A RE-EVALUATION OF THE USE OF GLYCERYL TRINITRATE TO ASSESS THE DEGREE OF ADRENERGIC BETA RECEPTOR BLOCKADE

T.J. STALLARD

Part-time External Candidate for M.Phil. Degree

The University of Aston in Birmingham

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SUMMARY

T.J. STALLARD, M.Phil. Degree, 1982

Adrenergic beta receptor blocking drugs have become an established part of medical practice. They are used primarily in the management of hypertension, ischaemic heart disease and cardiac arrhythmias. Since beta blockade is to a large extent dose dependent, it is important to determine whether sufficient of the drug has been given to inhibit the effects of endogenously released catecholamines. The most common test employed in clinical practice is to measure the heart rate response during dynamic exercise.

Unfortunately, formal exercise testing is not a simple procedure. In 1979, Fitzgerald introduced a new test of the degree of adrenergic beta receptor blockade using Glyceryl Trinitrate. From his studies, Fitzgerald concluded that the positive chronotropic effects of Glyceryl Trinitrate administration were due solely to sympathetically mediated reflexes. This conclusion, however, has been questioned by Hansson, Kofi Ekue and Zweifler; all suggesting that vagal withdrawal may also contribute a part in this response.

My studies have shown that both vagal withdrawal and sympathetic stimulation are involved in the heart rate response to sublingual Glyceryl Trinitrate administration. Also, as people age there appears to be a bigger fall in arterial blood pressure and relative blunting of the heart rate response, probably reflecting relative insensitivity of the adrenoceptors in the arterial tree and the heart, resulting in a failure of the sympathetic nervous system to compensate for the fall in pressure.

The Glyceryl Trinitrate induced tachycardia compares favourably with the tachycardia induced by sub-maximal bicycle exercise. The Glyceryl Trinitrate test was found to be very sensitive in detecting low levels of beta blockade, and would, therefore, be very useful, clinically, in outpatient departments or general practice to assess the degree of adrenergic beta blockade.

Key words: Glyceryl Trinitrate, Bicycle Exercise, beta blockade, hypertension.

ACKNOWLEDGEMENTS

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HISTORICAL REVIEW

I.1. Beta Adrenergic Blockade and its Clinical Assessment.

Adrenergic beta receptor blocking drugs (Beta-Blockers) have become an established part of medical practice. They are used primarily in the management of hypertension, ischaemic heart disease and cardiac arrhythmias. The discovery of beta-blockers vindicated Ahlquist's hypothesis (1948) that two adrenergic receptors existed, which would explain some of the actions of the catecholamines. Ahlquist proposed that the alpha receptor was associated with excitatory responses, except those of the heart; and the beta receptor with cardiac excitation and smooth muscle relaxation.

From the clinical viewpoint beta receptors are associated with positive chronotropic and inotropic adrenergic effects together with vasodilatation of skeletal muscle, relaxation of bronchial smooth muscle lipolysis and hepatic glycogenolysis, (whereas the alpha receptors are associated with vasoconstriction). Several years ago Lands, Arnold McAuliff, Luduena and Brown (1967) proposed the existence of at least two types of beta receptors, and adopted the terminology of Beta I for the myocardial receptors and Beta II for smooth muscle receptors. This idea did not receive the attention it deserved until the beta-blocking agent Practolol was discovered. The first generally recognised beta-blocker was dichloroisoproterenol (DCI), discovered by Powell and Slater (1958), and its action as a beta-blocker was proved by Moran and Perkins (1958), who also coined the name adrenergic beta receptor blocking agents. The use of beta blockade in the treatment of angina pectoris was first suggested by Black and Stephenson (1962); Pronethalol (naphthyl-isoproterenol) was the first specifically designed beta blocker, but was

proved to have carcinogenic action in mice by Paget (1963). Black, Duncan and Shanks (1965) were the first to introduce a beta-blocker, Propranolol, into clinical practice.

There are now many beta-blockers in clinical use, (Table 1), and a few others still being tested. Some of the beta-blockers are partial agonists, and so are said to have intrinsic sympathomimetic action (ISA); this is characterised by an ability to stimulate the beta receptors which have been blocked from extrinsic sympathetic influences. Some of the beta blockers have a local anaesthetic action, this is called the membrane stabilizing action (MSA) or the Quinidine-like action; and indicate a slowing of the rate of rise, and prolongation, of the action potential in myocardial cells. Other beta-blockers are cardio-selective, this being manifested in the blocking of cardiac beta receptors with little or no effect on the beta receptor sites of the bronchial tree and peripheral vasculature.

As far as can be determined all the beta-blockers are clinically equivalent, though they may differ in dosage schedules, and it appears that their therapeutic effectiveness results from their beta adrenoceptor blocking characteristic. Intrinsic sympathomimetic action and membrane stabilizing action do not appear to contribute to the effects of these drugs, although cardio selectivity may be of value in some patients (Conolly, Kerstiny and Dollery (1976); Hamer (1976)).

Pharmacologically, the beta-blockers act competitively by preventing the beta adrenergic receptors from responding to their natural transmitters. Thus, a beta-blocker would generally produce bradycardia, myocardial weakening, increased airways resistance, increase in peripheral resistance and some modification of the metabolic processes. Bradycardia always occurs, and a drug that does not slow a normal or fast heart rate, has no significant beta antagonist action. For any cardio-

vascular use the first dose of a beta-blocker is potentially the most dangerous, as this dose will induce an unknown amount of beta blockade in an unknown environment of adrenergic activity. Therefore, the first dose should be small and once started increases by up to 25% are not dangerous, and therefore the precipitation of heart failure is unlikely.

Since beta blockade is to a large extent dose dependent, it is important to determine whether sufficient drug has been given to inhibit the effects of endogenously released catecholamines. The activity of beta-blockers can easily be demonstrated in man; the inhibition of the tachycardia produced by an intra-venous infusion of Isoprenaline (Brick, Hutchinson, McDevitt, Roddie and Shanks (1968); Dollery, Paterson and Conolly (1969); and Aellig and Pritchard (1970)), or the inhibition of the tachycardia produced by exercise (Brick, Hutchinson, McDevitt, Roddie and Shanks (1968); and Coltart and Shand (1970)) have been widely used. However, the test most commonly employed in clinical practice is to measure the increase in heart rate during dynamic exercise. When the exercise induced tachycardia is used to test the effectiveness of beta adreno receptor blockade, it is important to achieve a level of exercise so much that vagal withdrawal is complete. In a subject exercised so that vagal withdrawal is complete, cardiac beta blockade will result in a heart rate equal to the so called "intrinsic heart rate" of that particular subject; in other words, a heart rate at which there are no sympathetic and parasympathetic influences.

Unfortunately, formal exercise testing (and the use of intra-venous Isoprenaline) is not a simple procedure, it requires specialised equipment and the presence of medical and para-medical personnel, and it is not easily applicable for use in outpatient clinics or General Practitioners surgeries. In 1970 Fitzgerald introduced a new test of the degree of beta receptor blockade using Glyceryl Trinitrate (GTN). From his

studies, under various conditions, Fitzgerald concluded that the positive chronotropic effects of GTN were due solely to reflex activation of sympathetic nerves. As the heart rate increase to a clinical test, which assesses the degree of beta blockade, must be due only to catecholamine release, and not to any other mechanism, such as vagal withdrawal, Fitzgerald suggested that advantage may be taken of the GTN reflex to determine the degree of beta adrenergic blockade. Also, he suggested this test as being suitable in patients who were unable to exercise, or to whom one did not wish to administer intra-venous Isoprenaline.

I.2. Glyceryl Trinitrate (GTN).

a. Chemistry.

GTN is a pale yellow oil at room temperature, its melting point being 13.2°C. The solubility of GTN in water is 1.2 to 1.8 grams per litre; and its solubility in ethyl alcohol is 350 grams per litre at 30°C.

In my studies I have used GTN in two forms:-

i) As tablets containing 0.5mg GTN, BP, for sublingual use. As GTN is inactivated by light the tablets are stored in a darkened bottle and at a cool temperature, the tablets being replenished monthly from the hospital pharmacy department.

ii) As an intra-venous solution of 5mg GTN, BP, in a ten mls solution (Lactose BP - 90% W/V and Alcohol BP - 10% V/V Arnar-Stone Laboratories), this being diluted with normal, sterile saline. The solutions are stored in sterile glass ampoules in a refrigerator and have a "shelf-life" of two years.

b. Chronology of Glyceryl Trinitrate Therapy.

GTN was first synthesised by Sobrero (1847) in 1846, by mixing cold

nitric acid and sulphuric acid with glycerine; on application of a small amount to his tongue, he reported severe throbbing of his temples. Later, Burton (1867) and Murrell (1879) described the ability of amyl nitrite and GTN respectively, to relieve the symptoms of angina pectoris.

The duration of the therapeutic effectiveness of sublingual GTN is measured in minutes, Davis and Wiesel (1958) used the concept of prolonged therapy by cutaneous application of GTN. Use of a 2% GTN ointment for the treatment of angina pectoris was reviewed by Reichek, Goldstein, Redwood and Epstein (1974), and for the treatment of acute myocardial infarction by Armstrong, Mathew, Doroomand and Parker (1976). Oral GTN preparations have found little favour, as their effectiveness is open to doubt, Needleman, Lang and Johnson (1972) postulated that oral GTN would undergo inactivation by the liver, thus precluding any possible clinical role for these agents. However, recent studies by Danahy and Aronow (1977), Glancy, Tichter, Ellis and Johnson (1977) and Danahy, Burwell, Aronow and Prahash (1977) have shown that oral GTN may be effective if the doses are adequate. Intra-muscular GTN administration during cardiac surgery was introduced by Viljoen (1968).

Intra-venous GTN has been used extensively by Flaherty, Reed, Kelley, Taylor, Weisfeldt and Pitt (1975); Armstrong, Walker, Burton and Parker (1975); Miller, Vismara, Williams, Amsterdam and Mason (1976); and Derrida, Sal and Chiche (1977), in the treatment of patient with acute myocardial infarction. It has also been used in relieving coronary artery spasm by Oliver, Potts and Pluss (1973), and in relieving angina pectoris by Kaplan, Dunbar and Jones (1976). Recently, intra-venous GTN has been used during coronary artery surgery to control angina, by Kaplan, Dunbar and Jones (1976); and Hemplemann, Piepenbach, Seitz and Karliczek (1977). Sublingual or topical GTN has been previously administered, however, neither of these therapeutic routes can be well controlled.

c. Action of Glyceryl Trinitrate.

The basic pharmacological action of GTN is to relax smooth muscle, this relaxation is non-specific and affects all smooth muscle. The most prominent and important actions of GTN, however, are on the vascular smooth muscle, and gives rise to the haemodynamic changes responsible for its therapeutic effectiveness.

Although there is no doubt about the effectiveness of GTN in angina pectoris, there is still controversy about its mechanism of action. Observations from a study by Boyer and Green (1941) led to the belief that the action was due to coronary vasodilatation. The most important clinical evidence that the coronary-dilator hypothesis is untenable came from Ganz and Marcus (1972). It was concluded that GTN probably relieves the pain of angina pectoris by reducing myocardial oxygen demands as coronary blood flow was decreased. It is now thought that the action of GTN is by dilatation of the venous peripheral circulation, with subsequent venous pooling, resulting in decreased venous return. Venous pooling causes a fall in systemic pressure, producing a reflex increase in heart rate (Ferrer, Bradley, Wheeler, Enson, Preisig, Brickner, Conroy and Harvey (1966)).

GTN is rapidly absorbed from the sublingual mucosa, its onset of action occurring within two to five minutes after administration. Maximal effects occur within three to fifteen minutes, and there is little residual activity after twenty to thirty minutes, post administration. GTN is rapidly and efficiently metabolised in the liver by the enzyme glulathione organic nitrate reductase; the metabolites having no vaso-dilator potency (Abrams, 1980).

I.3. Fitzgerald's Glyceryl Trinitrate Test.

Fitzgerald studied eight, normal, male subjects (age range -21 to 51 years); heart rate, determined by means of a cardio-tachometer, was observed for six minutes after each administration of sublingual GTN, and the highest heart rate obtained in any one minute was taken as being that subject's maximal response. The effects of GTN on heart rate responses was studied with the subjects in the standing position.

Fitzgerald found that there was little variation in heart rate increase to repeated GTN challenges for a given individual, (heart rate increase difference : range, 2 to 14 beats per minute; mean, 7 ± S.E.M. 1.7); but a considerable range of responses between subjects (range, 22 to 48 beats per minute; mean, 33 ± 1.6). When six subjects were challenged with GTN, ten minutes after receiving atropine sulphate (2.5mg infused intra-venously), he found that there was no significant difference in the mean rise in heart rate to GTN administration before and after atropine infusion, (before atropine: 32 ± 3.1; after atropine: 29 ± 3.9). He therefore concluded that vagal withdrawal did not play a significant role in the heart rate increase to GTN administration. Since progressive reduction in the tachycardia due to GTN administration followed increasing doses of beta-blockers in his studies, he suggested it supported the view that the GTN induced tachycardia was due, solely, to sympathetic activity.

However, Fitzgerald did not compare his GTN tests with either formal exercise testing or with Isoprenaline infusion; the commonest clinical tests for assessing the degree of beta blockade. Nor did he study the blood pressure response to GTN administration.

I.4. Nature of the Project.

The GTN test appears to be a relatively easy test to perform, as it does not require elaborate equipment, or specialised personnel; the heart rate can be simply recorded by radial palpation.

There is some debate (Hansson (1973); Kofi Ekue, Shanks and Walsh (1974); and Zweifler and Hansson (1976), as to the conclusion made by Fitzgerald, that the heart rate increase to sublingual GTN administration is due solely to sympathetic stimulation. I have therefore studied the effects of both partial cardiac autonomic blockade, using an intra venous solution of atropine sulphate, and total cardiac autonomic blockade, using intra venous solutions of atropine sulphate and practolol. The purpose of the first study is to try and reproduce Fitzgerald's tests and of the second study to determine if only the sympathetic system is involved in the heart rate increase to sublingual GTN administration or if vagal withdrawal is also involved.

I have also compared the heart rate increase and tachycardia produced by sublingual GTN administration to that produced by bicycle ergometry, the most common test of beta adrenergic blockade, before and after administration of Beta blockers. Also, I have studied the effects of an hypotensive agent, which is not a beta-blocker, but similar to a diuretic (Natrilix-Indapamide) to determine if the heart rate and blood pressure response to sublingual GTN alone are influenced by a decreased pressure. Lastly, I have studied the effects of age upon the heart rate and blood pressure responses to sublingual GTN administration.

CHAPTER II

METHODS

II.1. Patient Selection

Because I am not medically qualified, I have relied on the advice and clinical guidance of Professor W.A. Littler and his registrars in selecting subjects and patients for my work. The patients were all under the care of Professor Littler and the clinical diagnosis had been established before I studied them. Professor Littler had submitted the protocol of the studies, of which mine formed a part, to the East Birmingham Hospital Ethics Committee and had received its approval. The registrars were responsible for insertion of venous and arterial cannulae into the patients, since I was performing parallel studies on the patients being studied; however, all the measurements with regard to the GTN and bicycle exercise tests were made exclusively by myself.

II.2. Recording Technique

a) Electrocardiogram and Heart Rate

An electrocardiogram (E.C.G.) was recorded on all subjects and patients, during each test, using chest electrodes placed in a modified $V5_L$ recording position (three electrodes positioned such that one is over the apex of the heart so as to produce a strong signal, the second is positioned on the right shoulder but away from the muscle mass to eliminate interference from muscle activity, and the third electrode is used as an earth reference on the right lower side). The ECG is recorded throughout the test, for the majority of the time the speed is set to 2.5 MM/S , but for the last twenty seconds of each minute during the test, and for baseline measurements the speed is set to 10 MM/S for measurement and calculation purposes at a later time. The heart rate, during the

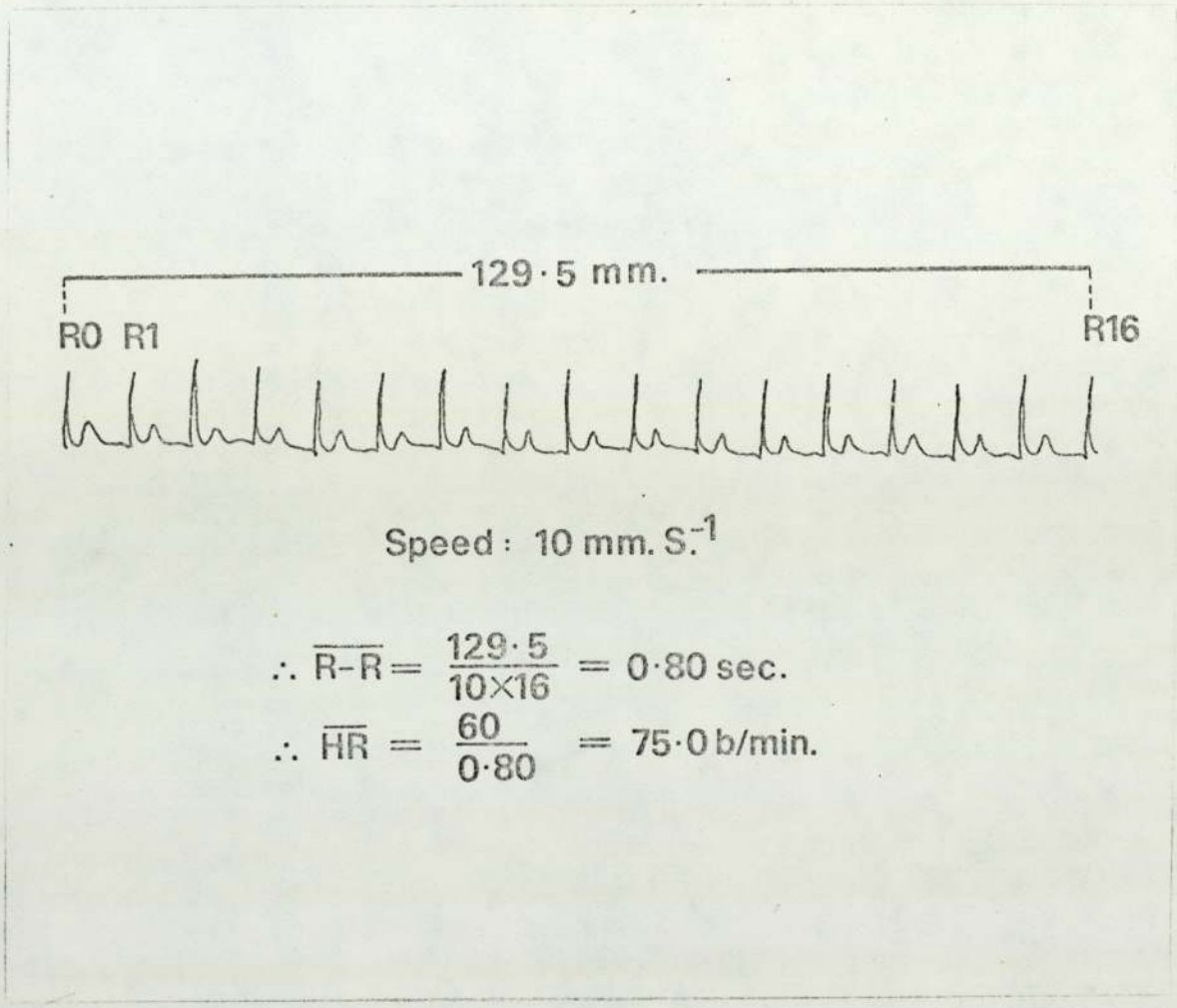


Figure 1. Calculation of mean heart rate from an E.C.G. recording.

faster recordings is then calculated over the whole twenty second period, so as to allow a truer estimate of the heart rate even if sinus arrhythmia is present. Time markers were recorded at the same time, enabling a check on the accuracy of the recorder speed setting.

The heart rate (HR) was then determined from the fast recording (Figure 1) as follows:

$$\text{Average Pulse Interval (msecs)} = \frac{\text{Distance between first and last R wave (mm)}}{\text{Paper speed (MM/S) x number of pulse intervals}}$$

$$\text{Average Heart Rate} = \frac{60}{\text{average pulse interval (ms)}} \text{ beats per minute}$$

The E.C.G. was recorded on a Grass multichannel recorder, which is a pen recorder (frequency response D.C. - 100 Hz (±3dB)), this recorder also has second time markers (accuracy -±1%).

II.2.b. Blood Pressure

Blood pressure was measured utilising either direct or indirect methods.

(i) Direct measurements - blood pressure was measured directly from the brachial artery in the patient's non-dominant arm. A "Seldicath Grand-Jean" teflon catheter (11cms long, 1mm bore), was inserted into the brachial artery using a micro-seldinger technique. After a good backflow of blood was obtained, and all air bubbles removed, the catheter was connected to a three-way stopcock and an "Intraflo" constant flushing device. The "Intraflo" maintains patency of the catheter by flushing slowly (approximately 3mls per hour) of heparined, sterile saline. The flushing system was parallely connected to a Gaeltec Luer 3e/A tip micro pressure transducer (Fig.2). Both pressure and ECG signals were then amplified, and recorded simultaneously onto a Grass Model 7 Polygraph

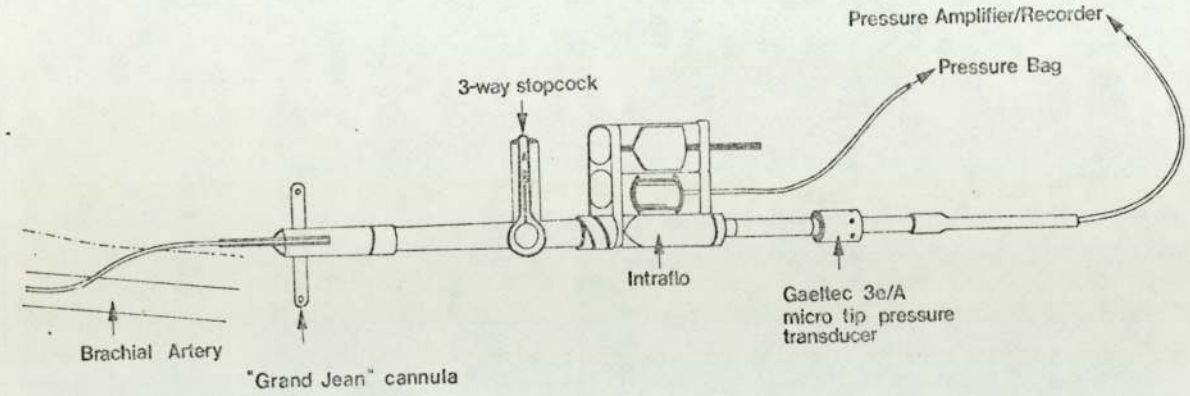


Figure 2. Pictorial representation of the equipment used for the measurement of direct arterial pressure from the brachial artery in the non-dominant arm of the patient.

Recording System, the paper speed was set to run at 10mm per second, during periods which were to be used for calculation of heart rate and blood pressure, otherwise the speed was set to 2.5mm per second for observations.

(ii) Indirect measurements - three types of sphygmomanometer were used for estimation of blood pressure.

