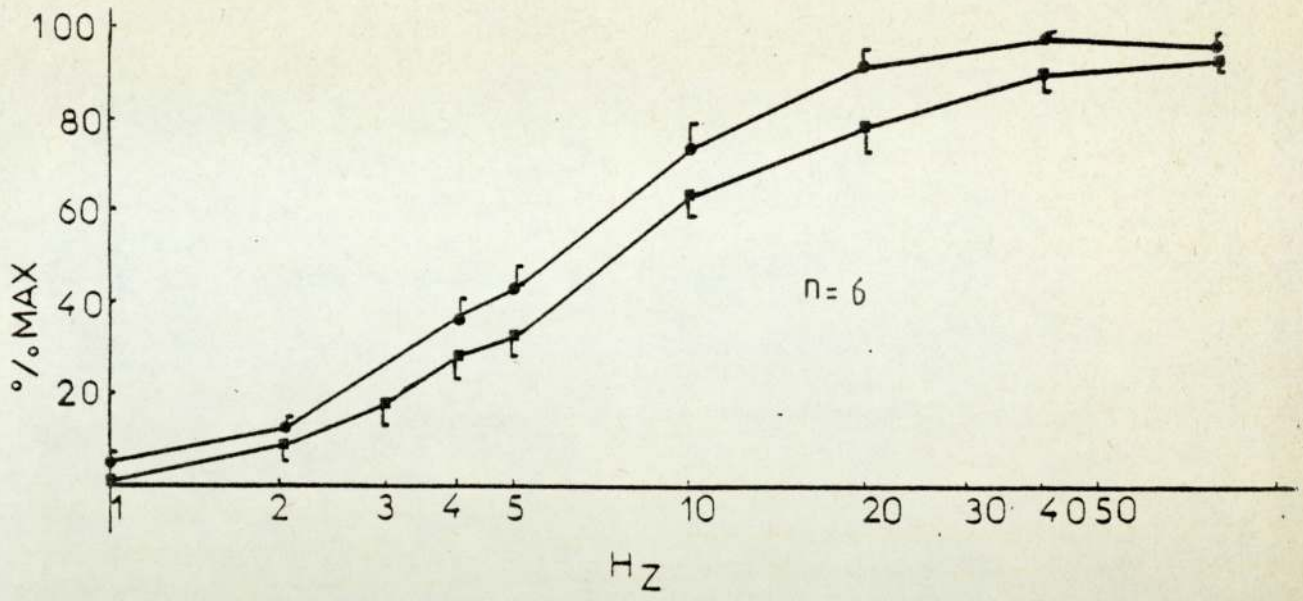


FIG 2.4

FIG 2.4. Frequency/effect curve for relaxations to vagal stimulation in the presence of drugs

- ● in the presence of 0.23μM hyoscine
- ■ in the presence of 15μM pempidine

The vertical bars indicate standard errors of the means for each point

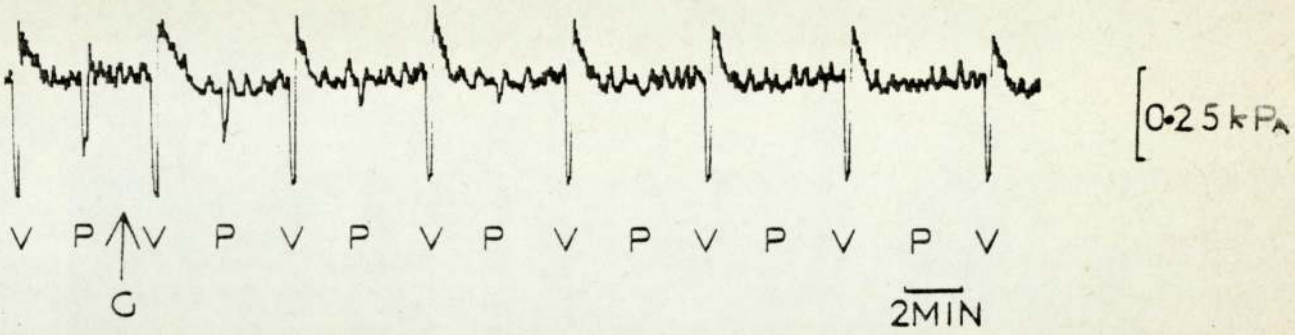


FIG 2.5

FIG 2.5. The effect of guanethidine on relaxations to supramaximal vagal and periarterial stimulation of the isolated whole guinea-pig stomach.

V = Supramaximal vagal stimulation

P = Supramaximal periarterial (sympathetic) stimulation

G = Guanethidine (16.9 μM)

15 μM pempidine present throughout.

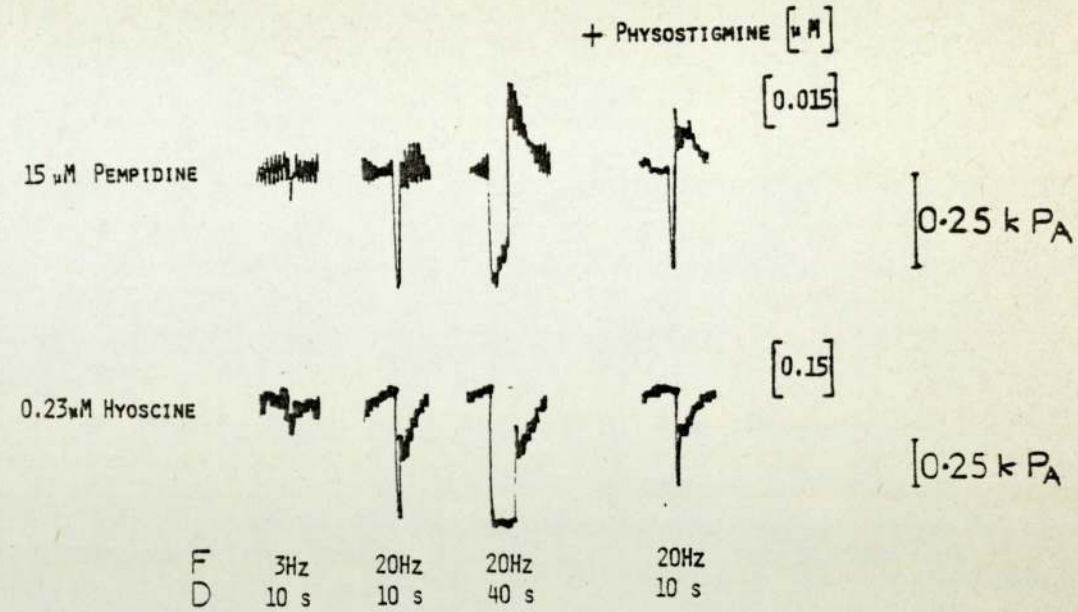


FIG 2.6

FIG 2.6. Comparison of the responses to vagal stimulation in the presence of hyoscine or pempidine

F = Frequency of stimulation

D = Duration of stimulation

In the presence of ganglion blocking drugs, following cessation of stimulation, the tone of the preparation quickly returned to the control (baseline) level. With stimulation frequencies of 30Hz or above, or with periods of stimulation greater than 20s, this rapid return to the baseline was followed by an immediate after contraction (FIG 2.6). The response in the presence of hyoscine differed in that following cessation of stimulation the tone of the preparation showed an initial fast recovery phase, followed by a slow recovery phase to control (baseline)level. Increasing the frequency or duration of stimulation had little or no effect on this 'recovery' phase.

The after contraction was also potentiated by low concentrations (0.015μM) of physostigmine. Even with 0.15μM physostigmine present, no after contraction was seen in the presence of hyoscine alone (FIG 2.6).

<u>Effect of hyoscine after ganglion blocking drugs on responses to vagal stimulation.</u>

It was seen in CHAPTER 1 that concentrations of hyoscine required for maximum sized vagal relaxations (0.23μM) caused a profound lowering of tone in the preparation.

When these concentrations were added to preparations already containing ganglion blocking drugs in the bathing medium it appeared that the response was reduced in size but it was impossible to be certain because of the fall in tone of the preparation. Lower concentrations (0.023μM) of hyoscine, however, did not cause this fall in tone. But relaxations to supramaximal vagal stimulations were clearly reduced (FIG 2.7). On average 0.023μM hyoscine produced a

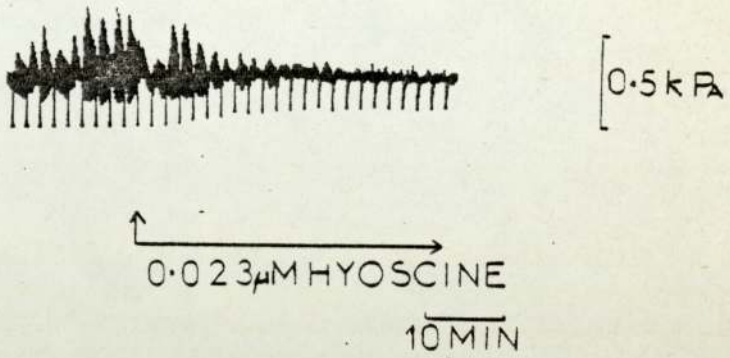


FIG 2.7

FIG 2.7. The effect of low concentrations of hyoscine, after pempidine, on responses to supramaximal vagal stimulation of the isolated whole guinea-pig stomach.

15 μM pempidine present throughout

53.6±5.2% (mean ± s.e. mean;n = 12) reduction of the response. As seen in CHAPTER 1 in the presence of both hyoscine and ganglion blockers at maximum concentrations, a small response to vagal stimulation still remained, despite the decrease in tone of the preparation.
The after contractions were also abolished by this low concentration (0.023μM) of hyoscine.
It was necessary to leave the drug in contact with the tissue for at least 20 minutes as at low concentrations the reduction in response was slow to develop.

Effect of physostigmine after ganglion blocking drugs on responses to vagal stimulation.

When physostigmine (0.015-0.062μM) was added to the organ bath an increase in tone of the preparation resulted. In FIG 2.8 it can be seen that at high rates of stimulation (40 Hz) physostigmine (0.062μM) potentiated the response to vagal stimulation. Actual values for vagal stimulation at 40 Hz were, in the presence of pempidine (15.0μM) alone 0.64±0.06 kPa (mean ± s.e. mean;n = 5) and after the addition of physostigmine (0.062μM) 0.65±0.10 kPa (mean ± s.e. mean;n = 5) when the size of response was corrected to account for tone changes. But when subjected to statistical analysis the responses after physostigmine when corrected for tone changes, were not significantly greater than before ($P > 0.01$).

Latency of the response to vagal stimulation in the presence of hyoscine or pempidine.

After commencement of stimulation in the presence of ganglion blockers alone, the latency before the response was seen was 1.65±0.08s (mean ± s.e. mean;n = 9), and

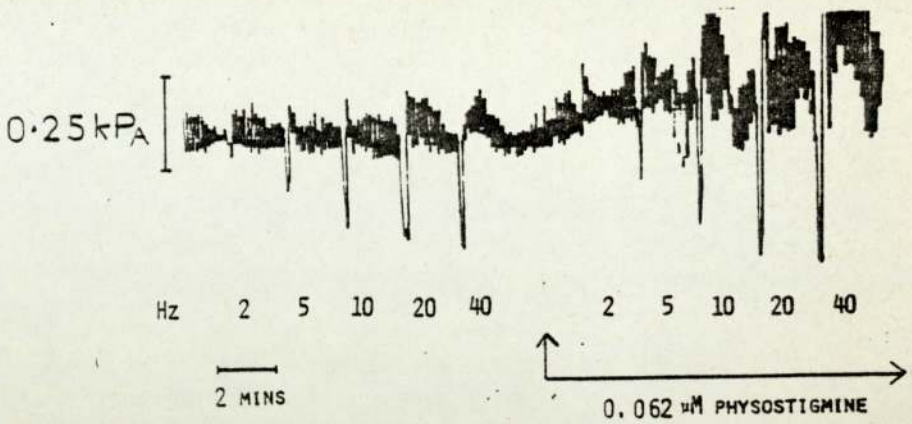


FIG 2.8

FIG 2.8. The effect of physostigimine on relaxations to supramaximal vagal stimulation of the isolated whole guinea-pig stomach in the presence of pempidine.

15 μM Pempidine present throughout.

10s duration of stimulation

1.23±0.11s (mean ± s.e. mean;n = 7) in the presence of hyoscine alone, and these values were significantly different (P<0.01).

The effect of hemicholinium (HC_3) on responses to vagal stimulation.

HC_3 causes a depletion of ACh in the cat superior cervical ganglion after bursts of nerve stimulation over a long period (MacIntosh, Birks and Sastry, 1956).
Over a period of 3 to 4 hours in the presence of HC_3, the response to transmural stimulation of the stomach changed from a contraction to a relaxation.
In the absence of drugs, transmural stimulation of the stomach excites both excitatory and inhibitory nerves. A contraction due to the release of ACh is normally seen, the relaxation being masked. Bursts of stimulation (here 10s every 3 mins. was used) over 3 to 4 hour periods in the presence of HC_3 will lead to depletion of ACh stores within the nerve fibres. The contraction then gives way to a relaxation. When all the ACh which is available for immediate release has been depleted, the relaxation is no longer antagonised by the release of ACh from the motor nerves and, therefore, the addition of drugs which block the motor response such as hyoscine or atropine, should not cause any increase in the size of the relaxation observed during transmural stimulation. This then can be used as a test to determine if ACh depletion has been complete. Furthermore, if one makes the assumption that the release of ACh from preganglionic nerves is affected similarly, then following chronic HC_3 treatment the remaining response to vagal stimulation should be a pure relaxation consisting only of events due to non-adrenergic inhibitory fibres

having no cholinergic ganglionic step. Under these conditions it should be theoretically possible to determine whether or not hyoscine, as used previously, has an inhibitory effect on this remaining response.

If the remaining response to vagal stimulation is still reduced by hyoscine, this would indicate that the reduction in the size of gastric relaxation caused by hyoscine after ganglion blocking drugs was not a specific effect on muscarinic ACh receptors.

Unfortunately in 6 experiments in the presence of 0.17 to 3.4 μM HC_3 even after 12 hours of 2 minute cycles of transmural stimulation at 40 Hz for 10 seconds, (pulse width 1 ms, 100 volts) it was not possible to achieve complete ACh depletion as measured by the action of hyoscine (0.23μM) on transmural stimulation. (The size of the relaxation to transmural stimulation was increased indicating that a residual ACh mediated component was still present: FIG 2.9). This reduction was not due to an effect on tone because during the prolonged period of the experiment the tone of the preparation had slowly fallen, with no additional fall in tone when hyoscine was added.

<u>The action of the muscarinic ganglion stimulant McN-A-343 on the isolated guinea-pig stomach.</u>

McN-A-343 is a compound that has been found to cause stimulation of muscarinic ACh receptors in sympathetic ganglia (Smith, 1966). The effect of this compound on the whole isolated guinea-pig stomach was, therefore, investigated.

