THE INFLUENCE OF THE SYMPATHETIC NERVOUS SYSTEM AND SYMPATHOMIMETIC AGENTS ON VASCULAR SMOOTH MUSCLE

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DECLARATION AND ACKNOWLEDGEMENTS

I declare that this thesis is of my own composition and that it is a record of original work conducted during the years 1966, 1967, 1968 and 1969 in the Department of Human Physiology and Pharmacology, University of Adelaide. The work described herein has not been submitted for any other degree, award or diploma.

I wish to record my gratitude to Professor R. F. Whelan for his assistance, guidance, invaluable discussion and constant encouragement during this work.

I record my gratitude to Dr I. S. de la Lande, Professor J. A. Downey, Dr J. Q. K. Ho, Dr L. B. Jellett, Dr G. C. Scroop, Dr Y. F. Teh, Dr J. A. Walsh and Dr J. G. Waterson with whom I have had the privilege of working during various stages of these investigations. I would also like to thank Mrs J. Cooper, Mrs M. A. Deutrom, Miss F. P. Katic, Mrs L. L. Kingston, Miss T. Mannik, Mr D. E. Smale and Mrs M. L. Wilkins for expert technical assistance, and to those colleagues, students and patients who acted as subjects for some of these studies.

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C O N T E N T S

INTRODUCTION

Historical Aspects

Galen (circa A.D. 130-200) was probably the first to distinguish the sympathetic trunks. He regarded them as 'costal branches' of the 'sixth' pair of cerebral nerves (now known as the tenth pair, or vagi), and he realised that they descended across the roots of the ribs, received connections from the thoracic and lumbar parts of the spinal cord and were distributed to the viscera. He also described three swellings on each 'costal' nerve, one just above the level of the larynx, another at the thoracic inlet and a third in the upper abdomen. There is little doubt that these were the superior and inferior cervical (or stellate) sympathetic ganglia and the coeliac ganglia of the coeliac plexus. He suggested that the 'costal' nerves and their branches were the pathways through which the viscera were endowed with sensitivity and motor power.

Galen is said to have originated the idea that 'sympathy', or 'consent', exists between all parts of the body, and that the brain and nerves played an essential role in this relationship. He believed that the brain generated 'animal spirits' from the 'vital spirits' in the blood, and that the former were conveyed throughout the body by the 'hollow nerves' and their interconnections, so that every part was influenced and brought into 'sympathy'. Galen's ideas dominated

all medical writing and teaching up to the middle of the 17th Century and it was not until that time that new information was added to the knowledge of the sympathetic nervous system.

Willis, in 1664, described, for the first time, the pre-vertebral sympathetic plexuses and their branches. He thought that the sympathetic trunks had an intracranial origin, but demonstrated the cervical ganglia and rami communicantes. He delineated more accurately the distribution of the thoracic splanchnic nerves. Willis adhered to the 'humoral theory', but differentiated 'voluntary' and 'involuntary' movements, ascribing to the cerebellum the special function of generating animal spirits which were responsible for involuntary activities such as the heart beat, respiration and gastrointestinal movements. The sympathetic ganglia he regarded as storage depots for animal spirits and considered that the rami communicantes strengthened, or reinforced, the sympathetic trunks, serving as channels through which involuntary spirits from the cerebellum and voluntary spirits from the cerebrum and cord could be brought together.

Winslow (1732) observed that interconnections existed between hepatic, renal and splenic plexuses. He was also the first to use the term 'great sympathetic' nerves to indicate their importance in effecting 'sympathy' between various organs.

Whytt's work (1751 and 1765) marked another conspicuous advance in knowledge of autonomic function. He adhered to the humoral

theory in the main, accepting Willis's suggestions about the cerebellum and animal spirits, but he had a clearer understanding of the part played by the nervous system in the phenomena of 'sympathy'. He elaborated Willis's conception of voluntary and involuntary activities, and regarded reactions such as the contractions of the intestines and bladder as responses resulting from local stimulation produced by distension of the muscular walls or irritation of the mucosa. Whytt's theories provided the authentic basis of all modern ideas of reflex action.

About the middle of the 19th Century there was much conflict with regard to the nature and destination of the different types of fibres of the sympathetic nervous system. Some of the theories postulated were that: (a) the sympathetic fibres arose exclusively in the ganglia, but passed either peripherally along spinal nerves or centrally along spinal nerve roots; (b) the rami communicantes contained sympathetic fibres passing in both directions between the cord and the sympathetic ganglia; and (c) the rami communicantes contained sympathetic fibres arising only from the cord.

Remak (1854) showed that the white rami contained myelinated fibres, and traced them to the cord through both ventral and dorsal spinal nerve roots. He supposed that a proportion arose within the cord and he followed some of them through ganglia to terminations in higher or lower ganglia in the sympathetic trunk.

He also observed that the grey rami were composed of fine myelinated and unmyelinated nerve fibres which were distributed with the spinal nerves.

Gaskell (1886), from a series of anatomical studies, was able to demonstrate the cranial, thoraco-lumbar and sacral outflows of the autonomic nervous system, and he later grouped them together with the sympathetic trunks, and prevertebral and other ganglia as the 'involuntary nervous system'. Langley subsequently separated the nerves into the sympathetic and parasympathetic systems.

About the middle of the 19th Century striking advances in the understanding of the physiology of the autonomic nervous system were also made and its relationship to the adrenal medulla more clearly established.

One of the earliest clues as to the function of the adrenal medulla and to the action of the adrenal amines on the circulation was provided by the experiment of Brown-Sequard, in 1856, which showed that stimulation of the adrenal gland was followed by constriction of the cerebral vessels. In 1894, Oliver, a general practitioner in the Yorkshire town of Harrogate, who was interested in the problem of high blood pressure and the possible aetiological role of oversecretion of the adrenal glands, administered an extract of the adrenal gland to one of his children, and noted a decrease in size of the radial artery and a rise in blood pressure. The results of the experiment

on his son impressed him so much that he travelled to University College, London, to discuss his findings with Professor E. A. Schafer. When he arrived in Professor Schafer's department, he found him engaged in an experiment in which the blood pressure of the dog was being recorded. When Schafer's experiment was finished, Oliver persuaded him to inject some of his suprarenal extract into the dog's vein. "They then stood amazed to see the mercury mounting in the manometer till the recording float was lifted almost out of the distal limb," (in Barcroft and Talbot, 1968). Oliver and Schafer together studied the nature of the adrenal extract in more detail over the next few years and showed, among other things, that the vasoactive principle of the adrenal gland was in the medulla and not in the cortex (Oliver and Schafer, 1895).

In parallel with these advances in knowledge of the physiological role of the adrenal medulla were the developments concerning sympathetic nerve function itself. Claude Bernard, in 1851 and 1852, showed that the division of the cervical sympathetic chain in the rabbit caused the ear of the same side to become flushed and warm. Stimulation of the sympathetic trunk had the reverse effects (Bernard, 1858). These observations indicated that the sympathetic nerves contained vasoconstrictor fibres and that under ordinary conditions the activity in these fibres kept the vessels in a partially constricted state.

The practice of cutting the sympathetic nerves to a part and thereby increasing its blood flow is one which is widely used today in surgical circles in the treatment of vascular insufficiency, particularly of the limbs. It owes its early beginnings to a chance observation made in 1923 on patients suffering from spastic paralysis who had been sympathectomised on the mistaken premise that it would alleviate this condition. It was found in this group that the limb on the 'treated' side felt warmer and appeared pinker than its fellow after the surgery (in Greenwood, 1967). Adson and Brown, at the Mayo Clinic, carried out lumbar sympathetic ganglionectomies on five spastic patients, beginning in May 1924, and carefully observed the changes in limb temperature, colour and sweat gland function. From this and subsequent work it became clear that following lumbar and thoracic sympathectomy there was warming of, and increased circulation through the feet and hands.

The possibility that the effects of stimulating the sympathetic nerve to an organ might be due to the liberation of a chemical substance from the nerve ending was suggested by the work of Lewandowsky in 1899. Elliott (1905) noted that the effects of adrenaline were almost identical with the effect of stimulating the sympathetic nerves to the part under study. However, despite the close similarity between the sympathetic transmitter and adrenaline in both chemical tests and biological action, there were certain

differences to be observed in most situations. These differences were commented on by Barger and Dale (1910) who pointed out that the action of amino- and ethylamino-bases of the catechol group corresponded more closely with that of the sympathetic nerves than did that of adrenaline.

To explain these differences, Cannon and Rosenbleuth, in 1933, postulated the formation of two different substances, 'Sympathin E' and 'Sympathin I', at different end organs by the combination of released adrenaline with different substances in the cells.

Bacq, in 1934, suggested that the effects of sympathetic nerve stimulation were due to the liberation from the nerve endings of a substance which behaved like noradrenaline rather than adrenaline - really restating the suggestion of Barger and Dale made 24 years earlier - and this was supported by the observations of Stehle and Ellsworth (1937) and Melville (1937).

In 1939, Lissak made the first extractions from sympathetic nerves and showed that such extracts had an adrenaline-like action. However, he was not able to define the precise nature of these extracted substances. In 1946, U. S. von Euler and his colleagues in Stockholm devised more refined methods for purification and biological and chemical analysis of catecholamines, and found that they could extract from the hearts of cattle, horses and cats a substance identical with noradrenaline in all its characteristics.

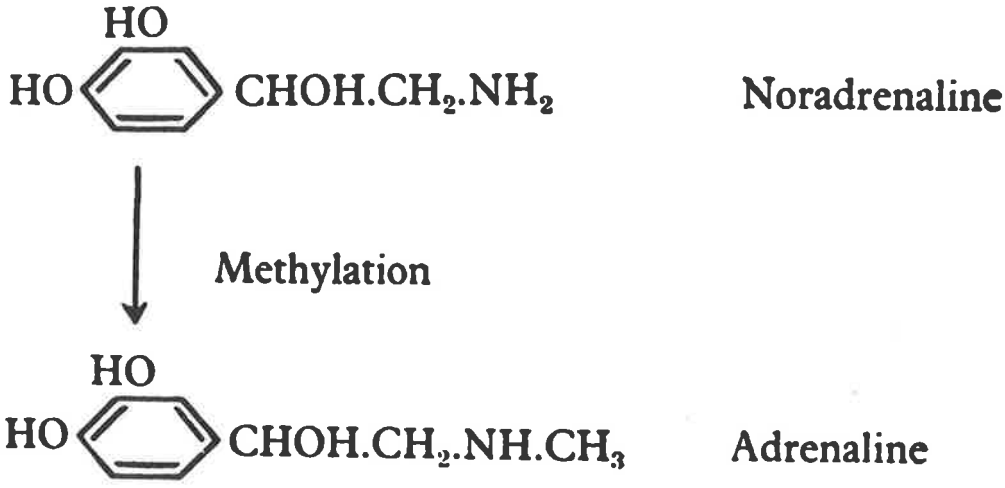


Fig. Intro. 1 - The structures of adrenaline and noradrenaline.

In 1947, Gaddum and Goodwin carried out experiments on the cat, in which they observed the blood pressure, pupil reactions and uterine contractions, and gave adrenaline and noradrenaline intravenously and compared their effects with those of stimulation of the hepatic nerves. They concluded that the substance released by the hepatic nerves was not adrenaline but noradrenaline or tyramine. Noradrenaline differs from adrenaline in its structure only in the absence of a methyl group (Fig. 1), but this difference confers quite different properties on the two substances.

The pharmacological actions of these substances on the cardiovascular system are still being elucidated, but an important step in this direction was provided in 1948, when Raymond Ahlquist hypothesised that there were two types of adrenotropic receptors on vascular smooth muscle, as determined by their relative responsiveness to the series of racemic sympathomimetic amines most closely related structurally to adrenaline. The α-receptors, he postulated, were associated with most of the excitatory functions (viz., vasoconstriction, stimulation of the uterus, nictitating membrane, ureter and dilator pupillae) and one important inhibitory function, intestinal relaxation. The β-adrenotropic receptor was associated with inhibitory functions (i.e., vasodilatation, relaxation of uterine and bronchial musculature) and one excitatory function (myocardial stimulation). The results of Ahlquist's experiments suggested that there was only one adrenergic

neurotransmitter, and its combination with one or other of the two receptors mentioned in the foregoing produced either an excitatory or an inhibitory effect.

In 1962, Cooper, Jellinek, Willman and Hanlon extracted noradrenaline from human heart biopsy specimens, while in 1963, Chidsey, Braunwald, Morrow and Mason found that the noradrenaline content of biopsy specimens of human heart was reduced by treatment with reserpine. It was inferred that the noradrenaline found in these tissues was derived from neurotransmitter stores.

The introduction of a fluorescent histochemical technique by which noradrenaline and adrenaline could be separately identified in tissues has helped in characterising further the presence and nature of the sympathetic transmitter. This technique was introduced by Falck in 1962, and in 1963, he and Rorsman studied punch-biopsy specimens of human skin using the method, and were able to demonstrate catecholamines at the outer border of arterial smooth muscle. Up to this time, this was probably the only direct evidence there was of the presence of these substances in arterial smooth muscle in man. In 1967, however, Waterson, using a modification of the Falck technique, was able to demonstrate noradrenaline in a proportion of blood vessels of fresh human dental pulp (Waterson, 1967) which was later confirmed by Kukletova, Zahradka and Lukas (1968).

The characterisation of the sympathetic neurotransmitter in

man and the mechanism of its release from sympathetic nerve endings is both of physiological and pharmacological significance, as a large number of therapeutic agents owe their action to interference with the synthesis, storage, release, re-uptake and inactivation of this substance. The sympathetic nervous system plays an important part in man's cardiovascular homeostasis, whether it be in response to a thermoregulatory stimulus, the need to main a constant blood pressure, or to meet the body's metabolic requirements. Using newer techniques, such as fluorescence histochemistry, electron microscopy and more refined assay methods, the nature of the sympathetic transmitter and its physiological role is now more apparent. However, there are certain areas in the human circulatory system, as, for example, the peripheral circulation, where the distribution of sympathetic nerves to the blood vessels, and the action of sympathomimetic substances and ions on these structures needs further elucidation. The material presented in this thesis examines some of these aspects of the human peripheral circulation.