Firstly, the standard clinical sphygmomanometer; which consists of a mercury manometer linked to an inflatable bag, surrounded by an inelastic cuff. The cuff is fitted snugly around the arm, neither loose nor touching any article of clothing, about two cms. above the antecubital space, with the rubber bag over the brachial artery. With the subject comfortable, preferably reclining, with the cuffed arm at the same level as the heart. The cuff is inflated by means of a rubber bulb, until the pulsations of the brachial artery can no longer be felt, the pressure is then raised by a further 20mm Hg., and then released at a rate of about 2mm Hg a second. With a stethoscope over the brachial artery, the systolic blood pressure (SBP) can then be determined as the point at which sounds (Korotkoff sounds) are first heard, as blood begins to pass through the artery which has previously been occluded. Diastolic blood pressure (DBP) is estimated at two phases; Phase IV is noted as the point when the sounds are muffled, and Phase V when the sounds disappear altogether, (systolic pressure is also known as Phase I). There has been much controversy as to whether the true diastolic pressure is better represented by the Phase IV or Phase V measurement; Phase V correlates best with intra-arterial readings, Phase V has a sharper end point and is therefore more reproducible.

Secondly, the random-zero sphygmomanometer; the principle is exactly the same as for the standard clinical sphygmomanometer, but the

zero position is varied in a random manner, by the raising and lowering of a mercury reservoir. Therefore, when the systolic and diastolic pressures have been recorded and noted, "zero" level, varying from 0 to 60mmHg is noted after the pressure in the cuff has been released. This figure is then subtracted from the noted blood pressure readings to give the true systolic and diastolic pressures. The zero reading cannot be determined until after the blood pressure has been taken, so that the determination is taken "blind".

Thirdly, the "Arterisonde" Blood Pressure Monitor; this operates in a similar way to the other two devices, but is automatic in its operation. It operates on a principle employing ultrasonic energy, systolic and diastolic determination are made electronically, by ultrasonically detecting arterial wall motion, the systolic and diastolic pressures are then displayed on two mercury manometers. The inflation of the cuff is automatically carried out, and therefore no personnel need be in attendance when blood pressure measurements are being made as the systolic and diastolic pressures can be displayed onto a chart recorder.

II.3. Statistical Analysis

Statistical analysis was performed using a Comucorp Programmable Statistics Calculator. Statistical significance for paired observations was analysed using Student's "t-test" Snedecor and Cochran (1976) - Statistical Methods, Sixth Edition. Linear regression and correlation coefficients for "goodness of fit", were also undertaken for paired data to test data for a linear relationship. Both tests also produced mean and standard deviation (SD) for each group, also the standard error of the mean (SEM) was calculated using:

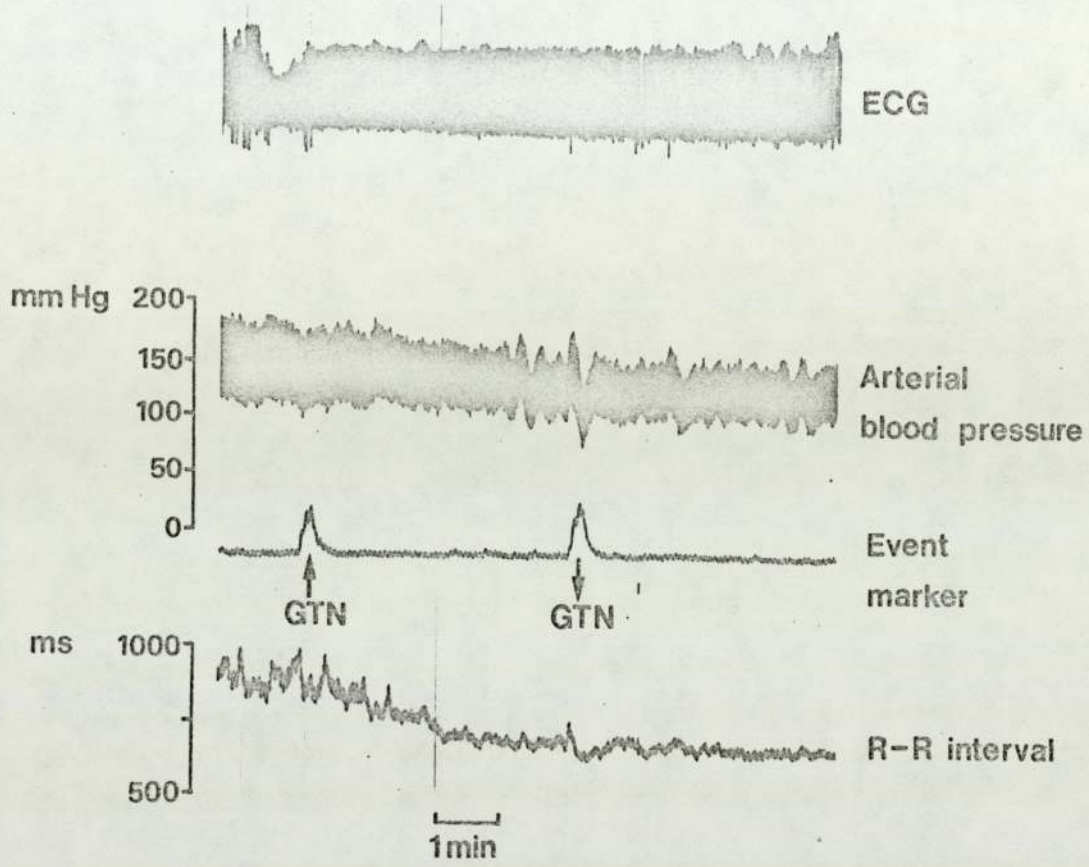


Figure 3. Typical tracing of cardiological responses to sublingual GTN administration, for four minutes, given to a hypertensive patient in the standing position. ↑ ↓ indicates when GTN was put into and taken out of the mouth respectively. It can be seen that the blood pressure falls after GTN administration with a concomitant increase in the heart rate (shown as the RR interval shortening).

$$S.E.M. = \frac{\text{standard deviation}}{\sqrt{\text{Number of values}}}$$

P values for statistical significance were obtained from standard tables (Ciba-Geigy).

II.4. The Glyceryl Trinitrate Test

After the subject has rested for twenty minutes in a supine position three baseline recordings of supine heart rate and blood pressure are made; the subject then stands, this position being maintained throughout the test, (or if the subject feels faint during the test, he/she is laid supine, in a feet-up posture to increase venous return). After a five minute equilibration period, several baseline recordings of standing heart rate and blood pressure are then made. When three consecutive measurements are fairly stable, one fresh 0.5mg tablet of GTN is given sublingually, without chewing, after four minutes absorption the tablet is removed. Recordings of heart rate and blood pressure are made at minute intervals, over the last twenty seconds, for eight minutes after administration of GTN. The subjects move their feet occasionally during the test, so as to prevent venous pooling, and therefore produce less risk of fainting occuring.

With reference to Figure 3, it can be seen that shortly after GTN administration (shown as event mark "GTN ↑"), there is a fall in arterial blood pressure, with a reflex tachycardia, shown as a shortening of the R-R (pulse) interval. For analysis purposes, I have taken as the subject's "response", the maximum tachycardia achieved and the maximum heart rate increase to GTN administration. In addition I have recorded the lowest systolic arterial pressure and the associated systolic pressure fall achieved after GTN administration.

For determining whether the response to GTN is affected by the amount absorbed, and for comparison to sublingual GTN, I have studied the heart rate and blood pressure responses to intra-venous GTN administration. For infusion of the GTN solution a "butterfly" cannula was inserted into a peripheral vein in all the normal subjects; the other patients already having a "Venflon" cannula inserted into a peripheral vein, as the cannulae were required for other procedures in a parallel study. All cannulations were performed by one of the registrars.

The concentrated GTN solution was made up to 100mls with sterile, normal saline for all doses up to 100µg; for doses above this the GTN solution was made up to 50mls, so as not to infuse too large a volume which would require a lengthy infusion time, and may have invalidated the results. Injections of the diluted GTN solution were infused at a rate of approximately 2mls of solution per minute, 5mls of sterile saline was then flushed through the cannula to remove the GTN solution, ensuring all the GTN was infused into the vein.

The ECG was recorded continuously throughout the test; blood pressure was measured directly on the patients, and with the "Arterisonde" on all the normal subjects.

II.5. Sub-maximal Bicycle Exercise Test

As stated previously, the most common clinical test to determine the activity of beta blockers, is the inhibition of the tachycardia produced by dynamic exercise. I have, therefore, used bicycle ergometry, on subjects and patients in the upright position, (using an electrically braked Elema Schoenander, EM-380 bicycle ergometer, with calibrated speedometer), with which to compare with responses from the GTN test.

The cardiac response to exercise is complex and involves the interaction of many variables, such as heart rate, stroke volume and ventric-

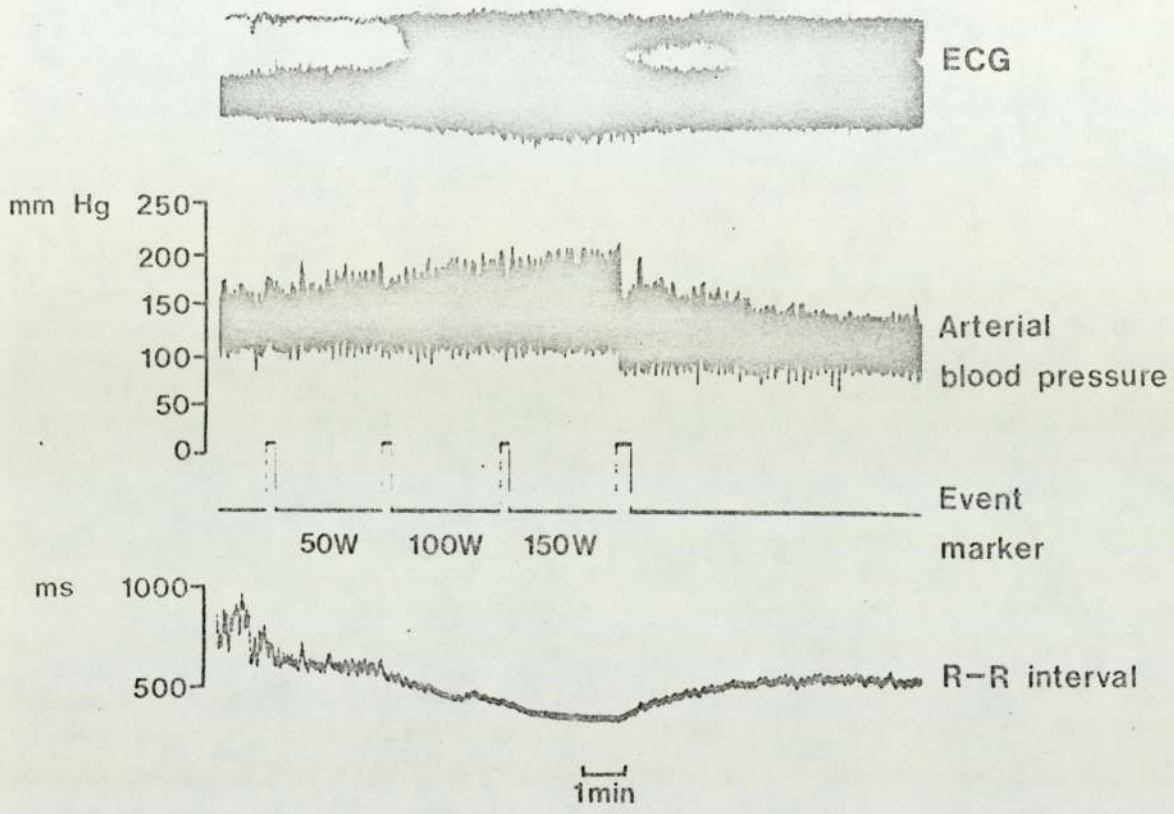


Figure 4. Typical tracing of cardiological responses to a graded, multi-stage, maximal bicycle exercise test, on a hypertensive patient. With increasing levels of exercise the blood pressure rises, particularly the systolic pressure, blood pressure abruptly falls once exercise ceases. The RR interval indicates the parallel increase in heart rate during exercise.

ular end-diastolic volume. During heavy exertion in the standing position, the stroke volume increases markedly, and contributes substantially to the increased cardiac output. The increased heart rate due to high levels of exercise is mainly a result of sympathetic activity, and partly to a release of vagal restraint; however, vagal activity is predominant at low levels of exercise. Both systolic and diastolic pressure rises as the severity of exercise increases, Braunwald, Sonnenblick, Ross, Glick and Epstein (1967) (Figure 4 shows a typical graded exercise test tracing).

To standardize the exercise test for subjects of different ages and different degrees of fitness, sub-maximal bicycle exercise was undertaken. This involves, firstly, the subject exercising to exhaustion using a progressive multi-stage exercise tolerance test. Prior to the test a 12 lead ECG is recorded, with the subject in the supine position; the resting ECG is then seen and checked by a clinician, who then allows the test to be performed. After authorisation has been given, the subject's skin is prepared by shaving, if necessary, the sites where electrodes are to be placed, the skin is then vigorously abraded with electrode gel to ensure good skin contact and low skin impedance. Electrode siting and the use of rubber straps for lead strapping to the subject's torso, have been used which minimise respiratory and electrode/lead movement artefacts on the ECG recording. The subject is then moved from the supine position to sit on the bicycle, making sure that the saddle and handlebar heights are suitable and comfortable for that subject. Instructions are then given to the subject on the protocol of the test, after a baseline ECG recording has been made:

(i) To maintain a constant pedal revolution, this being indicated on the speedometer, of between 50 - 70 revolutions per minute.

EAST BIRMINGHAM HOSPITAL

Department of Cardiology

EXERCISE No: 467/77

NAME: D.S. C

UNIT No: 139123

AGE: 48

DATE: 17/10/77

EST. MAX. HT. RATE: 152

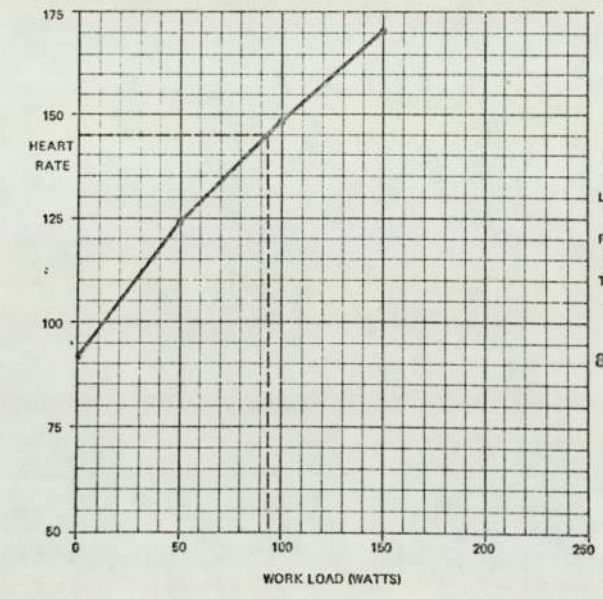
CONSULTANT: WAL

CLINICAL DIAGNOSIS: HYPERTENTION

WARD: OPD

CLINICAL FEATURES:

DRUGS & TREATMENT	NIL				
PAIN	—				
BREATHLESSNESS	—				
FATIGUE	at 150w(3')				
ARRHYTHMIA	NIL				
OTHER	—				



MINS AT EACH LOAD

	1	2	3	4	5
L	cont. 0	50 W	100 W	150 W	
R	92	124	148	170	
T	—	3	3	3	

85% max = 145 b/min
= 95 w

Figure 5. Work load/Heart Rate response curve for the determination of the sub-maximal (85%) bicycle exercise work load. This load would then be used on that particular patient, before and after beta-blocker therapy, to assess the degree of beta-blockade.

(ii) The workload would be increased after three minutes at that load; the increments and starting loads being the same (i.e. for men - 50W; for women - 30W). During the third minute an ECG recording would be made, the load would be increased until the subject is exhausted, or the test would be discontinued if cardiological signs were present (e.g. anginal pain).

(iii) To inform the operator when he/she is exhausted, so that a final ECG record of maximal exercise can be taken.

On completion of the exercise test the subject is quickly transferred from the ergometer to the couch, for post exercise ECG recordings.

To determine the load to be used for that subject, for sub-maximal exercise, a workload/heart rate response curve is drawn, as shown in Figure 5. From the maximum heart rate produced by that particular subject, the 85% level is calculated; from the response curve, the workload to produce 85% of the maximum tachycardia is interpolated. This load is then used in subsequent bicycle exercise tests at a sub-maximal level, for that particular subject.

85% of the maximum heart rate was used as this would standardize patients of different ages and fitness and allow them to complete eight minutes exercise at a near maximal level. The maximal response is taken as being the heart rate at which the subject or patient can no longer continue cycling. The heart rate may not plateau as the load is increased every three minutes, with exhaustion (Royal Infirmary, Edinburgh : protocol).

II.6. Beta Blocker Serum Drug Levels

In one of the studies, serum levels of beta-blocker (Acebutolol) were determined. Venous blood samples were taken from patients, spun down in a refrigerated centrifuge (at 2000r.p.m., - 4°C for 7 minutes).

The serum was then decanted off and stored in a deep freeze at -20°C, and later sent to May and Baker Ltd., Dagenham for assay.

The assay method measures the amount of pharmacologically active material (acebutolol and its acetyl metabolite, M & B 16,942), and is measured using a colorimetric method (Cuthbert and Collins, 1975). The reproducibility of the measurement is ±5%.

CHAPTER III

CARDIAC EFFECTS OF RESPONSES TO GLYCERYL TRINITRATE

III.1. Pilot Study

A pilot study was undertaken to assess the cardiac effect of responses to sublingual GTN administration in normotensive subjects and hypertensive patients during various anti-hypertensive (beta-blocker) therapies. This pilot study further served to assess the equipment available, and to design a suitable protocol.

Four normotensive subjects (age range: 29 to 46 years; mean 37 ± 4 years) and ten hypertensive patients (age range: 24 to 62 years; mean 45 ± 3 years), were studied; in all subjects and patients the blood pressure was measured directly. The GTN test was performed as previously described.

Table 2 lists the details of these individuals, their HR and SBP responses to sublingual GTN administration and their current (if any) drug therapy. Subject 3 was excluded from the grouped results, as she felt faint, and was laid supine two minutes after sublingual GTN administration, at this time her GTN induced tachycardia would not have been maximal.

These results demonstrate that the normotensive subjects (group A) had a heart rate increase and maximum tachycardia not significantly different ($p > 0.05$) to that seen in the hypertensive patients tested 27 hours after their last beta-blocker tablet (group D). The heart rate increase to sublingual GTN administration is clearly attenuated by beta blockade, if the test is made soon after treatment (Groups B and C). Therefore the beta-blocker duration of action is greater than five hours but less than 27 hours. Also, the GTN induced tachycardia is blunted, this being more marked in Group B (hypertensives tested 3 hours after

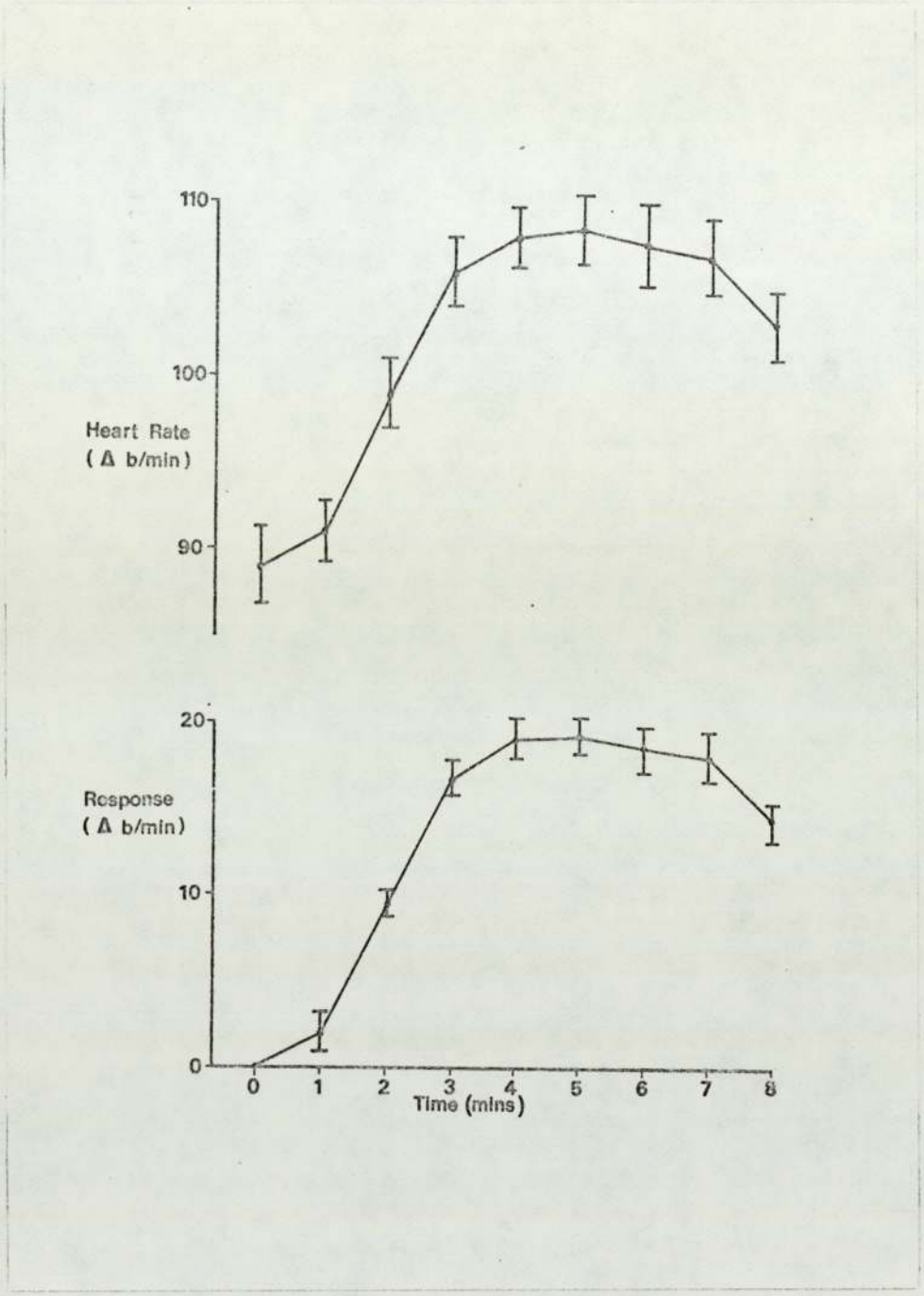


Figure 6. Mean (± S.D.) heart rate (upper) and heart rate increase (lower), responses to 20 challenges of sublingual GTN (for 4 mins.), to one subject over a twelve month period.

receiving their first dose of beta blockers). All the groups, except C (which was also the oldest group), had a similar systolic blood pressure fall to sublingual GTN administration.

These findings, of heart rate responses to sublingual GTN administration, are in agreement with those of Fitzgerald, who showed a similar attenuation of the heart rate responses, after beta-blocker therapy in normotensive subjects. The results of this pilot study encouraged me to proceed to evaluate the heart rate and blood pressure responses in more details. I also determined that this test was easy to perform and record.