This compound (2.0 to 200.0μM) caused only small contractions of the stomach without any evidence of relaxation, similar

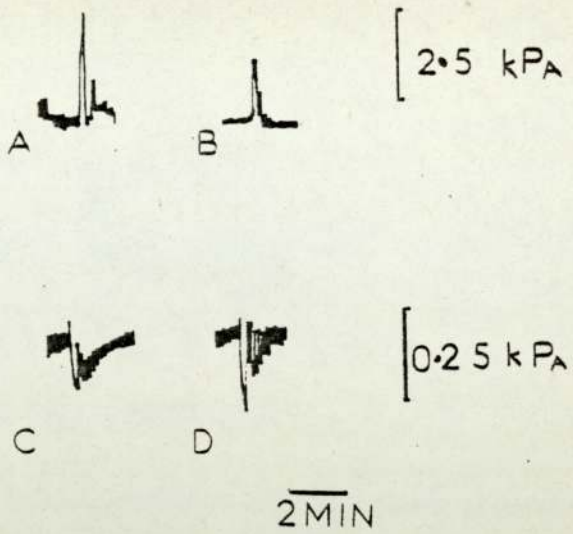


FIG 2.9

FIG 2.9. The effect of hyoscine on relaxations to supramaximal transmural stimulation of the isolated whole guinea-pig stomach after chronic HC_3 treatment.

A. Initial response to supramaximal transmural stimulation.

B. Response to supramaximal transmural stimulation after half an hour exposure to 1.7μM HC_3.

C. Response to supramaximal transmural stimulation after 6 hours exposure to 1.7μM HC_3.

D. Response to supramaximal transmural stimulation after an additional 20 minutes with 0.23μM hyoscine also present in the bathing fluid.

to but smaller than those observed with carbachol (FIG 2.10).

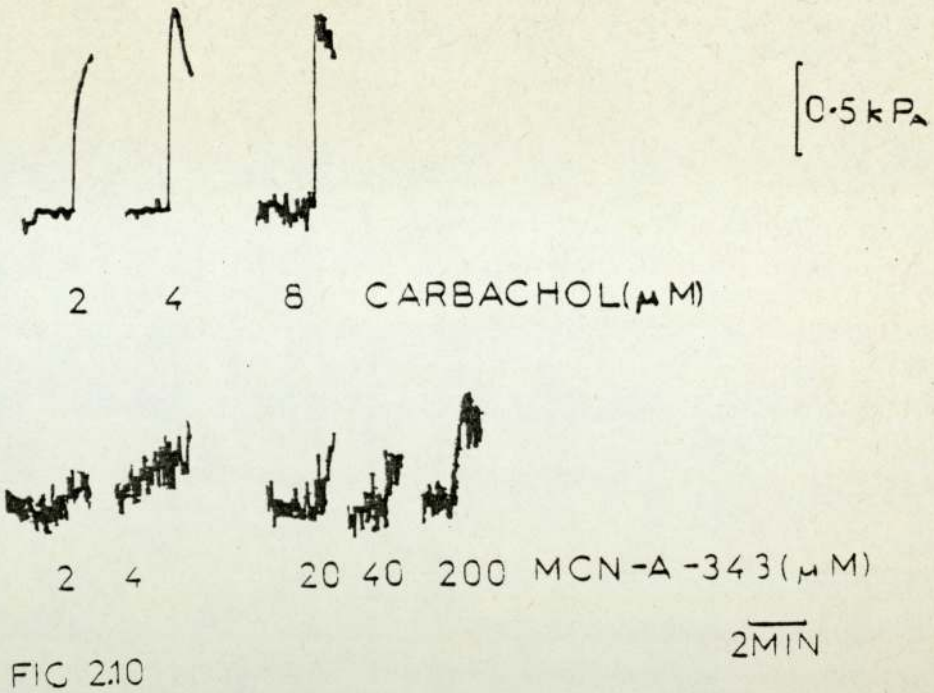


FIG 2.10

FIG 2.10. The effect of carbachol or McN-A-343 on the isolated whole guinea-pig stomach

DISCUSSION.

Relaxations of the guinea-pig stomach to supramaximal vagal stimulation, similar to those seen in the presence of hyoscine alone have been demonstrated in the presence of ganglion blocking drugs alone. Paton and Vane (1963) did not observe this phenomenon. Although the frequency effect curves for vagal stimulation in the presence of either hyoscine or ganglion blocking drugs alone showed no statistical difference, responses at low frequencies (<5 Hz) of stimulation in the presence of ganglion blocking drugs alone were less often observed than when hyoscine was used. As increasing the concentration of ganglion blocking drugs by up to ten fold or more caused no change in the size of the response, it can be argued that the relaxations observed were not due to the drugs selectively blocking the ganglia of the motor pathway before those of the inhibitory pathway.

The fact that these relaxations are not adrenergic in origin is shown by their resistance to guanethidine at a concentration sufficient to abolish responses to periarterial (sympathetic) stimulation.

In this study the relaxation in the presence of ganglion blocking drugs was sensitive to low concentrations of hyoscine. In electrophysiological studies on the rabbit superior cervical ganglion Eccles and Libet (1961) found that ganglionic potentials developed after preganglionic electrical stimulation consisting of three phases; an initial negative potential (N phase) was developed, followed by a small positive potential (P phase) and finally by a late negative (LN) phase where the response only slowly returned

to the baseline. The LN phase was susceptible to block by atropine which suggested the existence of muscarinic as well as nicotinic receptors. Since then a functional muscarinic transmission in sympathetic ganglia has been suggested by other workers; in cats (Jones 1963; Trendelenburg, 1966) and dogs (Flacke and Gillis, 1968; Gillis, Flacke, Garfield and Alper, 1968).

The characteristics of this type of transmission differ from those mediated by nicotinic receptors, apart from its sensitivity to atropine, (Jones, 1963; Flacke and Gillis, 1968; Gillis et al, 1968), the latency of the response during muscarinic transmission is longer than that during nicotinic transmission. In the dog stellate ganglion the latency was 0.3 to 0.5 s longer (Flacke and Gillis, 1968) and this figure agrees well with the figures of 0.2 to 0.4 s latency observed for LN phase in the rabbit superior cervical ganglion (Eccles and Libet, 1961).

The latency for muscarinic transmission observed by Jones (1963) in the cat was 6 s, whereas nicotinic transmission gave an almost immediate response. As with muscarinic transmission in sympathetic ganglia (Jones, 1963; Flacke and Gillis, 1968) the latency of the response to vagal stimulation in the presence of ganglion blocking drugs alone was found to be longer (1.65 ± 0.08 s C.f. 1.23 ± 0.11 s) than with hyoscine alone. This is in agreement with the latencies of 0.3 - 0.5 s longer for muscarinic transmission given by Flacke and Gillis (1968).

Another difference is that in the superior cervical ganglion of the cat it was found that the late atropine sensitive phase was potentiated by anti-cholinesterases, whereas the primary d-tubocurarine (d-TC) sensitive response was not

(Takeshige and Volle, 1962).

It was not possible to show, as Takeshige and Volle (1962) had, potentiation of the response by physostigmine because of the effect of this drug on spontaneous activity and tone, but in view of the slight potentiation of the response seen in FIG 2.8 a potentiation of the response may well have been present which could not be demonstrated conclusively with the organ bath technique.

Takeshige and Volle (1962) found that the late atropine sensitive response in sympathetic ganglia was 2 to 5 fold more sensitive to ACh than the early (d-TC sensitive) phase. This observation suggests that muscarinic ganglionic transmission is extremely sensitive to ACh, and it is likely, therefore, that this process is also extremely sensitive to muscarinic blocking drugs. The response to vagal stimulation in the presence of ganglion blocking drugs alone observed here was sensitive to low concentrations of hyoscine which would be in agreement with Takeshige and Volle (1962) for ganglionic muscarinic transmission.

A finding by Crema, Frigo and Lecchini (1970) that descending inhibition in the guinea-pig and cat colon is selectively inhibited by low concentrations of hyoscine could be a related effect.

No conclusions could be drawn from the experiments using HC_3. It was evident that HC_3 was not causing complete ACh depletion within the gastric nerve pathways as had been observed by MacIntosh et al (1956) in sympathetic ganglia. Compounds which selectively stimulate muscarinic receptors of sympathetic ganglia are available (Levy and Ahlquist 1962; Franko, Ward and Alphin, 1963; Smith, 1966) and one of these compounds, McN-A-343, was used in this

investigation, but in this tissue it was not possible to selectively stimulate the ganglia with McN-A-343, a contraction being observed similar to that of carbachol and probably due to stimulation of the muscarinic receptors on the smooth muscle.

However, in view of the fact that vagal relaxations in the presence of ganglion blocking drugs was reduced by a low concentration of hyoscine, and the latency of the response was longer that that in the presence of hyoscine alone, it seems probable that transmission through the ganglia of the vagal inhibitory nerves can take place by way of muscarinic receptors.

Eccles and Libet (1961) suggested that the larger latency indicates that the muscarinic receptors are further away from the point of ACh release than the nicotinic receptors. This principle could be applied to this pathway and is illustrated diagramatically in FIG 2.11.

It is worth noting at this point that stimulation of the vagus nerve in the absence of drugs caused a contraction, even though fibres causing relaxation must also be stimulated, this, therefore implying that the contractile component of the response is greater that the relaxation, thus masking the presence of a relaxation completely. If the motor component also contained a similar proportion of non-nicotinic pathways the status quo would not be disturbed by the addition of ganglion blocking drugs to the medium, and therefore, a contraction would also be seen in the presence of ganglion blocking agents. However, this is not so; a relaxation is seen. This implies that the motor pathway does not possess this ability to transmit without the use of nicotinic receptors, or if

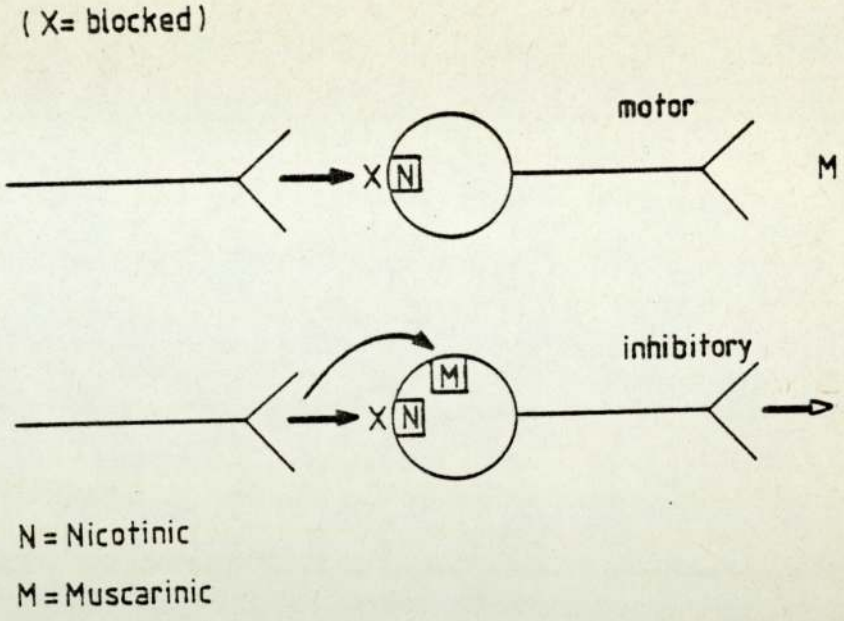


FIG 2.11

FIG 2.11. Schematic diagram of transmission in ganglia of the vagal inhibitory nerves in the presence of ganglion blocking drugs alone.

they do it is relatively unimportant.

The form of the response to supramaximal vagal stimulation in the presence of ganglion blocking drugs alone differed from that seen in the presence of hyoscine alone, insofar as with hyoscine alone following cessation of stimulation, the tone returned to the baseline slowly after an initial phase of rapid recovery, whereas in contrast, with ganglion blockers alone the relaxations were followed by an immediate return to the baseline; or a rebound contraction.

The after contraction seen after vagal stimulation to the stomach in the presence of ganglion blocking drugs were potentiated by low concentrations of physostigmine and blocked by low concentrations of hyoscine and this suggests that part of this response may involve a cholinergic step. As it is seen only in the presence of nicotinic blockade the effect probably involves stimulation of muscarinic receptors and may be due to ACh released preganglionically diffusing to muscarinic receptors on the smooth muscle cells.

Rebound excitation following non-cholinergic non-adrenergic nerve stimulation has been observed in other tissues. In an electrophysiological study of the guinea-pig taenia caecum, Bennett (1966) observed that stimulation of the intramural inhibitory nerves (field stimulation in the presence of atropine) with single pulses, gave an inhibitory junction potential which was followed by a burst of action potentials at intervals of about 0.5 s, lasting for up to 30 s. The tissue did give action potentials spontaneously in the absence of stimulation but these occurred less frequently, at intervals of about once every second.

The rate of firing of action potentials following inhibitory junction potentials, and the duration of this enhanced

rate of firing increased with an increase in the mean amplitude of the hyperpolarisation during the inhibitory junction potentials. The action potentials produced could not be due to ACh since the period of excitation persisted in the presence of atropine. He felt that the most likely explanation of rebound excitation was that as a result of hyperpolarisation of the cell membrane there follows a period in which the membrane tends to become depolarised beyond its normal rate of action potential firing. Burnstock et al (1966) had observed after contractions following stimulation of both the perivascular (sympathetic) and intramural inhibitory nerves (the after contractions were, however, greater following intramural nerve stimulation than perivascular stimulation, when relaxations of equal magnitude had been produced) and Bennett considered that the action potentials he had noted were responsible for this rebound contraction. However, the system may not be this simple; equal magnitudes of relaxation would presumably be indicative of equal magnitudes of hyperpolarisation in which case it would have been expected that equal magnitudes of after contractions would have been recorded if the hypothesis of Bennett was the only explanation required.

The rebound contractions following intramural inhibitory nerve stimulation in the guinea-pig taenia caecum is not mediated by ACh acting on muscarinic receptors because it persists in the presence of atropine (Burnstock et al, 1966; Campbell, 1966) and was abolished not potentiated by neostigmine (Campbell, 1966). It is clear that the rebound contraction of the guinea-pig stomach seen after vagal nerve stimulation in the presence of ganglion blocking drugs

in this work is caused by a different mechanism to that seen in the guinea-pig taenia caecum. Day and Warren (1968) observed that transmural stimulation of isolated intestine preparations from rabbits or kittens gave a biphasic response. They also concluded that the motor response they observed was not the same phenomenon as the rebound contraction described by Bennett (1966). The motor component was depressed when the bath temperature was lowered from 37 degrees centigrade to 28 degrees centigrade; the contractions to ACh were also inhibited. At the lower bath temperature hyoscine or atropine inhibited this motor response; the motor response seen in kitten intestine was abolished by a low concentration of hyoscine even at 37 degrees centigrade.

The motor component was also markedly enhanced by anticholinesterase agents. They, therefore, felt that the component seemed chiefly to result from activation of cholinergic nerve endings. The motor response could not be a rebound phenomenon because it sometimes preceded, and was sometimes seen in the absence of any inhibitory response, and was probably due to liberated ACh.