S E C T I O N 1

STUDIES ON ISOLATED BLOOD VESSELS

CHAPTER 1

The influence of sympathetic innervation on vascular sensitivity to noradrenaline.

Reference has been made in the introductory chapter to the early work of Claude Bernard on the vasoconstrictor role of the sympathetic nerves to the blood vessels of the rabbit ear (Bernard, 1851, 1852, 1858). More recent histological evidence for a sympathetic innervation of these vessels was found by Grant and Thompson (1963) who noted that many of the nerves accompanying the blood vessels of the perichondrium of the ear degenerated after removal of the homolateral superior cervical ganglion. The bioassay data of Burn and Rand (1958) indicated that the skin of the rabbit ear contained noradrenaline and that this was presumably associated with sympathetic nerves in the walls of the blood vessels.

de la Lande, Paton and Waud (1964), and de la Lande and Rand (1965) showed that small segments of the isolated major ear artery were highly sensitive to the effects of peri-arterial stimulation and the effects were characteristically those elicited by excitation of the sympathetic nerves. If the homolateral superior cervical ganglion in the rabbit was removed one to three weeks previously, these properties of the isolated artery were abolished.

The introduction of the fluorescent histochemical technique

of Falck, in 1962, made possible, for the first time, the visual localization of the sympathetic transmitter substance in its storage sites. The advantage of this method was that it detected localized concentrations of catecholamines and hence served to highlight those nerves which were rich in sympathetic neurotransmitter. The precise distribution of the sympathetic innervation in the central artery of the rabbit ear could thus be established. With the knowledge of the location of these nerve stores it became possible to carry out further experiments to elucidate the sympathetic nerve function in blood vessels and precisely correlate histochemical and physiological data.

In 1966, de la Lande, Cannell and Waterson showed that the central artery of the rabbit ear was much less sensitive to noradrenaline applied to the adventitia than to noradrenaline applied to the intima of the artery. In subsequent experiments, de la Lande and Waterson (1966, 1967) showed that cocaine, by selectively enhancing sensitivity to extraluminal noradrenaline, reduced or abolished this difference in sensitivity. In common with arteries in the brain, mesentery and muscle (Carlsson, Falck and Hillarp, 1962; Falck, 1962; Fuxe and Sedvall, 1965), noradrenergic structures in the ear artery are concentrated in the adventitia and there is no evidence of penetration of sympathetic nerve terminals into the media (Waterson and Smale, 1967). It was therefore proposed that the difference in sensitivity of the artery to intraluminal and

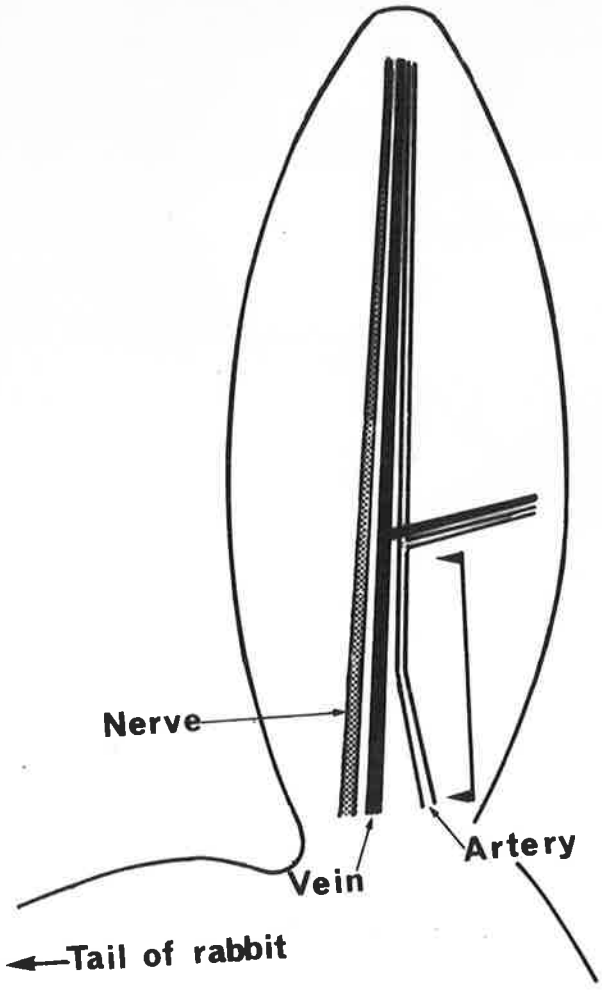


Fig. 1-1 A diagram of the erect left ear of a rabbit showing the convex surface of the ear as seen from the midline of the skull, portraying the relative positions of the ventral (great) auricular nerve, the central vein and the central (main) artery of the ear. The double arrows show the segment of the artery used in the perfusion experiments.

extraluminal noradrenaline was related to the uptake of noradrenaline into the adventitial noradrenaline stores (de la Lande and Waterson, 1967). In the present study, evidence of such a relationship has been further examined and the effects of denervation and cocaine compared. In addition, observations have been made on the time course of the action of cocaine, and the effects of cocaine on the response of the artery to nerve stimulation and to a non-adrenergic stimulant, namely, histamine.

METHODS

Preparation of rabbits:

Male and female semi-lop eared rabbits were used throughout this study. The weights of the animals varied from 1.5 to 3.0 Kg. Anaesthesia was induced with urethane, 8 ml/Kg of a 25% solution being injected intraperitoneally, with increments given as required. Before cannulation of the ear artery, heparin 1000 units/Kg was injected intravenously into an ear vein.

Perfusion of the isolated artery:

The method of perfusing the isolated central artery of the rabbit ear was that of de la Lande and Rand (1965). Small segments of the artery taken from the base of the ear (Fig. 1-1) were perfused

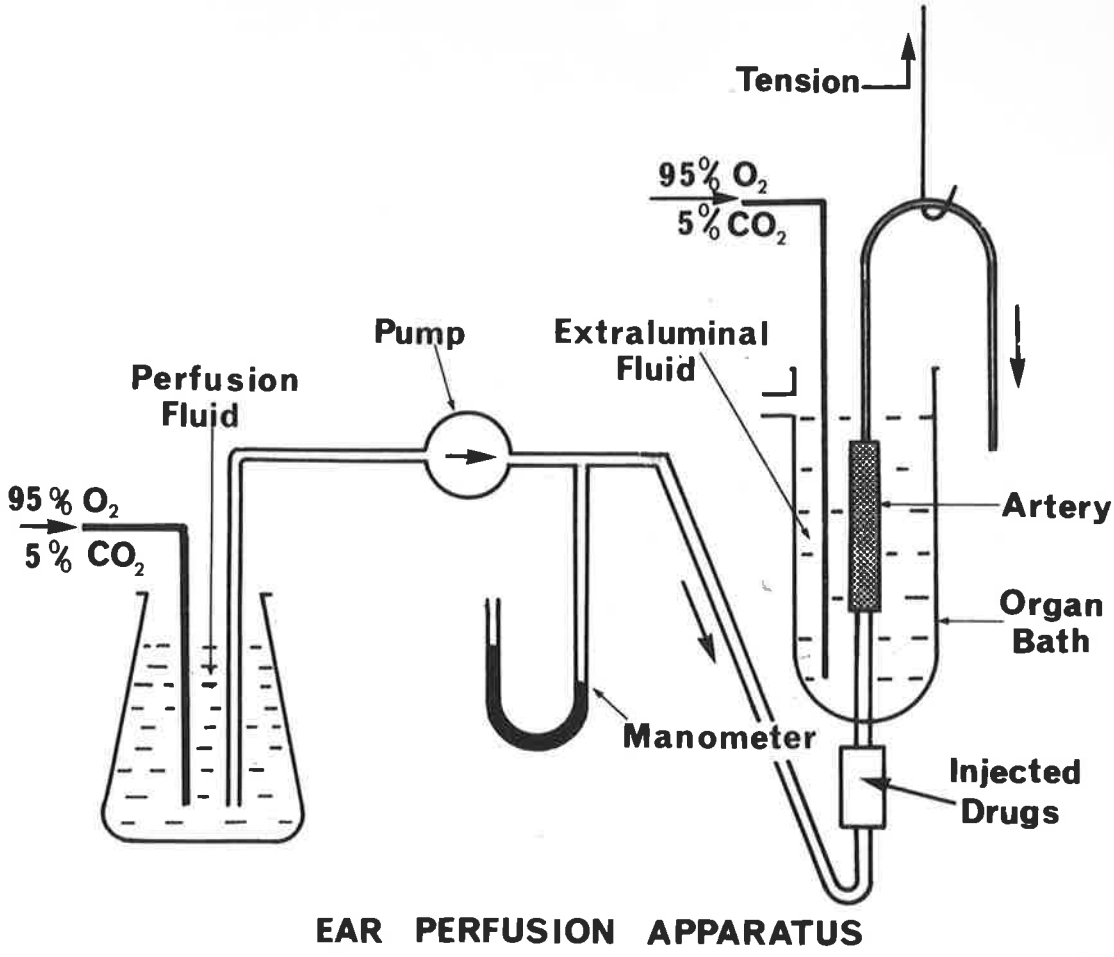


Fig. 1-2 Diagram of the apparatus used to perfuse the isolated central artery of the rabbit ear. Double cannula method.

at a constant rate with Kreb's bicarbonate solution at 37°C. To enable drugs to be applied to either the intima or adventitia, the artery was double cannulated so that the intraluminal and extraluminal perfusion media did not mix (Fig. 1-2). The absence of mixing between the two fluid compartments was tested both by the absence of volume changes in the extraluminal fluid and by the perfusion of dyestuff at the conclusion of the experiment (de la Lande *et al.*, 1966).

Histochemistry:

The technique used was that described by Falck, in 1962, and subsequently modified for use in our laboratory by Waterson and Smale (1967). The central artery of the rabbit ear was exposed by blunt dissection and a segment at least 1 cm long excised. This tissue was tied with cotton thread to a wire frame, stretching being avoided, and frozen in a mixture of acetone and dry ice (Fujiwara, Tanaka, Honjo and Okegawa, 1965). The frame and artery were rapidly transferred to a Freeze-Drying apparatus (Thermovac Model FD/3) which had been previously cooled to minimise the risk of thawing the tissue. Freeze drying was continued for from 12 hours (if the specimens were thin) to 72 hours (for the thickest specimens), at temperatures of -50°C to -35°C and pressures of 20-50 microns of mercury.

When this phase of the operation was complete, the tissue was removed from the freeze dryer and placed in a large glass jar which contained 5 grams of paraformaldehyde powder (Merck). The

paraformaldehyde had been stored over sulphuric acid for at least 1 week at a relative humidity of 70%. The jar was sealed with a rubber ring and a metal lid, placed in an oven preheated to 80°C and maintained at this temperature for either one hour or three hours. The selection of either one or three hours depended on the particular amine under investigation, i.e., noradrenaline or adrenaline, respectively. Falck and Owman (1965) showed that heating for one hour was necessary for the development of the noradrenaline fluorophore and for three hours for the development of the adrenaline fluorophore.

The tissue that had been treated with paraformaldehyde was vacuum infiltrated with paraffin wax and blocked in this medium. Transverse sections were then cut at 7 micron thickness and further prepared as follows:-

(a) For demonstrating the noradrenaline fluorophore, the tissue was mounted in an Entellan (Merck) and xylol mixture, while,

(b) For the adrenaline fluorophore, the tissue was mounted in liquid paraffin, since organic solvents such as xylol have been found to greatly reduce the adrenaline fluorescence (Falck and Owman, 1965).

The sections were then examined for fluorescence with a Leitz Ortholux microscope, using an HBO 200 mercury vapour lamp, a 3 mm Schott BG 12 excitation filter, and 490 to 530 millimicron barrier filter. A photographic record was made using a Leitz

Orthomat camera and Kodak Photoflure film. Photographic exposures were usually of 30-45 seconds' duration.

Tests for specificity of the fluorescence:

(a) Some of the sections of the formaldehyde treated tissue were floated on water before mounting as water eliminates catecholamine fluorescence (Falck and Owman, 1965).

(b) Some tissues were treated by the usual methods, with the exception that no paraformaldehyde was present in the jar during the heat treatment.

Nerve stimulation:

Sympathetic nerves in the artery wall were stimulated by means of field electrodes. In some preparations, the electrodes were placed only in the extraluminal bathing medium, and in others, one electrode was placed in each of the intraluminal and extraluminal perfusion media.

Denervation:

The ear artery in each of six rabbits was denervated by removing the homolateral superior cervical ganglion of the rabbit 14-24 days previously. Denervation was confirmed in each rabbit by the absence of noradrenergic structures in sections of the artery examined by the fluorescence histochemical method and compared with the normal control artery which was prepared simultaneously under the same conditions. The effectiveness of denervation was further

demonstrated in three of the rabbits by the absence of a constrictor response of the perfused arteries to field stimulation applied under identical conditions to those which caused massive constriction of the corresponding normal control arteries. Three arteries were not tested by this procedure.

Expression of results:

The constrictor response to noradrenaline was measured by the maximum rise in perfusion pressure recorded during sustained contact of the drug with the artery for periods varying from 3-10 min according to the nature of the response. The arteries showed some difference in their responses to this drug, some displaying a sustained constriction at or near the maximum value, while in others there was a pronounced fade after the maximum was reached. In addition, it was observed that the fade was extremely rapid and followed by a secondary constriction which was better sustained. The latter type of diphasic response was more pronounced with extraluminal than with intraluminal noradrenaline.

Concentration/response curves to noradrenaline were derived from responses recorded in duplicate or triplicate at two or three concentration levels. Changes in sensitivity to noradrenaline produced by drugs, or relative sensitivity to intra- and extraluminal noradrenaline, were measured in terms of concentrations producing equivalent maximum responses (sensitivity ratio). When the

two curves being compared were similar in shape and slope, the mean distance apart of the curves was used to estimate the sensitivity ratio, otherwise minimum and maximum values were calculated and the ratio expressed as a range.