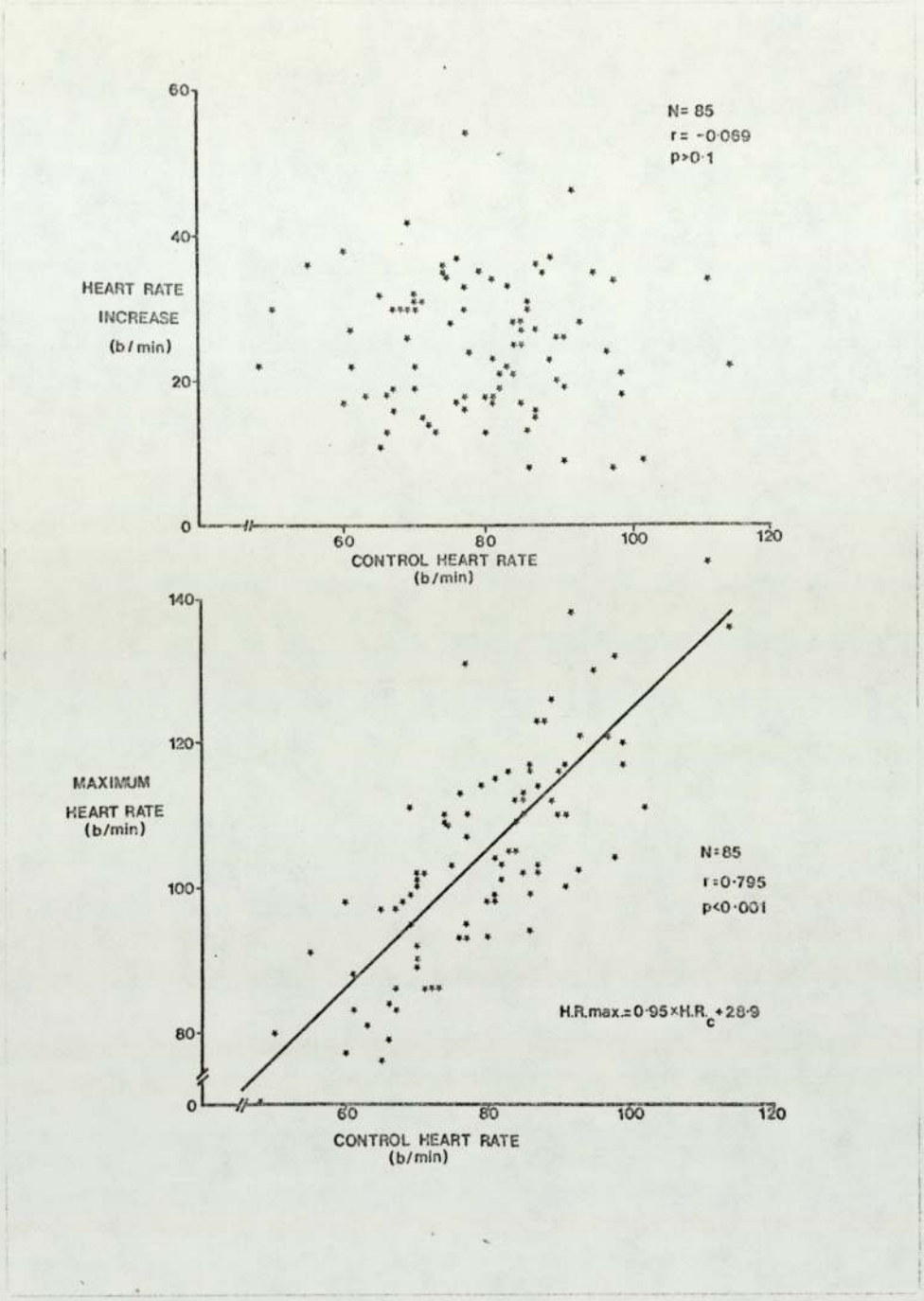
Conclusions

The heart rate response to sublingual GTN administration appears to be blunted by beta adrenoceptor blocking drugs, in hypertensive patients. This blunting appears to be more pronounced if the test is performed three to five hours after therapy, and returns to a "normal value" if the test is performed more than 24 hours after therapy. The reason for this is possibly that the beta-blocker is inactive after 24 hours.

III.2. The Glyceryl Trinitrate Response

III.2a. The Glyceryl Trinitrate Test

As a result of recording GTN tests on 102 subjects and patients (normotensive and hypertensive), over a wide age range (18 to 91 years), I have found that the heart rate increase to sublingual GTN administration is always maximal within eight minutes post administration of GTN (mean five minutes). Figure 6 represents the mean heart rate response to 20 challenges of sublingual GTN, carried out over a 12 month period, to one subject, Table 3 gives details of each response (no blood pressure being recorded). As can be seen there is an initial rapid rise in heart rate after the first minute, plateauing after four minutes post administration.



Figures 7 (lower) and 8 (upper) showing that there is no correlation ($p>0.1$) between standing, control heart rate and the heart rate increase to sublingual GTN administration (Figure 8), but a highly significant correlation ($p<0.001$) between control and maximum heart rate (Figure 7) to GTN administration.

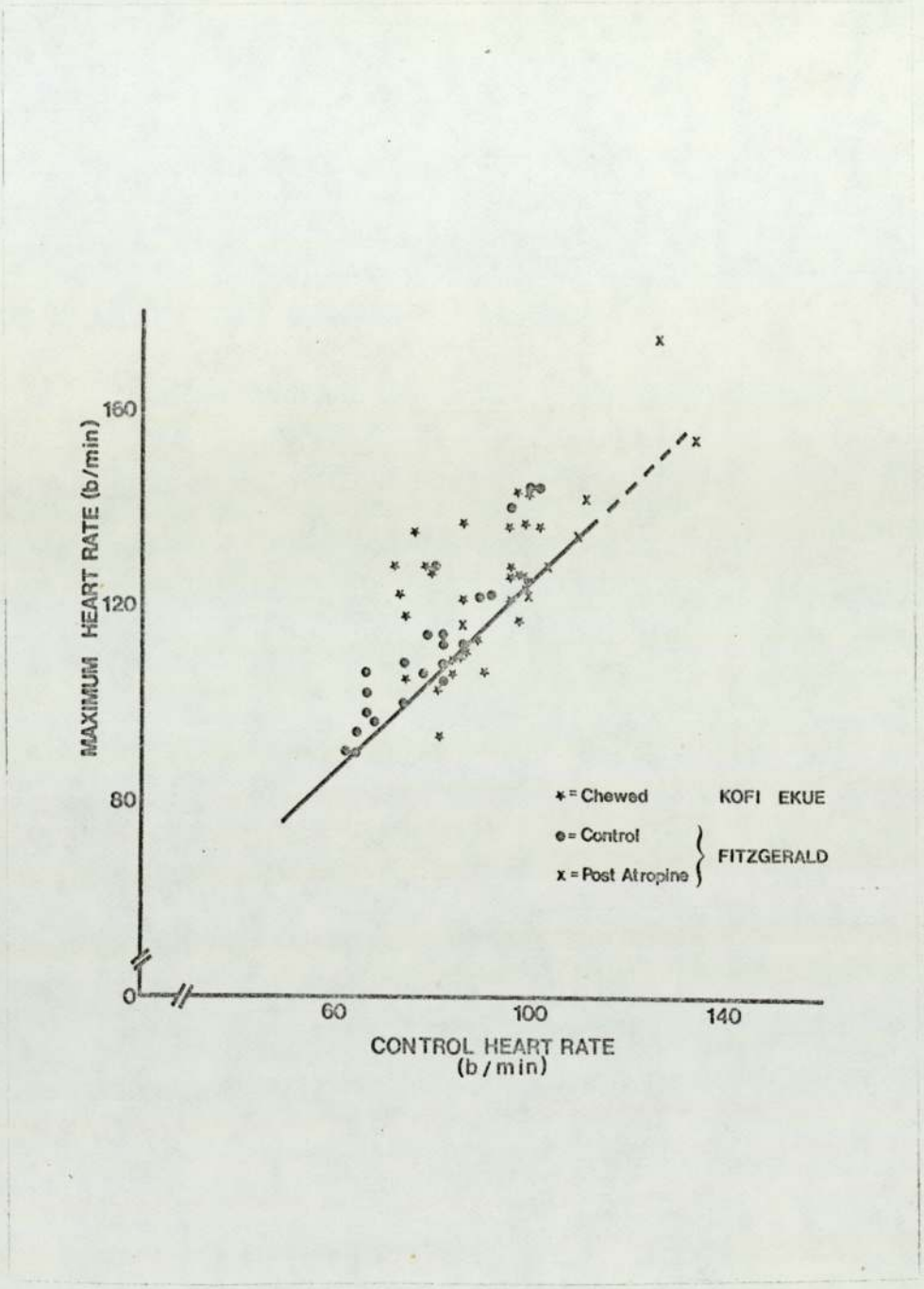


Figure 9. Results of other workers overlaid on the regression line from Figure 7, showing that their results behave in a similar manner to mine.

The heart rate then slowly declining after seven minutes, reaching the control heart rate after approximately 30 minutes.

In 85 subjects and patients, who had a GTN test carried out, on a day when no exercise had been performed, it was found that there was a significant correlation (Fig. 7: $r = 0.795$; $p < 0.001$) between the control standing heart rate before, and the maximum heart rate attained after sublingual GTN administration. However, there was no significant correlation (Fig. 8: $r = -0.069$; $p > 0.1$) between the control standing heart rate before, and the maximum heart rate increase due to, sublingual GTN administration. Moreover, there was a significant correlation ($r = -0.463$; $p < .001$) between the control standing heart rate before and the percentage maximum heart rate increase due to, sublingual GTN administration. This shows that as the control standing heart rate increases, the heart rate increase to sublingual GTN is virtually unchanged, therefore the heart rate increase cannot be determined by the control standing heart rate. Furthermore, this is substantiated by the fact that the slope of the regression line of control vs. maximum heart rate is virtually 1.0. Therefore the actual increase in heart rate due to sublingual GTN administration is a good indicator of the amount of sympathetic drive, as it is not dependent upon the initial heart rate.

Figure 9 illustrates other workers' results, including those of Fitzgerald after atropinisation, overlaid on my regression line showing that these results appear to behave in the same manner as mine. Also those of Fitzgerald after atropinisation lie on the line, showing that very high resting heart rates (greater than 110 beats/minute) do not attenuate to any significant level the heart rate increase to sublingual GTN administration.

In a few cases, I have noticed that there has been complete absorption of the GTN tablet within the first four minutes post

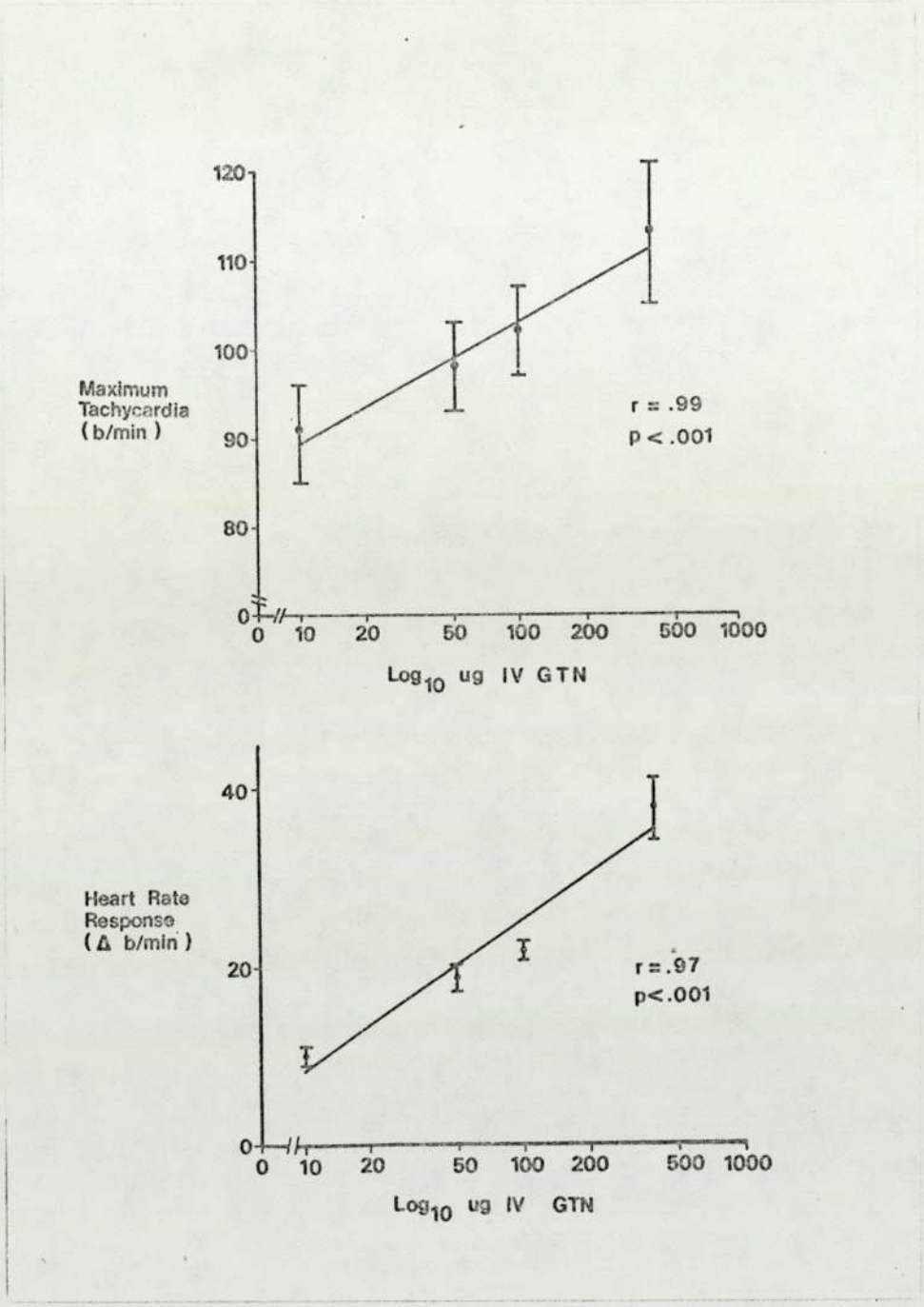


Figure 10. Regression of the dose of intra-venous GTN infused (shown as the subject's mean at each level of infusion), to the maximum tachycardia achieved (upper) and the heart rate increase (lower) to intra-venous GTN. In both cases there was a significant correlation ($p<0.001$).

administration, but in the majority of tests carried out, the GTN tablet has dissolved very little when it was removed after four minutes administration. In order to determine whether the cardiac effects of responses to sublingual GTN is an "all or none" response, or is in fact related to the dose of GTN received, (and if so, whether there is a threshold at which the response is maximal), I have studied the effects of intra-venous GTN solution upon heart rate and blood pressure responses.

III.2b. Responses to Increasing Doses of Intra-Venous Glyceryl Trinitrate

Eight normal, male subjects (age range: 25 to 31 years) were studied, blood pressure was measured using the "Arterisonde", the responses are detailed in Tables 4a and 4b. Bolus infusions at 2ml/min of intra-venous GTN were administered after control ECG and blood pressure records were recorded,the doses infused being 10µg, 50µg, 100µg and 400µg GTN made up to 2mls with sterile saline. Bolus doses were administered at intervals of at least eight minutes, or greater, until baseline recordings were stable. The bolus doses were not allocated in random order, but in order of increasing dose, as large doses of intra-venous GTN can cause severe hypotension in some subjects, therefore any subject feeling faint at a low dose was withdrawn from the study and received no more infusions. This occurred in two of the subjects; one subject four minutes after the 10µg infusion, and the other three minutes after the 50µg infusion; both subjects were immediately laid supine in a "feet-up" posture, until their symptoms had been relieved.

The remaining six subjects (age range: 25 to 31 years; mean 29 ± 1 years) have their mean results listed in Tables 4a and 4b, and shown diagramatically in Figure 10, and show that the heart rate increase ($r = 0.97$; $p < .001$) and maximum tachycardia ($r = 0.99$; $p < .001$) respectively, are related to the dose of GTN infused in a logarithmic

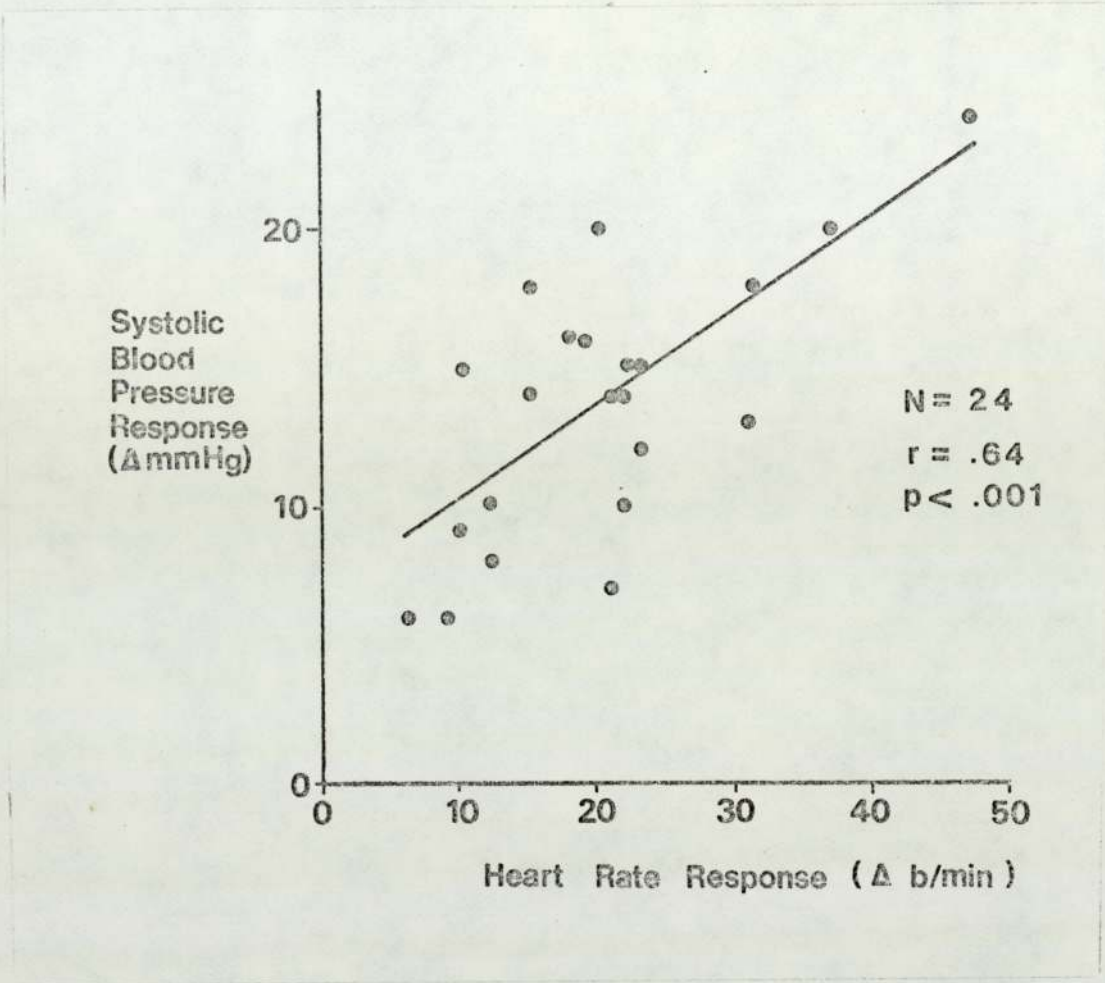


Figure 11. Regression of heart rate increase to maximum systolic pressure fall due in increasing doses of intra-venous GTN infusions, for each subject.

manner. There was also an association between the fall in the mean systolic blood pressure, and increase in heart rate responses, for the group as a whole, (Fig.11: $r = 0.64$; $p < .001$).

Abrams (1980) stated that GTN is a potent vascular dilator, exerting its strongest effect on the venous system; GTN acting at specific nitrate receptors in the vascular smooth muscle wall, to trigger vascular relaxation. My results do not show a plateau effect, but the dose I used may not have been large enough to reach this level. I decided not to use higher doses as this may have produced severe hypotension and extreme tachycardia in some subjects.

Conclusions

The heart rate and blood pressure responses to intra-venous GTN is not an "all or none" phenomenon. They demonstrate a linear relationship to the logarithm of the dose infused, and whether this eventually plateaux at higher doses was not studied because of the high tachycardias and hypotensive effects produced.

III.2c. Comparison of Intra-Venous (50µg) to Sublingual (500µg) Glyceryl Trinitrate Responses

The next objective was to determine the relationship between the response to a standard sublingual GTN tablet and an intra-venous dose. Christenson, Nordenfelt, Westling and White (1969), showed that an infusion of 50µg per minute of intra-venous GTN, produced haemodynamic responses similar to those obtained from sublingual GTN, in thirteen normal subjects in a supine position. Results from the previous study has shown that the 50µg and 100µg boluses of intra-venous GTN produced similar heart rate responses to that observed in subjects of the same age range (GTN IV: 50µg = 19; 100µg = 22; oral GTN =22) tested with sublin-

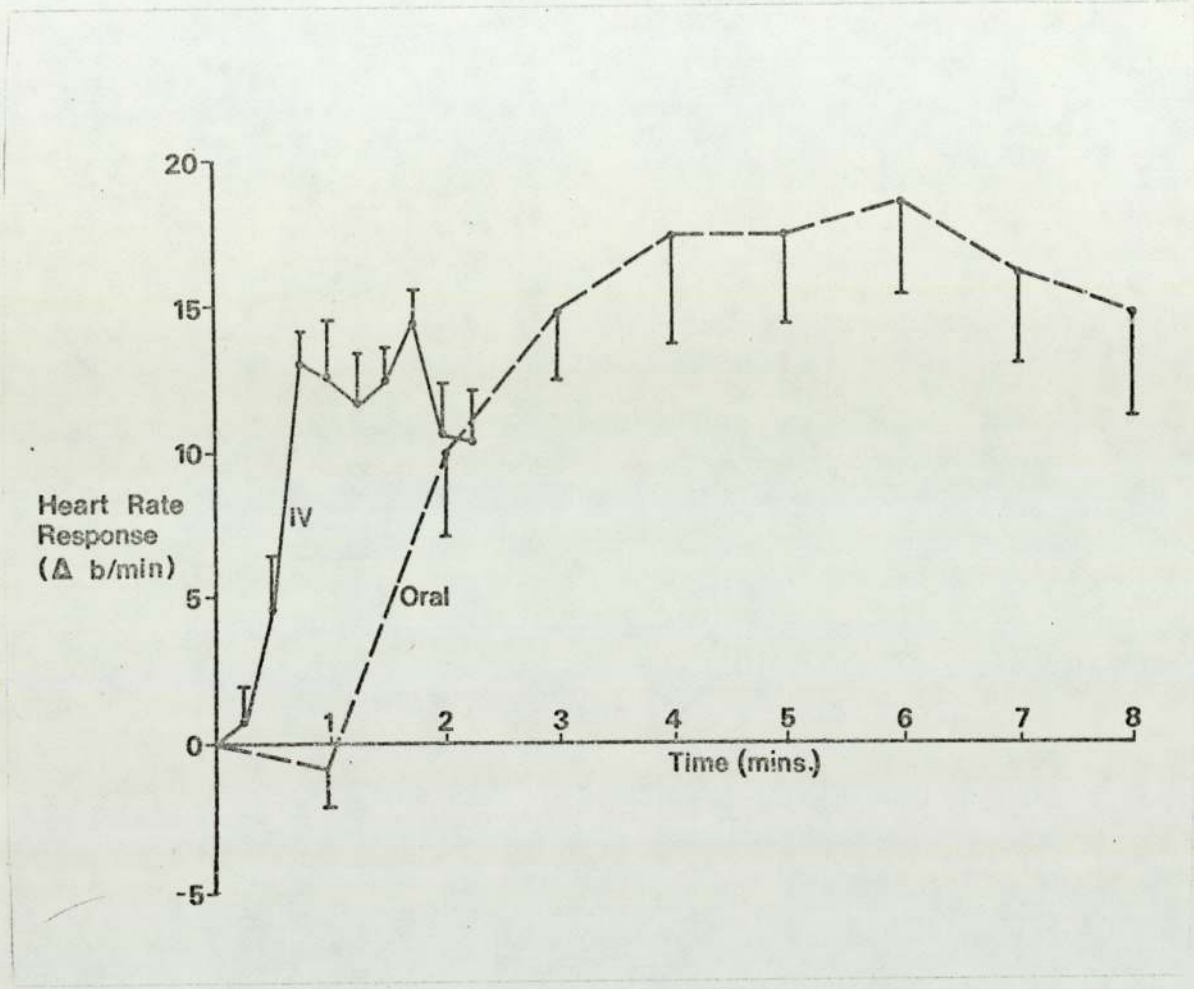


Figure 12. Mean time course of the heart rate increase to sublingual GTN and intra-venous GTN administration in six normal subjects. There was found to be no significant difference ($p>0.05$) between the maximum responses to either administration.

gual GTN. Therefore, I have compared the heart responses of sublingual GTN to those obtained from 50μg boluses of intra-venous GTN in the same subject.