Paton and Perry (1953) have described two phases of the ganglion blockade produced by nicotine. The first phase causes depolarisation and during this phase a blocked ganglion cell is unable to respond to any stimulation. Later a second phase of competitive block of the nicotinic receptors occurs and during this phase stimulants acting at sites other than the nicotinic receptor can elicit a response. This information is of relevance to an observation made by Bülbring and Gershon (1967) that after exposure to nicotine alone, the guinea-pig stomach showed relaxations to vagal stimulation

in the specific (2nd) phase of nicotine block. This observation of a non-nicotinic relaxation could be explained along similar lines to those used here, although Bülbring and Gershon explained this observation without drawing attention to the fact that vagal gastric inhibition had been produced in the presence of a ganglion blocking agent alone. They noted that during the first phase of nicotine block, responses to vagal stimulation and 5-HT were abolished, but during the second phase of specific antagonism to ACh, the inhibitory response to vagal stimulation recovered in parallel with a recovery of the relaxant effect of 5-HT and they felt that this indicated a role for 5-HT in the production of vagal gastric inhibition.

SUMMARY.

1) Vagal relaxations in the presence of ganglion blocking drugs alone similar to those in the presence of hyoscine alone have been demonstrated.

2) These relaxations were not adrenergic in nature.

3) The relaxation of the stomach seen in the presence of ganglion blocking drugs was reduced by low concentrations of hyoscine which did not lower the tone of the preparation; also the latency of transmission was longer with ganglion blocking drugs than with hyoscine. This suggests the presence of functional ganglionic muscarinic receptors.

4) The form of the response in the presence of ganglion blocking drugs alone differs from that seen in the presence of hyoscine alone insofar as upon cessation of stimulation an immediate return to the baseline or an after contraction was observed, whereas with hyoscine alone following cessation of stimulation the tone only slowly returns to the baseline.

5) The after contractions seen with ganglion blocking drugs alone, may involve a cholinergic process mediated through muscarinic receptors; it does not seem to be a myogenic rebound phenomenon as was described by Bennett (1966) for the guinea-pig taenia caecum.

CHAPTER 3.

Investigation into the role of 5-HT in vagal relaxation of the isolated whole guinea-pig stomach.

INTRODUCTION.

It was seen in CHAPTER 1 and 2 that after maximally effective concentrations of hyoscine and ganglion blocking drugs had been added to the bathing medium, small relaxations to vagal nerve stimulation could still be produced. This remaining response could be the result of stimulating nerve fibres which run uninterrupted to the smooth muscle of the stomach. Alternatively it is feasible that a ganglionic transmitter other than ACh is involved.

Bülbring and Gershon (1967) claimed that there were ganglia sensitive to neurally released 5-HT in the pathway, and it may well be that this remaining non-cholinergic relaxation was due to fibres involving 5-HT as the ganglionic transmitter.

One of the points of evidence for this viewpoint given by Bülbring and Gershon was that gastric relaxations to vagal stimulation were reduced when the tissue was desensitised to 5-HT. However, this observation could not be repeated by other workers (Beani et al, 1971). In view of this discrepancy the effect of 5-HT desensitisation on relaxation to vagal stimulation in the guinea-pig stomach was re-investigated.

It is now widely believed that tri-and tetra-cyclic anti-depressant drugs exert their action by inhibition of the neuronal re-uptake of amines in the C.N.S. (Schildkraut, 1965). Initially Schildkraut only considered nerves releasing

NA in this hypothesis but since then the theory has been extended to include central nerves releasing 5-HT (Carlsson, Fuxe and Ungestedt 1968; Carlsson, Corrodi, Fuxe and Hökfelt, 1969). In fact the tri-and tetra-cyclic anti-depressant drugs could be divided into two groups; those effective in blocking NA re-uptake, and those effective in blocking 5-HT re-uptake (Carlsson et al, 1969). Although the theory considers central amine releasing nerves, the same effects have been noticed in peripheral tissues, e.g. guinea-pig and mouse vas deferens (Hughes, Kreen and Main, 1974) and guinea-pig gut (Gershon, Robinson and Ross, 1976; Gershon and Jonakait, 1979).

It was, therefore, decided to investigate the effect of tri-cyclic anti-depressant drugs on the non-cholinergic, non-adrenergic gastric relaxation of guinea-pigs.

Two anti-depressant drugs were chosen for the study, desipramine which has a selective activity on the NA re-uptake process, and chlorimipramine which exerts a selective action on 5-HT re-uptake (Liderink, Jonsson and Fuxe, 1971).

RESULTS.

The effect of desensitisation to 5-HT on relaxations of the guinea-pig stomach to vagal stimulation.

After hyoscine (0.46 μM) plus pempidine (15.0 μM) a residual relaxation to supramaximal vagal stimulation was still present. The effect of desensitising the tissue to the action of 5-HT was then investigated.
The tissue was challenged by 5-HT (0.025 μM) which caused a relaxation. The 5-HT was then allowed to remain in the bath. 7 minutes later the tissue was challenged by a further addition of 0.25 μM 5-HT to the bath which did not produce a response. 7 minutes later a further addition of 0.25 μM 5-HT was again added to the bath, and again no response was seen. During this phase of desensitisation to 5-HT no reduction in the size of responses to supramaximal vagal stimulation was noted. (FIG 3.1). (n = 6).

The effect of desipramine and chlorimipramine on relaxations of the guinea-pig stomach to vagal, and sympathetic (peri-arterial) stimulation in the presence of hyoscine.

Hyoscine (0.4 μM) was present throughout the experiments. Desipramine or chlorimipramine was added to the bathing fluid starting with a concentration of 0.1 μM and increasing the concentration in a cumulative manner to give a final concentration of 20.0 μM in the case of desipramine and 2.0 μM in the case of chlorimipramine.
No potentiation or prolongation of relaxation was observed to supramaximal vagal stimulation, on the contrary, the responses were reduced as were responses to supramaximal sympathetic (periarterial) stimulations (FIG 3.2 and FIG 3.3)

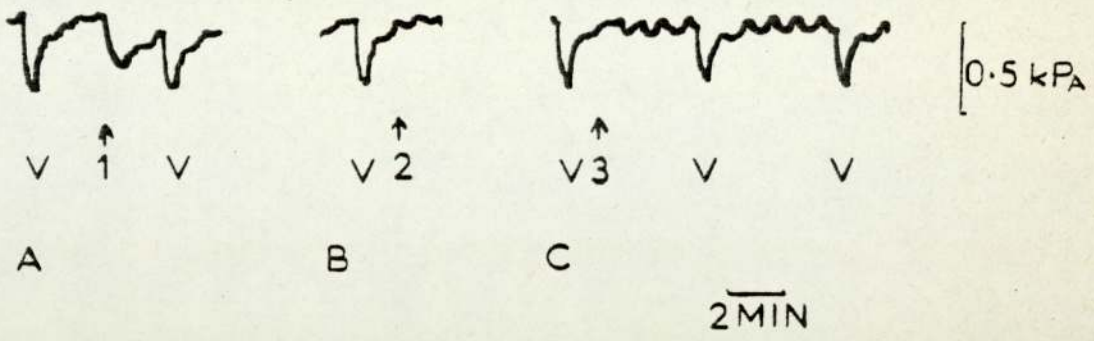


FIG 3.1

FIG 3.1. The effect of desensitisation to 5-HT on relaxations to supramaximal vagal stimulation of the isolated whole guinea-pig stomach.

0.46 μM hyoscine plus 15.0 μM pempidine present throughout. No washout of drugs between A, B and C.

A initial responses

B responses 7 minutes later

C responses after another 7 minutes

V = supramaximal vagal stimulation

1 = 0.025 μM 5-HT

2 = 0.25 μM 5-HT

3 = 0.25 μM 5-HT

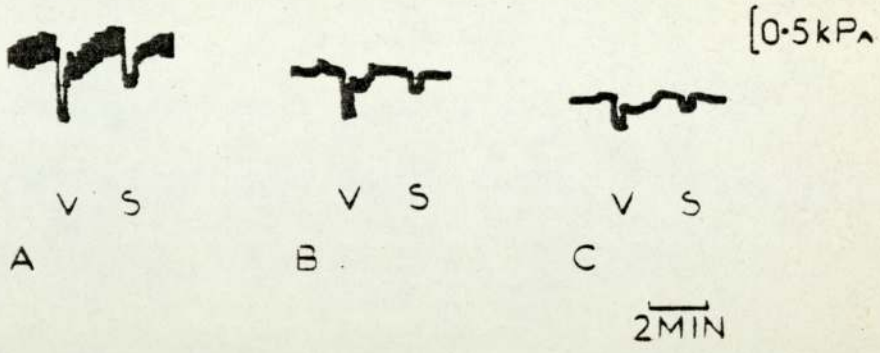


FIG 3.2

FIG 3.2. The effect of desipramine on responses of the whole isolated guinea-pig stomach to supramaximal vagal and sympathetic nerve stimulation.

A. Responses in the presence of 0.4 μM hyoscine

B. Responses 1 hour after the addition of 0.1 μM desipramine to the bathing fluid.

C. Responses 1 hour after a further addition of desipramine to give a final concentration of 1.0 μM in the bathing fluid.

V = responses to supramaximal vagal stimulation.
S = responses to supramaximal periarterial (sympathetic) stimulation.

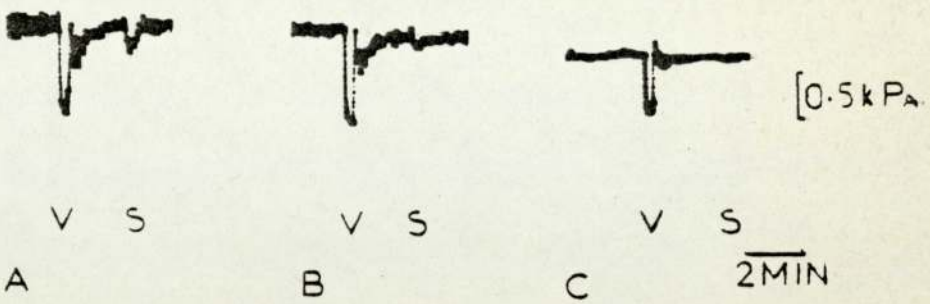


FIG 3.3

FIG 3.3. The effect of Chlorimipramine on responses of the whole isolated guinea-pig stomach to supramaximal vagal and sympathetic nerve stimulation.

A. Responses in the presence of 0.4 μM hyoscine.

B. Responses 1 hour after the addition of 0.1 μM chlorimipramine to the bathing fluid.

C. Responses 1 hour after a further addition of chlorimipramine to give a final concentration of 1.0 μM in the bathing fluid.

V = responses to supramaximal vagal stimulation

S = responses to supramaximal periarterial (sympathetic) stimulation

Tone was also lowered (as indicated by the fall in baseline FIG 3.1 and FIG 3.2). The reduction in the size of both vagal and periarterial responses could be accounted for by changes in tone alone. (Six experiments were conducted with each tricyclic anti-depressant drug).

DISCUSSION.

Desensitisation of the guinea-pig stomach to the effect of 5-HT failed to cause any reduction in the magnitude of the inhibitory response to vagal stimulation in the presence of hyoscine. This is in agreement with Beani et al (1971), who also found that methysergide, at a concentration that counteracted responses to 5-HT, had no effect on the inhibitory vagal response. In view of these results it is unlikely that 5-HT has a role in the production of vagal mediated gastric relaxation in the guinea-pig.

Neither of the tri-cyclic drugs caused a potentiation of responses to periarterial (sympathetic) stimulation. This suggests that the method employed was not detecting any uptake blocking action, and so no conclusions can be made from these experiments.

Although it seems unlikely that 5-HT has a role as a neurotransmitter within the non-cholinergic, non-adrenergic inhibitory nerve pathway Gershon and Ross (1966) have shown that radioactive 5-HT is accumulated throughout the gastro-intestinal tract in the myenteric plexus, confined to the nerve terminals, and the reason why this should be so is hard to imagine if 5-HT is not a neurotransmitter within the myenteric plexus.

Also 5-HT has been found to affect peristalsis of the guinea-pig ileum, stimulating peristalsis when applied to the lumen, but inhibiting it when applied serosaly furthermore 5-HT is released into the lumen during peristalsis (Bülbring and Lin, 1958).

Therefore, a role for 5HT in the control of gut motility is still a definate possability.

SUMMARY.

1. In the presence of 5-HT desensitisation, relaxations of the guinea-pig stomach to vagal stimulation were unaffected. It is, therefore, unlikely that 5-HT has a role in the production of non-cholinergic non-adrenergic relaxations of the guinea-pig stomach.

CHAPTER 4.

The role of prostaglandins in vagal gastric relaxation.

INTRODUCTION.

In CHAPTER 2 it was concluded that the after-contraction seen following vagal nerve stimulation in the presence of ganglion blocking agents was mediated by ACh acting upon muscarinic receptors.

It was therefore concluded that the origin of this after-contraction was different to that of the rebound contraction following non-cholinergic non-adrenergic nerve stimulation of the guinea-pig taenia caecum as observed by Bennett (1966). Prostaglandins (PGs) have been implicated as a factor involved in the rebound contraction of the guinea-pig taenia (Burnstock, Cocks, Paddle and Staszewska - Barzak, 1975).

The effect of the PG biosynthesis inhibitor, indomethacin (Vane, 1971), on inhibitory responses to vagus nerve stimulation was therefore examined, to see if any part of the responses seen in the presence of hyoscine, or ganglion blocking drugs was mediated by PGs, and whether an explanation for the difference in the responses between those seen in the presence of hyoscine, and those seen in the presence of ganglion blocking drugs could be offered.

Indomethacin reduces the tone of the rat stomach strip preparation (Eckenfels and Vane, 1972), so the effects of sodium nitroprusside and papaverine, two other tone reducing drugs were also examined and compared with those of indomethacin.

RESULTS.

The effect of indomethacin on the isolated whole guinea-pig stomach preparation.

In the experiments described here, either hyoscine (0.46μM) or pempidine (15.0μM) was already present in the bathing medium and the preparations were giving relaxations to vagal nerve stimulation. Stimulation was supramaximal and for periods of 20s in a 5 minute cycle, in order to increase the certainty of an after contraction being produced (CHAPTER 2).

Indomethacin was then added to the organ bath in a cumulative manner (1 hour periods between additions), until no further change in the responses to vagal stimulation could be produced. The tissues varied in their individual sensitivity to indomethacin. Maximally effective concentrations of indomethacin fell within the range, 2.8μM - 28.0μM.

Indomethacin caused a fall in tone both with hyoscine or pempidine present as is indicated by the fall in the baseline of FIG 4.1. When indomethacin had been added to the preparation at a concentration which produced no further changes in the response to vagal stimulation, the maximum fall in tone was also achieved. At this point indomethacin (the concentration required varied with individual preparations between 2.8 and 28μM) lowered the tone by 0.46$\pm$0.07 kPa (mean $\pm$ s.e. mean;n = 18). Before the addition of indomethacin the response to vagal stimulation in the presence of hyoscine consisted initially of a relaxation which was followed, on cessation of stimulation, by a phase of rapid but incomplete recovery

of tone, this in turn being followed by a slow return to the original baseline (e.g. FIG 1.1, FIG 1.4, FIG 4.1A). Indomethacin reduced the rapid recovery phase of the response (FIG 4.1C). Before the addition of indomethacin the responses to vagal stimulation in the presence of pempidine consisted of relaxations and on cessation of stimulation an immediate return to the baseline, with an after contraction.