Arteries tended to gain sensitivity during the course of an experiment. In the majority of arteries, the spontaneous increase in sensitivity was not sufficient to prevent quantitative assessment of cocaine's effect on noradrenaline sensitivity. The latter effect was measured in two ways, i.e.,

Method 1 - The responses to intraluminal and to extraluminal noradrenaline were compared before and in the presence of cocaine applied to one or both surfaces of the artery.

Method 2 - Cocaine was applied to the artery by intraluminal perfusion or injection, or by adding it to the extraluminal fluid during the sustained phase of constrictor response to noradrenaline. The further increase in the response was recorded.

Doses of drugs:

Dose response curves to noradrenaline were based on rises in perfusion pressure in the range of 10 to 200 mm Hg. In the majority of arteries, the required concentrations of intraluminal noradrenaline were in the range of 0.005 to 0.1 µg/ml and those of extraluminal noradrenaline in the range 0.02 to 5 µg/ml. All concentrations of noradrenaline refer to the base; those of cocaine

T A B L E 1

EFFECT OF COCAINE ON NORADRENALINE SENSITIVITY

Ratio of sensitivities $\frac{\text{NA during cocaine}}{\text{NA without cocaine}}$

Admin. of noradren.	Concn. of cocaine (µg/ml)	Experiment No. 1	2	3	4	5	6	7	8	9
Intra-luminal	1	1.1	1-1.5	1.2		1-3.2			1.8-2.2	1.3
	5	1.1		1.7		1-4.2 (1-1.2)	1.8 (2.2-2.5)	1.7-2.0 (1.4)		
	10				1.5-1.8				2.2-2.8 (1.5-1.6)	
Extra-luminal	1	4-9.8	4.8-5.3	6.6-13		3.6-5.1			9.2-9.4	7-10 (7-10)
	5	18				4.5-7.0 (7.8)	10 (10)	6.3-6.6 (8.4)		
	10		9.6-12.0	16.5	(4.7-5.2)				9-12.8 (3.8-4.2)	

Ratio of sensitivities $\frac{\text{NA extraluminal}}{\text{NA intraluminal}}$

Cocaine absent	0.03	0.02	0.12	0.2	0.5	0.14	0.23	0.09	0.025
Cocaine present	0.4	0.3	1.2	0.6	1.0	0.8	1.2	1.4	0.15

1. The sensitivity ratios were determined by method (1) for experiments 1 to 8 and by method (2) for experiment 9. A ratio of, for example, 5.0 means that cocaine caused a five fold increase in sensitivity to noradrenaline. The ratio is expressed as a range where the concentration response curves under comparison differed in shape.
2. Cocaine was applied to the same surface as the noradrenaline; the ratio obtained when cocaine was applied to the opposite surface as the noradrenaline is shown in brackets.
3. The lower table refers to the ratios of the sensitivity of extraluminal to intraluminal noradrenaline in the absence of cocaine (top row) and the maximum ratio observed in the presence of cocaine (bottom row), for each of the experiments 1 to 9. A ratio of less than one means that extraluminal noradrenaline was less active than intraluminal noradrenaline.

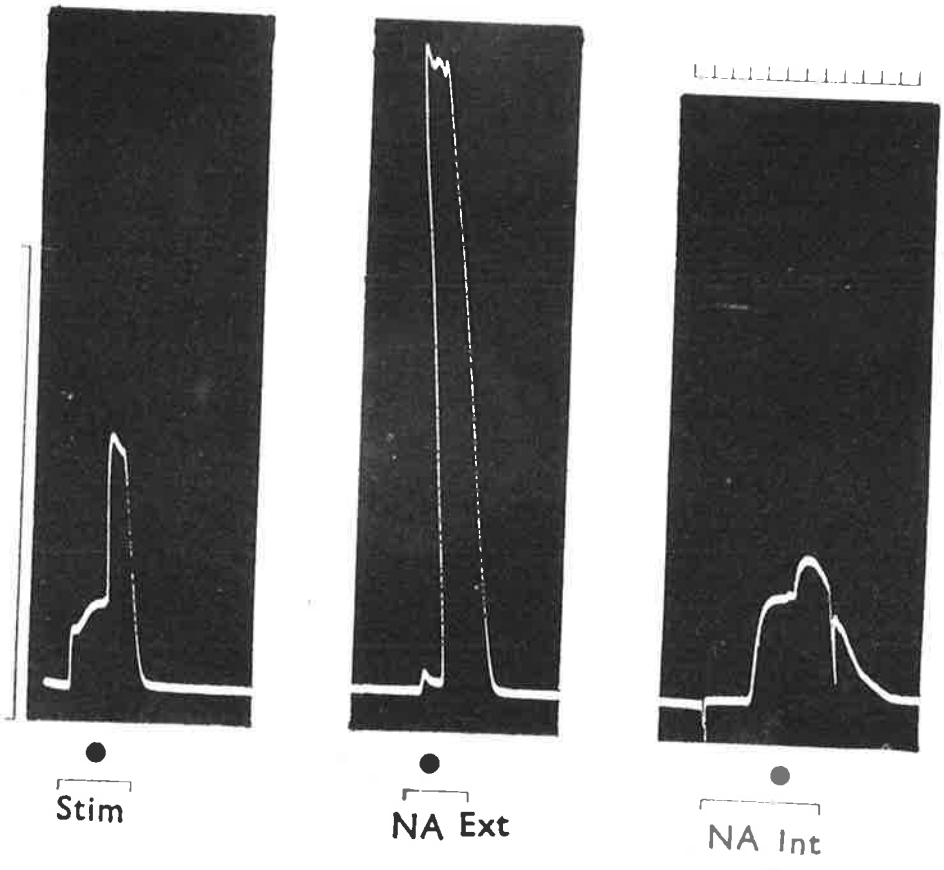


Fig. 1-3a

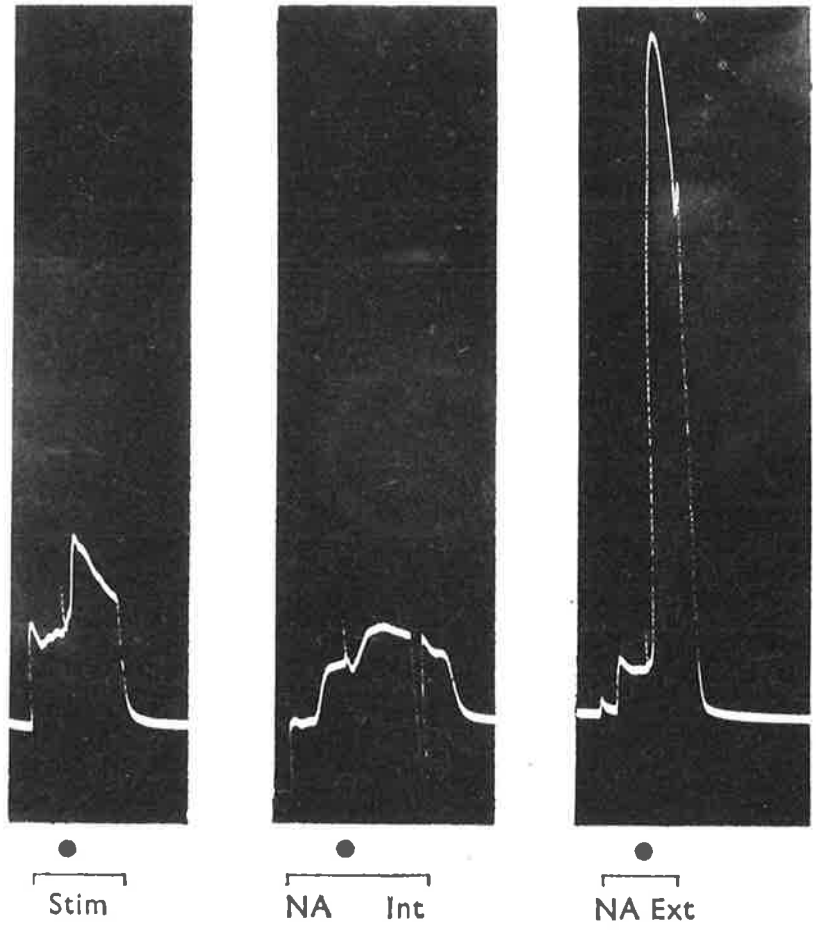


Fig. 1-3b Comparison of cocaine's action on the constrictor responses to field stimulation (Stim) 0.3 msec, 0.4 pulses/sec, extraluminal noradrenaline (NA Ext) and intraluminal noradrenaline (NA Int), applied for the periods shown by line. The lag between the onset of perfusion and response to intraluminal noradrenaline represents the time required for the noradrenaline to reach the artery from the perfusion reservoir (dead space). (1-3a). Cocaine 0.5 µg/ml applied extraluminally at black dot and washed out simultaneously with the noradrenaline, or with termination of stimulation. Note that, although the response to extraluminal noradrenaline is considerably less than those to intraluminal noradrenaline and to stimulation, cocaine produces much greater augmentation of the extraluminal response. Stim 0.4/sec, 0.3 msec. NA Ext 200 ng/ml, NA Int 10 ng/ml. Time scale, minutes. Ordinate, perfusion pressure, 100 mm mercury. (1-3b). A second artery in which cocaine 5 µg injected intraluminally at black dots. Stim 0.8/sec, 1 msec. NA Ext 50 ng/ml, NA Int 5 ng/ml. Time scale and ordinate as for 1-3a.

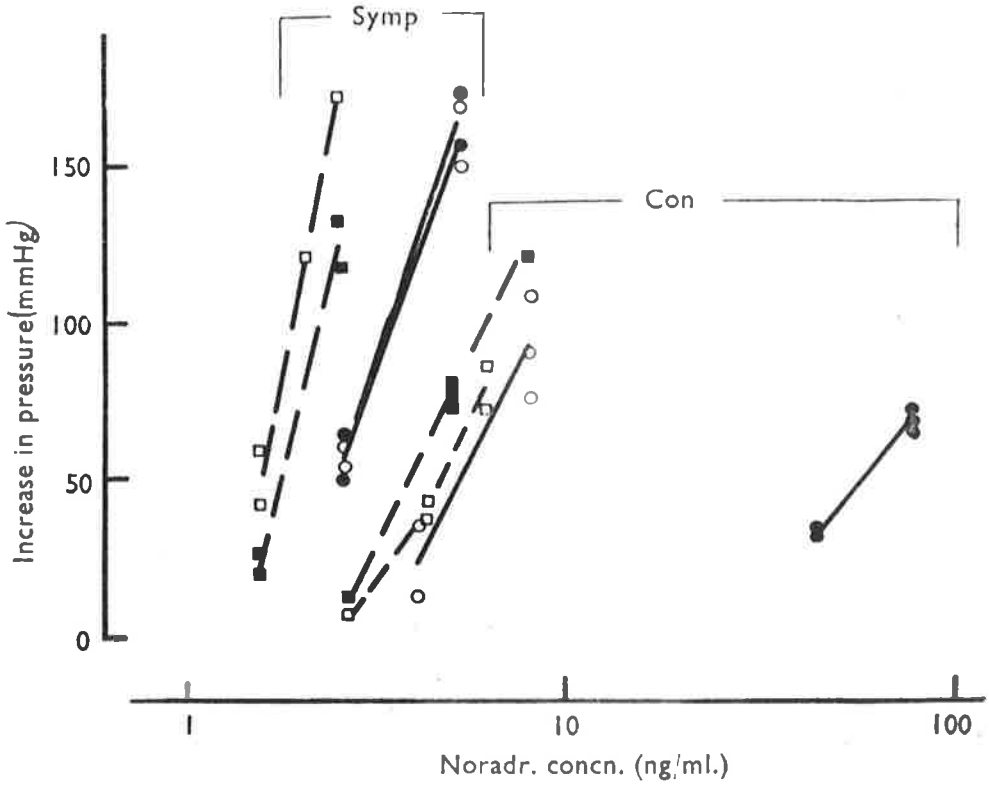


Fig. 1-4 Concentration-response curves to noradrenaline in a denervated artery (Symp), and a normal innervated artery (Con) from the opposite ear. Closed and open symbols refer to the response to extraluminal and intraluminal noradrenaline, respectively.
——— = cocaine absent. - - - = cocaine (10 µg/ml) present.

refer to the hydrochloride salt, and those of histamine to the acid phosphate salt.

RESULTS

The ability of cocaine to cause marked and selective enhancement of extraluminal noradrenaline is illustrated in Figs. 1-3 and 1-4 and by quantitative data on nine arteries summarized in Table 1. Fig. 1-3 shows the effects of applying cocaine during the sustained constrictor response to noradrenaline (Methods) and Fig. 1-4 the effect of cocaine on the concentration/response curves to noradrenaline (Method 1). It will be observed that, in each of the experiments in Table 1, extraluminal cocaine caused only a small increase in sensitivity to intraluminal noradrenaline compared with that to extraluminal noradrenaline, and that intraluminal cocaine, although less active in one artery (number 8), exerted a qualitatively similar action to that of extraluminal cocaine. A tendency for cocaine to be less active by the intraluminal route was also observed in three of a further seven arteries in which the relative sensitizing potencies were estimated solely by the magnitudes of the increased response resulting from the application of cocaine during noradrenaline-induced sustained constriction (Method 2). This

T A B L E 2

TIME COURSES OF ACTION OF NORADRENALINE AND COCAINE

		Noradrenaline (NA)		Cocaine during intraluminal NA		Cocaine during extraluminal NA		Infused dye	
		Intra-luminal	Extra-luminal	Intra-luminal	Extra-luminal	Intra-luminal	Extra-luminal	Time to reach artery	Interval between appearance of dye and maximum concn.
Experiment 1	Onset of response	17,17	3,3	34	15	30 (20)	5		
Flow rate=8 ml/min	Onset to maximum	107	11* & 102	102	85	90	65	47	10-20
		122	12* & 232						
Experiment 2	Onset of response	21,23	4,6	78	37	42 (29)	20		
Flow rate=5.6 ml/min	Onset to maximum	204,188	12* & 120	170	160	213	125	70	30
			20* & 110						

1. All numbers are times, in sec.
2. * onset of first peak of diphasic response.
3. In order to allow for perfusion of "dead space", the times of onsets of action of intraluminal noradrenaline and of intraluminal cocaine are calculated from the difference between the observed times of onset after commencing perfusion and the time required (shown) for Evan's blue dye 1% perfused by the same route to reach the artery. Nevertheless, the intraluminal times of onset are still probably overestimated, since perfused dye did not attain its peak sustained concentration in the intraluminal outflow for a further 10 to 20 sec, and 30 sec, for arteries 1 and 2 respectively. For this reason, the onset of action of intraluminally injected cocaine was also estimated (shown in parentheses). The drug injection was made immediately proximal to the artery so that the artery was exposed to a maximum concentration of the drug within 2 sec.