Four normal subjects and two patients with suspected hypertension were studied, (Table 5: age range: 18 to 31 years; mean: 28 ± 2 years). None of the subjects or patients felt faint with either sublingual or intra-venous GTN; slight throbbing in the temple was noticed in three of the group when studied with sublingual GTN, but no side effects were noted during testing with intra-venous GTN. My results indicate that their heart responses to this intra-venous dose and sublingual GTN are similar; no significant ($p > 0.05$) difference being found between the GTN induced tachycardias (sublingual = 100: i.v. = 99 beats per minute $p > 0.5$), heart rate increase (19 : 21 beats per minute $p > 0.5$) but a significant difference in systolic arterial blood pressure fall (- 21 : - 16 mm Hg $p < .02$).Differences were also observed in the time course of the responses; with sublingual GTN the response is maximum at six minutes, with intra-venous GTN maximum response occurred at 105 seconds (Figure 12). The effects of sublingual GTN (500μg) persist longer than those of intra-venous GTN (50μg) (sublingual 10 to 25 minutes : i.v. 4 to 7 minutes).

The maximum responses shown in Figure 12 appears to differ from those recorded in Table 4, as the mean group time course is illustrated; each individual having a maximal response at varying times after administration of GTN. This difference is more noticeable in the response to intra-venous GTN, where a wide range of maximal response times (range: forty-five seconds to two minutes) compared to the time course existed; whereas, with sublingual GTN, all the subjects' maximal responses occurred between four to six minutes after administration, illustrated in Figure 12 as a plateauing effect (this being similar to the mean response

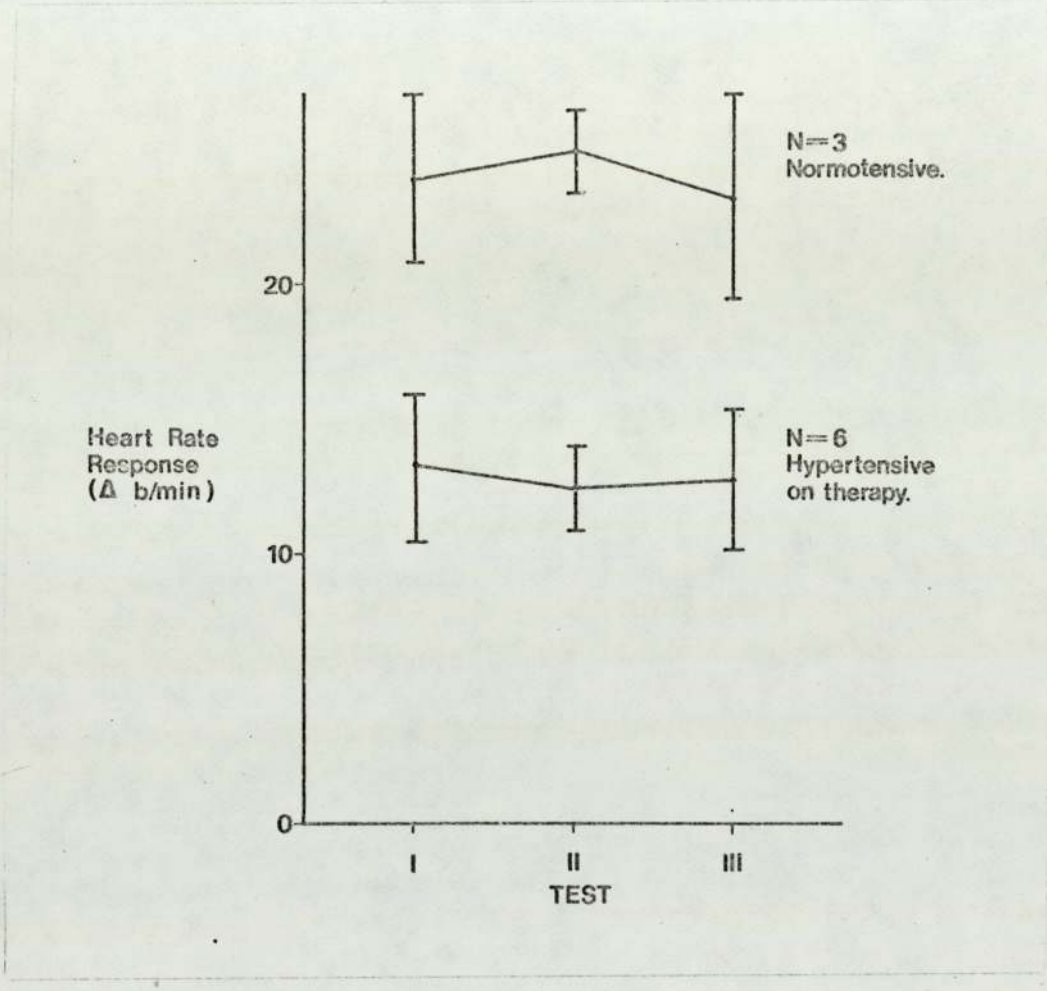


Figure 13. Mean (± S.D.) heart rate responses of sublingual GTN challenges on three different occasions to three normotensive subjects (upper) and to six hypertensive patients (lower) receiving hypo-tensive therapy.

to repeated challenges of GTN, in one individual - see Figure 6).

Conclusion

The heart rate and blood pressure responses to a standard, sublingual GTN (500µg) tablet are similar to those produced by an intra-venous bolus of 50µg of the drug. The maximum heart rate response to sublingual GTN occurs later than it does with the intra-venous form and persists longer.

III.2d. Reproducibility of the Glyceryl Trinitrate Response

There is a large inter-subject variation of heart rate increase and maximum tachycardia responses to sublingual GTN, in the patients and subjects I have tested. This variation in response is less noticeable in any one individual, and is even less noticeable if repeated challenges of sublingual GTN are made over a short period of time; (Table 2: responses over twelve months : range 16 to 33 beats per minute; mean 23 ± 1 beats per minute; responses over twenty-four hours : range 20 to 25 beats per minute; mean 22 ± 1 beats per minute). Figure 13 illustrates that there is little change in responses when repeated challenges of sublingual GTN are made to individuals (Table 6) and also holds true if the individual is receiving beta-blocker therapy, where the heart rate increase is blunted due to therapy blocking the sympathetic system.

Conclusion

Despite the fact that the heart rate response to GTN increases with the logarithm of the dose infused, the response elicited with a standard sublingual GTN tablet appears to be reproducible in both normal subjects and hypertensive patients receiving treatment.

III.3. The Role of the Autonomic System in the Glyceryl Trinitrate Response

III.3a. Introduction

In his original studies Fitzgerald concluded that the heart rate response to sublingual GTN administration was solely due to sympathetically mediated reflexes. However, this conclusion has been questioned by Hansson (1973); Kofi Ekue, Shanks and Walsh (1974); Zweifler and Hansson (1976).

Both Kofi Ekue and Hansson confirmed results of Fitzgerald, in that administration of sublingual GTN to normal subjects in the standing position, produced an increase in heart rate, that was attenuated by beta-blockers. Kofi Ekue stated that the large doses of beta-blockers used in his and Fitzgerald's studies should have produced almost complete blockade of the increased sympathetic drive to the heart, and suggested that the residual increase in heart rate to sublingual GTN may result from a reduction in parasympathetic activity. Kofi Ekue also suggested that the effects of combined blockade of sympathetic and parasympathetic nervous systems would be required to settle this point. The conclusion was drawn that the increase in heart rate produced by sublingual GTN can be used to demonstrate the activity of beta-blockers, but suggests that the increase in heart rate probably does not result entirely from increased sympathetic activity.

Hansson also suggested that the tachycardia induced by sublingual GTN in his group of hypertensive patients appears more likely to be the result of both sympathetic stimulation and vagal withdrawal. He concluded that the response to GTN is of limited value in the assessment of beta adrenergic blockade, as its responses are determined in some part by the parasympathetic system.

George (1975) commented that the GTN test could be used as a test of

beta-adrenoceptor function, but there was conflicting evidence concerning the value of the test as a quantitative measure of beta-adrenoceptor blockade and that fainting occurs not infrequently (Hansson, 1973). George also stated that sub-maximal bicycle exercise provides a reliable and precise test of beta-adrenoceptor function, if the level of exercise used would normally be sufficient to increase the heart rate to at least 130 beats per minute.

Therefore, to re-evaluate Fitzgerald's claim that there was no vagal component associated with sublingual GTN administration, I have studied the responses to GTN after partial and total autonomic cardiac blockade.

Cardiac autonomic blockade was achieved using the protocol of Jose and Taylor (1969). These authors demonstrated that their doses of Atropine (0.04mg/kg) and Propranolol (0.2mg/kg), given intravenously, achieve total cardiac autonomic blockade. These observations have been subseqently confirmed by Korner, Shaw, Uther, West, McRitchie and Richards (1973), in both normotensive and hypertensive men. Jose and Taylor showed that there was no significant difference ($p > 0.5$) in the heart rate change between a dose of 0.04mg/kg and 0.05mg/kg atropine infused intravenously. This technique of cardiac autonomic blockade is now well accepted.

Many years ago Crawford (1923) showed that following atropinisation, the heart rate increase was dependent on age, the increase in heart rate becoming less as age increased. Crawford demonstrated that one was not able to foretell the increase in heart rate after parasympathetic blockade from the initial heart rate. These observations were confirmed by Leon, Shaver and Leonard (1970) using the doses of atropine as recommended by Jose and Taylor. In normal individuals, the percentage increase in heart rate with parasympathetic blockade ranged from 62% to 130%; and the maximal heart rate achieved varied from 122 to 142 beats

per minute, with no strict relationship between either of these rates and the initial rate. Jose and Taylor showed that in normal people the resting heart rate was mainly controlled by an excess of vagal activity over sympathetic activity. However, there was progressively less dominance of vagal activity over sympathetic activity with increasing age. Korner's group showed that in hypertensive subjects there was diminished vagal activity and thus these subjects tended to have higher resting heart rates, when compared with normotensive subjects.

The conclusion of these studies would seem to indicate that the heart rate response after parasympathetic blockade is variable, reflecting the different amount of vagal activity in individuals and there is clearly no "maximal" heart rate which is common to all subjects after parasympathetic control has been removed. With hypertension or increasing cardiac disease, the vagal activity is diminished. Thus in carrying out the GTN test in hypertensive subjects it is unlikely that we would be underestimating the sympathetic component.

III.3b. Effect of Parasympathetic Blockade on the Glyceryl Trinitrate Response.

In order to determine the time course of the parasympathetically blocked tachycardia, five normal subjects (age range 22 to 28 years; mean: 26 ± 1 years) were studied in the standing position. ECG was recorded every minute, and throughout the infusion period; the blood pressure was measured using the "Arterisonde". After a stable control period, Atropine Sulphate (0.04mg/kg) was slowly infused over a 1½ to 2 minute period. ECG and blood pressure was recorded every minute, for twenty minutes after the end of the infusion.

Table 7 lists the individuals responses to parasympathetic blockade, the results from subject 3 are not included in the mean results, or

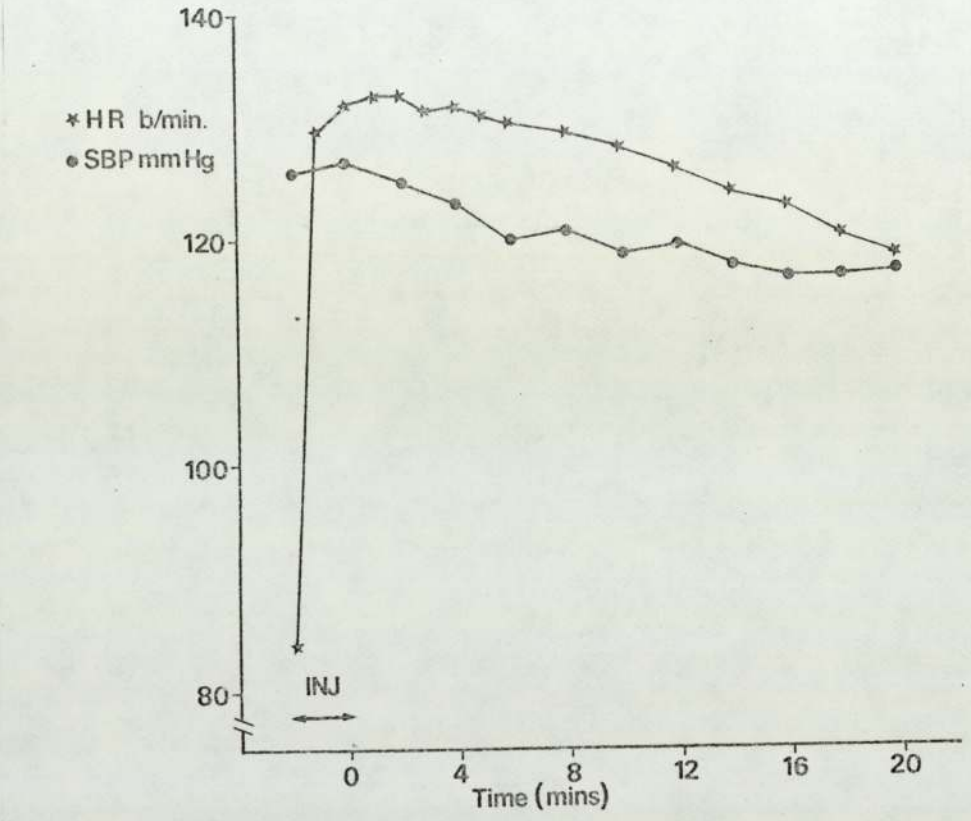


Figure 14. Time course of the mean heart rate (HR) and mean systolic pressure (SBP) response to an intra-venous infusion of atropine sulphate (0.04mg/Kg) in four normal subjects

Figure 14, as he felt faint a few minutes after administration of the Atropine Sulphate, and was laid supine. Figure 14 illustrates the mean time course of the parasympathetically blocked tachycardia. Maximum tachycardia was always achieved within three minutes after administration of the Atropine Sulphate, (usually occuring at the first minute after infusion), maximum heart rate remained relatively constant for the ten minutes after the infusion, the mean heart rate fall being -4 ± 1 beats per minute (range: -2 to -6 beats per minute). Heart rate then fell quite quickly during the next ten minutes, which is in agreement with results from Jose (1969); also blood pressure (systolic) changes were minimal (range: +4 to -10mmHg; mean: -4 ± 3mmHg).

As the GTN response has been found to be generally maximum within six minutes after administration of sublingual GTN, I decided to administer sublingual GTN three minutes after the end of infusion of Atropine Sulphate. This would allow for control measurements to be recorded after parasympathetic blockade, and for the maximum heart rate response to sublingual GTN to occur in a relatively stable period. In this study, five normal subjects (age range 22 to 31 years; mean 27 ± 2 years) were studied to determine the effect of parasympathetic blockade on the GTN response. One hour before parasympathetic blockade, a control GTN response was elicited; GTN was again administered three minutes after the end of infusion of Atropine Sulphate, to determine the responses to GTN administration after parasympathetic blockade. Blood pressure was recorded every minute using the "Arterisonde".

The group mean GTN heart rate increase after parasympathetic blockade was attenuated to approximately one half of the control value obtained (Table 8: control response = 19 beats per minute: parasympathetic blocked response = 9 beats per minute $p < .02$); whereas the group

mean systolic blood pressure fall was identical in both cases (- 21mmHg p = 1.0). These results are in disagreement with those of Fitzgerald, who showed no significant difference in heart rate responses to sublingual GTN administration before and after parasympathetic blockade (32 : 29 beats per minute). My results also confirm the claims of Kofi Ekue and others, in that the heart rate response to sublingual GTN administration is most probably partly due to vagal withdrawal as well as sympathetic stimulation. This then led me to study the effect of total cardiac autonomic blockade on normal subjects, to determine if the response to sublingual GTN administration was further reduced, thereby indicating that both the sympathetic and parasympathetic nervous systems were involved.

Conclusion

The heart rate response to sublingual GTN administration appears to be blunted by parasympathetic blockade, in normal subjects. This suggests that vagal withdrawal is important in the heart rate response to GTN administration.

III.3c. Effect of Partial and Total Autonomic Blockade on the Glyceryl Trinitrate Response

Six normal male volunteers (age range: 25 to 35 years; mean 29 ± 2 years) received bolus injections of either Atropine Sulphate (0.04mg/Kg) or practolol (0.6mg/Kg), to produce partial cardiac blockade, the order of administration being allocated at random. Twenty minutes later a "booster dose" of either 0.008mg/Kg atropine sulphate or 0.012mg/Kg practolol, plus the other blocking agent, were administered to produce total cardiac autonomic blockade, all infusions were given over two minutes. These doses have been shown by Jose and Taylor (1969); and Korner, Shaw, Uther, West, McRitchie and Richards (1973) to produce

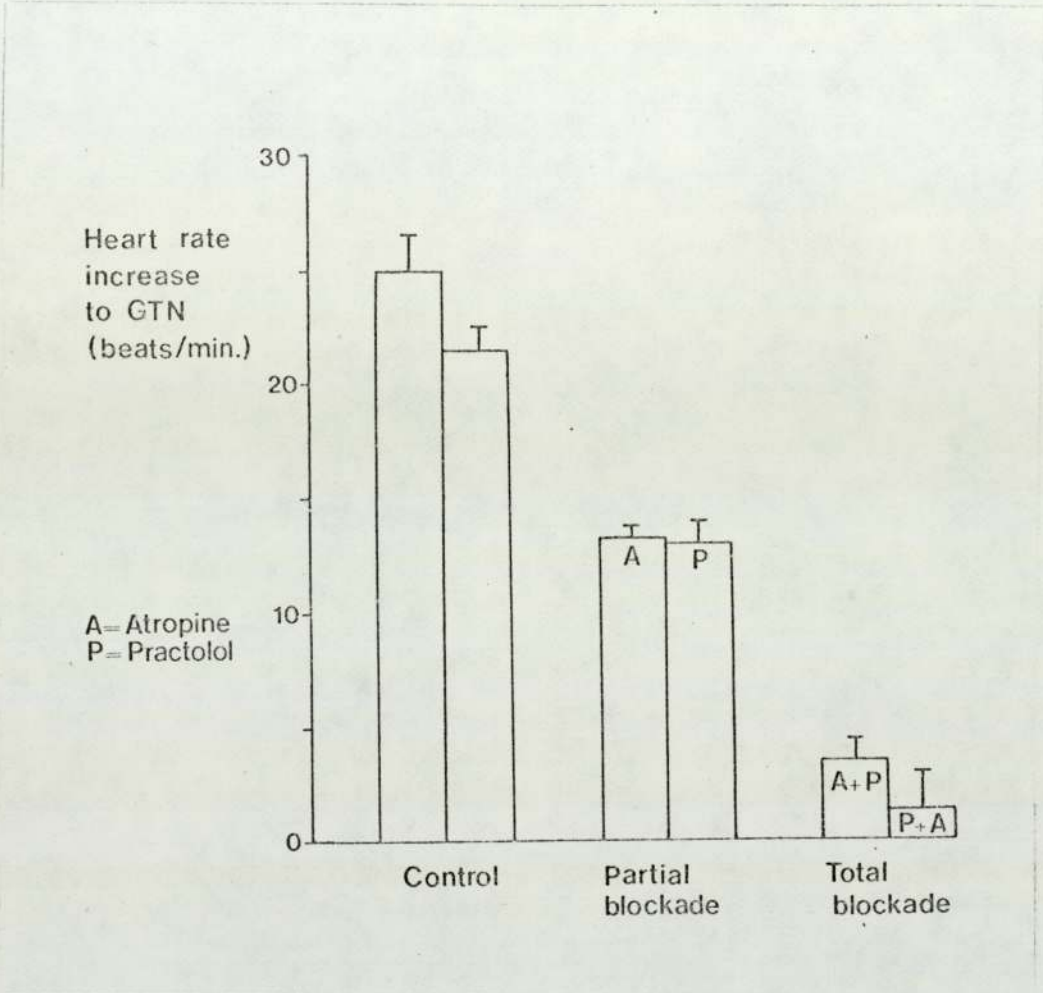


Figure 15. Group data of the heart rate increase to sublingual GTN administration at various levels of cardiac blockade. There is no significant difference ($p>0.05$) between each group at control, partial or total blockade, but both partial blockade ($p<0.05$) and total blockade ($p<0.001$) were significantly lower than control.

partial and total cardiac autonomic blockade.

Control GTN tests were performed thirty minutes before venous cannulation; GTN responses were then elicited three minutes after the end of the first infusion, to determine the heart rate and blood pressure response to GTN during partial cardiac autonomic blockade; and then three minutes after the second infusion to determine these responses during total cardiac autonomic blockade. Blood pressure was recorded every minute using the "Arterisonde" recorder. Practolol was selected as the sympathetic blocking agent as it is cardio selective, and would therefore, not affect the peripheral vessels, which might affect the response to GTN administration. Jose has shown that a dose of 0.2mg/Kg propranolol produces complete sympathetic blockade, and as propranolol is three times more potent than practolol I have used 0.6 mg/Kg as the dose to be used to block the sympathetic system.

Tables 9a and 9b list the individual responses; Figure 15 illustrates the two group's mean heart rate responses to sublingual GTN administration at control and during partial and total cardiac autonomic blockade. There was found to be no significant difference ($p > 0.05$) between groups during control, partial or total blockade; however, the heart rate response to sublingual GTN administration was markedly attenuated after partial blockade (that is at sympathetic $p < 0.05$ and at parasympathetic blockade $p < .02$) in all subjects, moreover, the heart rate response was almost abolished in all subjects during total cardiac blockade $p < .001$.