When indomethacin was then added to the bath the after contraction was abolished (FIG 4.1F).

PGE_2 (5.67μM) restored both the tone (causing a rise of 0.49±0.08 kPa (mean ± s.e. mean;n = 9)) and (FIG 4.2 A,B,) the shape of the response after indomethacin. $PGF_{2\alpha}$ (4.21μM) had no action after indomethacin treatment (FIG 4.3).

The effect of sodium nitroprusside and papaverine in concentrations giving similar tone changes to indomethacin.

In order to be certain that changes in the response to vagal stimulation caused by indomethacin were not due to depression of tone alone, it was necessary to compare the effects of indomethacin with drugs which lower the tone by the same amount, but by a different mechanism.

Sodium nitroprusside lowered the tone of the preparation when hyoscine (0.46μM) or pempidine (15.0μM) were already present, but the individual preparations varied in their sensitivity to the drug. The concentration of sodium nitroprusside was, therefore, adjusted in each case to give the desired fall in tone. Concentrations between 2.0 to 8.0μM sodium nitroprusside were used and the tone was lowered by 0.44±0.08 kPa (mean ± s.e. mean;n = 13).

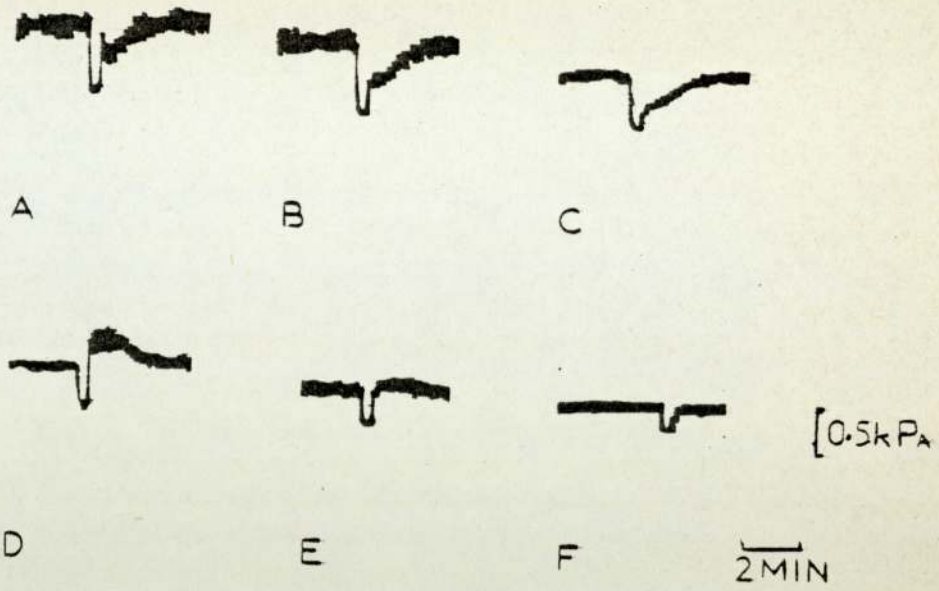


FIG 4.1

FIG 4.1. The effect of indomethacin on responses of the whole isolated guinea-pig stomach to supramaximal vagal stimulation.

Responses to supramaximal vagal stimulations

A in the presence of 0.46 μM hyoscine

B the same preparation as A after 1 hour exposure to 14.0 μM indomethacin

C the same preparation as A after 1 hour exposure to 28.0 μM indomethacin

D in the presence of 15.0 μM pempidine

E the same preparation as D after 1 hour exposure to 5.6 μM indomethacin

F the same preparation as D after 1 hour exposure to 28.0 μM indomethacin

FIG 4.2. The effect of PGE_2 on responses of the whole isolated guinea-pig stomach to supramaximal vagal stimulation, after various drug treatments.

Responses to vagal stimulation:

A in the presence of 0.46 μM hyoscine

B the same preparation as A 1 hour after the addition of 28.0 μM indomethacin

C in the presence of 0.46 μM hyoscine

D the same preparation as C after the addition of 28.2 μM papaverine

E in the presence of 0.46 μM hyoscine

F the same preparation as E after the addition of 4.0 μM sodium nitroprusside

The arrows represent the addition of 5.67 μM PGE_2.

• Supramaximal vagal stimulation.

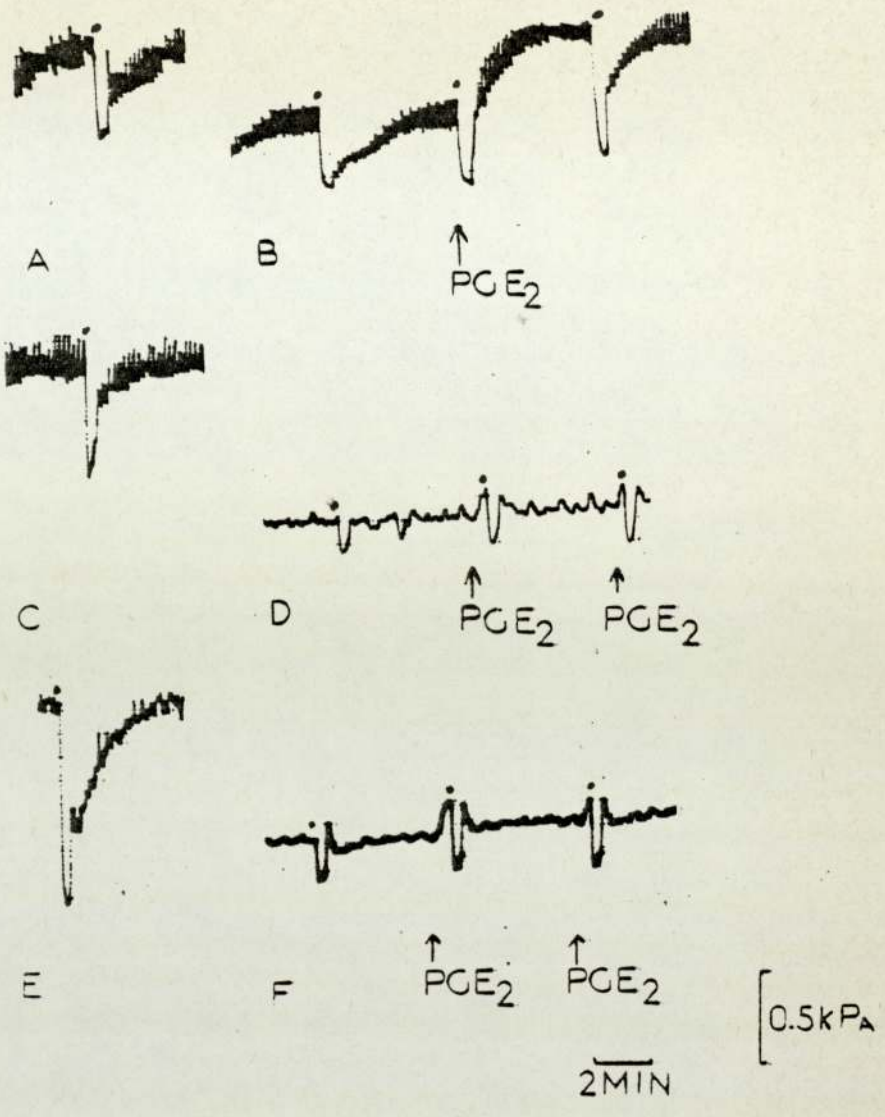


FIG 4.2

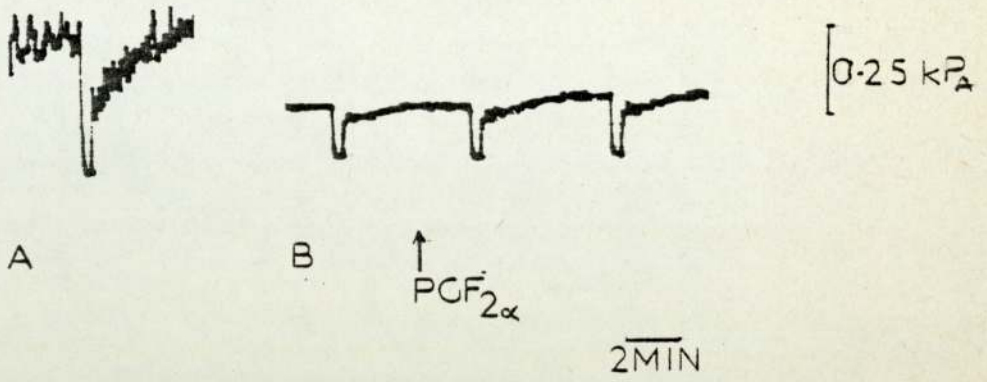


FIG 4.3

FIG 4.3. The effect of $PGF_{2\alpha}$ on responses of the whole isolated guinea-pig stomach to supramaximal vagal stimulation, after indomethacin treatment.

Responses to supramaximal vagal stimulation:

A in the presence of 0.46 μM hyoscine

B the same preparation as A after 1 hour exposure to 11.2 μM indomethacin

The arrow represents the addition of 4.21 μM $PGF_{2\alpha}$

Compare this with FIG 4.2B.

Papaverine also lowered the tone of the preparations when either hyoscine (0.46μM) or pempidine (15.0μM) was present. The preparations varied in individual sensitivety to the drug and a concentration rangeof 14.1 to 42.6μM was employed, which gave a fall in tone of 0.44$\pm$0.12 kPa (mean $\pm$ s.e. mean;n = 9).

The falls in tone produced by sodium nitroprusside or papaverine were not significantly different from those caused by maximally effective concentrations of indomethacin ($P > 0.01$).

Unlike the experiments with indomethacin, PGE_2 (5.67μM) did not fully restore the tone following sodium nitroprusside or papaverine. In the case of sodium nitroprusside tone was raised by 0.21$\pm$0.03 kPa (mean $\pm$ s.e. mean;n = 13) and in the case of papaverine tone was raised by 0.19$\pm$0.02 kPa (mean $\pm$ s.e. mean;n = 9). These small increases in tone were significantly smaller ($P < 0.05$) than those seen with PGE_2 after indomethacin (FIG 4.2).

In the presence of hyoscine, sodium nitroprusside (2.0 - 8.0μM) did not affect the rapid recovery phase of the response; in fact by lowering the tone it appeared as if an after-contraction had become visible (FIG 4.4C). Similarly a lowering of the baseline by sodium nitroprusside when added to preparations in the presence of pempidine, made the after-contraction appear more pronounced (FIG 4.4F). These observed effects could be explained as being purely due to tone depression, with no real changes in the form of the response.

The tone slowly recovered after sodium nitroprusside but could be again lowered by a further addition of the drug.

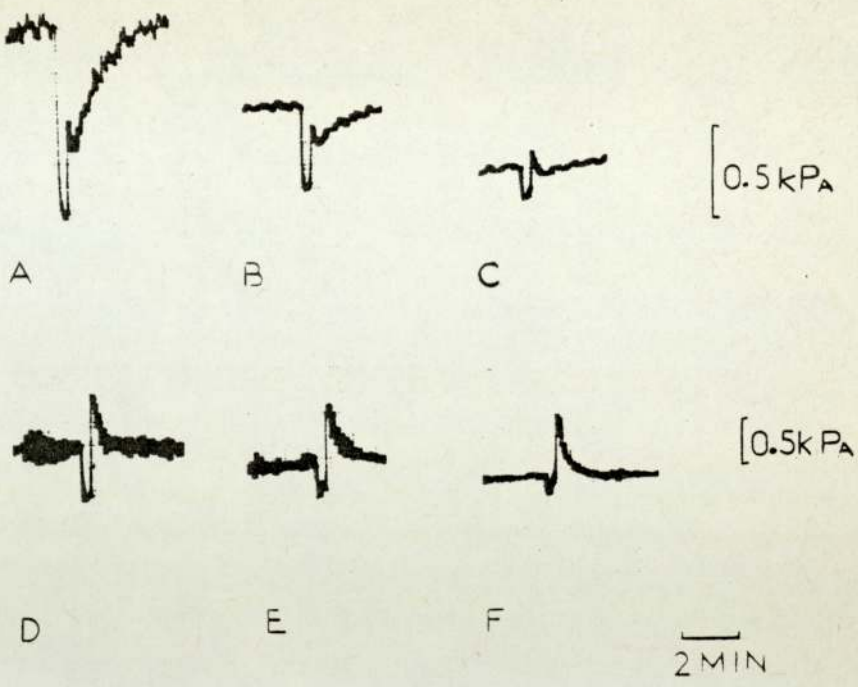


FIG 4.4

FIG 4.4. The effect of sodium nitroprusside on responses of the whole isolated guinea-pig stomach to supramaximal vagal stimulation.

Responses to supramaximal vagal stimulation

A in the presence of 0.46 μM hyoscine

B the same preparation as A after exposure to 0.2 μM sodium nitroprusside

C the same preparation as A after exposure to 4.0 μM sodium nitroprusside

D in the presence of 15.0 μM pempidine

E the same preparation as D after exposure to 0.1 μM sodium nitroprusside

F the same preparation as D after exposure to 2.0 μM sodium nitroprusside

Exposure time for sodium nitroprusside 5 minutes.

When papaverine (14.1 to 42.6μM) was added, the slow return of tone to the baseline seen after vagal atimulation in the presence of hyoscine appeared to have been abolished (FIG 4.5C) but this is accounted for by depression of tone with no reduction of the rapid phase of recovery. The response to vagal stimulation in the presence of pempidine showed a reduction in the size of the after-contraction (FIG 4.5F). This reduction is real and not an effect simply of tone reduction as had been seen in FIG 4.4F with sodium nitroprusside (here the reduction in tone without an effect on the after-contraction made the after-contraction appear to be more pronounced). FIG 4.5 shows the effect of increasing the concentration of papaverine until no further change in tone of the response to vagal stimulation could be produced. It can be seen that a rapid recovery to the baseline still occurred and this could not be abolished by papaverine, the after-contraction, however, was almost completely abolished. When corrected for tone the relaxation to vagal stimulation was not reduced by papaverine.

PGE_2 (5.67μM) did not fully restore the responses to vagal stimulation after treatment with sodium nitroprusside or papaverine (FIG 4.2).