Doses of drugs: Experiment 1: cocaine 1 µg/ml, by injection 2 µg; intraluminal noradrenaline 0.005 µg/ml, extraluminal noradrenaline 0.01-0.2 µg/ml. Experiment 2: cocaine 0.4 µg/ml, by injection 2 µg; intraluminal noradrenaline 0.05 µg/ml; extraluminal noradrenaline 0.2-1.0 µg/ml.

tendency was evident, not only by the greater response to extraluminal noradrenaline, but in two arteries by depression of the response to intraluminal noradrenaline by intraluminal, but not extraluminal, cocaine. However, the striking and consistent feature of cocaine's action on all arteries was the selective enhancement of extraluminal noradrenaline. The net effect was that, regardless of its route of application, cocaine reduced, or abolished, the difference between the intra- and extraluminal sensitivities to noradrenaline. This effect of cocaine is evident also in the control arteries used in the studies on denervation (Table 3).

Applying the drug during sustained constriction to noradrenaline permitted analysis of cocaine's time course of action. The onset and attainment of maximum sensitization was always rapid (Fig. 1-3), being complete within 2 to 4 min in all arteries examined. The onset of action of cocaine was slower than that of noradrenaline, but the lag was never greater than 60 sec and was only of the order of 5 to 20 sec in the case of extraluminal applications. These features of cocaine's action are illustrated by data from two experiments shown in Table 2. Attention is drawn to the speed of onset of intraluminal cocaine's sensitizing action on extraluminal noradrenaline, which, in the two experiments, was only 9 and 15 sec slower than that of extraluminal cocaine.

T A B L E 3

EFFECT OF DENERVATION ON SENSITIVITY TO NORADRENALINE

Artery	Route of application of noradrenaline	Experiment No. 1	2	3	4	5	6
Control	Intraluminal	1.0	1.0	1.0	1.0	1.0	1.0
	Extraluminal	.07	.09	.1	.18	.09	.16
Control + Cocaine	Intraluminal	1.3	2.0	1.8	3.1	4.4	2.3
	Extraluminal	1.4	0.9	1.3	1.8	1.9	0.9
Denervated	Intraluminal	1.9	2.4	9.5	1.3	1.0	5.2
	Extraluminal	2.0	1.5	6.0	0.3-1.0	0.2-0.5	3.8
Denervated + Cocaine	Intraluminal	3.7	-	-	1.8	-	13
	Extraluminal	3.0	-	-	0.8	-	7

Each figure is the ratio of extraluminal noradrenaline sensitivity to intraluminal noradrenaline sensitivity. In each control artery the latter sensitivity is arbitrarily assigned a value of one. Only the mean value of the ratio is shown, except in experiments 4 and 5 where the ratio is expressed as a range to include an increase in sensitivity to extraluminal noradrenaline which occurred spontaneously during perfusion.

Values for cocaine in the sympathectomized artery in experiments 2, 3 and 5 are not shown, since the arteries progressively decreased in sensitivity to intra- and extraluminal noradrenaline once perfusion with cocaine was commenced.

Concentration of cocaine, 10 µg/ml.

Denervation:

Six arteries, which had been denervated 14 to 24 days previously, showed greatly enhanced sensitivity to extraluminal noradrenaline, but a smaller increase in sensitivity to intraluminal noradrenaline. Comparison was made in each case with the artery from the opposite ear which had not been denervated. An example of the concentration/response curves to noradrenaline in the two arteries is shown in Fig. 1-4. The results are summarized in Table 3, which also shows the effect of cocaine on each control and sympathectomized artery. It will be noted that the denervated artery closely resembles the cocaine-treated artery in its relative sensitivities to intra- and extraluminal noradrenaline, that is, denervation, like cocaine, tends to abolish the difference between these sensitivities. The effect of cocaine on the sensitivity of the denervated arteries to noradrenaline varied between relatively slight enhancement (three arteries) and depression (three arteries).

Nerve stimulation:

In six arteries, responses of approximately equivalent magnitude were elicited by field stimulation, intraluminal noradrenaline and extraluminal noradrenaline. During the sustained phase of the responses cocaine was applied extraluminally, or intraluminally, by injection or perfusion, and its effect measured by the increase in constriction. The results of the six experiments are

T A B L E 4

EFFECT OF COCAINE ON NORADRENALINE AND ON FIELD STIMULATION

	Experiment No. →	1	2	3	4	5	6
Intraluminal noradrenaline	Int. cocaine	$\frac{0}{3}$ $\frac{0}{34}$		$\frac{8}{12}$ $\frac{20}{12}$	$\frac{20}{30}$	$\frac{8}{38}$	$\frac{10}{33}$
	Ext. cocaine	$\frac{8}{22}$ $\frac{7}{13}$	$\frac{30}{85}$ $\frac{20}{55}$ $\frac{8}{26}$		$\frac{36}{45}$	$\frac{10}{45}$	
Stimulation	Int. cocaine	$\frac{32}{20}$		$\frac{20}{18}$ $\frac{10}{15}$	$\frac{15}{5}$ $\frac{20^{*}}{25}$	$\frac{105}{25}$	$\frac{30}{30}$ $\frac{30}{28}$
	Ext. cocaine	$\frac{35^{*}}{20}$ $\frac{25}{5}$	$\frac{14}{10}$ $\frac{12^{*}}{21}$		$\frac{59^{*}}{20}$ $\frac{40^{**}}{36}$	$\frac{136^{*}}{17}$	
Extraluminal noradrenaline	Int. cocaine	$\frac{41}{9}$ $\frac{115}{12}$		$\frac{128}{12}$ $\frac{150}{10}$	$\frac{170}{20}$	$\frac{180}{10}$	$\frac{48}{40}$
	Ext. cocaine	$\frac{66}{4}$ $\frac{136}{2}$	$\frac{135}{5}$		$\frac{160}{20}$	$\frac{180}{10}$	
Stimulation characteristics	Pulse duration (msec)/pulse frequency per sec	0.3/2 0.3/0.4*	1/3 1/8	1.0/0.8	0.3/5 *1/3 **1/1.5	1/1 *1/5	1/0.35

1. Each ratio is the $\frac{\text{Increase in response (in mm) elicited by cocaine.}}{\text{Response (in mm) prevailing immediately before adding cocaine.}}$

2. Stimulation characteristics shown at foot of table marked * refer to the response marked * in the same column.

3. Concentration of cocaine: Experiment 1: 0.5 µg/ml; 2: 2 µg/ml; 3: 5 µg injection; 4: 1 µg/ml (extraluminal), 5 µg injection; 5: 0.5 µg/ml (extraluminal), 5 µg injection; 6: 5 µg injection.

T A B L E 5

EFFECT OF COCAINE ON HISTAMINE

Route of application	Ratio of Sensitivities		
	Expt. 1	Expt. 2	Expt. 3
Intraluminal histamine	0.46-0.56	0.6-0.9	.66-1.2
Extraluminal histamine	1.25	.7-1.0	1-1.3
Extraluminal noradrenaline	10	6-10	20
Sensitivity ratio in the absence of cocaine; $\frac{\text{Extraluminal histamine}}{\text{Intraluminal histamine}}$	0.5	0.5	0.9-1.0

The ratios refer to the sensitivity to histamine, or noradrenaline (extraluminal only), in the presence of cocaine, compared with the sensitivity in the absence of cocaine. Concentrations: cocaine 1 µg/ml, histamine 0.1-1 µg/ml.

presented in Table 4. The main feature is that, in five of the arteries, potentiation of field stimulation is much less marked than that of extraluminal noradrenaline and corresponds more closely to that of intraluminal noradrenaline. These actions of cocaine are also illustrated in Fig. 1-3.

Histamine:

The effect of cocaine on histamine-induced constriction was examined in three arteries. The response to histamine, perfused intraluminally or applied extraluminally, resembled that to noradrenaline in that it was prompt in onset, reached a maximum within 1 to 4 min and was well-sustained. The ratios of the sensitivity of the arteries to histamine before, and in the presence of, cocaine (1 µg/ml) were derived from concentration/response curves and are shown in Table 5. The data points to a slight depressant action of cocaine on intraluminal histamine. However, the main feature is cocaine's lack of effect on extraluminal histamine, which contrasts with the marked potentiation of extraluminal noradrenaline. The latter was measured in each artery following the observations on histamine by applying noradrenaline immediately before, and after, cocaine wash out.

Attention may be drawn also to the relatively small difference between the sensitivity to intra- and extraluminal histamine. The maximum difference was twofold, which may be compared

with the five- to tenfold difference commonly observed with noradrenaline.

DISCUSSION

In other tissues, particularly heart and cat nictitating membrane, it has been demonstrated that cocaine prevents uptake of noradrenaline into the storage sites and that chronic denervation achieves the same effect by causing the storage sites to deteriorate (Trendelenburg, 1963). Hence the ability of cocaine and of denervation to increase the sensitivity of the artery to extraluminal noradrenaline to a level approaching that to intraluminal noradrenaline indicates that the marked differences between these sensitivities in the normal untreated artery is probably related to uptake of noradrenaline into storage sites and not simply to diffusion barriers in the artery. The observations that the sensitizing action of serotonin (de la Lande *et al.*,1966) and the constrictor potency of histamine (present study) are little affected by their routes of application to the artery are further evidence that diffusion barriers are unlikely to play a major role in the differences in sensitivity to intra- and extraluminal noradrenaline.

It has been shown (de la Lande and Waterson, 1967; Waterson

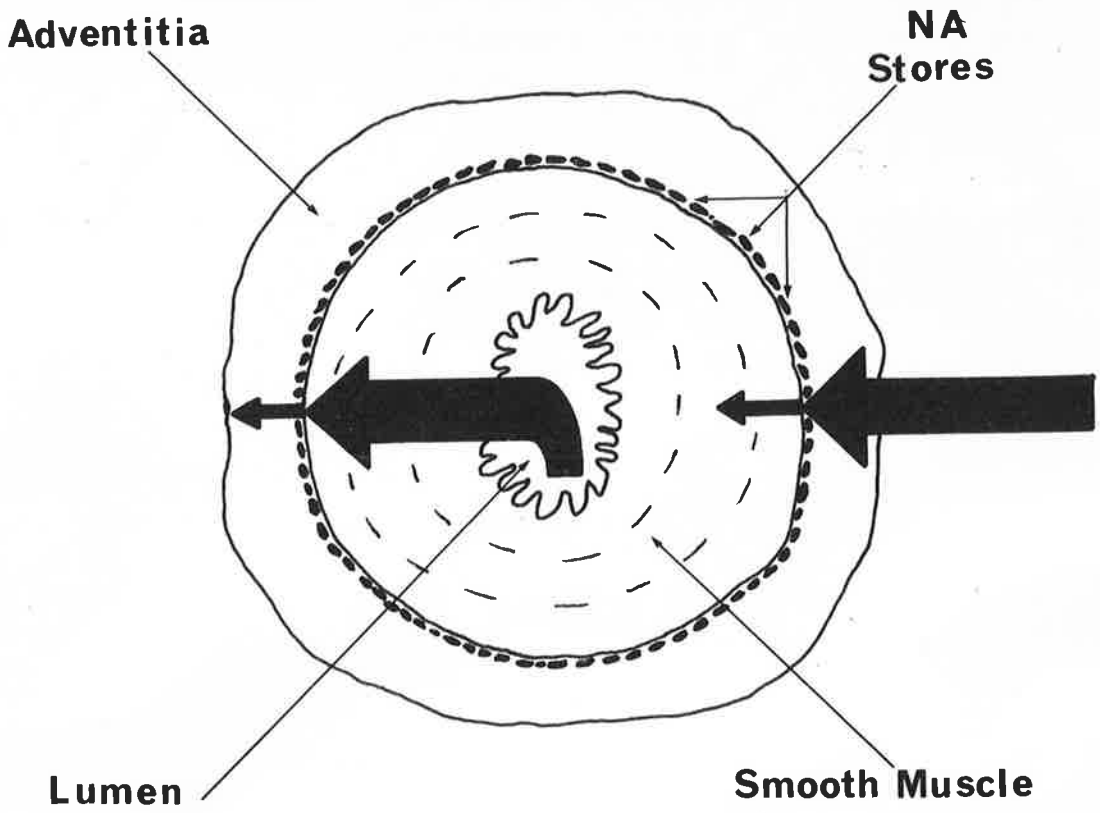


Fig. 1-5 Diagrammatic representation of the influence of the sites of uptake (shown as NA Stores) on the concentration of noradrenaline in the smooth muscle of the artery. The direction of the arrow indicates the direction of diffusion of noradrenaline, and its thickness the concentration of noradrenaline.

and Smale, 1967) that the noradrenaline storage sites in this artery are closely packed structures located in the adventitia immediately outside and completely surrounding the outer border of the smooth muscle layer. The position of the storage sites is consistent with the hypothesis, advanced previously (de la Lande and Waterson, 1966, 1967), that noradrenaline applied to the adventitia undergoes considerable loss by uptake into the storage sites before it reaches the underlying smooth muscle, and that cocaine and denervation exert their dramatic effect on sensitivity to extraluminal noradrenaline by preventing this loss. The relatively slight potentiation of intraluminal noradrenaline may be explained in two ways: either low uptake, or an inability of uptake to influence the concentration of noradrenaline in the smooth muscle. The second explanation is favoured by the speed with which intraluminal cocaine exerts its potentiating and inhibitory effects on extraluminal noradrenaline. The effect of cocaine commences within 15 to 30 sec of application to the perfusion medium, and is maximal or near maximal within a further 2 to 4 min. The time course implies rapid penetration of cocaine from the intima to the storage sites in the adventitia, and it is a reasonable assumption that intraluminal noradrenaline penetrates to the storage sites at a rate at least comparable with that of cocaine. The hypothesis is presented diagrammatically in Fig. 1-5. The storage sites are assumed to represent major sites of loss for both

intraluminal and extraluminal noradrenaline, so that loss of intraluminal noradrenaline occurs, but only after it has diffused through the smooth muscle, that is, after it has exerted its physiological action. Hence the extremely high sensitivity of the artery to intraluminal noradrenaline, which has provided the basis for its application to catecholamine bioassay (de la Lande and Harvey, 1965), is explained, not by the absence of uptake, but by the inability of uptake to affect this sensitivity; similarly, the sensitivity is little affected when uptake is prevented or abolished by cocaine or denervation.