Conclusion

The heart rate response to sublingual GTN administration appears to be due to both vagal withdrawal and sympathetic stimulation, each contributing approximately 50% to the total heart rate response.

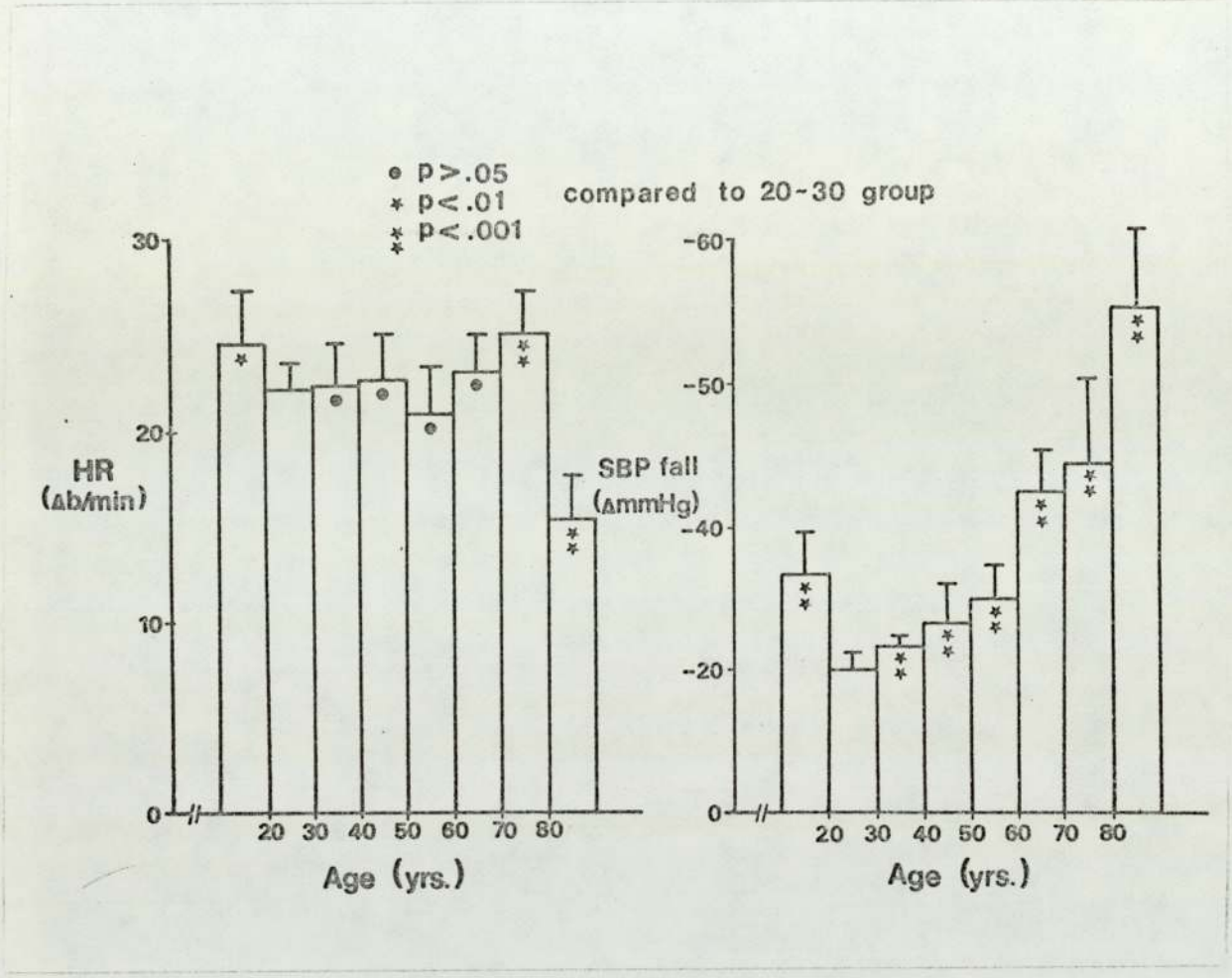


Figure 16. Association of heart rate increase and systolic blood pressure fall with age (arranged in decade groups) to sublingual GTN administration.

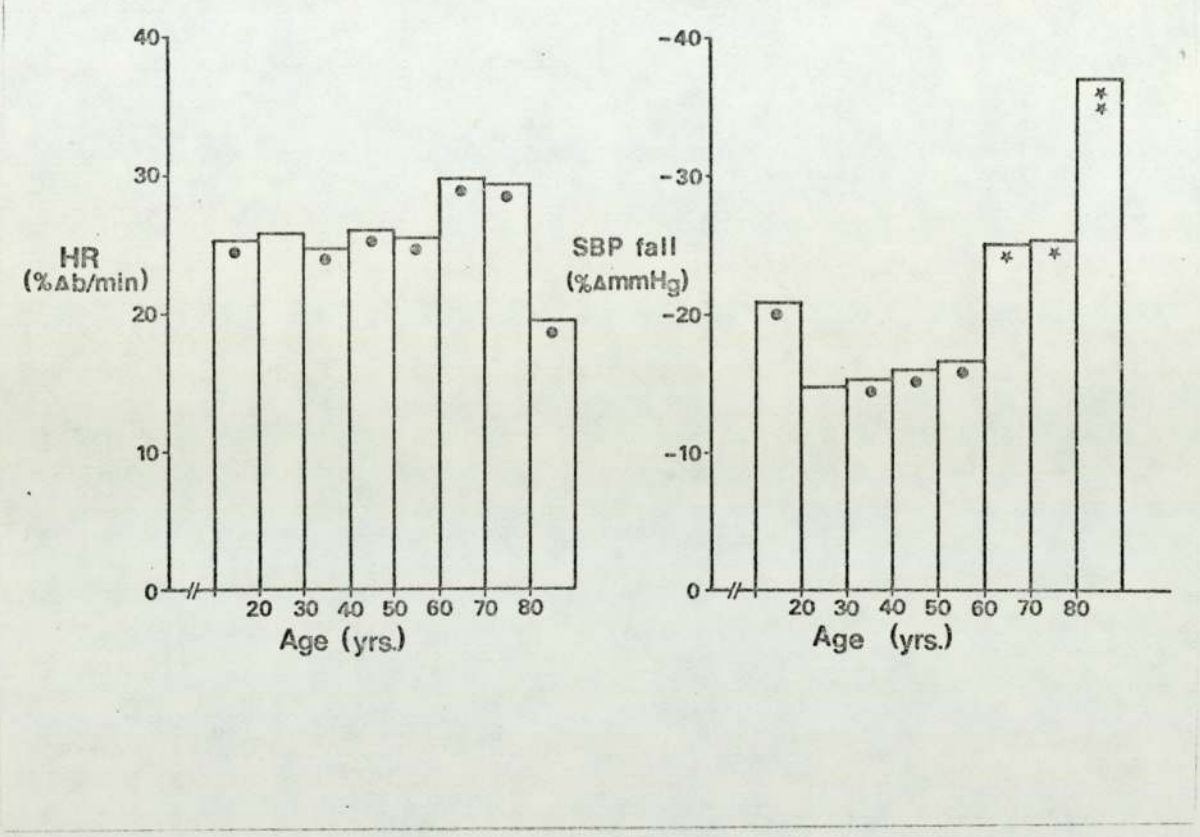


Figure 17. Association of % heart rate increase and % systolic blood pressure fall with age (arranged in decade groups) to sublingual GTN administration. The p values are as shown in Figure 16, this shows that when the % increase in heart rate is used, which would allow for different resting, control heart rates, there was no significant difference between each decade group. Similarly for blood pressure, the significantly different responses found in Figure 16, are now only significant in patients aged over 60 years.

III.4. Effect of Age Upon the Glyceryl Trinitrate Response

I have studied the heart responses to sublingual GTN administration in a population of normotensive subjects and patients, and hypertensive patients, (age range 17 to 91 years), to determine whether these responses are affected by age. Jose and Collinson (1969) have shown that the so-called "intrinsic heart rate" or the heart rate of the heart which is isolated from autonomic influences declines linearly with age (at a rate of six beats per minute per decade), whereas the behaviour of the resting heart rate remains essentially unchanged in man throughout adult life. Therefore, I was interested to determine whether there was any change of heart responses (heart rate increase, maximum tachycardia or systolic blood pressure fall) to sublingual GTN administration with age. Also, as age and blood pressure go so closely together in so many situations, would this manifest itself in the GTN response. All tests were performed on subjects receiving either no therapy, or therapy had been discontinued at least four weeks prior to study.

Table 10 lists the mean heart responses of the subjects arranged in decade groups, also illustrated in Figures 16 and 17. There appears to be a slight decrease in heart rate responses (heart rate increase and maximum tachycardia) to sublingual GTN administration as age increases. The most profound difference, however, was seen in the systolic blood pressure response, where there is a very significant fall in systolic pressure with increasing age, this could not be related to the higher resting pressures, as the percentage systolic blood pressure fall in the group aged over 60 years (Figure 17) was also more marked.

Figure 18 illustrates the relationship between the fall in systolic blood pressure and the increase in heart rate to sublingual GTN administration of three different age groups, showing that with increasing age the slope decreases (that is there is a bigger fall in

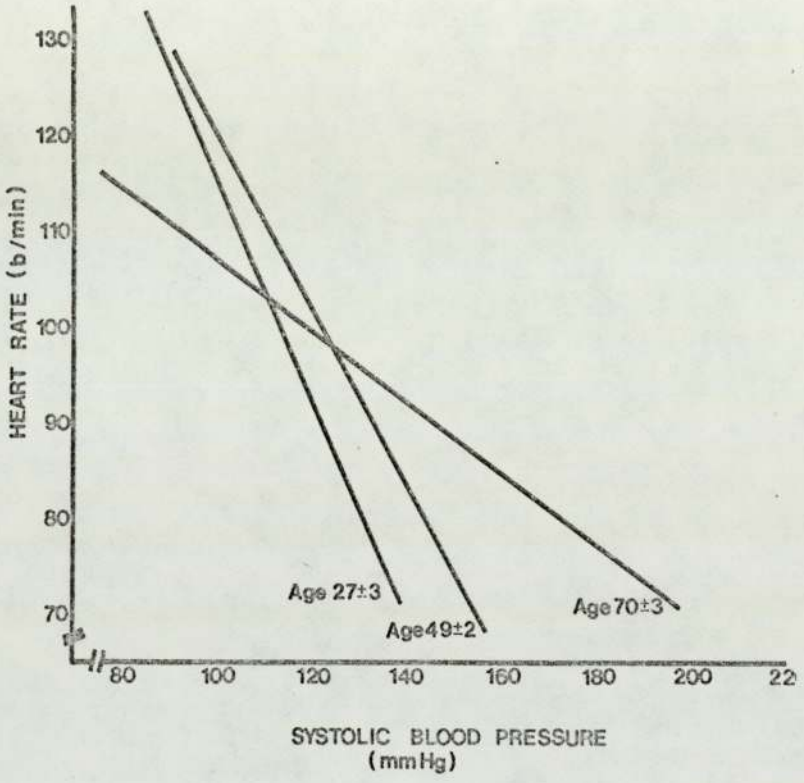


Figure 18. Regression lines of systolic blood pressure to heart rate responses to sublingual GTN administration in three groups of ten patients (young: mean age 27±3 years; middle age: mean age 49±2 years; and elderly: mean age 70±3 years).

systolic blood pressure for a similar heart rate increase in the older group). This is in agreement with results of Gribben, Pickering, Sleight and Peto (1971) who showed a similar decrease in slope with increasing age when subjects were infused with bolus injections of Phenyl Ephrine - decrease of baroreflex sensitivity.

Conclusion

As people age there appears to be a bigger fall in systolic blood pressure and an attenuation of the heart rate response, probably reflecting relative insensitivity of the adrenoceptors in the arterial tree and the heart resulting in a failure of the sympathetic nervous system to compensate for the fall in arterial blood pressure induced by sublingual GTN administration.

III.5. Discussion

From my studies, I can confirm the results of Fitzgerald in which he showed that GTN given sublingually, to a standing subject, produces an increase in heart rate, moreover this increase is attenuated by beta blockers. However, I have found that the sympathetic system is not the only mechanism involved in producing the GTN induced tachycardia.

In my first study of parasympathetic blockade on the GTN responses, I found that the heart rate increase to GTN administration before parasympathetic blockade was reduced to approximately one half after parasympathetic blockade by Atropine Sulphate, whereas Fitzgerald found no significant difference between GTN responses elicited before and after parasympathetic blockade. After a personal communication with Dr. Fitzgerald (1978), I have received the protocol of his study; in this the GTN test was performed last in a series of physiological manoeuvres. As the GTN response was elicited ten minutes after para-

sympathetic blockade and a standard dose of 2.5mg Atropine Sulphate was administered, it is possible that parasympathetic blockade was not complete at the time of testing; from my results the heart rate after parasympathetic blockade is falling fairly quickly after ten minutes, also Jose and Taylor (1969) suggested using an infusion dose of 0.04mg per Kg of Atropine Sulphate for complete parasympathetic blockade; 2.5mg, as used by Fitzgerald, would therefore be unsuitable for subjects who were heavier than 60Kg. Jose and Collinson (1969) stated that the effect of complete parasympathetic blockage (90%) was stable for only ten minutes after infusion of Atropine Sulphate. Incompleteness of parasympathetic blockade is also shown in Fitzgerald's results relating to the heart rate after parasympathetic blockade, whereas his subjects' mean control heart rate, was 110 beats per minute (rising to 139 beats per minute after GTN administration), the subjects in my study had a mean control heart rate of 129 beats per minute (rising to 138 beats per minute after GTN administration).

From my studies with intra-venous GTN administration, it appears that the heart rate and blood pressure responses to GTN administration are not an "all or none" phenomenon, in fact they show a linear relationship to the logarithm of the dose infused. However, heart rate and blood pressure responses to a sublingual GTN tablet are similar to those produced by an intra-venous bolus of 50µg of the drug; the maximum heart rate response occurs later, and the effect lasts longer, than it does with the intra-venous form.

The heart rate response to sublingual GTN administration was always found to be maximal within eight minutes after administration, usually occurring within three to six minutes after administration (mean of five minutes). Despite the logarithmic dose response curve, the response is reproducible in both normal subjects and hypertensive patients receiving

treatment; however, there is a large inter-subject variation in heart rate responses which is not related to the subject's resting heart rate. There is also a large variation in systolic blood pressure responses, the fall generally being greater as the subject gets older in any one individual, but when groups of patients and subjects are arranged in decade groups there is a definite large fall particularly in the groups over 60 years old. These variations of heart rate and blood pressure responses within an individual may be due to variations in autonomic tone and receptor response.

GTN dilates vascular smooth muscle throughout the body, the dominant action being venodilation, leading to a reduction in venous tone and redistribution of the circulating blood volume. Decreased venous return causes systemic blood pressure to fall, this may be considerable in some patients when standing; these factors stimulate the low pressure baroreceptors which act by "turning" the vagus off and the sympathetic system on. Decreased vagal tone and increased sympathetic drive result in the increased heart rate, and vasoconstriction which attenuates the blood pressure fall.

There appears to be a slight attenuation of the heart rate increase after sublingual GTN administration in older subjects; a profound fall in systolic arterial blood pressure was found in the older subjects which was not related to their higher resting pressures, as the percentage systolic blood pressure fall was still marked. As people get older there is increasing "autonomic failure", thought largely to be due to insensitivity of the adrenergic receptors, therefore the GTN induced systolic blood pressure fall may be due to poor sympathetic function in addition to decreased vagal tone. The larger systolic blood pressure fall might have been expected to produce a greater tachycardia, the inappropriate heart rate response is probably further evidence of the relative insensitivity

of the cardiac beta-adreno receptors in these older patients. The GTN test would be a very useful test of B-blockade in these older patients, as the majority would not be able to exercise such that a heart rate above 140 beats per minute could be attained, or the infusion of isoprenaline would be possibly harmful.

GTN is generally well tolerated, the most disturbing side effects are headaches and postural dizziness related to orthostatic hypotension. The headaches, typically throbbing in the temples, occurred frequently but generally were mild and disappeared after ten to thirty minutes; a few subjects reported these headaches lasting for up to thirty-six hours.

Some subjects (7½%) had a profound hypotensive response to GTN administration sometimes associated with bradycardia, and they reported being dizzy or feeling faint. However, these symptoms were relieved immediately they were laid supine, with their legs elevated in severe cases.

There appears not to be any tolerance of the response to sublingual GTN administration, this being demonstrated by repeated challenges to an individual which showed the response to be variable but not determinable from resting heart rate. Tests performed on patients with angina pectoris, taking sublingual GTN to relieve their symptoms showed similar heart rate responses to normal subjects and hypertensive patients of the same age. Therefore, the GTN would appear to give a reproducible challenge, unlike bicycle exercise where the "training effect" reduces the subject's maximum tachycardia.

CHAPTER IV

A COMPARISON OF THE HEART RATE RESPONSES TO GLYCERYL TRINITRATE AND SUB-MAXIMAL EXERCISE BICYCLE

IV.1. Introduction

To assess the usefulness of the heart rate responses to sublingual GTN administration as a test of the degree of beta blockade, I have compared these responses to those obtained from sub-maximal bicycle exercise performed in the same subject. I have used bicycle ergometry since this is the usual way that beta blockade is assessed clinically, furthermore both tests increase sympathetic drive and both involve vagal withdrawal.

Sub-maximal bicycle exercise and GTN tests were performed as previously described (Chapter II: Methods); the exercise test was always performed first, the subject was then rested supine for twenty to thirty minutes, the GTN test then being performed when baseline recordings were stable.

I have compared the heart rate responses from the GTN test to those of sub-maximal bicycle exercise in hypertensive patients and patients with ischaemic heart disease who were being treated with beta blocking drugs. Also, I have compared the heart rate responses to GTN in the same subject on two different occasions; once, on a day when sub-maximal bicycle exercise had been performed, twenty to thirty minutes beforehand, and then on a day when no exercise had been undertaken, to determine if exercise before the test would influence the heart rate and blood pressure responses.

IV.2. Hypertensive Patients: Once and Twice Daily Treatment Trial.

Thirteen patients, (age range: 35 to 58 years; mean 49 ± 2 years) with essential hypertension and average outpatient blood pressure greater

than 150/110mm.Hg. completed the trial; patients who had evidence of target organ damage (that is evidence of retinal fundal changes, left ventricular hypertrophy or renal changes) or asthma were excluded. Their mean outpatient blood pressure had been recorded as 177/113mm.Hg. and their personal details are listed in Table 11. Each patient underwent three trial periods double-blind for four weeks in random order:-

(i)	Placebo	2 capsules placebo taken in the morning and evening.
(ii)	Acebutolol 400mg once daily	2 capsules Acebutolol (200mg) taken in the morning, and 2 capsules placebo taken in the evening.
(iii)	Acebutolol 200mg twice daily	2 capsules Acebutolol (100mg) taken in the morning and evening.

Morning and evening doses were taken from separate containers, between 07.00 and 09.00, and between 19.00 and 21.00 respectively.

At the end of each four week period, the patients would return to the hospital between 08.00 and 10.00, having taken no capsules on that day; therefore, observations were made at least twelve hours (for twice daily treatment), or twenty-four hours (for once daily treatment) after the last dose of active treatment. After the patient had rested supine, in a quiet room, for fifteen minutes, blood pressure was recorded using the Random Zero sphygmomanometer; heart rate was measured by radial palpation over thirty seconds. Venous blood was then obtained for measurement in serum of pharmacologically active material (acebutolol plus its acetyl metabolite, M & B 16,942). Sub-maximal bicycle exercise and GTN tests were then performed as previously described; blood pressure being measured with a standard clinical sphygmomanometer.

Table 12 lists the patients' mean supine blood pressure and heart

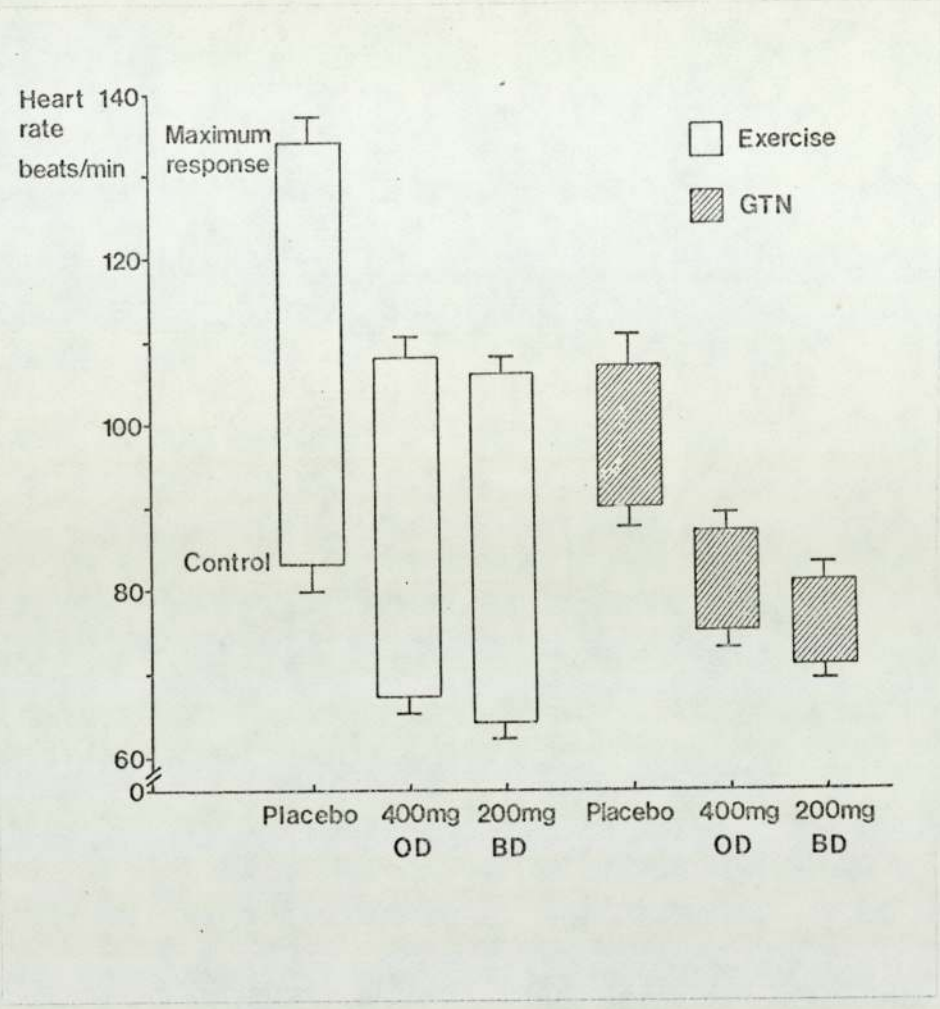


Figure 19. Heart rate responses of sublingual GTN administration (shaded) and sub-maximal bicycle exercise (open) during each trial period. There is a significant reduction in bicycle exercise tachycardia ($p<0.005$) and resting heart rate ($p<0.001$) at both once daily and twice daily therapies, the two treatment periods being similar ($p>0.05$). Also, there was a significant reduction ($p<0.001$), in the GTN induced tachycardia at once and twice daily therapies, however, there was also a significant reduction ($p<0.02$) from once to twice daily therapies.

rate, during each treatment period; also the mean serum level during active treatment is shown. Table 13 lists the patients' mean heart responses to sub-maximal bicycle exercise and GTN testing during each treatment period, whilst Figure 19 illustrates these responses. There is a significant reduction in resting blood pressure ($p < .001$) and resting heart rate at each active treatment period, when compared to the placebo treatment period; differences in systolic blood pressures between active treatments were not significant, but the reduction in resting heart rate at twice daily was significantly different ($p < .02$) from that at once daily (Fig. 19). There was also a significant decrease in exercise tachycardia ($p < .005$) and pre-exercise control heart rates ($p < .001$) during active treatment periods compared to the placebo treatment period, the reduction being similar for both active treatment periods (Fig. 19). The reduction of the GTN induced tachycardia was also significant ($p < .001$) during active treatment when compared to the placebo period; furthermore, the reduction of the GTN induced tachycardia to twice daily treatment was significantly lower ($p < .05$) than that to once daily treatment.