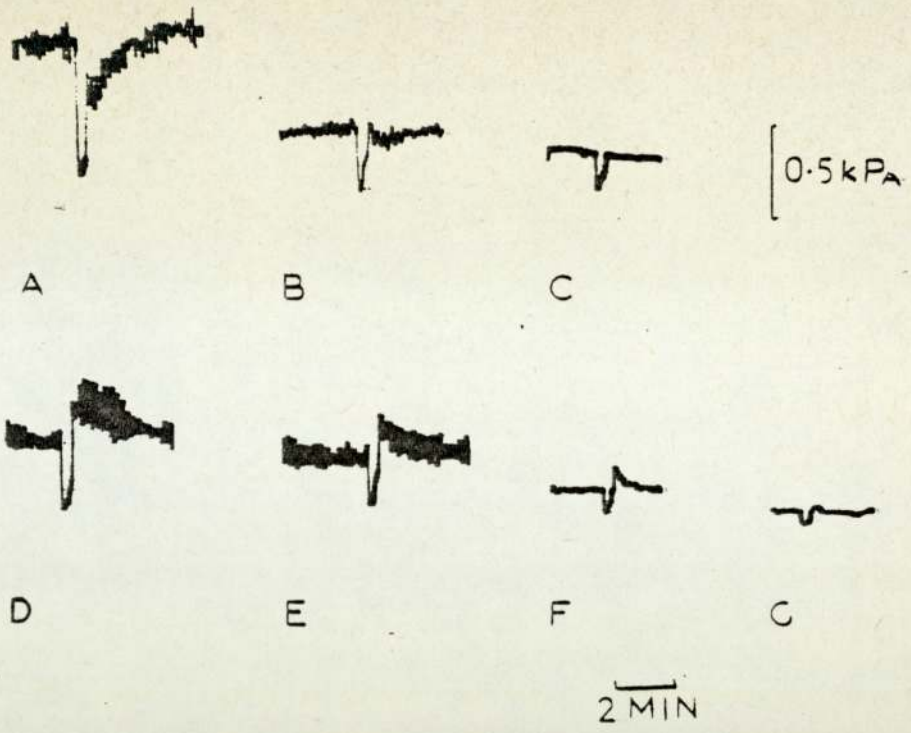


FIG 4.5

FIG 4.5. The effect of papaverine on responses of the whole isolated guinea-pig stomach to supramaximal vagal stimulation.

Responses to supramaximal vagal stimulations:

A in the presence of 0.46 μM hyoscine

B the same preparation as A after exposure to 10.7 μM papaverine

C the same preparation as A after exposure to 42.6 μM papaverine

D in the presence of 15.0 μM pempidine

E the same preparation as D after exposure to 2.7 μM papaverine

F the same preparation as D after exposure to 14.1 μM papaverine

G the same preparation as D after exposure to 56.4 μM papaverine

Exposure time for papaverine; ten minutes.

DISCUSSION.

Indomethacin inhibits PG biosynthesis (Vane, 1971). PGs were considered to be involved in the maintenance of smooth muscle tone in the isolated rabbit jejunum (Ferreira, Herman and Vane, 1972) after it had been observed that the tone of the preparation gradually increased with time, and fell after washing. During this period of investigation PGs were released and this release was abolished by indomethacin. Furthermore, the tone increase was also abolished by indomethacin.

Indomethacin also caused a gradual fall in tone of the rat stomach strip (Eckenfels and Vane, 1972) and these authors concluded that PG generation maintained the smooth muscle tone. The results of this study are in agreement with this. Indomethacin gradually caused a fall in tone and this could be reversed by PGE_2. $PGF_{2\alpha}$ had little effect so it can be concluded that tone was maintained by PGE_2 generation.

The aim in this section of the work was to investigate the action of indomethacin on the inhibitory response to vagal stimulation and therefore sodium nitroprusside and papaverine which also lowered tone, were included in the study. Sodium nitroprusside and papaverine caused falls in tone which could not be restored by PGE_2 confirming that the mechanism of tone reduction of these drugs was different to that of indomethacin.

Of the drugs used, only the effects of sodium nitroprusside could be accounted for purely on the basis of tone reduction. In order to explain the effects of indomethacin and papaverine additional events must have occurred. The rapid phase of recovery seen after vagal nerve stimulation in the presence

of hyoscine was reduced by indomethacin, an effect which could not be accounted for by a reduction in tone (cf. sodium nitroprusside), indicating that this effect of indomethacin seems to be inhibition of PGE_2 biosynthesis since the response was restored by PGE_2 but not $PGF_{2\alpha}$. The after-contraction seen with vagal nerve stimulation in the presence of pempidine was also abolished by indomethacin and restored by PGE_2, but not $PGF_{2\alpha}$. This could not be accounted for by tone changes as a fall in tone would tend to enhance the after-contraction, and not reduce it.

It is possible to conclude at this point, therefore, that PGE_2 plays a role in the mediation of both the rapid phase of recovery following inhibitory vagal nerve stimulation in the presence of hyoscine, and in the rapid after-contraction following vagal nerve stimulation in the presence of ganglion blocking agents.

In CHAPTER 2 it was concluded that the after-contractions seen following vagal nerve stimulation in the presence of ganglion blocking drugs involved a cholinergic (muscarinic) step. This is by no means at variance with the suggestion for a role of PGE_2 in the production of the after-contraction, since Bennett, Eley and Stockley (1975) concluded that PGs modulate nerve mediated cholinergic contractions of the guinea-pig ileum after noting that indomethacin substantially reduced contractions of guinea-pig isolated ileum to nerve stimulation with electrical pulses or nicotine, and that PGE_2 restored the excitatory responses.

The after-contraction following vagal nerve stimulation in the presence of pempidine was also reduced by papaverine,

the rapid phase of recovery following vagus nerve stimulation in the presence of hyoscine was, however, unaffected. The main difference between these two phenonema is that the after-contraction seen following vagal stimulation in the presence of ganglion-blockers involves acetylcholine acting upon muscarinic receptors, as it is abolished by low concentrations of hyoscine and potentiated by low concentrations of physostigmine (CHAPTER 2) whereas the rapid phase of recovery following vagal stimulation in the presence of hyoscine is clearly completely non-muscarinic, as it is present with hyoscine in the bath. Papaverine seems only to be affecting the response which involves a muscarinic step. This is not due to an atropine-like action of papaverine because no reduction of the size of vagal relaxation in the presence of pempidine (FIG 4.5) similar to that caused by hyoscine (CHAPTER 2) was produced by the drug. In this series of experiments, papaverine reduced processes that were mediated by PGE_2 if they also involved a muscarinic step. This was not due to an action on PG biosyntheses, or receptors to PG as the response to vagal inhibitory nerve stimulation was not fully restored by PGE_2 which did restore the response after indomethacin treatment.

Processes mediated by PGE_2 which were not muscarinic were unaffected. If these observations can be repeated in other tissues, papaverine may prove to be a useful tool for making this distinction. If after vagal nerve stimulation in the presence of hyoscine a rapid recovery phase, mediated by PGE_2 (but not muscarinic) occurs, this is also likely to be present when vagal relaxations are produced in the presence of ganglion blocking drugs. The recovery of the

response and the after-contraction, therefore, are probably two events: a rapid recovery of the response mediated by PGE_2 which is non-muscarinic, and an after-contraction mediated by PGE_2 but which also involves a role for muscarinic receptors.

Therefore, two separate roles for PGE_2 are postulated

(i) where PGE_2 is involved in a non-muscarinic process

(ii) where PGE_2 is involved as a modulator for the effects of ACh at muscarinic sites.

This is confirmed by the experiments using higher concentrations of papaverine (FIG 4.5G) when after abolition of the after-contraction following vagal nerve stimulation in the presence of pempidine, a phase of rapid return to the baseline was still present.

These conclusions are diagramatically represented in FIG 4.6, which shows the various phases of the inhibitory response of the guinea-pig stomach to vagus nerve stimulation.

The rapid phase of recovery, which is non-muscarinic, is most likely to be the equivalent process in the guinea-pig stomach to the rebound contraction seen after inhibitory nerve stimulation in the guinea-pig taenia caecum reported by Burnstock et al (1975), which was also abolished by indomethacin but was seen in the presence of atropine.

A related observation has been made by Kadlec, Masek, and Seferna(1974). They reported an increase in the height of the rebound contraction shown by the guinea-pig ileum following co-axial stimulation in the presence of atropine, caused by PGE_2.

The reason for the presence of the slow phase of recovery

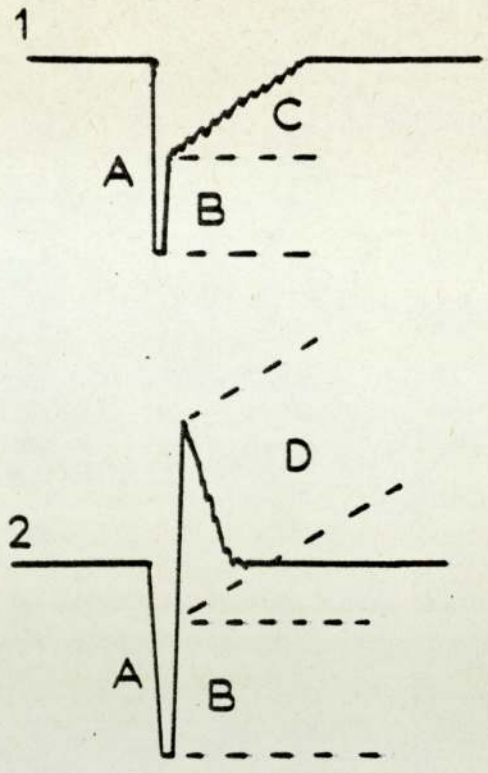


FIG 4.6

FIG 4.6. Diagramatic representation of the various phases involved in vagal relaxation of the guinea-pig stomach.

1 in the presence of hyoscine

2 in the presence of ganglion blocking drugs

Phases of the inhibitory response to vagal stimulation:

A non-cholinergic, non-adrenergic relaxation.

B rapid recovery phase, mediated by PGE_2 which has no muscarinic component, not affected by papaverine.

C slow recovery phase seen in the presence of hyoscine of unknown mediation.

D after-contraction seen in the presence of ganglion blocking drugs, mediated by PGE_2 and also having a muscarinic component, blocked by papaverine.

seen after vagal stimulation in the presence of hyoscine is still unknown.

SUMMARY.

1. PGE_2 biosynthesis maintains the tone of the guinea-pig stomach. When this is blocked by indomethacin the tone falls.

2. Sodium nitroprusside and papaverine lower the tone of the guinea-pig stomach by a process not involving PGs.

3. The inhibitory response to vagus nerve stimulation of the guinea-pig stomach can be divided into various phases as shown in FIG 4.6;

   A non-cholinergic, non-adrenergic relaxation.

   B rapid recovery phase, mediated by PGE_2 which has no muscarinic component, not affected by papaverine.

   C slow recovery phase seen in the presence of hyoscine of unknown mediation.

   D after-contraction seen in the presence of ganglion blocking drugs, mediated by PGE_2 and also having a muscarinic component, blocked by papaverine.

CHAPTER 5.

Non-cholinergic, non-adrenergic relaxations in various tissue preparations.

INTRODUCTION.

Up until this point this work has been concerned with the non-cholinergic, non-adrenergic relaxation of the whole guinea-pig stomach.

Non-cholinergic, non-adrenergic relaxations of the rat and mouse stomach have also been reported (Heazell, 1974;1975, Bülbring and Gershon, 1967). It was, therefore, decided to examine the response of the rat and mouse whole stomachs to see whether a hyoscine sensitive component of ganglion transmission would be demonstrated as for the guinea-pig.

Burnstock et al (1966) have described a preparation of the guinea-pig taenia caecum where the taenia is removed with a flap of caecal wall attached.

Electrical stimulation of this flap is supposed to excite preganglionic inhibitory nerves running to the taenia. This preparation as an alternative to the stomach, from guinea-pigs was therefore, also examined.

It was decided to perform experiments using the longitudinal muscle coat with vagi attached from the guinea-pig stomach in order to discover whether the longitudinal smooth muscle layer of the guinea-pig stomach also receives inhibitory non-cholinergic, non-adrenergic innervation from the vagi.

A demonstration of non-adrenergic innervation to the circular muscle of the guinea-pig stomach having already been made (Beani et al, 1971).

RESULTS.

Responses of the isolated whole rat stomach to vagal stimulations.

In untreated preparations stimulation of the vagi with similar parameters (10 - 40 Hz, pulse width 200μs, supramaximum voltage) to those used for the guinea-pig stomach, resulted in a contraction.

This contraction could be converted to a relaxation by hyoscine which was maximally effective at a concentration of 0.40μM (FIG 5.1).

As for the guinea-pig these relaxations were reduced by pempidine (Maximally effective at 15.0μM concentrations) (FIG 5.1).

As has been shown with the guinea-pig, relaxations to vagal stimulation could also be produced in the presence of pempidine alone (FIG 5.2). Maximal relaxations were observed in the rat with 15.0μM.

It can be seen in FIG 5.2 that unlike the response of the guinea-pig stomach, after-contractions were not seen in the presence of pempidine alone. The shape of the response resembled that seen in the presence of hyoscine alone.

During the period of the experiments, the tone of the preparation changed greatly. In FIG 5.2 it can be clearly seen that the tone was falling even before the addition of hyoscine (in fact the tone of this preparation rose after the addition of hyoscine).

Relaxations in the presence of pempidine were reduced by subsequent additions of low concentrations of hyoscine

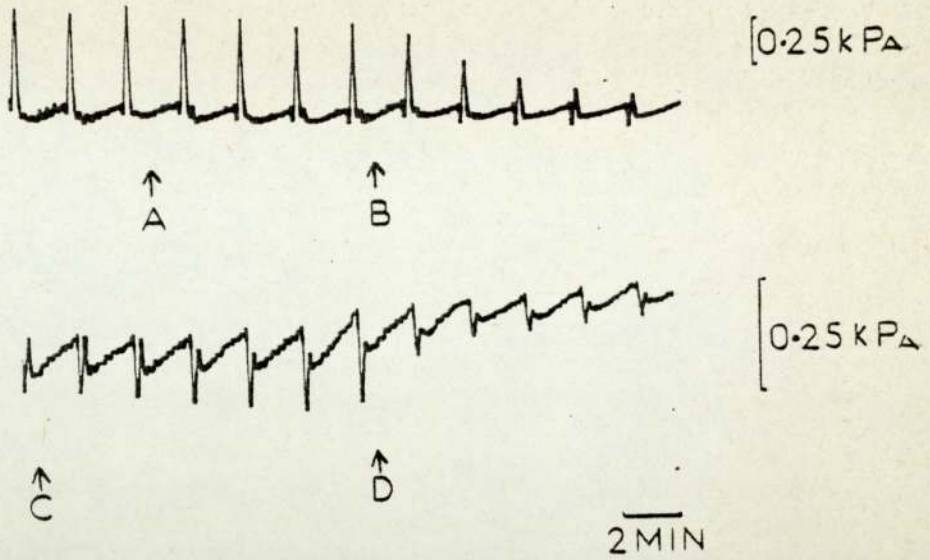


FIG 5.1

FIG 5.1. The effect of drugs on responses to supramaximal vagal stimulation of the isolated whole rat stomach.

0.02μM hyoscine added at A, increased to 0.20μM at B and to 0.40μM at C, 15.0μM pempidine added at D.