However, the first explanation - that is, low uptake - is not excluded by our data since, despite rapid penetration of intraluminally applied drugs to the adventitia, it is possible that their concentration is markedly reduced in the outer region of the artery wall by a diluting effect of the extraluminal bathing solution. If such an effect extends to the region of the adventitial storage sites, the concentration of noradrenaline in this region may be considerably less than in the lumen and uptake of noradrenaline will be correspondingly less. Recent studies (de la Lande and co-workers, unpublished) have shown that the concentration of intraluminally applied noradrenaline which finally reaches the nerve stores is, in fact, reduced as a result of metabolism by the enzyme catechol-O-methyl transferase which is probably located entirely in the media.

Although the potentiation by cocaine of the constrictor responses to stimulation tended to be greater than that to intraluminal noradrenaline, in accord with previous observations (de la Lande *et al.*, 1966), the magnitudes of the increases were much smaller than those to extraluminal noradrenaline. The results were unexpected, since it was anticipated that endogenously released noradrenaline would be exposed to considerable re-uptake in the storage sites before it diffused from the adventitia into the underlying smooth muscle, that is, its response to cocaine would resemble that of extraluminal rather than intraluminal noradrenaline. However, it has been noted that in guinea-pig and rat vas deferens preparations, where noradrenergic storage sites are distributed throughout the smooth muscle layer, augmentation of nerve-induced responses is nevertheless slight compared with that of responses to exogenous noradrenaline (Bentley, 1966). Furthermore, Trendelenburg (1966) has observed supersensitivity to noradrenaline in the cat nictitating membrane during sustained nerve stimulation, and a similar effect has been observed on the rabbit ear artery (de la Lande, unpublished). Hence the possibility emerges that cocaine's ability to cause supersensitivity by inhibiting uptake of noradrenaline may be modified in some way by the state of supersensitivity prevailing during nerve stimulation.

SUMMARY

1. The sensitivity of the isolated rabbit ear artery to extraluminal noradrenaline, but not to intraluminal noradrenaline, is greatly enhanced by cocaine. The net effect is that the difference between the intraluminal and extraluminal sensitivity to noradrenaline is greatly reduced, or abolished.

2. The effects of denervation closely resemble those of cocaine.

3. Cocaine also enhances the constrictor response of the artery to field stimulation, but the effect is much less pronounced than that on extraluminal noradrenaline.

4. Cocaine has little effect on the constrictor response to histamine.

5. It is concluded that the position of the noradrenaline stores in the medial adventitial border of the artery determines the low sensitivity of the artery to extraluminal noradrenaline. Cocaine and denervation, by eliminating the effects of uptake, reduce the loss of noradrenaline by uptake into the storage sites as it diffuses from the adventitia to the media.

CHAPTER 2

The histochemical localisation of sympathetic nerve endings in human blood vessels.

Falck and Rorsman (1963) were the first to report specific fluorescence enclosing the smooth muscle layer of arterial vessels in the deeper layers of the corium in human skin. Over four years elapsed before another report of specific fluorescence in human vascular smooth muscle appeared and this was on the presence of such fluorescence in the blood vessels of human dental pulp (Waterson, 1967; Waterson and de la Lande, 1967). Owman, Rosengren and Sjoberg, in the same year, using a combined fluorescent histochemical and fluorimetric assay technique, observed that the organs of the human female reproductive tract were supplied with an adrenergic innervation of varying density. Part of the innervation was related to the blood vessels and the only amine present in significant amounts was noradrenaline. A further histochemical study on the presence of monoaminergic nerve fibres in the blood vessels of human dental pulp was published by Kukletova, Zahradka and Lukas (1968), while more recently, Gannon and his co-workers have reported specific fluorescence around the blood vessels in the submucosa of the human rectum (Gannon, Burnstock, Noblett, and Campbell, 1969).

Despite the demonstration of catecholamine fluorescence in

the blood vessels to skin, dental pulp, the female reproductive tract and the rectum of man, there is little direct evidence on the distribution and localisation of sympathetic nerve terminals in peripheral human limb blood vessels despite the fact that these are the vessels whose reflex and pharmacological responses have been most extensively studied in man. Studies on the functions of these nerves are largely indirect and rely on the actions of various sympathomimetic agents on the blood vessels of normal and sympathectomized limbs. The present study was undertaken to examine some peripheral human vessels histochemically to ascertain the distribution of their sympathetic nerve supply. Human gingival and mesenteric vessels were also studied, and an attempt made to evaluate whether a relationship existed between the intensity of the fluorescence and the age of the patients from whom the vessels were obtained.

METHODS

The techniques used and the tests for specificity of the fluorescence were described in Chapter 1. When specific fluorescence was observed, it was arbitrarily classified into three groups, viz., strong, moderate, or weak, as based on brilliance.

Tissue for study was obtained from several sources. As

TABLE 2-1

CATECHOLAMINE FLUORESCENCE IN HUMAN BLOOD VESSELS

	Source	Vessels	Details of Specimen	Age of Patient	Fluorescence
1.	Lower limb	Dorsalis pedis artery	Autopsy 2 hrs after death. (Myocardial infarction)	74 yrs	No specific fluorescence.
2.	Hand	Digital artery	Autopsy 4½ hrs after death. (Myocardial infarction)	68 yrs	No specific fluorescence.
3.	Foot	Digital artery	Amputation for long-standing vascular insufficiency and associated gangrene.	63 yrs	No specific fluorescence.
4.	Hand	Digital artery	Autopsy 26 hrs after death. (Carcinoma of the stomach)	60 yrs	No specific fluorescence.
5.	Large intestine	Branches of the inferior mesenteric artery	Abdomino-perineal resection for a carcinoma of the rectum.	Approx. 60 yrs	No specific fluorescence.
6.	Gingival tissue	Gingival arteries	Following surgery for an impacted wisdom tooth.	35 yrs	No specific fluorescence.
7.	Gingival tissue	Gingival arteries	- do -	23 yrs	Moderate specific fluorescence.
8.	Gingival tissue	Gingival arteries	- do -	21 yrs	Moderate specific fluorescence.
9.	Hands Gingival tissue	Digital arteries Gingival arteries	Autopsy 18 hrs after death. (Pulmonary embolism following a fractured femur)	19 yrs	No specific fluorescence.
10.	Gingival tissue	Gingival arteries	Autopsy 2 hrs after death. (Sub-arachnoid haemorrhage)	9 yrs	Moderate specific fluorescence.
11.	Gingival tissue	Gingival arteries	Autopsy approx. 5-10 hrs after death. (Hepatoblastoma)	17 mths	Moderate specific fluorescence.
12.	Hands Gingival tissue	Digital arteries Gingival arteries	Autopsy 5 hrs after death. (Sudden death syndrome)	5 mths	Strong specific fluorescence.
13.	Hands	Digital arteries	Autopsy 3½ hrs after death. (Intestinal obstruction)	17 wks	Strong specific fluorescence.
14.	Hands Gingival tissue	Digital arteries Gingival arteries	Autopsy 8 hrs after death. (Sudden death syndrome)	7 wks	Strong specific fluorescence.
15.	Hands Gingival tissue	1. Cutaneous vessels 2. Digital arteries 3. Gingival arteries	Autopsy 12 hrs after death. (Heart failure and septicaemia)	17 days	1. Weak specific fluorescence present. 2. Occasional granule of weak specific fluorescence seen. 3. No specific fluorescence.

catecholamines probably diminish with autolysis, efforts were made to get material as soon as possible after operation or post-mortem. The most readily available source of fresh, healthy tissue was gingival tissue obtained at oral surgery, especially at the extraction of impacted wisdom teeth.

Several attempts were made to study arteries obtained from amputated human lower limbs. In these patients, the need for amputation had arisen due to long-standing vascular insufficiency leading to gangrene, and in all save one of the digits studied the gangrenous process was well advanced and the tissues were necrotic. The result from the one digit without gangrene is included in Table 2-1. Tissues obtained at diagnostic skin biopsy were found to be inadequate for histochemical study.

When the tissues became available from any source, they were placed immediately into freshly prepared, oxygenated Kreb's bicarbonate solution and subjected to the histochemical study within thirty minutes.

RESULTS

Table 2-1 lists the blood vessels studied and the extent of the fluorescence seen in them, arranged in order of decreasing age of

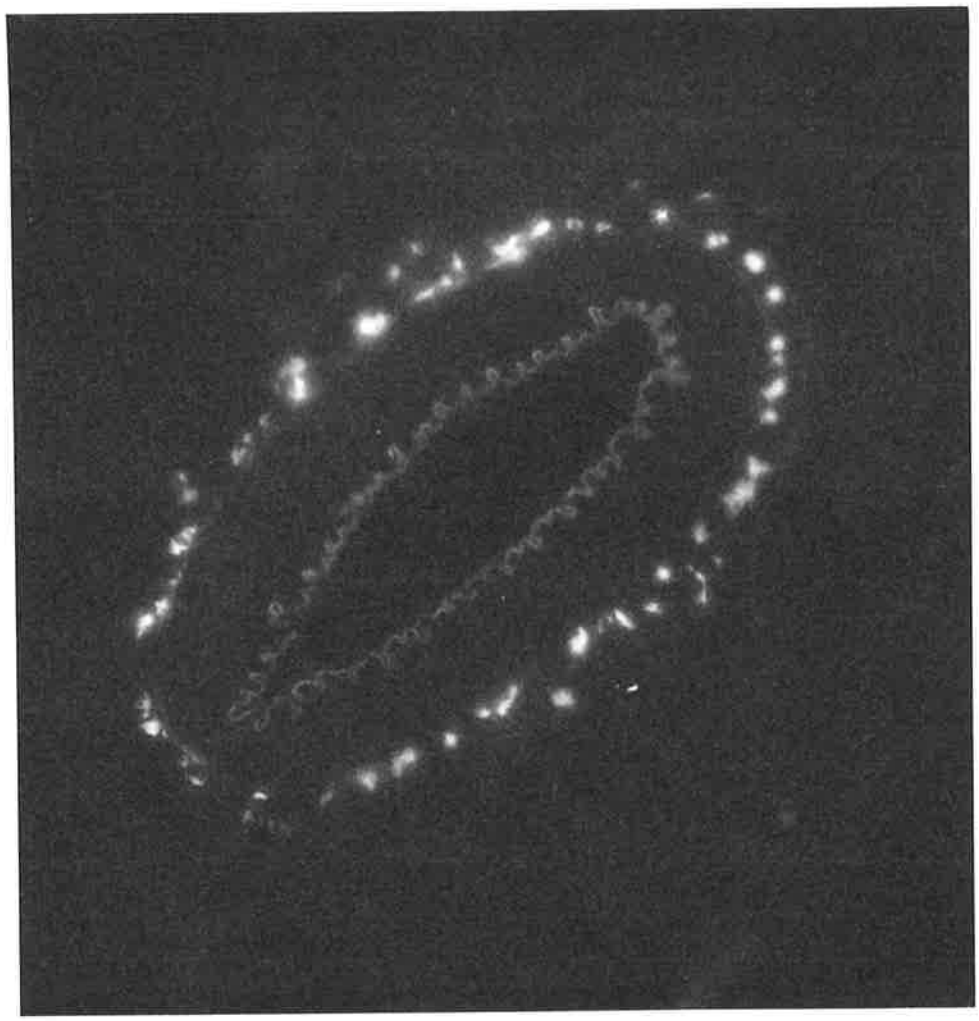


Fig. 2-1 Transverse section of a digital artery from a 17-week old infant. There are two areas of fluorescence - an outer ring of specific (adrenergic) fluorescence at the medio-adventitial border, and an inner ring of autofluorescence caused by the internal elastic lamina. Magnification X100.

Fig. 2-2(a) A digital artery from a 7-week old infant, in transverse section, showing the same pattern of fluorescence as in Fig. 2-1. Magnification X100.