Maximum exercise systolic blood pressure was significantly ($p < .005$) reduced to similar levels during each active treatment period. There was no significant differences found in systolic blood pressure fall to GTN administration at either active treatment period.

Both tests were therefore able to detect beta blockade at twenty-four hours and twelve hours after the last dose of active drug. From my data the GTN test appears to be more sensitive than sub-maximal bicycle exercise in detecting these low levels of beta-blocking drugs.

Figure 20 illustrates the individual's maximum responses of heart rate to GTN administration against their exercising tachycardia, during each treatment period. There was found to be a significant correlation

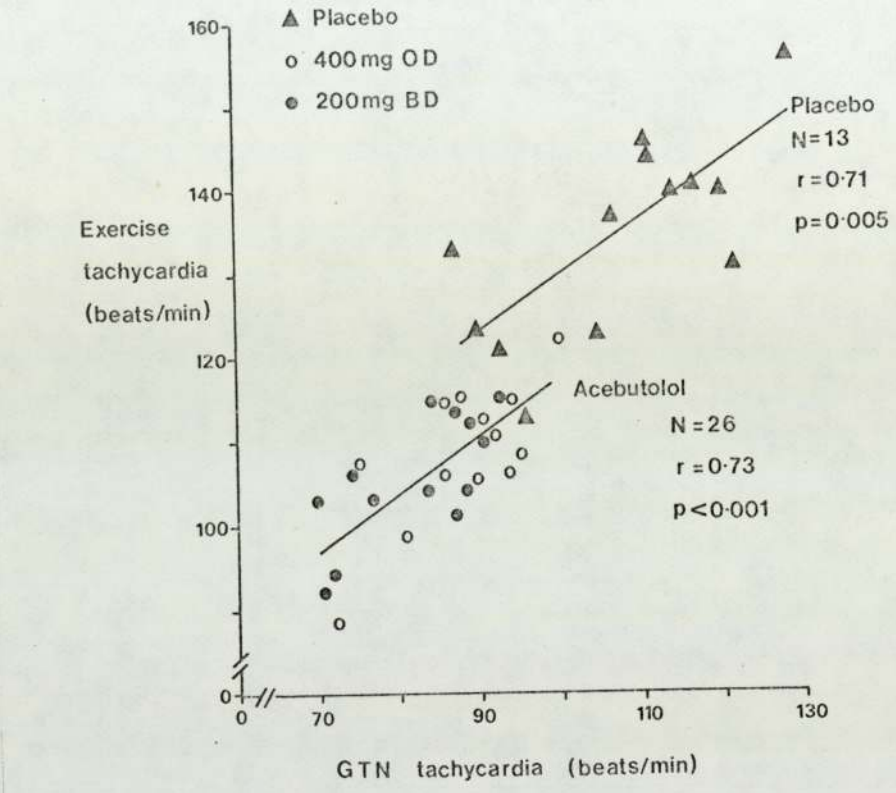


Figure 20. Correlation of the GTN induced tachycardia to the sub-maximal bicycle exercise tachycardia during placebo and active treatment periods.

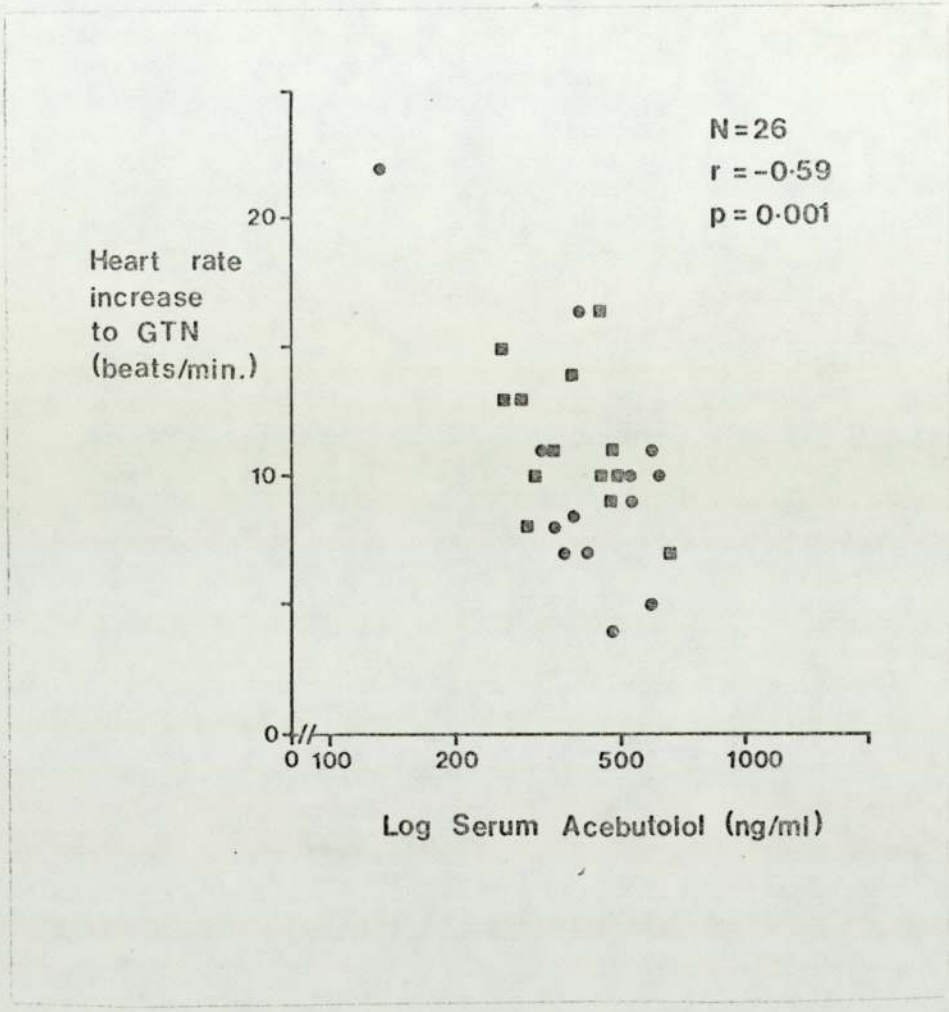


Figure 21. Correlation of Heart Rate increase to sublingual GTN administration to the log of the serum level of Acebutolol. There is a significant correlation ($p<0.001$) between the log serum Acebutolol level and the heart increases, showing that as the serum level increases, the heart rate increase to sublingual GTN administration decreases.

between each test during placebo ($r = 0.71$ $p = .005$) and active treatment periods ($r = 0.73$ $p < .001$). The difference between bicycle exercise tachycardia and GTN tachycardia is due to the degree of sympathetic stimulation, this being greater in bicycle exercise, producing a larger tachycardia. There was also a significant correlation ($r = .59$ $p = .001$) between the maximum heart rate increase to sublingual GTN administration and the logarithm of the serum acebutolol level (Figure 21); this response being reduced as the log serum level increases.

Conclusion

The GTN test appears to be at least as effective as sub-maximal bicycle exercise in detecting low levels of beta adrenoceptor antagonism in hypertensive patients; as there is a significant relationship between exercise and GTN induced tachycardias, as well as a significant correlation between GTN heart rate increase and serum blood levels of acebutolol.

IV.3. Hypertensive Patients: Titrated Treatment Trial.

Unlike the last study where hypertensive patients received a fixed dose of acebutolol, in this study hypertensive patients were titrated with timolol against their resting, supine blood pressure and maximum bicycle exercise heart rate, to achieve the best blood pressure control for that particular patient.

Six essential hypertensive, male patients (age range: 37 to 58 years; mean: 48 ± 3 years) were studied; Table 14 lists their personal details. Patients were first studied using a twenty-four hour ambulatory, intra-arterial pressure recording monitor (Littler, Honour, Sleight and Stott, 1972) to diagnose hypertension; sub-maximal bicycle exercise and GTN tests were also recorded for control responses, blood pressure being measured directly.

Two weeks after receiving 20mg timolol, taken between 07.00 and 09.00, once daily, the patients returned to hospital between 08.30 and 09.30, having taken no capsules on that day; therefore, observations were made at least twenty-four hours after the last dose of active treatment. As before, blood pressure was measured using the Random Zero sphygmomanometer after a fifteen minute supine rest in a quiet room. Exercise and GTN tests were then performed, no blood pressure measurements being made, as titration is against maximum induced tachycardia. If the supine blood pressure was above 150/90mm.Hg. or the maximum exercise tachycardia was above 130 beats per minute, the dose of timolol was increased; all patients then returned after a further two weeks, undertaking the same testing protocol. The dose of timolol was then titrated at two weekly intervals until adequate blood pressure control or maximum exercising tachycardia was achieved; at the end of the titration period patients returned at four weekly intervals for observations to ensure that there was still adequate blood pressure control or maximum exercising tachycardia was still controlled. After sixteen weeks treatment with timolol, the patients returned for a second twenty-four hour intra-arterial ambulatory recording, the capsules being taken between 09.00 and 10.00 on that morning. Sub-maximal bicycle exercise and GTN tests were then performed three hours and twenty-four hours after this dose of active treatment, blood pressure being measured directly.

Tables 15a and 15b lists the patients' heart responses at control and after sixteen weeks treatment (three hours and twenty-four hours after the last active dose), where direct blood pressure measurements had been recorded. As can be seen there is a marked attenuation of the heart rate increase to sublingual GTN administration after sixteen weeks therapy (+ 3 hrs : $p < .01$; + 24 hrs $p < .05$) compared to the control response; the reduction being greater at three hours (when the beta blocking agent,

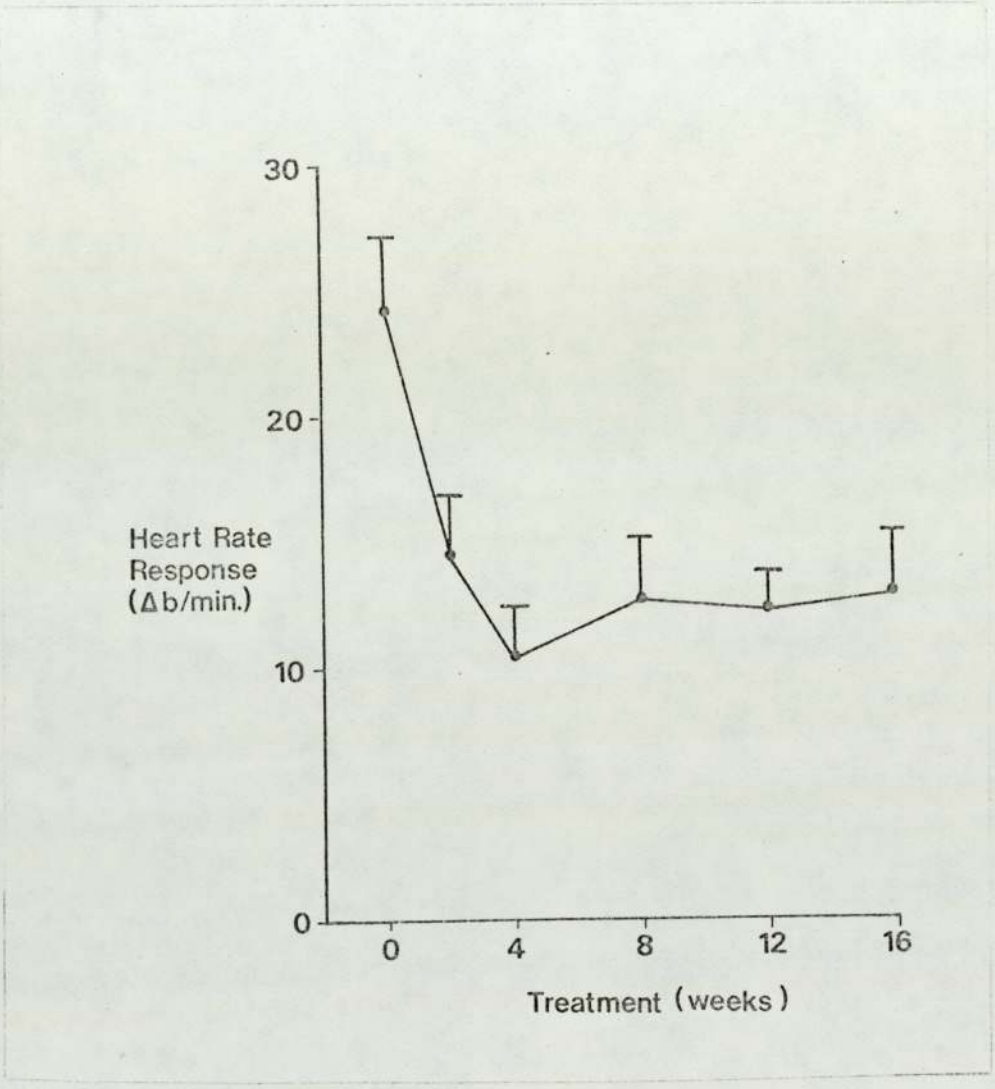


Figure 22. Heart Rate increase to sublingual GTN administration during the titration period to Timolol in six hypertensive patients, showing a significant reduction in the heart rate response after therapy with Timolol once daily.

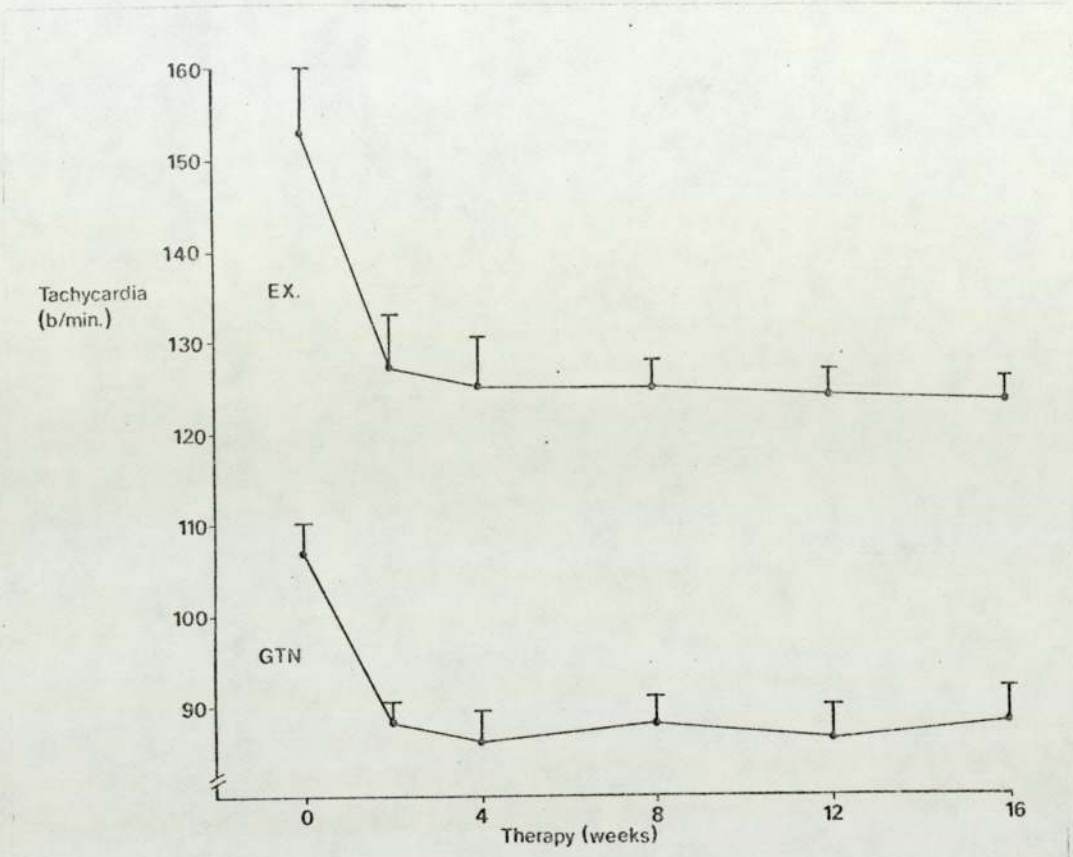


Figure 23. GTN induced and sub-maximal exercise tachycardias during the titration period of Timolol, showing parallel changes in the induced tachycardias of bicycle exercise and GTN administration. The difference being explained by the difference in the sympathetic stimulation.

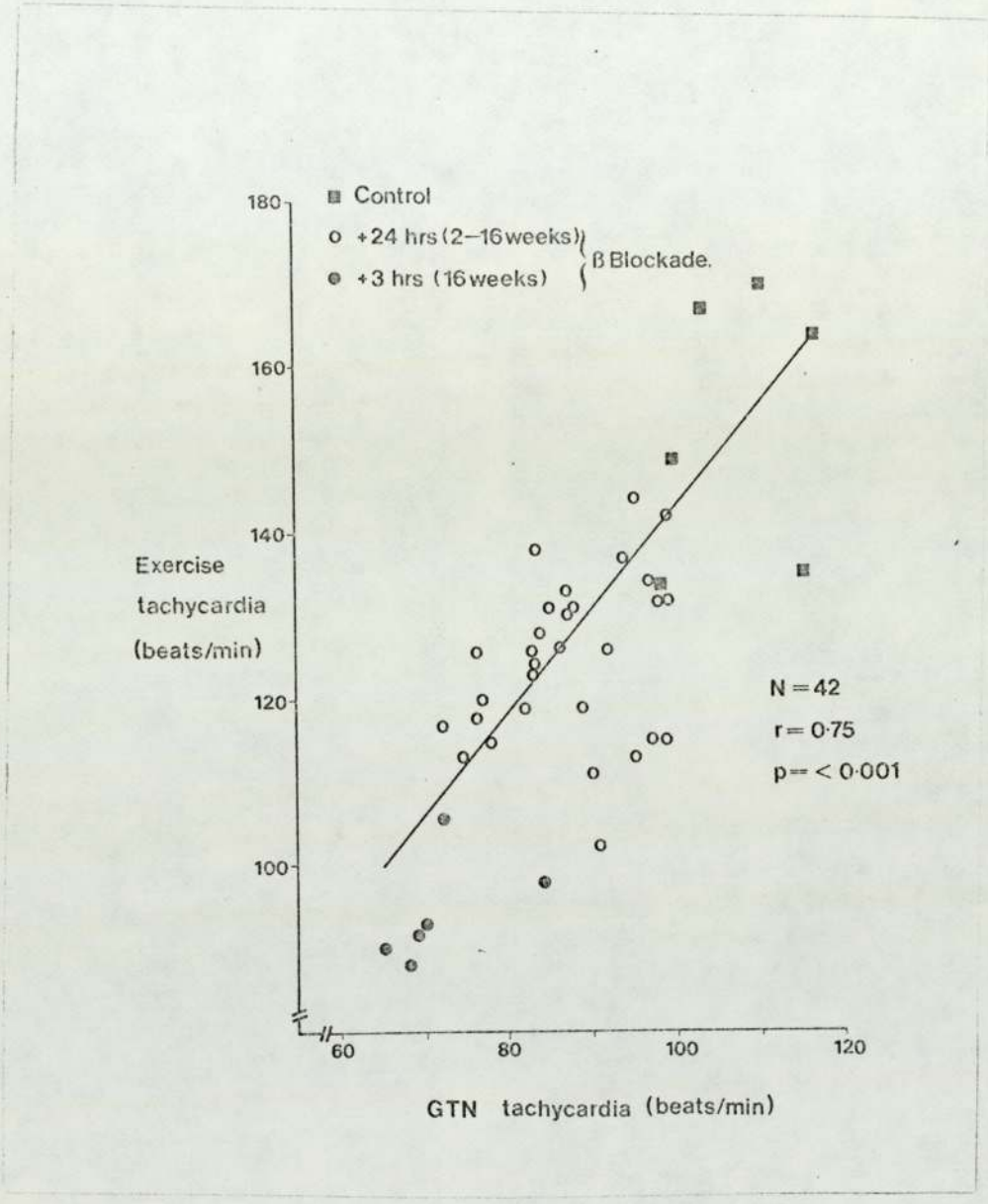


Figure 24. Correlation of GTN induced tachycardia to sub-maximal bicycle exercise tachycardia before and after Timolol therapy.

timolol, is exerting its greatest beta adrenoceptor antagonism effect) after the last dose than at twenty-four hours after the last dose. There was no significant difference in the mean systolic blood pressure fall of the group during active treatment (three hours or twenty-four after the last dose) compared to the control response. Maximum heart rate and heart rate increase to sub-maximal bicycle exercise was also clearly attenuated during therapy.

Figure 22 (Table 16 lists the individuals' results), shows the effect of titrating therapy to timolol; the heart rate increase to sublingual GTN administration is decreased to its minimum value after four weeks therapy and then stabilises at a slightly higher but not significantly so, level eight to sixteen weeks after the start of therapy, despite the groups mean daily dosage of timolol (Table 17) has been increased by 20%. This slight increase in heart rate response could be due to the patients' sensitivity to the GTN decreasing or more probably the patients' sensitivity to the beta blocker decreasing. This is despite the fact that the groups mean exercising tachycardia (Fig. 23) is still falling slightly (but not significantly so) after eight weeks therapy, this could be due to the increased dosage of timolol being used causing more sympathetic blockade, or more likely, it is a "training effect". Figure 24 illustrates the individual's responses of maximum GTN induced tachycardia compared against their maximum sub-maximal exercising tachycardia; as shown in the last study, there is again a significant correlation between each test, showing that the GTN test is at least as effective as sub-maximal bicycle exercise in detecting low levels of beta blockade.

Conclusion

The GTN induced tachycardia shows a significant linear relationship with that of bicycle exercise, during different dosages of therapy. It

has also shown that titration of dosage of beta blocker against effect is advisable; as it is likely that optimal effect only occurs with higher doses in some patients.

IV.4. Hypertensive Patients: Once daily treatment with a diuretic.

Unlike the last two studies, where hypertensive patients were treated with a B-blocker, this study was designed to determine if the effect of lowering the blood pressure with a different hypotensive drug produced a different cardiac response to sublingual GTN administration. Hypertensive patients were thus treated with a diuretic-type hypotensive agent, Indapamide (Natrilix: Servier).