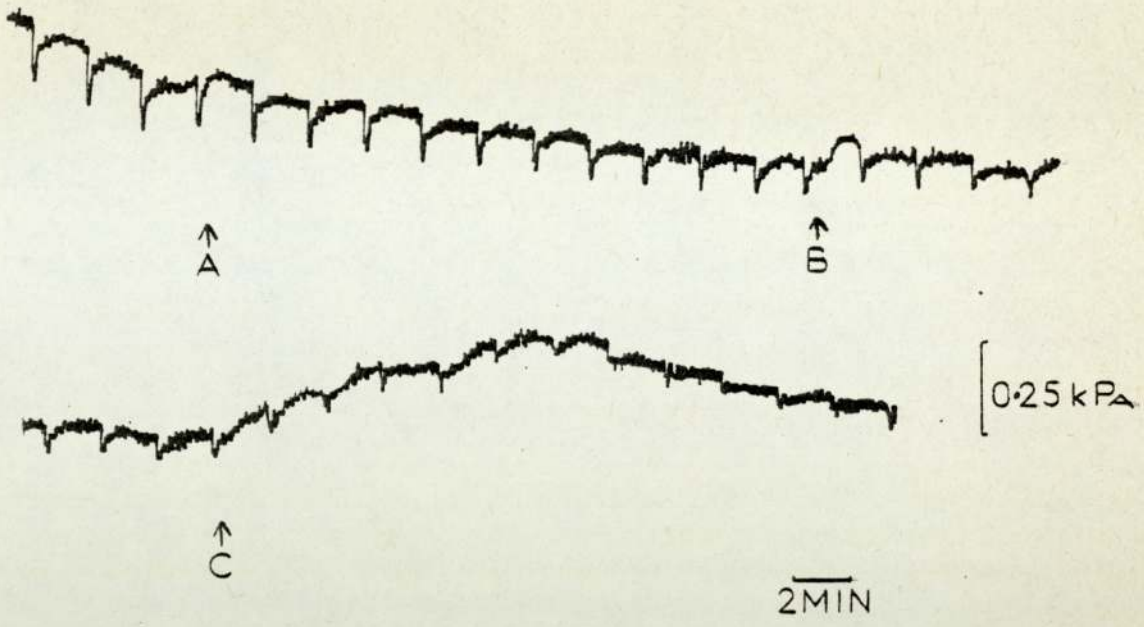


FIG 5.2

FIG 5.2. The effect of hyoscine on relaxations to supramaximal vagal stimulation of the isolated rat stomach in the presence of pempidine.

15μM pempidine present throughout, 0.01μM hyoscine added at A, increased to 0.02μM at B and to 0.04μM at C.

(0.02μM) to the bath, but because the tone of the preparation was changing by a large extent it became meaningless to make measurements of the responses corrected for tone changes. (This problem is discussed in CHAPTER1). If the responses were measured without reference to tone changes, relaxations produced in the presence of pempidine (15.0μM) were reduced by 57.58±5.29% (mean± s.e. mean:n=6) by 0.02μM hyoscine, and this reduction was significant ($P<0.01$).

Relaxations to vagal stimulations could be seen in the presence of phentolamine (10.0μM) plus propranolol (10.0μM) (n=6) both with hyoscine or pempidine present.

Responses of isolated whole mouse stomach to vagal stimulation.

In the absence of drugs, the mouse stomach responded to supramaximal vagal stimulation with a rapid relaxation followed after cessation of stimulation by an after contraction, which in turn was followed by a second small relaxation (FIG 5.3). Maximum sized relaxations were produced with a stimulation rate of 10 Hz or above (pulse width, 200μs, supramaximal voltage).

Hyoscine 0.23μM which abolished the aftercontraction had little effect on the relaxation observed during stimulation (FIG 5.4).Much higer concentrations (>4.0μM) caused a complete abolition of the response.

Pempidine (30.0μM) completely abolished all phases of the response to vagal stimulation (FIG 5.5).

The initial relaxation of the mouse stomach to vagal stimulation was unaffected by phentolamine (26.5μM) or propranolol (33.8μM).

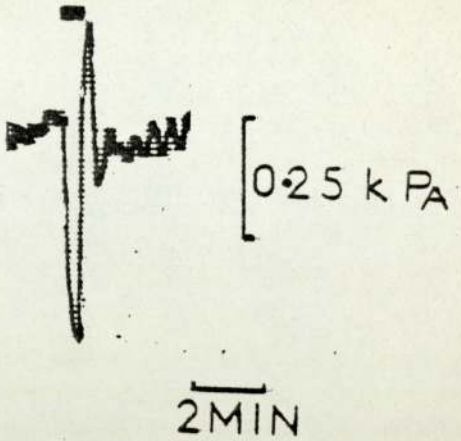


FIG 5.3

FIG 5.3. The response of the isolated whole mouse stomach to supramaximal vagal stimulation in the absence of drugs.

Upper bar represents duration of stimulation.

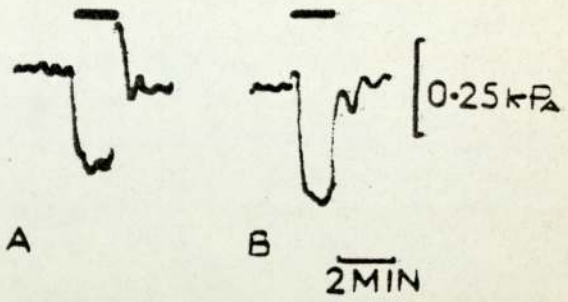


FIG 5.4

FIG 5.4. The effect of hyoscine on the responses of the isolated whole mouse stomach to supramaximal vagal stimulation.

Responses to supramaximal vagal stimulation:

A Untreated preparation

B After the addition of 0.23 μM hyoscine to the bathing fluid.

Upper bars represent duration of stimulation.

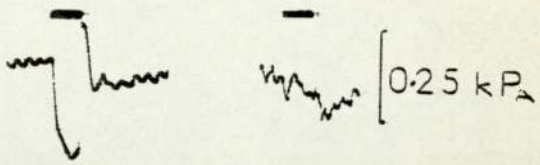


FIG 5.5

FIG 5.5. The effect of pempidine on responses of the isolated whole mouse stomach to supramaximal vagal stimulation.

Responses to supramaximal stimulation.

A Untreated preparation.

B After 30.0μM pempidine.

Upper bars represent duration of stimulation.

Relaxation of the guinea-pig taenia caecum with caecal wall attached.

Whenthe preparation of the taenia caecum with a flap of caecal wall attached was set up in the organ bath according to the method of Burnstock et al (1966) large pulse widths (1 m s.) were needed in order to produce a response to caecal wall stimulation; a stimulation strength of $\geq$60v was also required.

In untreated preparations supramaximal caecal flap stimulation gave rise to a contraction. Hyoscine (maximally effective at 0.1 μM) caused these responses to change to relaxations with a large and prolonged after contraction (FIG 5.6). Pempidine (15.0 - 45.0 μM) had no effect on these relaxations (FIG 5.6). Furthermore, pempidine (15.0 - 45.0 μM) had no affect on the control contractions. All responses were abolished by Ttx (15.7 μM n=7). Field stimulation (30 Hz, 1 μs. 100v) was identically affected by the drugs used above, and resembled caecal flap stimulation in every way.

Responses of the innervated longitudinal muscle strips from the guinea-pig stomach.

In 26 of the total 27 experiments performed, this preparation showed a great deal of spontaneous activity (FIG 5.7). This spontaneous activity was unaffected by hyoscine (0.23 μM) pempidine (15.0 μM) propranolol (5.0 μM) phentolamine (5.0 μM) or Ttx (15.6 μM). Because of this spontaneous activity it was difficult to distinguish any changes due to vagal stimulation.

In the one experiment that did not show spontaneous activity contractions were seen to supramaximal vagal stimulation.

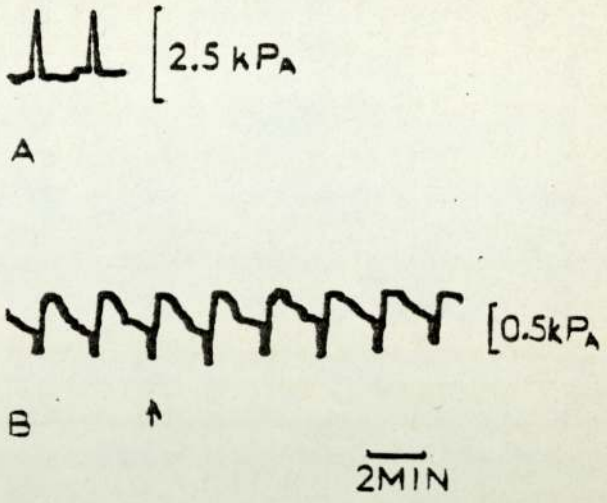


FIG 5.6

FIG 5.6

The response of the guinea-pig taenia caecum to caecal wall stimulation.

A. In the untreated preparation

B. In the presence of 0.1 μM hyoscine, 30.0 μM pempidine added at arrow

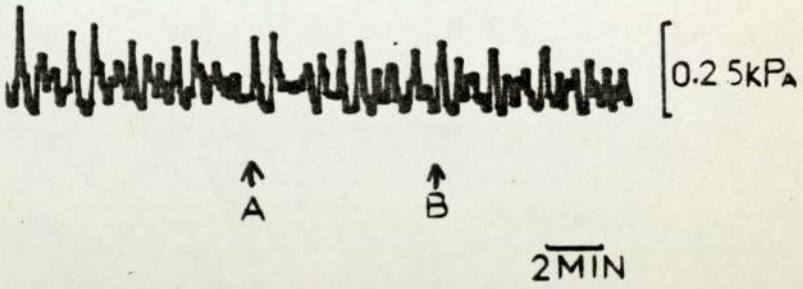


FIG 5.7

FIG 5.7. The effect of hyoscine on spontaneous activity of the longitudinal muscle from the guinea-pig stomach.

0.20μM hyoscine added at A

increased to 0.40μM at B

These contractions were abolished by large concentrations (0.2 - 0.8 μM) of hyoscine; no relaxations were seen (FIG 5.8).

Adrenaline (2 μM) caused a contraction of the preparations (n=6). This action was blocked by phentolamine (5.0 μM) but not by propranolol (5.0 μM) (FIG 5.9).

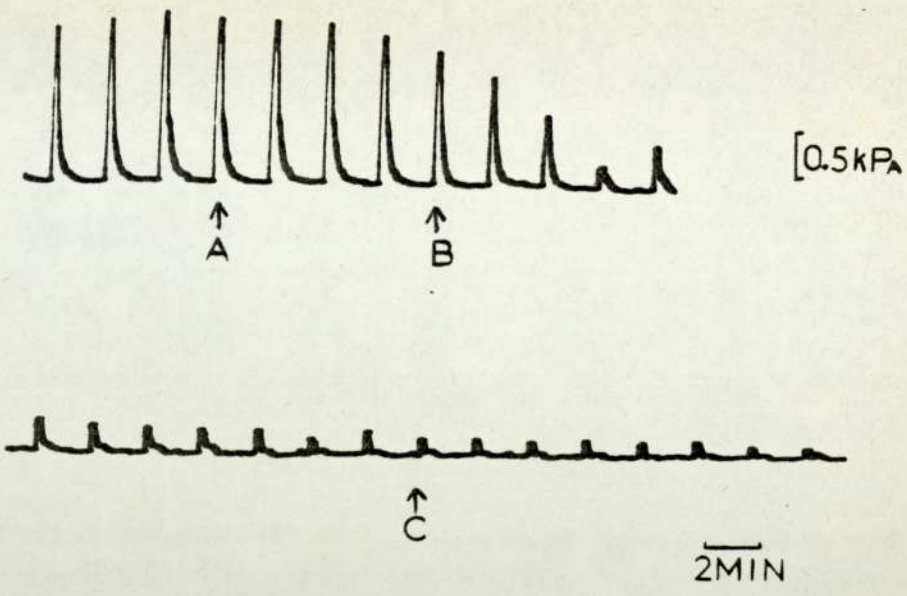


FIG 5.8

FIG 5.8. The effect of hyoscine on responses of the longitudinal muscle from the guinea-pig stomach to supramaximal vagal stimulation.

A 0.20 μM hyoscine added

B Increased to 0.40 μM hyoscine

C Increased to 0.80 μM hyoscine

FIG 5.9

FIG 5.9. Contractions of the longitudinal muscle from the guinea-pig stomach to adrenaline.

A 2μM adrenaline

B 2μM adrenaline after 5μM propranolol

C 2μM adrenaline after 5μM phentolamine (no propranolol present)

DISCUSSION.

Non-adrenergic relaxations of the rat stomach in the presence of muscarinic receptor blockade have been demonstrated previously (Heazell, 1974). Here it was confirmed that untreated rat stomachs contracted in response to vagal nerve stimulation and these contractions were converted to relaxations by the addition of hyoscine to the bathing fluid. Furthermore, the relaxations were seen in the presence of a combination of phentolamine and propranolol and were thus non-adrenergic in nature. It must be pointed out that the effect of phentolamine and propranolol on periarterial (sympathetic) stimulations was not investigated here. However, the drugs were used in concentrations (5μM phentolamine plus 10μM propranolol) which are almost double those sufficient to block responses mediated by catecholamines in other tissues (Day and Warren 1968).

In the rat stomach relaxations to vagal stimulation in the presence of pempidine alone, or pempidine plus phentolamine and propranolol, were also observed. As has been seen earlier with the guinea-pig stomach, these gastric relaxations, seen in the presence of pempidine alone were reduced by low concentrations (0.02μM) of hyoscine.

Because of the large changes in tone throughout the course of the experiments it was not possible to measure the responses corrected for changes in tone, but in view of the similarity of these results to those reported in CHAPTER 2 of this thesis, for the guinea-pig stomach, a genuine reduction in the size of the response had probably

been observed after the addition of hyoscine. It therefore, seems that the ganglia within the inhibitory nerve pathway of the vagi in rats, are also capable of transmission via muscarinic ACh receptors.

The mouse stomach differed from that of the guinea-pig and rat insofar as the response to vagal stimulations in untreated preparations showed a large relaxation. A similar response of the mouse stomach to field stimulation was shown by Bülbring and Gershon (1967 , FIG 10 inset). Furthermore, this relaxation was unaffected by large concentrations of phentolamine (26.5 μM) plus propranolol (33.8 μM).(at approximately ten times those used by Day and Warren (1968)).

Although this relaxation could be abolished by hyoscine, large concentrations (4.0 μM) of the drug were required to achieve this. It is likely therefore, that this reduction of the response was due to a non-specific action of hyoscine, especially since hyoscine has already been shown to have a non-specific activity at this concentration in the guinea-pig (CHAPTER 1). This relaxation during vagal stimulation is therefore, probably due to stimulation of non-cholinergic, non-adrenergic inhibitory nerves with the vagi similar to those already described in the guinea-pig and rat. The secondary motor response seen in the mouse after vagal stimulation was blocked by low concentrations of hyoscine. Pempidine completely abolished all phases of the response to vagal stimulation in the mouse. This is indicative of the presence of ganglia within the various vagal pathways but it is clear that these ganglia are only capable of transmission by nicotinic ACh receptors.

It was difficult to produce a response of the guinea-pig taenia caecum by stimulation of an attached flap of caecal

wall, and the stimulation parameters required were similar to those needed for field stimulation rather than direct nerve stimulation (in these experiments flap stimulation was at 10 - 40 Hz, 1 m s pulse width, 60 to 100 volts; compare this to vagal nerve stimulation at 10 - 40 Hz, 200 μs pulse width, 10 to 30 volts). The effect of drugs on responses of this preparation to caecal flap stimulation was in all ways identical to the effect of drugs on responses to field stimulation. It seemed, therefore that with the electrodes used, which were unshielded platinum rings, the responses observed were due to field stimulation and not preganglionic nerve stimulation. The spontaneous activity in the innervated longitudinal muscle preparation from the guinea-pig stomach was clearly myogenic being unaffected by all the drugs tried, including Ttx. This amount of spontaneous activity was not observed in the whole stomach (Chapter 1 and 2) so it is possible that in the intact stomach the longitudinal muscle received an inhibitory input from within the layers of circular muscle which are removed in the dissection.