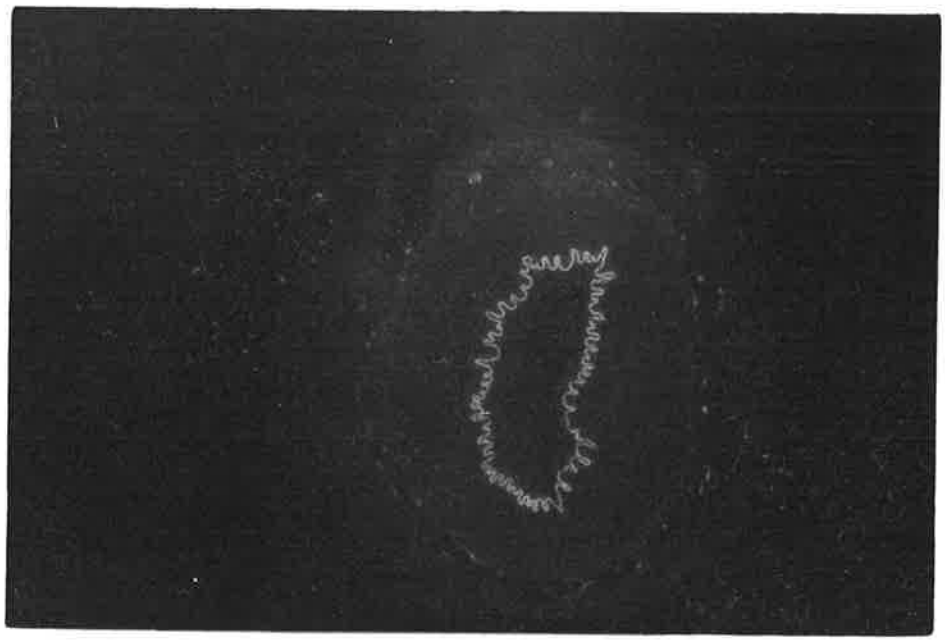


Fig. 2-2(b) An adjacent section to that shown in Fig. 2-2(a) after it had been floated on water. The specific fluorescence has disappeared, while the intimal autofluorescence remains. Magnification X100.

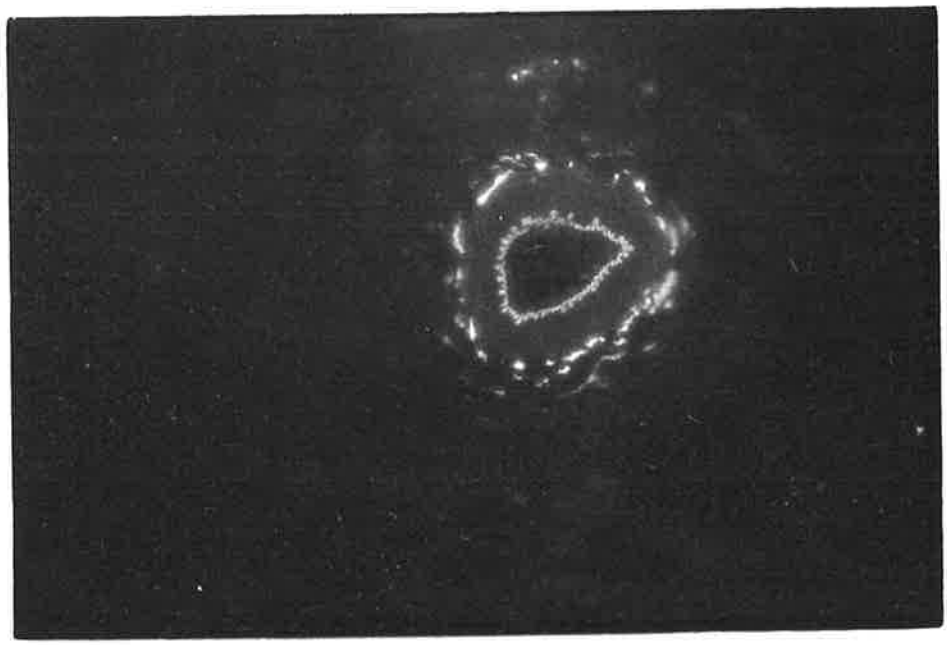


Fig. 2-3(a) A transverse section of a gingival artery from the 7-week old infant, showing specific fluorescent structures at the medio-adventitial junction and intimal autofluorescence. Magnification X100.

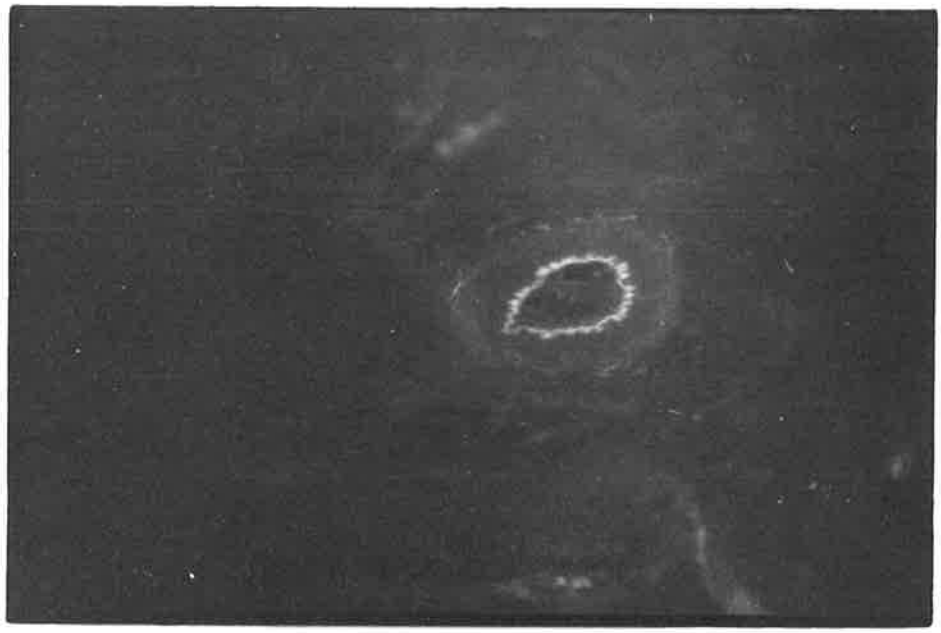


Fig. 2-3(b) An adjacent section to that shown in Fig. 2-3(a) after it had been floated on water. The specific fluorescence has disappeared, while the intimal autofluorescence remains. Magnification X100.

the patients. The first six arteries listed, obtained from subjects aged from 35 to 74 years, showed no specific fluorescence. The remaining blood vessels all showed specific fluorescence, except those from the 19 year old, where the autopsy was performed eighteen hours after death. The specific fluorescence seen in the vessels of the 17 day old infant was scanty. In this case, the autopsy was performed twelve hours after death.

Figures 2-1 and 2-2(a) show the specific fluorescent structures in digital arteries from the 17 week and the 7 week old infants, respectively. Figure 2-2(b) is a control section of the digital artery from the 7 week old treated by floating on water to destroy specific fluorescence. Figure 2-3(a) shows the specific fluorescence in a gingival artery from the same subject and 2-3(b) the control section after floating on water. The specific fluorescence has an intense green appearance and is located at the medio-adventitial junction in the vessel.

DISCUSSION

The results of this study indicate that specific catecholamine fluorescence can be demonstrated in human digital and gingival arteries. The fluorescence was observed to lie near the outer border

of the smooth muscle layer of the blood vessel and had an intense green colour. It was seen in great abundance in the vessels of all but one of the children and young adults. Specific fluorescence was not seen in the blood vessels of the adults aged thirty-five years and over.

The above findings point to an inverse relationship between age and the intensity of specific fluorescence in human blood vessels and, though only a qualitative estimate of sympathetic nerve stores, supports the findings of Frolkis (1968) who found a decline in sympathetic nerve activity with increasing age. Owman *et al.* (1967), in their study on the adrenergic innervation of the human female reproductive tract, also found that fluorescent nerves to the 'cranial' portion of the vagina and the stroma of the ovary were more prominent in the newborn. In the study by Gannon *et al.* (1968) on the rectal mucosa, in which they demonstrated perivascular fluorescence, all the subjects were under twelve years of age. Adams, Horton and Zilkha (1968) found scanty endogenous catecholamine fluorescence in biopsies from temporal arteries obtained at craniotomy from patients aged 38 to 59 years.

In the study on the neonate in our series, in whose vessels only weak fluorescence was seen, a period of twelve hours had elapsed between death and the performance of the autopsy. In the case of the nineteen year old in whom no fluorescence was demonstrated, eighteen

hours elapsed between death and autopsy. In view of the abundance of specific fluorescence which was present in the vessels of the other infants, children and young adults, it is possible that the longer post-mortem autolysis to which the vessels were exposed destroyed the sympathetic neurotransmitter. We have observed that specific fluorescence in the central artery of the rabbit ear is considerably diminished if the artery remains in the cadaver for more than three hours after death at 26°C. If the human cadavers were exposed to this temperature for more than a few hours, autolysis with loss of specific fluorescence could have occurred.

It is not clear why the amount of fluorescence should diminish with increasing age. Our observations are compatible with a decline in the noradrenaline content of the sympathetic nerves with age and also with a diminution in the density of the nerve network itself. Either or both of these changes could contribute to the findings of Frolkis (1968) who reported that against a background of a decline in responsiveness to sympathetic stimulation in older animals there was an increase in sensitivity of the cells and tissues to the transmitter. This is consistent with the increased sensitivity of smooth muscle known to occur after denervation and loss of neurotransmitter substance (Trendelenburg, 1963).

SUMMARY

1. The Falck histochemical technique has been applied to human digital, gingival and mesenteric arteries to localise the sympathetic nerves in these vessels.

2. Specific catecholamine fluorescence, located at the medio-adventitial junction, was observed in digital and gingival arteries obtained from young adults, children and infants, but not in the vessels obtained from older adults.

3. These findings suggest a relationship between the age of the subject and the quantity of transmitter present in sympathetic nerve endings, or in the density of the nerve network.

S E C T I O N 2

STUDIES ON THE CIRCULATION IN MAN

GENERAL METHODS

Venous occlusion plethysmography:

In many of the human studies performed for this thesis the assessment of blood flow in a limb segment, hand, or forearm was made by the technique of venous occlusion plethysmography.

Francis Glisson (1677) was one of the first to use the technique of plethysmography. His plethysmograph was a large glass cylinder, into which the whole arm could be placed, the open end being sealed to the upper arm and the instrument completely filled with water. An open tube at one end, into which the water had risen, enabled him to observe the changes in limb volume during muscular activity.

Buison, in 1862, modified the technique considerably, and the instrument he designed could record volume changes graphically, which is embodied in the name of the technique (*plethysmos* .... increase, *graphein* .... to write).

By the application of intermittent venous occlusion, as suggested by Brodie and Russell in 1905, the rate of blood flow in an organ could be determined quantitatively. This modification was applied for use in the human limb by Hewlett and van Zwaluwenburg in 1909. The latter workers used an air filled glass tube, into which the hand or forearm was placed, and venous occlusion was applied

with a pneumatic cuff placed proximal to the plethysmograph. Subsequent improvements to the technique consisted of sealing the instrument, using a rubber sleeve or glove attached to the plethysmograph (Krogh, Landis and Turner, 1932), and the correct water temperature was determined for both the hand and forearm segments to allow an accurate measurement of resting blood flow (Barcroft and Edholm, 1946).

The principle embodied in venous occlusion plethysmography is that the pneumatic congesting cuff, which encircles the limb proximal to the segment being studied, is inflated to a pressure below diastolic, usually 60-70 mm Hg. When the pressure is applied, arterial blood still flows into the segment and becomes pooled in the capacity vessels. The rate of increase of the part tends to be linear until the capacity vessels are distended and then some blood escapes back under the cuff. Three phases have been described during venous occlusion (Greenfield and Patterson, 1954).

Phase 1 - When there is unimpeded arterial inflow, the increase in volume of the limb segment is equal to the arterial inflow.

Phase 2 - The capacity vessels have filled sufficiently and this leads to a progressive rise of pressure within them, but the pressure is, as yet, insufficient to cause blood to leak past the

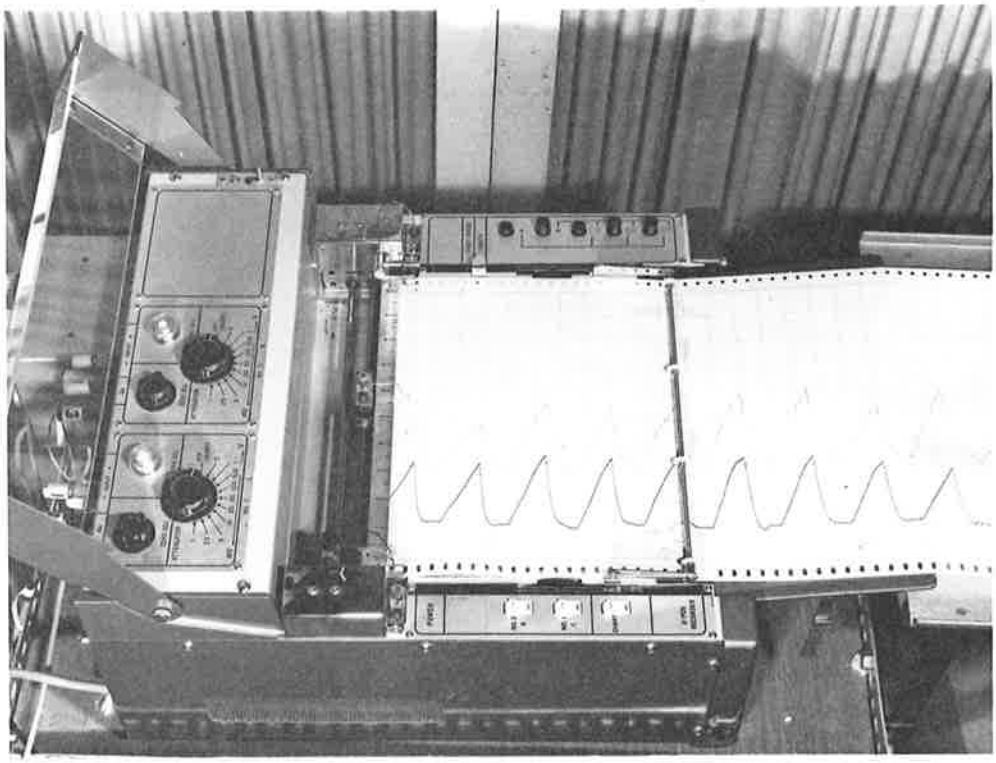


Fig. Method 1 The dual pen-recorder which was used in some of these studies. The record shown on the tracing is of bilateral hand blood flow.