Thirteen essential hypertensive patients (5 male, 8 female: age range 34 - 78 years; mean 58 ± 16 years) with no evidence of target organ damage were studied. Table 18 lists their ages and blood pressures taken before and after treatment with Natrilix (blood pressure was measured by the same observer under similar conditions using a random-zero sphygomomanometer). A twenty-four hour ambulatory intra-arterial recording was undertaken to diagnose hypertension, and a GTN test was elicited before recording began.

The patients were then treated with Natrilix, 2.5mg once daily and returned to the hospital, monthly, when their blood pressure was checked. After four months treatment they remained in hospital for a repeat intra-arterial tape recording and repeat GTN test. Table 19 lists the individual's heart rate and blood pressure responses to sublingual GTN administration before and after treatment.

As shown in Table 18 there is a significant reduction ($p < .001$) in both systolic and diastolic pressure after four months treatment with the diuretic (190/105 before and 162/90 after treatment). There was also a significant reduction of the control, standing systolic pressure ($p < .01$),

after treatment which is also reflected in the minimum systolic pressure attained after sublingual GTN administration ($p < .02$), however, there was no significant difference ($p > .05$) between the systolic pressure falls to sublingual GTN administration before and after treatment. The heart rate response was also significantly reduced at control ($p < .05$) and at maximum after sublingual GTN administration ($p < .02$), but no significant difference ($p > .05$) between before and after treatment test heart rate increases to sublingual GTN administration (Table 19).

Both cuff pressures and intra-arterial control pressures were significantly reduced showing adequate control of blood pressure using once daily diuretic treatment. The significant reduction in standing heart rate could possibly be due to the patient being less anxious on the second visit to hospital.

Conclusion

The heart rate and blood pressure changes to sublingual GTN administration was not significantly reduced to a diuretic, although the drug produced significant control of blood pressure and heart rate.

We would not expect a diuretic to significantly affect sympathetic function or beta adrenoceptors and these observations support our previous conclusions from our studies with beta blockers; namely that the GTN test is able to assess cardiac beta blockade and is not simply dependent on the blood pressure reduction.

IV.5. Patients with Angina Pectoris

Seven male patients with classical symptoms of angina pectoris (age range: 36 to 57 years; mean: 49 ± 3 years), and currently on therapy, were studied. These patients were undergoing tests in another study in which graded, maximal bicycle exercise was carried out; in addition

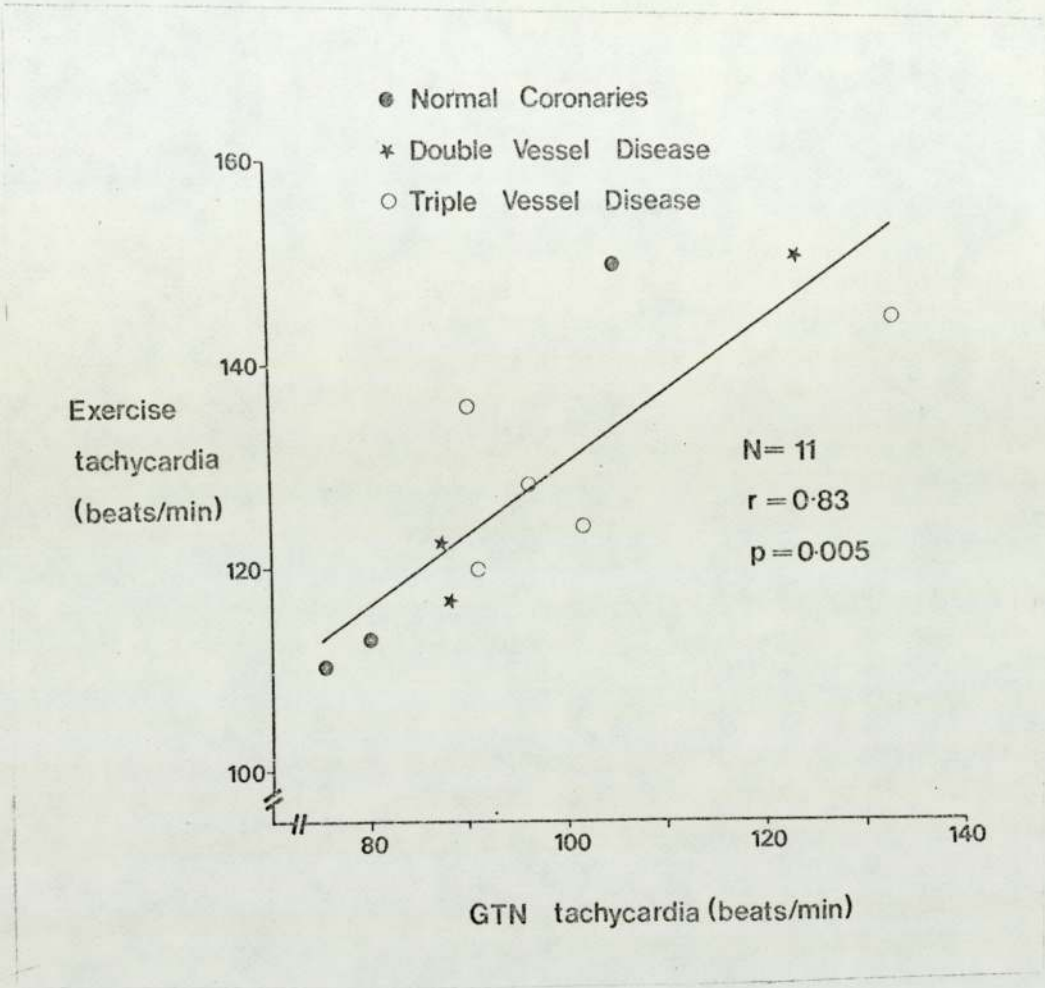


Figure 25. Correlation of GTN induced tachycardia and sub-maximal bicycle exercise tachycardia in patients diagnosed with angina pectoris, on beta-blocker therapy.

diagnostic coronary angiography was undertaken to establish the severity of coronary artery stenosis. Four of the patients (age range: 36 to 57 years; mean: 50 ± 5 years), were found to be inadequately beta blocked and so were restudied after their therapy dosage had been increased. Table 20 lists the patients personal details, and Table 21 lists the individual heart rate responses to GTN and bicycle exercise testing; as can be seen two of the patients had in fact normal coronary arteries and left ventricular function, despite classical symptoms of angina, the other five having moderate to severe double or triple vessel disease. The heart rate responses of the patients with angina pectoris were similar to those seen in the hypertensive groups, and are also similar to those of the normotensive subjects tested.

Figure 25 demonstrates a significant correlation ($r = 0.83$) between the GTN induced tachycardia and maximal exercising tachycardia, showing that the GTN test may be very useful in patients with angina pectoris, to determine whether their beta blocker therapy is adequate enough, rather than using bicycle exercise which may exacerbate their symptoms, producing angina.

Conclusion

The heart rate response to sublingual GTN administration in patients with angina pectoris is similar to that seen in hypertensive patients and normotensive subjects who were similarly beta blocked. The GTN test is safe in such patients as it does not appear to exacerbate angina pain.

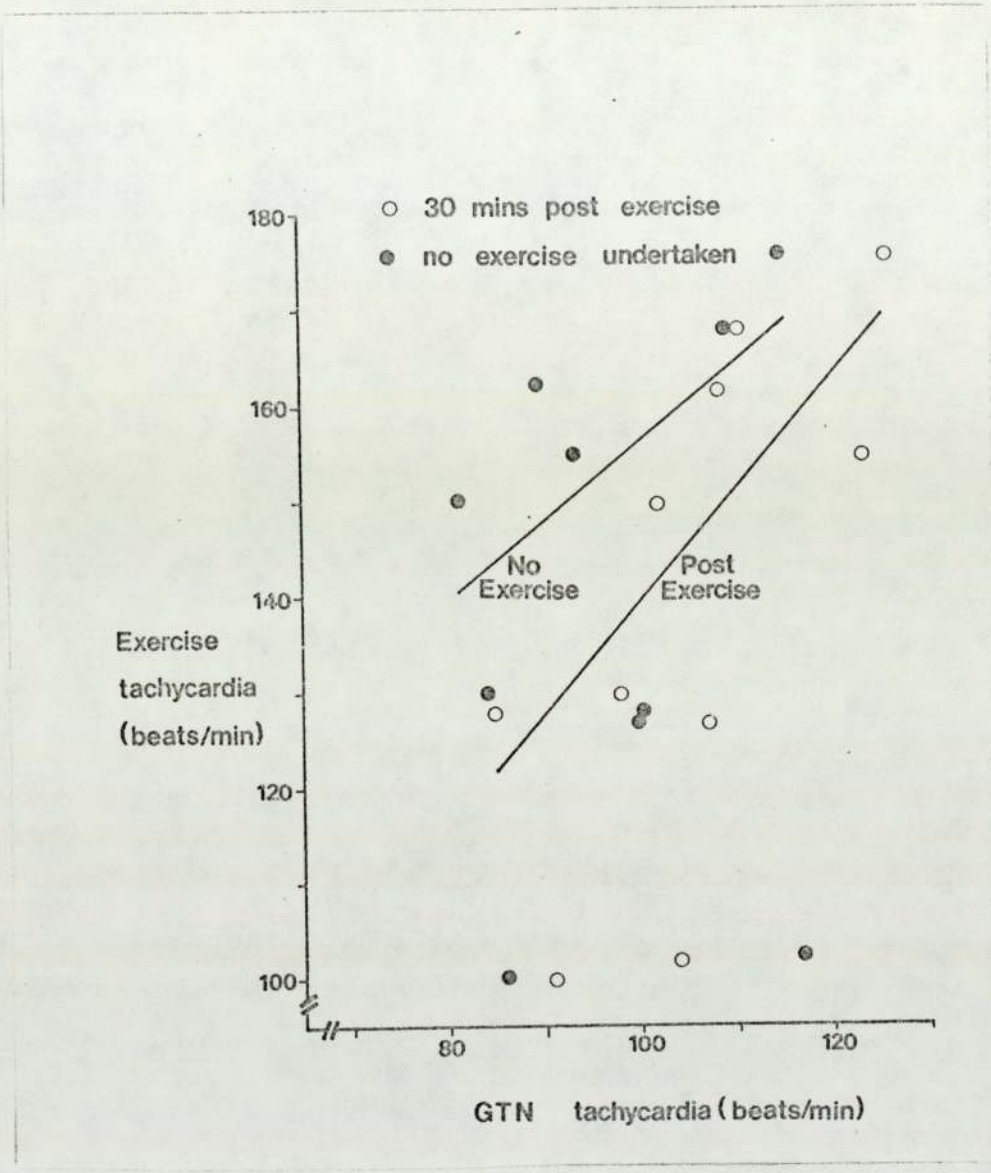


Figure 26. Regression of GTN induced tachycardia to sub-maximal bicycle exercise on the day no exercise was undertaken, and also on the following day 30 mins. after sub-maximal bicycle exercise.

IV.6. Study to compare the heart responses to Glyceryl Trinitrate before and after bicycle exercise.

Ten hypertensive and normotensive patients (age range: 19 to 63 years; mean: 42 ± 5 years) were studied to determine whether the GTN heart responses were affected by either residual sympathetic drive or inadequate vagal tone as a consequence of the previous bicycle exercise. All patients had their blood pressure measured directly on both occasions; on the first day no exercise was undertaken, whilst on the second day a GTN test was performed after twenty to thirty minutes supine rest following sub-maximal bicycle exercise.

Table 22 documents the individual's responses; it shows that there is no significant difference in heart rate responses or systolic blood pressure fall to sublingual GTN administration, whether the GTN test was performed after exercise or on a day when no exercise had been performed (heart rate increase:- post 21: pre 24 beats per minute ($p < 0.2$); systolic blood pressure fall:- post-21: pre-23mm.Hg ($p < 0.5$); tachycardia:- post 105: pre 97 beats per minute ($p < 0.1$) no response being statistically significant). This small heart rate difference is unlikely to make any significant difference in the interpretation of the test of beta blockade using GTN. It almost certainly is due to the fact that the cardiovascular system, particularly the autonomic control of the heart rate, has not always completely returned to control levels.

Figure 26 illustrates the two regressions of GTN induced tachycardia against that subject's exercise tachycardia; it shows that the slopes are similar with the pre-exercise GTN test offset above the post-exercise GTN test. This accounts for the higher GTN induced tachycardia elicited after strenuous exercise.

Conclusion

There is no significant difference between the systolic blood pressure

and heart rate responses to GTN administration when the test is performed before a standard exercise test or performed twenty to thirty minutes after this exercise test has been completed. This suggests that the GTN test can be performed, reproducibly in the outpatient clinic or surgery after a brief rest on each occasion.

IV.7. Discussion

The GTN induced tachycardia compares favourably, in normotensive subjects, hypertensive patients and patients with angina pectoris, with sub-maximal bicycle exercise induced tachycardia in detecting varying levels of beta blockade. The most probably explanation for this relationship is that both tests increase sympathetic drive to the heart and both involve vagal withdrawal, both being more marked for bicycle exercise. Therefore, Kofi Ekue and Hansson were correct in their assumption that vagal withdrawal is associated with the GTN test, but because of the significant relationship of the GTN induced tachycardia to that of sub-maximal bicycle exercise tachycardia, statements that the clinical usefulness of the test is questionable are not valid. The fact that the heart rate increase to sublingual GTN administration bears a logarithmic relationship to the blood serum level of a beta blocker, similar to the results obtained with bicycle exercise (Watson and Littler, 1979), further upholds the GTN test as a useful clinical assessment of beta adrenergic blockade.

I have found the GTN test to be very sensitive to low levels of beta blockade, in fact, more sensitive than bicycle exercise, as determined in the study of hypertensive patients in a once daily versus twice daily therapy trial, where the GTN test was more blunted twelve hours after twice daily therapy, than twenty-four hours after the once daily therapy. Also, I have shown that the test is reproducible in normotensive

subjects and hypertensive patients receiving therapy; there is no loss of sensitivity to GTN as shown in the repeated challenge study, and the study with the patients with symptoms of angina pectoris who produced similar heart rate responses to the normotensive subjects and hypertensive patients. The GTN test is probably a more "standard" stimulus than bicycle exercise, since there is a training effect with bicycle exercise, and also difficulty in calculating a "standard" load which takes into account the physical fitness and motivation of the subject.

The GTN test would be clinically useful in out-patient departments or surgeries as it does not require elaborate equipment; since heart rate changes can be simply measured by radial palpation. In addition the test can be performed after a short rest when strenuous physical activity has been undertaken without compromising the result. I have found that for adequate beta blockade a heart rate of 95 beats per minute should not be exceeded when the test is performed twenty-four hours after the last dose of beta blocker, or of 90 beats per minute if the test is performed two to eight hours after the last dose of beta blocker. The GTN test would be useful in those patients who cannot or are not motivated to bicycle, especially the elderly. The fact that the GTN test appears to be better "standardised" than bicycle exercise is especially useful in this context.

Although, side effects were reported in 50% of the subjects tested, the most common was only slight temple throbbing, which was relieved five to ten minutes after completion of the test; a few felt faint or giddy and were laid supine, upon which the majority's symptoms were relieved. Headaches persisted for up to thirty-six hours in a few cases, but no side effects were reported in any of the patients with coronary artery disease. I believe that the GTN test is safe and acceptable to the majority of patients.

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Beta Blocker	Cardio-selective	I.S.A.	M.S.A.	Lipophilic	Hydrophilic
Propranolol	-	-	✓	✓	-
Oxprenolol	-	✓	✓	✓	-
Practolol*	✓	✓	-	-	✓
Metoprolol	✓	-	-	✓	-
Sotalol	-	-	-	-	✓
Timolol	-	-	-	✓	-
Pindolol	-	✓	✓	✓	-
Acebutolol	✓	✓	✓	✓	-
Atenolol	✓	-	-	-	✓

Table 1. Some Beta-Blockers in common clinical use and their pharmacological properties (*Practolol is forintravenous use only).

	Subject(Age)	Therapy		Heart Rate (b/min)			Systolic Blood Pressure (mmHg)		
				Control Standing Heart Rate	Maximum Heart Rate Post GTN	Heart Rate Increase	Control Standing Systolic	Minimum Systolic Post GTN	Systolic Pressure Fall
Normo-tensive	1 GB (33)	Nil	A	89	114	25	155	124	-31
	2 PB (46)	Nil	A	94	111	17	128	102	-26
	3* JL (39)	Nil	A	94	104	10	135	99	-36
	4 SP (29)	Nil	A	100	125	25	137	118	-19
Hypertensive	5 RL (47)	Acebutolol	B	76	83	7	159	125	-34
	6 CG (42)	Acebutolol	B	66	83	17	170	157	-13
	7 BH (39)	Acebutolol	C	96	108	12	145	106	-39
	8 RR (50)	Acebutolol	C	91	100	9	155	119	-36
	9 JD (47)	Acebutolol	D	57	77	20	139	105	-34
	10 JG (24)	Acebutolol	D	85	107	22	164	150	-14
	11 DG (49)	Propranolol	B	68	74	6	193	178	-15
	12 JM (42)	Propranolol	D	117	148	31	184	172	-12
	13 ER (34)	Propranolol	D	116	150	34	138	122	-16
	14 BJ (63)	Acebutolol & Methyl Dopa	C	111	119	8	115	72	-43
Mean ±S.E.M.	N=3 . 36±5	Normotensive	A	94± 2	117± 4	22± 3	140± 8	115± 7	-25± 3
	N=3 . 46±2	+3HR First Dose	B	70± 3	80± 3	10± 4	174±10	153±15	-21± 7
	N=3 . 51±7	+5HR Chronic Therapy	C	99± 6	109± 6	10± 1	138±12	99±14	-39± 2
	N=4 . 37±5	+27HR "	D	94±14	121± 8	27± 3	156±11	137±15	-19± 5

Table 2. Cardiac effects of responses to sublingual GTN administration to normotensive patients and hypertensive patients on hypotensive therapy (*JL not included as she fainted after two minutes).

Note: The heart rate increase to sublingual GTN administration in the acutely treated hypertensives (B and C), is less than that in the untreated normotensives (A) and chronically treated hypertensives (D). This suggests that the sympathetic component of the response is attenuated by beta blockade.

Heart Rate (b/min)			
Standing Control Heart Rate	Maximum Heart Rate Post GTN	Heart Rate Increase	
83	114	31	
77	106	29	
112	130	18	
69	93	24	
93	114	21	
105	126	21	
96	116	20	
92	112	20	
84	106	22	
84	104	20	
89	111	21	24 hr
85	106	21	
90	114	24	
90	110	20	
85	110	25	
99	117	18	
99	123	24	
82	98	16	
90	112	22	
75	108	33	
Mean	89 ± 2	112 ± 2	23 ± 1	All
± S.E.M.	88 ± 2	110 ± 1	22 ± 1	24 hr

Table 3. Heart responses to repeated challenges of sublingual GTN in one subject. The tests were carried out over twelve months (with several carried out over a twenty-four hours period).

	Heart Rate (b/min)											
	10µg	I.V.	GTN	50µg	I.V.	GTN	100µg	I.V.	GTN	400µg	I.V.	GTN
Subject (Age)	Control	Maximum	Increase	Control	Maximum	Increase	Control	Maximum	Increase	Control	Maximum	Increase
1 MD (30)	82	94	12	80	102	22	81	104	23	92	139	47
2 TS (31)	99	109	10	94	115	21	91	110	19	75	112	37
3 SO (31)*	76	96	20	81	102	21	----------		F A I N T	----------		
4 DR (30)	67	76	9	68	83	15	67	89	22	61	92	31
5 RM (27)	89	101	12	91	106	15	94	120	26	80	122	42
6 SN (25)	75	85	10	69	90	21	68	91	23	75	121	46
7 NH (25)*	87	94	7	----------		F A I N T	----------			----------		
8 RW (30)	74	80	6	74	92	18	76	96	20	64	90	26
Mean 29	81	91	10	79	98	19	80	102	22	75	113	38
±S.E.M. 0.9 N=6	4.7	5.2	0.9	4.5	4.8	1.3	4.6	4.9	1.0	4.6	7.7	3.4
t→10				1.25	-6.65	-6.10	0.72	-4.01	-15.36	1.41	-3.36	-10.53
p				<0.5	<.01	<0.01	<0.5	<.01	<.001	<0.5	<.02	<.001

Table 4a. Heart rate responses to increasing boluses of intra-venous GTN to normotensive subjects.
(* SO and NH are not included as they fainted before the completion of the study)

	Systolic Blood Pressure (mmHg)											
	10µg I.V. GTN			50µg I.V. GTN			100µg I.V. GTN			400ug I.V. GTN		
Subject	Control	Minimum	Fall	Control	Minimum	Fall	Control	Minimum	Fall	Control	Minimum	Fall
1 MD	115	107	- 8	120	110	- 10	117	105	- 12	128	104	- 24
2 TS	107	92	- 15	108	94	- 14	106	90	- 16	114	94	- 20
3 SO *	132	124	- 8	134	100	- 34	----------F A I N T----------					
4 DR	113	107	- 6	118	104	- 14	118	104	- 14	117	104	- 13
5 RM	118	108	- 10	117	99	- 18	111	96	- 15	114	96	- 18
6 SN	125	116	- 9	121	114	- 7	122	107	- 15	120	114	- 6
7 NH *	109	96	- 13	----------F A I N T----------								
8 RW	130	124	- 6	131	115	- 16	134	114	- 20	120	93	- 27
Mean	118	109	- 9	119	106	- 13	118	103	- 15	119	101	- 18
±S.E.M. N=6	3.4	4.4	1.4	3.0	3.5	1.6	3.9	3.5	1.1	2.1	3.3	3.1
t→10				-0.82	1.42	1.98	0.0	3.44	3.52	-0.24	1.65	2.61
p				>0.9	<0.5	<0.2	>0.9	<0.05	<0.05	>0.9	<0.2	<0.05

Table 4b. Systolic blood pressure responses to increasing boluses of intra-venous GTN to normotensive subjects (* SO and NH not included).

		Heart Rate (b/min)						Systolic Blood Pressure (mmHg)					
		50µg I.V. GTN			500µg Sublingual GTN			50µg I.V. GTN			500µg Sublingual GTN		
Subject	(Age)	Control	Maximum	Increase	Control	Maximum	Increase	Control	Minimum	Fall	Control	Minimum	Fall
1 MW	(30)	70	94	24	68	98	30	170	152	- 18	176	156	- 20
2 TS	(31)	94	115	21	85	108	23	108	94	- 14	108	90	- 18
3 IM	(18)	127	148	21	116	140	24	125	89	- 36	123	82	- 41
4 DR	(30)	57	74	17	60	74	14	116	112	- 4	118	109	- 9
5 MI	(29)	66	81	15	62	75	13	124	120	- 4	125	110	- 15
6 RM	(27)	73	89	16	79	99	20	116	98	- 18	110	90	- 20
Mean	28	81	100	19	78	99	21	127	111	- 16	127	106	- 21
±S.E.M. N=6	2.0	10.4	11.1	1.4	8.5	9.9	2.6	9.1	9.5	4.8	10.2	11.0	4.4
t		1.05	0.40	-1.17				-0.10	2.30	3.58			
p		<0.5	<0.9	<0.5				>0.9	<0.1	<0.05			

Table 5. Cardiac effects of responses to 50µg intra-venous GTN and 500µg sublingual GTN administration to determine if the responses were similar.