Adrenaline causes a relaxation of the whole stomach (CHAPTER 6), but contracted the longitudinal muscle preparation, this action being mediated by alpha- adrenoceptors (blocked by phentolamine but not propranolol). The strip of longitudinal muscle chosen lies between the two sphincters of the stomach and it may be that the muscle strip taken has some resemblance to sphincter tissue, at least in its response to catecholamines. Contractions to adrenaline have also been observed in the rabbit stomach serosal strip preparation (Khayyal, Tolba and El-Hawary, 1976); these contractions also being mediated by alpha-adrenoceptors.

SUMMARY.

1. Non-cholinergic, non-adrenergic inhibitory fibres have been shown to occur in the vagal supply to the stomach of the rat. There are ganglia within this pathway which are capable of transmission by ACh via both nicotinic and muscarinic receptors.

2. Non-cholinergic, non-adrenergic inhibitory fibres have been shown to occur in the vagal supply to the stomach of the mouse. There are ganglia within this pathway but transmission can only occur by ACh acting on nicotinic receptors.

3. Strips of longitudinal muscle from the lesser curvature of the guinea-pig stomach contract to adrenaline and this response is mediated by alpha-adrenoceptors.

CHAPTER 6.

The role of Bradykinin in non-cholinergic non-adrenergic gastric inhibition.

INTRODUCTION.

The identity of the transmitter released from non-cholinergic, non-adrenergic inhibitory nerves remains controversial. A considerable amount of evidence has been presented by Burnstock (1972) that the most likely candidate is an adenine nucleotide such as ATP (see general introduction for details).

In 1975 Fasth, et al., reported that Bk injected intravenously into the anaethetised cat, produced a relaxation of the stomach which was similar to the effect of stimulating vagal non-cholinergic, non-adrenergic nerves. More recently Gillespie and McKnight (1978) have suggested that Bk or a related peptide may play a part in the inhibitory response to nerve stimulation in the anococcygeus muscle of the rat, cat and rabbit.

In view of these results the actions of Bk have here been re-examined in the isolated whole guinea-pig stomach, and the responses compared to relaxations caused by vagus nerve stimulation and ATP.

RESULTS.

Relaxation of the isolated guinea-pig stomach to BK.

When the stomach was bathed in McEwen's (1956) solution (containing 1.1mM calcium chloride) the responses to Bk were variable in form and size. In 57 out of 69 experiments a contraction was observed which was preceded by a transient relaxation. In the remaining 12 experiments only a contraction was seen. The stomach gave responses to Bk within a concentration range of 0.4 to 9.6 nM. There was a wide variation in the size of the relaxation seen with the lower concentrations, such that it was found impossible to construct a meaningful concentration/response curve. Maximum relaxations to Bk were produced with concentrations ranging from 6.4 to 9.6 nM.

Hyoscine (0.23 μM) had no effect on the size or form of the response to Bk but enabled a comparison to be made between relaxations produced by Bk and those seen during vagal stimulation. In the presence of hyoscine (0.23 μM) the maximum relaxation produced by Bk was 30.2±2.8% (mean ± s.e. mean; n = 18) of that produced by supramaximal vagal stimulation.

Seven experiments were performed with the stomach bathed in Krebs' solution containing 2.6 mM calcium chloride. Responses to Bk similar to those seen in McEwen's were obtained. If the calcium chloride concentration of the Krebs' solution was raised to 5.2 mM a relaxation to Bk was seen in all (n = 35) experiments, in only 14 of these was the relaxation followed by a contraction. FIG 5.1 illustrates the form of the response in the various bathing solutions. A table summarising the type and incidence of the form of response observed in the different bathing

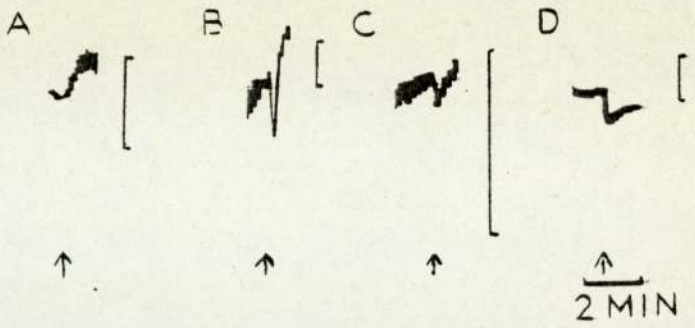


FIG 6.1

FIG 6.1. Examples of responses of the isolated whole guinea-pig stomach to bradykinin in various bathing solutions.

A contractile response to 4.0 n M bradykinin in McEwen's ringer solution.

B biphasic response to 8.0 n M bradykinin in McEwen's ringer solution

C biphasic response to 3.2 n M bradykinin in Krebs' ringer solution containing 2.6 m M calcium.

D relaxation to 3.2 n M bradykinin in Krebs' ringer solution containing 5.2 m M calcium

Drugs added at points indicated by arrows

Vertical bars are 0.5 kPa.

The responses shown were obtained from different preparations.

Bathing Solution	Type of Response	Number of times observed	% of total responses observed in each bathing solution
McEwen's	contraction	12	17.39
	biphasic	57	82.26
	relaxation	0	0
Krebs' containing 2.6 mM calcium	contraction	0	0
	biphasic	7	100.00
	relaxation	0	0
Krebs' containing 5.2 mM calcium	contraction	0	0
	biphasic	14	40.00
	relaxation	21	60.00

TABLE 6.1. Type and incidence of responses of the isolated whole guinea-pig stomach to bradykinin in different bathing solutions.

solutions is given in TABLE 6.1.

As before hyoscine (0.23 μM) had no effect on the size or form of the responses. In those experiments performed in 5.2 mM calcium chloride Krebs' with hyoscine (0.23 μM) present, the maximum relaxations produced by Bk were 40.8±3.5% (mean ± s.e. mean; n = 8) of those seen during supramaximal vagal stimulation. There was no significant ($P>0.01$) difference in size to those produced in McEwen's (1956) ringer.

As with McEwen's solution there was a large variation in the size of the responses to the lower concentrations of Bk making it impossible to construct a meaningful concentration/response curve.

Maximum sized relaxations were obtained with concentrations from 6.4 to 9.6 n M Bk in either Krebs' solutions.

All experiments subsequently described in this chapter were performed separately in both McEwen's (1956) solution (1.1 mM Ca^{2+}) and Krebs' solution containing 5.2 mM Ca^{2+}. There was no qualitative difference in the results obtained using either bathing solution.

Effect of drugs on relaxations of the isolated guinea-pig stomach produced by Bk.

Relaxations of the stomach produced by Bk were seen in the presence of Ttx (n = 16) in a concentration (5.7 μM) which abolished relaxations produced by vagal stimulation, (FIG 6.2).

Combinations of phentolamine (5.0 to 50.0 μM) and propranolol 15.0 to 50.0 μM) greatly reduced submaximal responses to adrenaline (2.0 to 4.0 μM) but were ineffective in preventing relaxations to Bk (FIG 6.3), reducing the response

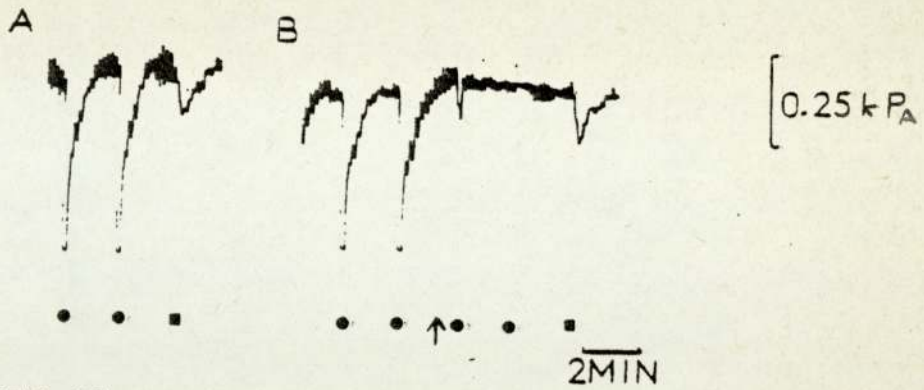


FIG 6.2

FIG 6.2. The effect of tetrodotoxin on responses of the isolated whole guinea-pig stomach to vagal stimulation and bradykinin. 0.23μM. hyoscine present throughout.

Experiment performed in McEwan's solution

Closed circles indicate point of supramaximal vagal stimulation.

Closed squares indicate point of addition of 3.2 n M bradykinin.

A = initial responses

B = the effect of addition of 5.7μM tetrodotoxin. Point of addition is shown by the arrow.

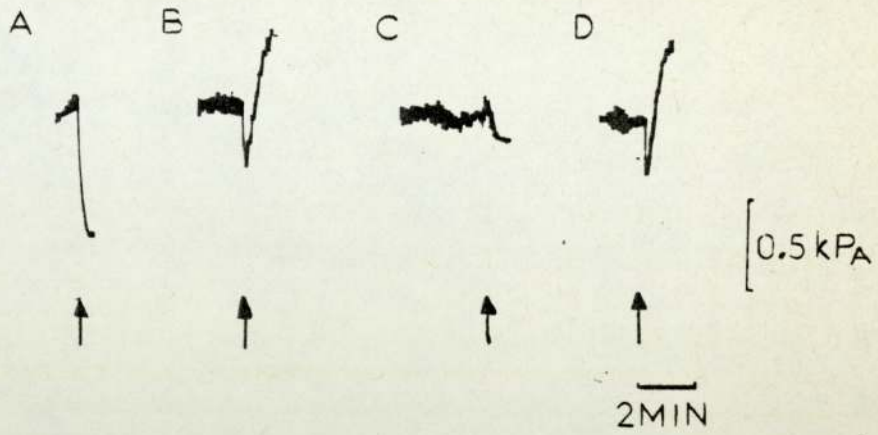


FIG 6.3

FIG 6.3. The effect of phentolamine plus propranlolol on responses of the isolated whole guinea-pig stomach to adrenaline and bradykinin.

Experiment performed in McEwans ringer solution

Arrows indicate points of addition of drugs.

A initial response to 5.0 μM adrenaline

B initial response to 4.0 nM bradykinin

C response to 5.0 μM adrenaline in the presence of 10.0 μM phentolamine plus 10.0 μM propranolol

D response to 4.0 nM bradykinin in the presence of 10.0 μM phentolamine plus 10.0 μM propranolol

to Bk by 38.5±2.6% (mean ± s.e. mean;n = 5). This difference was significant (P<0.01). Indomethacin (14.0 and 28.0μM) did reduce the size of the relaxations to Bk and nerve stimulation, (FIG 6.4) but this reduction could be accounted for by the reduction in the tone of the preparation caused by indomethacin.

Desensitisation to Bk.

Hyoscine (0.23μM) was present in all experiments. A virtually complete desensitisation could be achieved by exposing the preparation to three additions of Bk, (90.6±5.2% (mean ± s.e. mean;n = 13) reduction of the relaxation caused by Bk, each addition (6.4 nM) given at two minute intervals in a cumulative manner either in McEwen's (1956) solution (when a relaxation to Bk was present) or double (5.2 mM) calcium Krebs' At the same time it was noted that relaxations to ATP (10μM) were reduced by 89.4±3.7% (mean ± s.e. mean;n = 13) and this reduction did not significantly differ from that observed for Bk (P>0.05).

Immediately following this demonstration of Bk desensitisation and with Bk still present, relaxations to vagal stimulations were reduced by 24.2±7.7% (mean ± s.e. mean;n = 11) and those to adrenaline (2.0μM) by 25.9±7.6% (mean ± s.e. mean;n = 5). The reduction of the response to vagal stimulation did not differ significantly to that seen with adrenaline (P>0.05), but did differ from the reductions of the responses to Bk and ATP (P<0.01). (FIG 6.5).

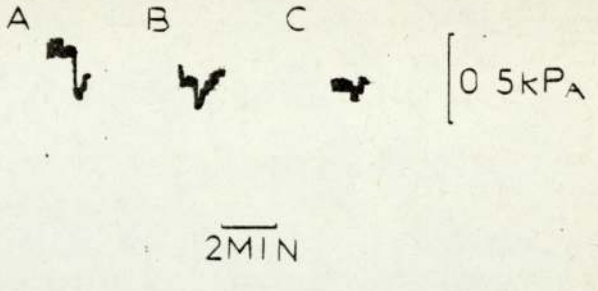


FIG 6.4

FIG 6.4. The effect of indomethacin on relaxation of the isolated whole guinea-pig stomach to bradykinin.

Experiment performed in Krebs' ringer solution containing 5.2mM calcium.

A initial response to 6.4 nM bradykinin

B response to 6.4 nM bradykinin after 1 hour exposure to 14.0 μM indomethacin

C response to 6.4 nM bradykinin after 1 hour exposure to 28.0 μM indomethacin

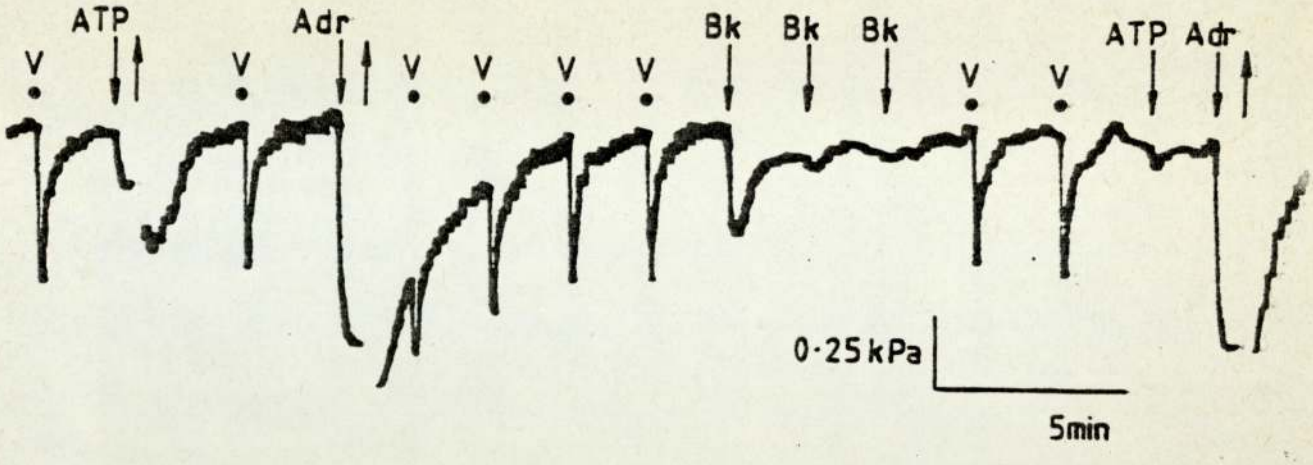


FIG 6.5

FIG 6.5 The effect of densensitation to bradykinin on responses to adrenaline, ATP, bradykinin and supramaximal vagal stimulation of the isolated whole guinea-pig stomach.