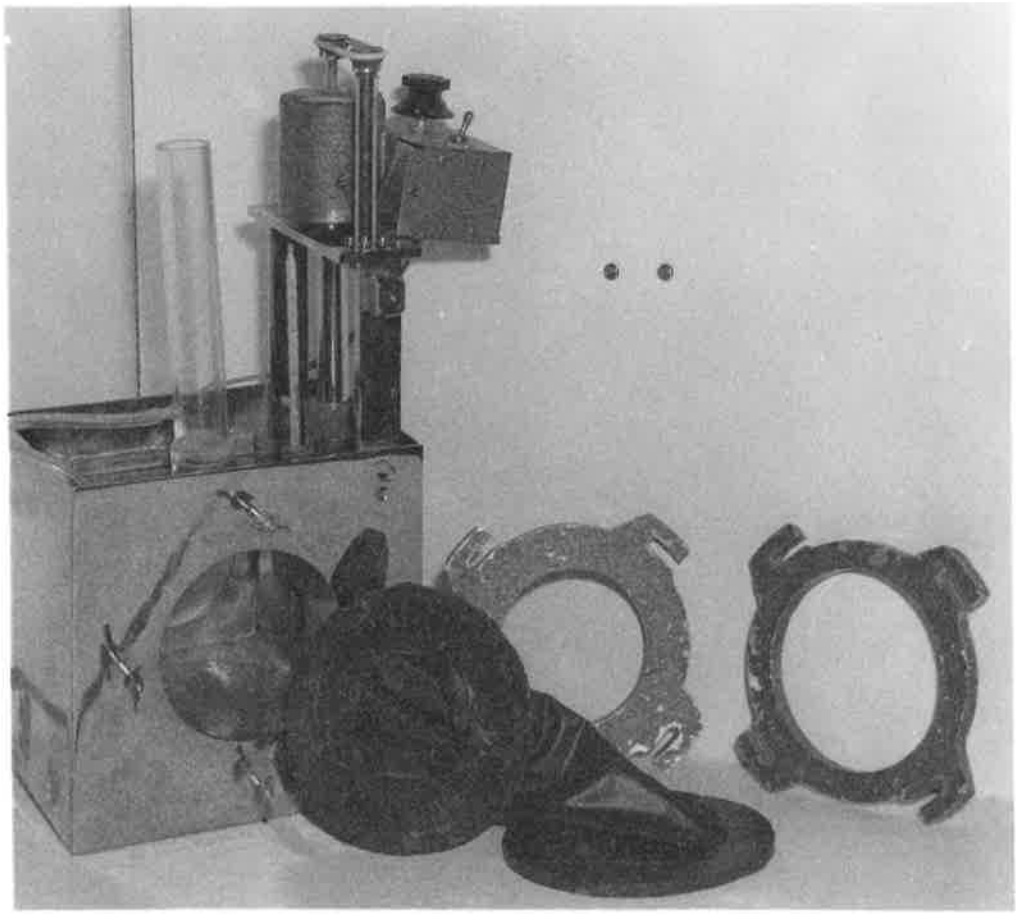


Fig. Method 2 The water-filled, temperature-controlled plethysmograph with the rubber sleeve used for forearm plethysmography.

collecting cuff.

Phase 3 - In addition to the above, the pressure in the capacity vessels has risen sufficiently for blood to leak back under the collecting cuff.

Greenfield and Patterson calculated that arterial inflow would be unaltered by venous back pressure until the resting volume exceeded 2%, and this usually takes much longer than the 10-15 sec collection time used in the present studies.

The increase in the volume of the segment against time is recorded using a kymograph or a pen writer (Fig. Method 1) and the arterial inflow in ml/100 ml/min can be directly deduced from the slope of the record. The technique has been critically examined on several occasions and shown to give an accurate measurement of blood flow (Landowne and Katz, 1942; Formel and Doyle, 1957; Greenfield, 1960).

The plethysmograph which has been used in these experimental studies is one which was designed by Greenfield (1954) and incorporates a temperature control mechanism (Fig. Method 2).

In the measurement of forearm blood flow by this technique a second pneumatic cuff was applied to the wrist immediately distal to the plethysmograph and inflated above systolic pressure, usually to 200 mm Hg. This cuff excluded the hand circulation from the field of study and thereby made the measurement of forearm blood flow

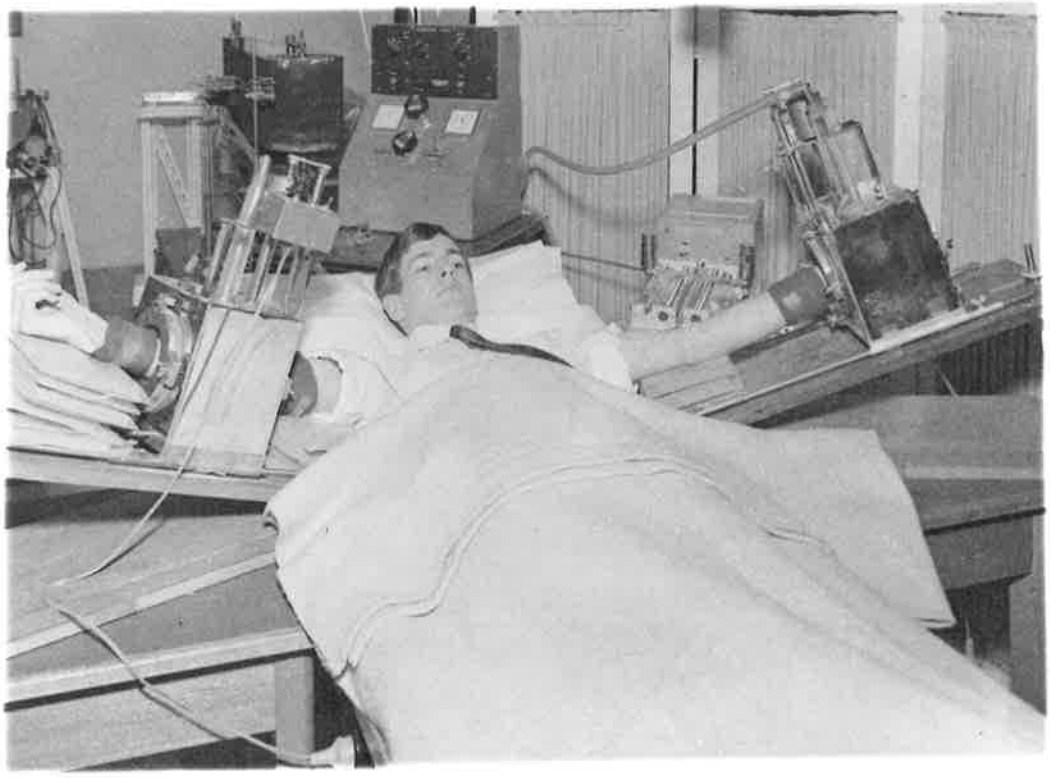


Fig. Method 3 The general laboratory set-up showing a kymograph with smoked drum attached, sequence timer, and automatic collecting and occlusion cuff inflating apparatus. Hand blood flow is being measured on the (L) and forearm blood flow on the (R).

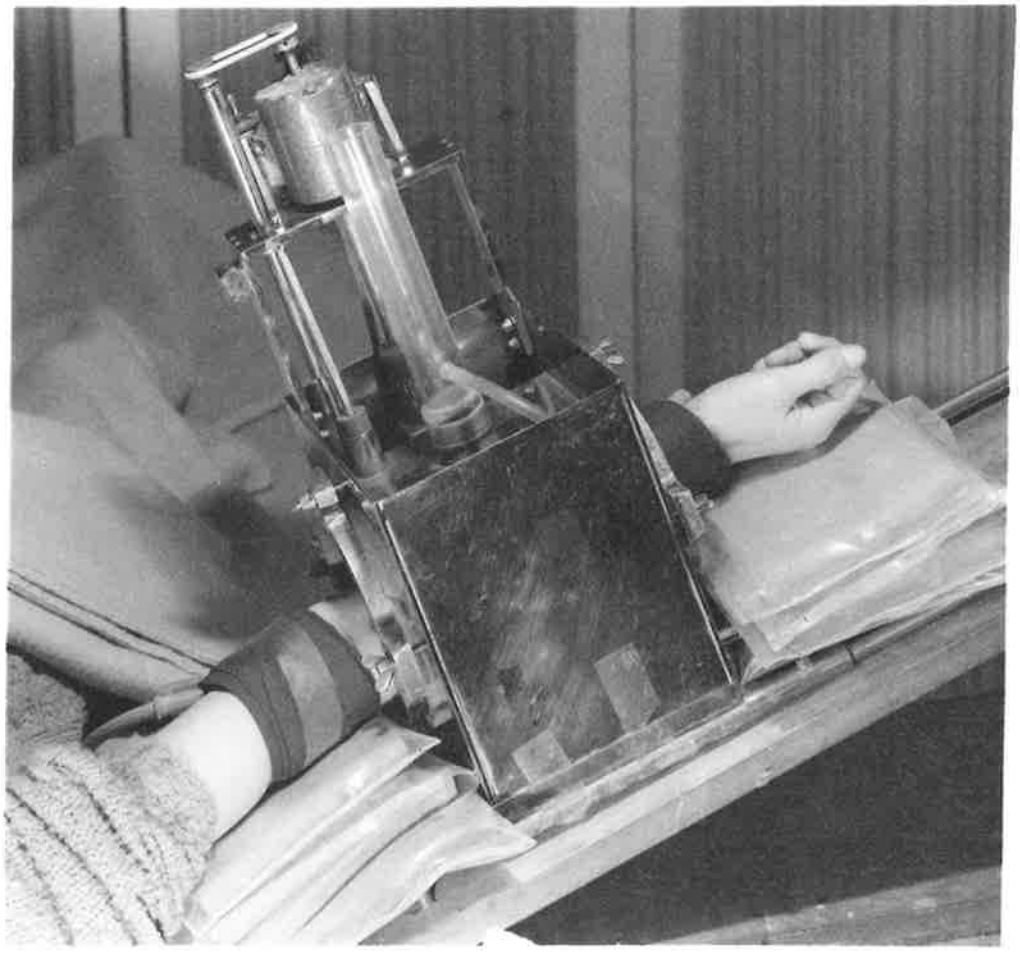


Fig. Method 4 A close up view of a plethysmograph measuring forearm blood flow.

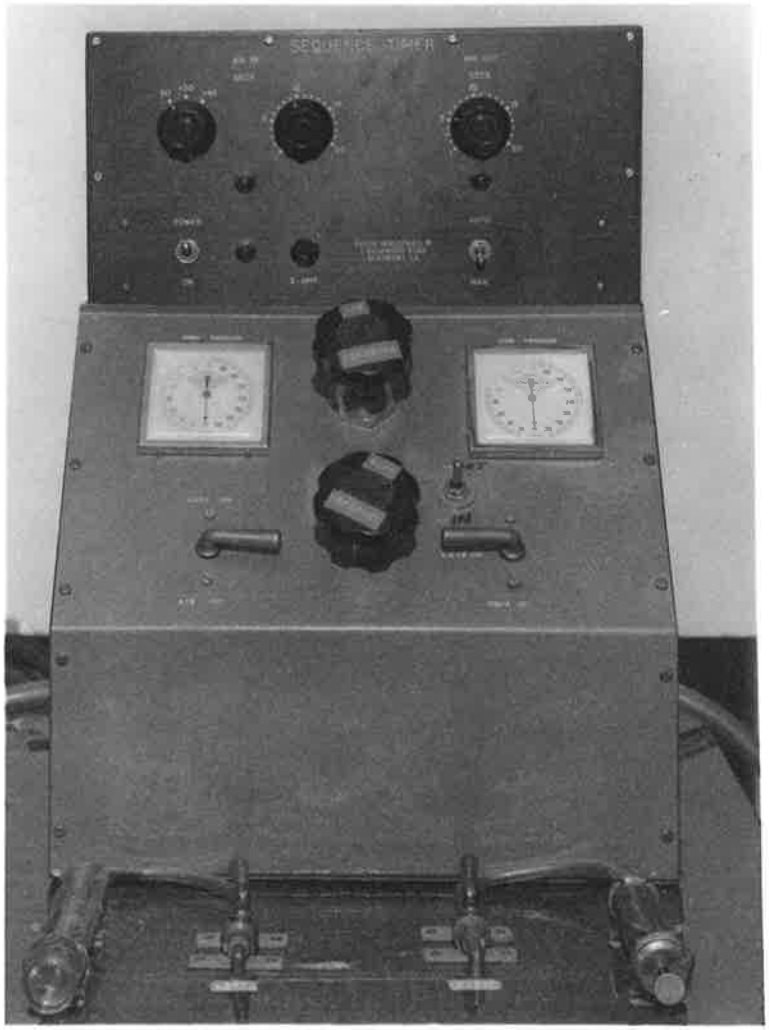


Fig. Method 5 The apparatus used to automatically inflate and deflate the pneumatic cuffs during venous occlusion plethysmography.

accurate. The arterial occlusion cuff was applied for 60-90 seconds before flow measurements were commenced, as suggested by Kerslake (1949).