		Heart Rate (b/min) Test Occurrence (Heart Rate Increase)		
	Subject (Age)	I	II	III
Normo-tensive	1 TS (31)	26	24	16
	2 DR (30)	18	28	23
	3 EW (29)	28	23	29
	Mean 30	24	25	23
	±S.E.M. 1.0	3.1	1.5	3.8
	N = 3			
Hypertensive	1 WR (58)	11	15	10
	2 DT (51)	18	14	14
	3 TF (37)	7	9	10
	4 HJ (39)	24	18	25
	5 PK (52)	12	8	12
	6 JM (53)	8	11	8
	Mean 48	13	12.5	13
	±S.E.M. 3.4	2.7	1.6	2.5
	N = 6			

Table 6. Heart rate increase to sublingual GTN administration, on three separate occasions to three normotensive subjects and six hypertensive subjects on hypo-tensive therapy.

Subject (Age)	Heart Rate (b/min)				Systolic Blood Pressure (mmHg)				ATROPINE DOSE
	Control	Maximum	Increase	Δ 4-10mins	Control	Minimum	Fall	Δ 4-10mins	(at 0.04mg/Kg) mg
1 BP (22)	76	129	53	- 6	128	97	- 31	+ 4	3.0
2 MJ (23)	85	138	53	- 3	114	113	- 1	- 3	3.2
3 RS (24)*	97	146	49	-	113	?	?	-	2.4
4 MD (28)	90	143	53	- 2	128	114	- 14	- 6	2.2
5 MI (27)	74	129	55	- 6	138	118	- 14	-10	3.2
Mean 25	81	135	54	- 4	127	111	- 15	- 4	2.9
±S.E.M. 1.5 N = 4	3.8	3.5	0.5	1.0	4.9	4.6	6.2	3.0	0.24

Table 7. Heart rate and systolic blood pressure responses in normotensive subjects to parasympathetic blockade, using an intra-venous infusion of Atropine Sulphate (0.04 mg/Hg).

(* RS is not included as he felt faint immediate after infusion)

	Heart Rate (b/min)			Systolic Blood Pressure (mmHg)									Atropine
	Control GTN Test			GTN Test after Parasympathetic Block			Control GTN Test			GTN Test after Parasympathetic Block			
Subject (Age)	Control	Maximum	Increase	Control	Maximum	Increase	Control	Minimum	Fall	Control	Minimum	Fall	mg
1 TS (31)	75	108	33	123	138	15	118	101	-17	118	101	-17	2.4
2 AP (30)	69	88	19	107	114	7	130	105	-25	106	86	-20	2.8
3 MK (23)	81	99	18	146	154	8	108	100	- 8	88	75	-13	2.6
4 PL (22)	81	96	15	121	128	7	103	66	-37	108	86	-22	2.4
5 DR (28)	58	70	12	133	141	8	135	117	-18	127	94	-33	2.8
Mean 27	73	92	19	126	135	9	119	98	-21	109	88	-21	2.6
± SEM 1.8	4.3	6.4	3.6	6.5	6.7	1.5	6.1	8.5	4.8	6.5	4.4	3.4	0.09
N = 5													
t	-7.35	-4.93	4.49				1.68	1.10	0.0				
p	<.01	<.01	<.02				<0.2	<0.5	1.0				

Table 8. Heart rate and systolic blood pressure responses to sublingual GTN administration, before and after parasympathetic blockade using an intra-venous solution of Atropine Sulphate, to normal subjects.

		HEART RATE (b/min)								
					CARDIAC BLOCKADE					
		CONTROL			PARTIAL			TOTAL		
	Subject (Age)	Control	Maximum	Increase	Control	Maximum	Increase	Control	Maximum	Increase
I Atropine First	1 PC (35)	91	113	22	137	149	12	108	112	4
	2 FD (25)	94	121	27	136	148	12	98	103	5
	3 DR (28)	83	109	26	133	149	16	113	115	2
	Mean 29	89	114	25	136	149	13	106	110	4
	±S.E.M. 3.0	3.3	3.5	1.5	1.2	0.3	1.3	4.4	3.6	0.9
	N = 3									
II Practolol First	1 MP (26)	62	84	22	57	70	13	95	94	- 1
	2 TS (30)	73	96	23	72	84	12	113	117	4
	3 MD (28)	86	106	20	79	94	15	115	116	1
	Mean 28	74	95	22	69	82	13	108	109	1
	±S.E.M. 1.2	6.9	6.4	0.9	0.5	7.0	0.9	6.4	7.5	1.5
	N = 3									
TOTAL I & II	Mean 29	82	105	23	103	116	13	107	110	3
	±S.E.M. 1.5	4.9	5.4	1.1	15.1	15.1	0.7	3.5	5.3	0.9
	N = 6									

Table 9a. Heart rate responses to sublingual GTN administration before and after partial (with either atropine or practolol intra-venous infusion) and total (with both atropine and practolol intra-venous infusion) blockade in normal subjects.

		SYSTOLIC BLOOD PRESSURE (mmHg)								
					CARDIAC BLOCKADE					
		CONTROL			PARTIAL			TOTAL		
	Subject	Control	Minimum	Fall	Control	Minimum	Fall	Control	Minimum	Fall
I Atropine First	1 PC	123	98	-25	111	87	-24	100	87	-13
	2 FD	121	109	-12	120	98	-22	102	95	- 7
	3 DR	121	98	-23	110	98	-12	98	88	-10
	Mean	122	102	-20	114	95	-19	100	90	-10
	±S.E.M. N = 3	0.7	3.7	4.0	3.2	3.7	3.7	1.2	2.5	1.7
II Practolol First	1 MD	123	105	-18	110	99	-11	108	100	- 8
	2 TS	115	94	-21	96	88	- 8	95	88	- 7
	3 MD	118	108	-10	110	100	-12	108	103	- 5
	Mean	119	103	-16	106	96	-10	104	97	- 7
	±S.E.M. N = 3	2.3	4.3	3.3	4.7	3.8	0.9	4.3	4.6	0.9
Total I & II	Mean	120	102	-18	110	95	-15	102	94	- 8
	±S.E.M. N = 6	1.3	2.5	2.5	3.1	2.4	2.8	2.2	2.8	1.1

Table 9b. Systolic blood pressure responses to sublingual GTN administration before and during partial and total cardiac blockade in normal subjects.

Age Group	Heart Rate (b/min)			Systolic Blood Pressure (mmHg)		
	Control	Maximum	Increase	Control	Minimum	Fall
<20 N = 5: 18 ± 0.4	95 ± 8.9	120 ±10.5	25 ± 3.0	155 ±13.4	121 ±16.7	-33 ± 0.4
21 - 30 N = 26: 27 ± 0.5	84 ± 3.0	107 ± 3.9	22 ± 1.4	137 ± 6.5	117 ± 4.5	-20 ± 2.0
31 - 40 N = 18: 35 ± 0.7	90 ± 2.5	113 ± 9.0	22 ± 2.2	151 ± 8.6	128 ± 9.0	-23 ± 1.7
41 - 50 N = 14: 46 ± 0.8	84 ± 3.9	106 ± 4.3	23 ± 2.4	164 ± 5.8	136 ± 8.0	-28 ± 5.2
51 - 60 N = 20: 54 ± 0.6	81 ± 2.0	102 ± 2.8	21 ± 2.5	169 ± 3.7	138 ± 7.2	-30 ± 4.6
61 - 70 N = 9: 67 ± 0.9	78 ± 2.9	101 ± 2.8	23 ± 2.0	180 ±10.1	135 ± 9.4	-45 ± 5.7
71 - 80 N = 5: 75 ± 0.8	87 ± 1.6	112 ± 1.1	25 ± 2.2	191 ±15.4	143 ±24.7	-48 ±12.1
>81 N = 5: 88 ± 1.4	80 ± 7.1	96 ± 5.6	16 ± 2.2	183 ±18.5	112 ±15.8	-71 ±10.9

Table 10. Cardiac effect of responses to sublingual GTN administration, in normotensive and hypertensive subjects and patients (undergoing no hypotensive therapy) arranged in decade age groups. (There is a significant difference in blood pressure falls ($p < .001$) between those < 60 (N = 74) and those > 60 (N = 18))

Subject (Age)	Mean of 3 outpatient blood pressures before treatment	Sub-maximal bicycle (85%) Exercise Load (W)
1 VB (41)	220/115	65
2 DS (48)	179/118	95
3 BR (35)	163/100	35
4 JJ (55)	197/116	75
5 PD (49)	172/130	60
6 EM (48)	158/117	60
7 VP (37)	180/105	75
8 MF (55)	182/112	90
9 GG (58)	164/109	100
10 RB (45)	153/110	115
11 WB (52)	190/120	80
12 HB (58)	163/107	110
13 MT (57)	170/110	60
Mean 49	177/113	78
±SEM 2	5 2	6

Table 11. Patient details of hypertensive patients undergoing a once daily against twice daily administration of Acebutolol trial.

Administration Period	Blood Pressure mmHg	Heart Rate (b/min)	Serum Acebutolol Level (mg/ml)
I PLACEBO N = 13	169 ± 3/112 ± 3	79 ± 3	-
II ONCE DAILY N = 13	157 ± 4/105 ± 3	68 ± 2	349 ± 42
III TWICE DAILY N = 13	156 ± 3/103 ± 2	64 ± 2	510 ± 64

Table 12. Heart rate, blood pressure and serum Acebutolol levels (mean value: N = 13), of hypertensive patients during placebo; once daily and twice daily treatment with Acebutolol.

		Heart Rate (b/min)			Systolic Blood Pressure (mmHg)		
Administration Period		Control	Maximum	Increase	Control	Maximum	Increase
EXERCISE TEST	I N = 13	82 ± 3	134 ± 3	52 ± 3	167 ± 8	234 ± 9	67 ± 5
	II N = 13	68 ± 2	108 ± 2	40 ± 2	154 ± 7	212 ± 6	58 ± 3
	III N = 13	65 ± 2	106 ± 2	41 ± 2	155 ± 7	209 ± 5	54 ± 3
					Control	Minimum	Fall
GTN TEST	I N = 13	90 ± 3	107 ± 4	17 ± 2	171 ± 6	152 ± 6	-19 ± 3
	II N = 13	75 ± 2	87 ± 2	12 ± 1	155 ± 8	138 ± 8	-17 ± 2
	III N = 13	72 ± 2	81 ± 2	9 ± 1	151 ± 6	133 ± 6	-18 ± 2

Table 13. Heart rate and blood pressure response (mean values) to bicycle exercise and GTN tests in hypertensive patients undergoing placebo; once daily and twice daily treatment with Acebutolol.

Subject (Age)	Mean of 3 outpatient blood pressures before treatment	Sub-maximal bicycle (85%) Exercise Load (W)
1 WR (58)	183/113	100
2 DT (51)	153/105	135
3 TF (37)	158/106	150
4 KJ (39)	175/115	120
5 PK (52)	165/92	120
6 JM (53)	182/11	90
Mean 48	169/107	119
± SEM 3	5 / 3	9

Table 14. Patient details of hypertensive patients undergoing a once daily administration of Timolol, being titrated to their blood pressure, trial.

	Heart Rate (b/min)								
	Control Test			16 weeks treatment					
				+ 24hrs			+ 3hrs		
Subject	Control	Maximum	Increase	Control	Maximum	Increase	Control	Maximum	Increase
1 WR	77	110	33	62	72	10	60	65	5
2 DT	82	103	21	86	99	13	75	84	9
3 TF	83	116	33	82	92	10	66	71	5
4 KJ	89	115	26	70	95	25	58	68	10
5 PK	83	98	15	70	82	12	57	68	11
6 JM	83	100	17	78	85	7	66	70	4
Mean GTN	83	107	24	75	88	13	64	71	7
± S.E.M.	1.6	3.2	3.2	3.6	4.0	2.6	2.8	2.7	1.2
1 WR	59	170	111	48	117	69	45	90	45
2 DT	76	167	91	77	132	55	63	98	35
3 TF	82	164	82	64	126	62	63	106	43
4 KJ	74	135	61	60	114	54	44	88	44
5 PK	64	130	66	54	119	65	44	92	48
6 JM	74	149	75	76	131	55	52	93	41
Mean Ex.	72	153	81	63	123	60	52	95	43
± S.E.M.	3.4	7.0	7.4	4.8	3.1	2.6	3.7	2.7	1.8

Table 15a. Heart rate responses, in hypertensive patients, to sublingual GTN administration and sub-maximal bicycle exercise before and after therapy with once daily Timolol.

	Systolic Blood Pressure (mmHg)								
	Control Test			16 weeks therapy					
				+ 24hrs			+ 3hrs		
Subject	Control	Minimum	Fall	Control	Minimum	Fall	Control	Minimum	Fall
1 WR	155	103	-52	163	154	- 9	155	148	- 7
2 DT	175	135	-40	131	105	-26	135	80	-55
3 TK	170	130	-40	140	75	-65	130	90	-40
4 HJ	178	152	-26	168	132	-36	161	137	-24
5 PK	167	149	-18	148	128	-20	162	135	-27
6 JM	193	169	-23	131	122	- 9	113	90	-23
Mean GTN	173	140	-33	147	119	-28	143	113	-29
± S.E.M.	5.2	9.2	5.3	6.5	11.0	8.6	8.1	12.2	6.7
	Control	Maximum	Increase	Control	Maximum	Increase	Control	Maximum	Increase
1 WR	175	253	78	170	234	64	178	214	36
2 DT	172	248	76	128	216	88	131	153	22
3 TF	178	249	71	152	230	78	146	198	52
4 HJ	154	208	54	139	197	58	160	196	36
5 PK	164	237	73	138	231	93	182	218	36
6 JM	188	244	56	128	218	90	121	206	85
Mean Ex.	172	240	68	143	221	79	153	198	45
± S.E.M.	4.8	6.7	4.2	6.6	5.7	6.0	10.1	9.6	9.0

Table 15(b). Systolic blood pressure responses in hypertensive patients, to sublingual GTN administration and sub-maximal bicycle exercise before and after therapy with once daily Timolol.

Heart Rate (b/min)

	Control Test			Once Daily Timolol Therapy														
				+2 Weeks			+4 Weeks			+8 Weeks			+ 12 Weeks			+ 16 Weeks		
Subject	Cont.	Max.	Incr.	Cont.	Max.	Incr.	Cont.	Max.	Incr.	Cont.	Max.	Incr.	Cont.	Max.	Incr.	Cont.	Max.	Incr.
1 WR	77	110	33	67	83	16	63	74	11	65	76	11	62	77	15	62	72	10
2 DT	82	103	21	73	87	14	77	94	17	80	98	18	74	98	14	86	99	13
3 TF	83	116	33	76	83	7	79	84	5	76	83	7	74	83	9	82	92	10
4 HJ	89	115	25	66	91	25	71	90	19	75	99	24	79	97	18	70	95	25
5 PK	83	98	15	72	86	14	66	78	12	72	84	12	68	76	8	70	82	12
6 JM	83	100	17	88	99	11	80	95	15	79	87	8	76	87	11	78	85	7
Mean GTN	83	107	24	74	88	15	73	86	13	75	88	13	72	86	13	75	88	13
± SEM GTN	1.6	3.2	3.2	3.3	2.5	2.5	2.9	3.5	2.0	2.2	3.7	2.7	2.5	3.9	1.6	3.6	4.0	2.6
1 WR	59	170	111	52	138	86	54	113	59	49	126	77	49	120	71	48	117	69
2 DT	76	167	91	60	130	70	64	137	73	70	134	64	70	132	62	77	132	55
3 TF	82	164	82	62	124	62	66	128	62	62	123	61	64	126	62	64	126	62
4 KJ	74	135	62	54	102	48	63	111	48	63	115	52	68	115	47	60	113	54
5 PR	64	130	66	57	126	69	55	115	60	58	119	61	53	118	65	54	119	65
6 JM	74	149	75	72	142	70	73	144	71	65	133	68	66	130	64	76	131	55
Mean EX.	72	153	81	60	127	68	63	125	62	61	125	64	62	124	62	63	123	60
± SEN EX.	3.4	7.0	7.4	2.9	5.7	5.1	2.9	5.6	3.7	2.9	3.1	3.4	3.5	2.8	3.3	4.8	3.2	2.6

Table 16. Heart rate responses to sublingual GTN administration and sub-maximal bicycle exercise in hypertensive patients before and during titrated treatment with Timolol, once daily. The patients being tested twenty-four hours after their last dose of Timolol.

	Timolol Dosage (mg/day)					
		Once Daily Timolol Therapy				
Subject	Control Test	+2 weeks	+4 weeks	+8 weeks	+12 weeks	+16 weeks
1 WR	-	20	30	30	30	30
2 DT	-	20	20	20	25	25
3 TF	-	20	20	25	25	30
4 HJ	-	20	20	20	25	25
5 PK	-	20	30	30	30	30
6 JM	-	20	30	40	40	40
Mean	-	20.0	25.0	27.5	29.2	30.0
± SEM		0.0	2.2	3.1	2.4	2.2

Table 17. Dosage schedule for hypertensive patient, in a titrate trial of timolol.

	Random Zero Blood Pressure	
	Once Daily Natrilix	
Subject (Age)	Before Treatment	After Treatment
1 ES (69)	220/118	184/102
2 DR (77)	222/106	184/ 84
3 MT (78)	180/100	174/ 96
4 HH (67)	220/104	200/ 92
5 KL (75)	230/120	200/108
6 SB (69)	170/ 91	132/ 86
7 PO'M (62)	209/111	200/ 96
8 JG (60)	203/109	176/ 90
9 LW (38)	173/104	116/ 76
10 RM (39)	153/ 95	122/ 78
11 DH (40)	155/ 94	132/ 80
12 CC (45)	160/ 95	133/ 81
13 PW (39)	177/119	151/105
Mean 58	190/105	162/ 90
±S.E.M 4.6	7.8/2.8	8.8/2.9
T		7.80 8.42
p		<.001 <.001

Table 18. Patient details of hypertensive patients undergoing a once daily administration trial with Natrilix (Indapamide).

	Before Treatment						After 16 weeks Treatment					
	Heart Rate (b/min)			Systolic Pressure (mmHg)			Heart Rate (b/min)			Systolic Pressure (mmHg)		
Subject	Control	Maximum	Increase	Control	Minimum	Fall	Control	Maximum	Increase	Control	Minimum	Fall
1 ES	97	121	24	194	151	-43	83	108	25	175	144	-31
2 DR	86	99	13	205	159	-46	72	87	15	197	152	-45
3 MT	87	102	15	180	146	-34	84	103	19	174	138	-36
4 HA	91	117	26	230	182	-48	83	97	14	160	113	-47
5 KC	87	123	36	222	142	-80	80	115	35	202	138	-64
6 SB	68	101	33	170	117	-53	72	103	31	178	126	-52
7 P'OM	60	97	37	190	151	-39	64	95	31	183	136	-47
8 JG	72	86	14	189	156	-33	66	79	13	182	162	-20
9 CW	77	107	30	170	142	-28	67	105	38	154	126	-28
10 RM	81	99	18	154	140	-14	62	78	16	150	130	-20
11 PH	85	113	28	182	130	-52	91	116	25	154	107	-47
12 CC	50	80	30	182	154	-28	55	80	25	149	131	-18
13 PW	83	105	22	190	177	-11	71	101	30	170	150	-20
Mean	79	104	25	189	150	-39	73	97	24	171	135	-36
± SEM	3.6	3.5	2.3	5.8	4.8	5.0	2.9	3.6	2.3	4.8	4.2	4.1
p	<.05	<.02	>0.05	<.01	<.02	>0.05						

Table 19. Cardiac effect of responses to sublingual GTN administration in hypertensive patients before and after sixteen weeks treatment with Natrilix, 2.5mg, once daily.

Subject (Age)	Disease of Coronary Arteries (number of vessels involved)	Left Ventricular Function	Treatment
1 FB (53)	severe (2)	normal	Metoprolol (100mg bd)
2 FM (57)	moderate (3)	normal	Metoprolol (100mg bd)
3 BM (42)	severe (3)	hypertrophy	Metoprolol (100mg bd)
4 AR (57)	severe (2)	dyskinsia	Metoprolol (200mg bd)
5 NS (50)	severe (3)	moderate impairment	Atenolol (100mg bd)
6 DS (36)	normal (0)	normal	Propranolol (80mg ds)
7 DW (45)	normal (0)	normal & mild mitral regurgitation	Metoprolol (100mg bd)
Mean 49 ± SEM 3.0			

Table 20. Patient details of patients diagnosed as having angina, and the diagnostic cardiac catheterisation data.

Subject		Heart Rate (b/min)					
		GTN Test			Sub-Maximal Bicycle Exercise Test		
		Control	Maximum	Increase	Control	Maximum	Increase
1 FB	II	70	87	17	60	122	62
2 FM	I	100	133	33	65	144	79
	II	87	102	15	67	124	57
3 BM	II	77	91	14	65	120	55
4 AR	I	87	123	36	62	150	88
	II	72	88	16	46	117	71
5 NS	I	77	90	13	70	136	66
	II	74	96	22	52	128	76
6 DS	I	96	105	9	78	150	72
	II	65	75	10	52	110	58
7 DN	II	71	80	9	51	113	62
I not adequately blocked							
	Mean	90	113	23	69	145	76
N = 4	± SEM	5.1	9.5	6.9	3.5	3.3	4.7
II Adequately blocked							
	Mean	74	88	15	56	119	63
N = 7	± SEM	2.6	3.5	1.7	2.8	2.4	2.9

Table 21. Heart rate responses to sublingual GTN administration and sub-maximal bicycle exercise in patients with angina on beta-blocker therapy.

Subject (Age)	Heart Rate (b/min)						Systolic Blood Pressure (mmHg)						Exercise Heart Rate (b/min)
	No Exercise that Day			20 min post Exercise			No Exercise that Day			20 min post Exercise			
	Control	Maximum	Increase	Control	Maximum	Increase	Control	Minimum	Fall	Control	Minimum	Fall	
1 TC (52)	63	81	18	81	102	21	174	153	- 21	159	148	- 11	150
2 PK (52)	66	84	18	73	98	15	186	162	- 24	167	149	- 18	130
3 JU (63)	69	99	30	84	107	23	162	125	- 37	137	108	- 29	127
4 DW (44)	70	100	30	70	85	15	156	132	- 24	164	151	- 13	128
5 TD (30)	76	93	17	99	123	24	158	145	- 13	144	124	- 20	155
6 MS (25)	79	114	35	100	125	25	177	161	- 16	172	150	- 22	176
7 ST (50)	67	86	19	75	91	16	166	119	- 47	157	141	- 16	100
8 DR (27)	84	109	25	93	110	17	157	142	- 15	172	147	- 25	168
9 PS (54)	90	116	26	83	104	21	179	164	- 15	175	152	- 23	102
10 DO (19)	70	89	19	80	108	28	188	169	- 19	185	150	- 35	162
Mean 42	73	97	24	84	105	21	170	147	- 23	163	142	- 21	140
± SEM 4.8	2.7	4.0	2.0	3.3	4.0	1.4	3.8	5.5	3.4	4.6	4.6	2.3	8.4

Table 22. Cardiac effects of responses to sublingual GTN administration in hypertensive patients, on a day when no sub-maximal exercise was performed and on a day when exercise had been performed, the patient having rested supine for at least twenty minutes post exercise.