Arrows pointing downwards represent the addition of drugs to the organ bath. Arrows pointing upwards represent wash-out of the organ bath.
Adr = 2μM adrenaline added to the bath
ATP = 10μM ATP added to the bath
Bk = 6.4nM bradykinin added to the bath
V = supramaximal vagal stimulation

DISCUSSION.

Bk causes a contraction of most gastro-intestinal smooth muscle, but relaxations have been observed in the rat duodenum and biphasic responses (relaxations followed by a contraction) in rabbit duodenum, rat colon (Elliot, Horton and Lewis, 1960) and guinea-pig ileum (Hall and Bonta, 1973).

In this work either a biphasic (relaxation followed by a contraction) response, a contraction or a relaxation was observed in the guinea-pig stomach to Bk. The nature of the response was sensitive to the calcium concentration in the bathing medium. Raising the calcium concentration from 1.1mM (McEwen's) to 5.2mM (Krebs') increased the certainty with which a relaxation would be observed, and reduced the frequency with which an after contraction was observed.

The changing form of the response could be due to an action of calcium stabilising the membrane to depolarising stimuli (Bulbring and Kuriyama, 1963) and hence reducing the contraction to Bk and allowing the inhibitory component to be uncovered. Raising the concentration of calcium from 1.1mM (McEwen's) to 2.6mM (Krebs') was insufficient to have any demonstrable effect on the form of the response although only a relatively small number of experiments were performed in the latter solution. There have been other reports that relaxation to Bk in gastro-intestinal smooth muscle is sensitive to variations in the calcium concentration of the bathing solution. In rat duodenum lowering the calcium concentration from 4.0mM to 0.5mM caused a contraction to appear, when only a relaxation

had been observed before (Antonio, 1968). Aarsen and Van Caspel de Bruyn (1970) have also reported that the initial relaxation phase of the biphasic response of guinea-pig taenia caecum to Bk was abolished by lowering the calcium concentration.

Bk has the ability to stimulate nerves (Della Bella, Benelli and De Pauli, 1972) but in view of the persistance of the response to Bk in the presence of Ttx, which abolished relaxations to vagal stimulation, Bk must have been acting directly on the smooth muscle cells.

Bk is known to be capable of releasing catecholamines, at least from the adrenal medulla (Feldberg and Lewis, 1964) but the relaxations described here were produced in the presence of high concentrations of phentolamine and propranolol, which greatly reduced the response to adrenaline and were therefore, not due to the release of catecholamines by Bk.

Prostaglandins have been suggested to play a role in the relaxant action of Bk in the rat duodenum (Walker and Wilson, 1979). However, the relaxation to Bk was not reduced by indomethacin in the experiments described here. The response to Bk was unaffected by hyoscine, and it was therefore, possible to compare the relaxation produced by Bk to that produced by vagal nerve stimulation.

The small size of the relaxation to Bk compared to that of vagus nerve stimulation in the presence of hyoscine could be taken as evidence that Bk or a similar peptide is unlikely to be the inhibitory transmitter. It could be argued that exogenous drug does not have access to all the sites available to the endogenous transmitter. In contrast

to the observations described here Gillespie and McKnight (1978) found that the magnitude of the Bk response was similar to that produced by nerve stimulation in the anococcygeus muscle of rats, rabbits and cats

During desentisation to Bk, relaxations to Bk were almost completely abolished, whereas the diminution of responses to vagal nerve stimulation was small, and similar reductions of the response to adrenaline were noted. This suggests that the reduction of the response to nerve stimulation, and adrenaline was a non-specific effect.

The persistance of the nerve mediated relaxations, in the presence of Bk desensitisation making it improbable that Bk could be the gastric non-cholinergic non-adrenergic inhibitory transmitter.

The occurrence of cross-desensitisation between Bk and ATP suggest that they share a similar mechanism which is not utilised by adrenaline to produce gastric relaxation. As with Bk, relaxations to ATP were small compared to those due to vagal stimulation being similar in magnitude to those produced by Bk. Furthermore, relaxation to vagal nerve stimulation persisted when the response to ATP was greatly reduced.

These limited observations therefore, do not support the concept of ATP as the inhibitory transmitter in the pathway.

An observation that desensisation to ATP did not reduce the inhibitory response to intramural nerve stimulation in rabbit duodenum was made by Weston (1973) and he favoured the opinion that ATP could not be the inhibitory transmitter in view of this finding.

Burnstock, Campbell, Satchel and Smythe (1970) could not observe desensitisation to ATP in the guinea-pig taenia caecum but did see desensitisation in Finkleman (rabbit intestine) preparations but in this case, in three preparations the inhibitory response to transmural stimulation was abolished.

It may be that exogenous 'transmitter' substances do not have access to the same receptor sites as the endogenous transmitter and therefore, desensitisation to exogenous transmitter substances may not significantly affect the receptors activated by inhibitory nerve stimulation. It seems hard to imagine how this could be so and indeed in the guinea-pig ileum desensitisation to the transmitter substance ACh prevents the cholinergic excitatory component of the peristalic reflex (Schaumann, Jochim, and Schmidt, 1953).

SUMMARY.

1. Bk is capable of causing relaxations of the isolated guinea-pig stomach, and the certainty of observing the inhibitory response is increased in solutions containing higher than normal calcium concentrations, (5.2 Of 2.6 mM in normal Krebs').

2. The action of Bk is directly onto the smooth muscle, and is not due to an action on nerve fibres, or due to the release of catecholamines; nor is it mediated by prostaglandins.

3. The relaxation to Bk is smaller (30 - 40%) than that seen to vagal stimulation in the presence of hyoscine. The relaxation to ATP is similar in magnitude to that produced by Bk.

4. When desensitisation to Bk was produced in the guinea-pig stomach a reduction in response to ATP was also observed, whereas responses to inhibitory nerve stimulation (in the presence of hyoscine) and adrenaline were little affected. It seems likely therefore, that Bk and ATP share a common step in the production of gastric relaxation which is not utilised by adrenaline or the inhibitory transmitter released from non-cholinergic non-adrenergic nerves.

CONLUDING REMARKS

Many of the studies of non-sympathetic inhibition of the gut have been performed using field or transmural stimulation to demonstrate the non-adrenergic inhibitory response.This type of stimulation exites post-ganglionic nerves.By utilising the isolated whole guinea-pig stomach with the vagi attached,one is enabled to study the pathway through the ganglia.

When the vagi are stimulated,nerves which are both motor and inhibitory to the stomach are excited. The traditional method of demonstrating the inhibitory reponse has been to prevent the motor response with muscarinic receptor blocking drugs such as hyoscine or atropine and hence uncover the inhibitory response which is normally masked by the greater motor response. This method has been used by many workers (e.g. Campbell, 1966; Bülbring and Gershon, 1967; Beani et al, 1971; Heazell, 1974; Spedding, 1977; CHAPTER.I). These responses have been shown not to be due to stimulation of adrenergic nerves since the response was resistant to adrenergic neurone blocking agents (Campbell, 1966; Beani et al, 1971; Heazell, 1974; CHAPTER I) or by alpha and beta adrenoceptor blocking agents (Martinson , 1965b; Beani et al, 1971).

The relaxation of the stomach seen to vagal stimulation in the presence of muscarinic receptor blocking drugs is reduced by ganglion blocking drugs (Bülbring and Gershon, 1967; Beani et al, 1971: CHAPTER.1). This observation implies that ganglia are present within the inhibitory nerve pathway. The discovery in CHAPTER.2 that relaxations of the guinea-pig stomach could also be demonstrated in the presence of ganglion

blocking drugs alone has given an alternative method for demonstrating the vagal inhibitory response.

If it can be assumed that the adrenergic nerves were not involved in these responses (the response was found in CHAPTER 2 to be resistant to guanethidine), then they must have been due to either: (a) fibres traversing the ganglia without synapsing (or have synapses distal to the point of stimulation) or (b) fibres containing a ganglionic synapse utilising ACh as the transmitter but acting on different receptors or (c) fibres containing a ganglionic synapse but not utilising ACh as a transmitter at this synapse. The work in CHAPTERS 2 and 3 of this thesis support the concept of ganglionic muscarinic transmission and it is argued that such transmission seems to be unimportant in the ganglia of the motor pathway.

The ganglia contained within the sympathetic nerve pathway are capable of transmitting impulses via muscarinic ACh receptors (Eccles and Libet, 1961; Jones 1963; Trendelen - berg, 1966; Flacke and Gillis, 1968; Gillis et al, 1968). The main points of evidence that the inhibitory fibres to the guinea - pig stomach contain ganglionic muscarinic receptors is based on a comparison between the inhibitory vagal response in the presence of ganglion blocking drugs alone compared to responses to muscarinic transmission in sympathetic nerves.

The inhibitory response to vagal stimulation in the presence of ganglion blocking agents alone was found to be sensitive to low concentrations of hyoscine. Further more the latency at the response in the presence of ganglion blockers alone was found to be longer than that

seen with hyoscine alone as had been observed for muscarinic transmission in sympathetic nerves (Flacke and Gillis, I968) . Takeshige and Volle (I962) have shown that muscarinic transmission is selectively potentiated by physostigmine. It was not possible to conclusively show potentiation at the response to vagal stimulation in the presence at ganglion blocking drugs alone due to the in - crease in tone of the guinea - pig stomach produced by this drug, but if the responses were examined without reference to tone changes a potentiation of the response was evi - dent.

Inhibitory reponses to vagal stimulation in the presence of ganglion blocking drugs alone was seen in CHAPTER 5 in the isolated whole rat stomach and this was also sensitive to hyoscine. This was not the case in the mouse where no role for ganglionic muscarinic transmission could be advanced. Work in other species is required to see if this type of transmission is common,or whether it is peculiar to guinea - pigs and rats.

Final proof of transmission via muscarinic receptors in the non-adrenergic inhibitory ganglia and its physio - logical significance must await detailed electrophysio- logical study. Because of the nature of these ganglia in the form of a plexus, this will involve micro - electrode penetration of the ganglion cells.

Bülbring and Gershon (I967) had claimed a role for 5HT as a ganglionic transmitter within the inhibitory vagal path- way to the stomachs of guinea - pigs and mice. However desensitisation of the stomach to the action of 5-HT caused no reduction of the inhibitory responses to vagal stimulation (CHAPTER 3), an observation in agreement with

Beani et al (I97I). It therefore seems that 5-HT has no part to play in the inhibitory response of the stomach to vagal stimulation. Gershon and Ross (I966) have shown 5-HT is present in the myenteric plexus. Further investigations into the purpose of this store of 5-HT are required, before 5-HT can be entirely dismissed as a neurotransmitter in the non-cholinergic nervous pathways.

The form of the response to vagal stimulation differed with ganglion blockers alone to that seen with hyoscine alone. With hyoscine alone, following cessation of stimulation after an initial fast recovery phase the tone returns to the baseline slowly. In contrast, with ganglion blockers alone, the relaxations were followed by an immediate return to the baseline or an after contraction. The after contraction was potentiated by low concentrations of physostigmine and abolished by low concentrations of hyoscine. It was therefore suggested that the after contraction seen could be due to ACh released pre-ganglionically diffusing to muscarinic receptors on the smooth muscle. The after contraction was therefore a different phenomenon to the rebound contraction seen after stimulating intramural inhibitory nerves described by Bennett (I966).

Burnstock et al (I975) had suggested that PGs may be involved as a factor in the rebound contractions following intramural nerve stimulation seen in the guinea - pig taenia caecum, therefore the effect of indomethacin and PGs upon the responses to vagal stimulation in the pre - sence of hyoscine or pempidine alone was examined in CHAPTER 4 to see if any explanation for the differences in the form of the response seen with ganglion blockers alone to that seen with hyoscine alone could be offered. As a

result of these investigations it was possible to divide the inhibitory response to vagus nerve stimulation of the guinea-pig stomach into four component phases (Fig 4.6):

a). a non - cholinergic, non-adrenergic relaxation seen with hyoscine or ganglion blocking drugs alone.

b). a rapid recovery phase mediated by PGE_2, but with no muscarinic component and not affected by papaverine, seen with both hyoscine or ganglion blocking drugs alone.

c). a slow recovery phase seen in the presence of hyoscine alone, but not in the presence of ganglion blocking drugs alone of unknown mediation.

d). an after contraction seen in the presence of ganglion blocking drugs alone but not hyoscine alone, mediated by PGE_2, and also having a muscarinic component blocked by papaverine.

Phase B most closely resembles the rebound contraction seen in the guinea-pig taenia caecum and is probably a similar phenomenon.

PGE_2 therefore appeared to play a role in two distinct processes, one having a muscarinic component, and the other being non-muscarinic.

Papaverine was seen only to inhibit those processes involving PGE_2 if they also involved a muscarinic component. The drug may therefore prove to be useful for making this distinction in the future. This is of special interest in view of recent reports that PGs can act as modulators in responses to ACh at muscarinic receptors involved in the nerve mediated response of the guinea - pig ileum (Bennett Eley and Stockley, 1975).

The slow recovery phase C seen only in the presence of hyoscine is of unknown origin, but it could be due to a

long acting inhibitory substance released during nerve stimulation or it would be the normal rate of recovery of the smooth muscle from inhibitors unaffected by humoral agents.

Burnstock (I972) has produced considerable evidence to support the hypothesis that this final transmitter is ATP. However , Fasth et al, (I975) and Gillespie and McKnight (I978) have suggested that Bk or a related peptide may play a part in non - cholinergic , non - adrenergic relaxations. The affect of Bk on the guinea - pig stomach compared to ATP was therefore examined in CHAPTER 6. Bk could have produced relaxation by stimulating the non - sympathetic inhibitory ganglion cells or nerve terminals but it was shown to produce relaxations of the isolated whole guinea-pig stomach by an action directly on the smooth muscle cells since the response was unaffected by Ttx. Relaxations to Bk were also unaffected by combinations of alpha- or beta-adrenoceptor blocking compounds and was therefore unlikely to be due to a release of catecholamines or sympathetic nerve stimulation. PGs do not appear to play a role in the relaxation to Bk in the guinea - pig stomach as has been observed for the contraction of rat isolated ileum (Crocker, Walker and Wilson, I978)and the relaxation of rat duodenum (Walker and Wilson, I979), since the response to Bk was un-affected by indomethacin at similar concentrations to those used by Crocker et al (I978).

Relaxations produced by Bk and ATP were much smaller (~30 - 40%) than those seen to vagal stimulation in the presence of hyoscine. It may be argued that exogenous transmitter substances do not have access to all the sites that endog-enous transmitter has, and hence the response is smaller.

However in CHAPTER 6 other observations were made that do not support the view of Bk being the transmitter sub - stance. Desensitisation to Bk gave little reduction of the responses to inhibitory vagal stimulation (in the presence of hyoscine) or Adr, but cross desensitisation to ATP was produced. It seems likely from these observations that Bk and ATP share a common step in the production of gastric relaxation which is not utilised by Adr, or the inhibitory vagal transmitter. Weston (1973) has made a similar observation insofar as he saw no reduction in the response to intramural nerve stimulation of the rabbit duodenum following desensitisation to ATP.

It seems unlikely that Bk or ATP could be the non-cholinergic non-adrenergic transmitter in view of these results.

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