Fig. Method 3 shows the equipment in operation, and Fig. Method 4 a close up view of forearm blood flow measurements. The high pressure "wrist" cuffs were inflated to 200 mm Hg by air obtained from a compressed air cylinder through a two-stage reducing valve. A two-way gas stop-cock permitted the cuff to be inflated and deflated when required. The low pressure venous occlusion cuffs were inflated and deflated 3-4 times each minute from a reservoir of air maintained at a constant pressure (usually 60-70 mm Hg) by a compressed air cylinder through two reducing valves. A sequence timer (Paton Industries, Adelaide) operated solenoid inlet and outlet valves, and could be set to function automatically with inflation periods from 1-60 seconds and deflation periods from 1-20 seconds. It was also possible to operate this machine manually so that the duration of inflation and deflation could be adjusted at will. The average "collecting period" used in these experiments ranged from 9-15 seconds, while the "air out" phase was in the region of 4-5 seconds. A close up of the Sequence Timer is shown in Fig. Method 5. Care was taken to position the limb segment above heart level during the flow measurements so that adequate venous drainage during the "air out" phase ensured that the capacity vessels were relatively

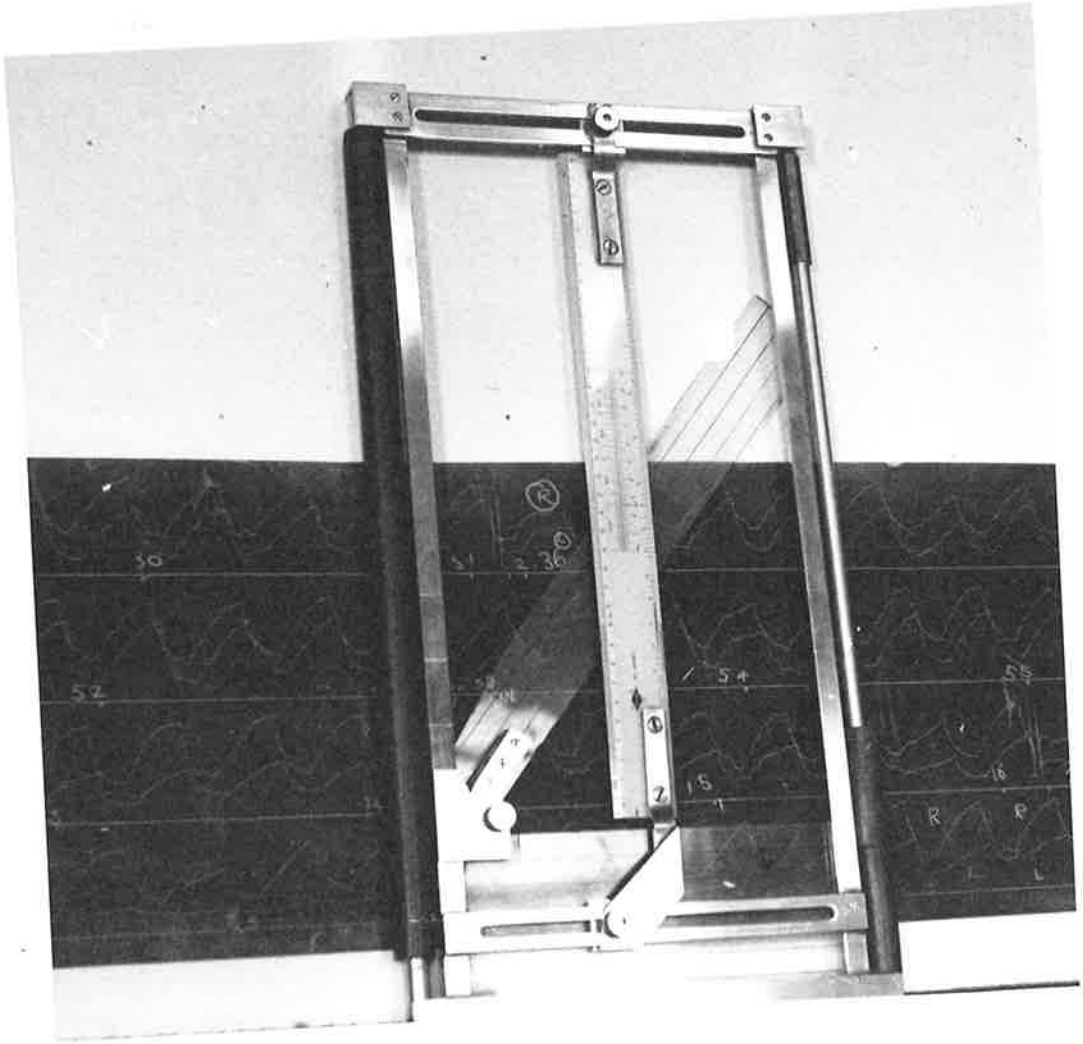


Fig. Method 6 The slide caliper which was used to measure the recorded blood flow.

empty for the subsequent flow estimation.

The increase in volume of the part under study during venous occlusion was converted to a value for blood flow in ml/100 ml/min using the slide caliper shown in Fig. Method 6. The instrument was placed on the record so that one of the horizontal lines on its base was parallel to the time trace line on the recording. One of the parallel lines on the movable perspex arm was placed over the recorded blood flow measurement, and the height in cm read at the point of intersection of the lower border of the perspex arm and the inner border of the vertical centimetre rule. The distance between the pivot of the perspex arm and the inner edge of the vertically placed centimetre rule could be varied and represents the time base (Tx) which had to be calculated for each experiment. This value for (Tx) was calculated from:

1. The vertical movement of the recorder writing point in cm for a volume increment of 1.0 ml(h).
2. The volume of the limb segment/100 (V) and given by the formula $Tx = T60/h.V$.

where T60 was the distance in cm travelled by the recording trace in 60 secs. Once the value for Tx was derived, the slide caliper was set and each cm on the vertical scale read corresponded to a blood flow of 1 ml/100 ml/min.

Superficial and deep venous blood O_2 saturation estimations:

The plethysmograph gives a satisfactory measurement of total blood flow through a limb segment, yet it gives little information about changes in flow in the different vascular beds within the segment itself. Since reflex phenomena and many vasoactive substances have qualitatively and quantitatively different effects on different vascular beds, skin and muscle in particular, a method of differentiating changes in blood flow through each of these vascular beds is most useful.

Iontophoresis of adrenaline to exclude skin blood flow (Cooper, Edholm and Mottram, 1955) was one of the early methods described for "separating" skin and muscle circulations; clearance of radio isotopes injected intradermally or intramuscularly (Winsor and Hyman, 1965), application of counter pressure to minimize skin blood flow over a short segment of forearm (Hyman, Greeson, Clem and Winsor, 1964), and estimation of muscle blood flow with a Hensel needle and skin blood flow with a heat flow calorimeter (Bock, Dengler, Kuhn and Matthes, 1957) are some of the other methods available for this purpose. In the present study, however, the technique used was that of estimating superficial and deep venous blood O_2 saturation and using these values as an index of flow through skin and muscle.

Mottram (1954) made certain observations on muscle vessel

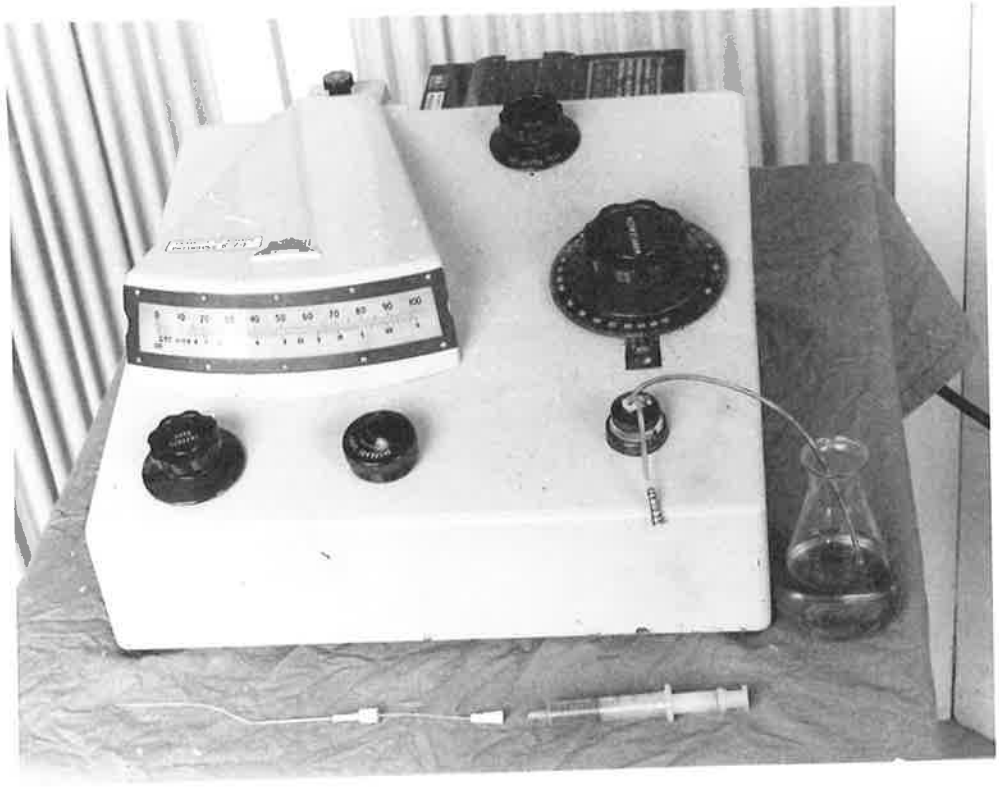


Fig. Method 7 The Unicam prism absorptiometer used to estimate the oxygen saturation of samples of venous blood from skin or muscle.

blood 0_2 saturations, and his technique of retrograde catheterization and blood sampling from a deep (muscle) vein in the cubital fossa was extended by Roddie, Shepherd and Whelan (1956, 1957b). In this technique polyethylene catheters (Intracath No. 17, Bardic) were inserted centrifugally into a deep (muscle) and a superficial (skin) vein in the cubital fossa, and following the inflation of a wrist cuff to 200 mm Hg to exclude hand flow, sampling of venous blood from skin and muscle was commenced, and the effect of a particular procedure, e.g. the infusion of magnesium sulphate into the brachial artery, studied.

Samples of blood (1.0 ml) were collected anaerobically into heparinized plastic syringes and haemolysed by a saponin in sodium carbonate solution. The 0_2 saturation of each sample was rapidly determined by estimating the percentage of light transmission through each sample with a Unicam SP 1400 prism absorptiometer (Fig. Method 7), using a wavelength of 660 mμ and a self-flushing cuvette. A fully oxygenated and a fully reduced standard were prepared from the subject's blood so that the percentage saturation of each test sample could be derived.

The assumption made is that, during the test procedure the metabolic activity, and therefore the 0_2 requirement, of a tissue remains constant, and the changes in 0_2 saturation of the returning venous blood would reflect changes in blood flow through that tissue.

A rise in O_2 saturation of the sample from the deep catheter would indicate increased muscle flow and a decrease in O_2 saturation indicates stasis in these vessels. The same principle would apply to the superficial catheter. The method does not permit quantitative estimation of skin and muscle flow but allows qualitative assessment of the circulatory changes in these vascular beds.

Intravascular infusions:

General considerations: The effects of drugs on the cardiovascular system are a combination of a direct or local action, usually mediated through specific receptors, and indirect or general effects, such as baroreceptor stimulation, interaction with the autonomic nervous system and the release of various hormones.

The route of administration and the dose of drug used determine the relative contribution of the above effects to the total cardiovascular response to a particular drug. When an intravenous infusion is given a combined response is usually seen. However, it is possible to examine the direct action alone, without complicating systemic effects, by using the intra-arterial route of administration and giving a dose of drug that does not produce effects on the general circulation. In the study of hand and forearm blood flow it is possible to confine drug effects to the infused limb alone, and this enables the opposite uninfused limb to act as a control during the infusions and detect spontaneous variations in

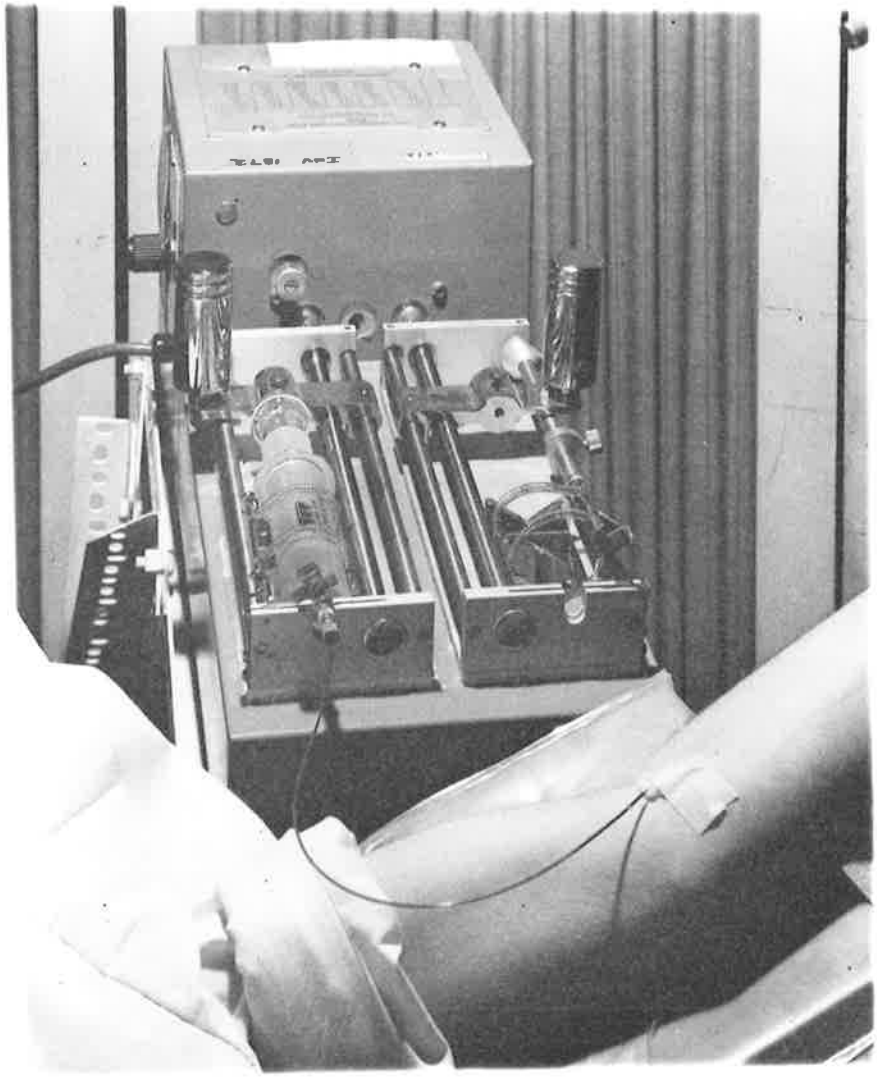


Fig. Method 8 An intra-arterial infusion being administered through a needle inserted centrifugally into the (L) brachial artery of a subject. The constant infusion device is shown in the background.

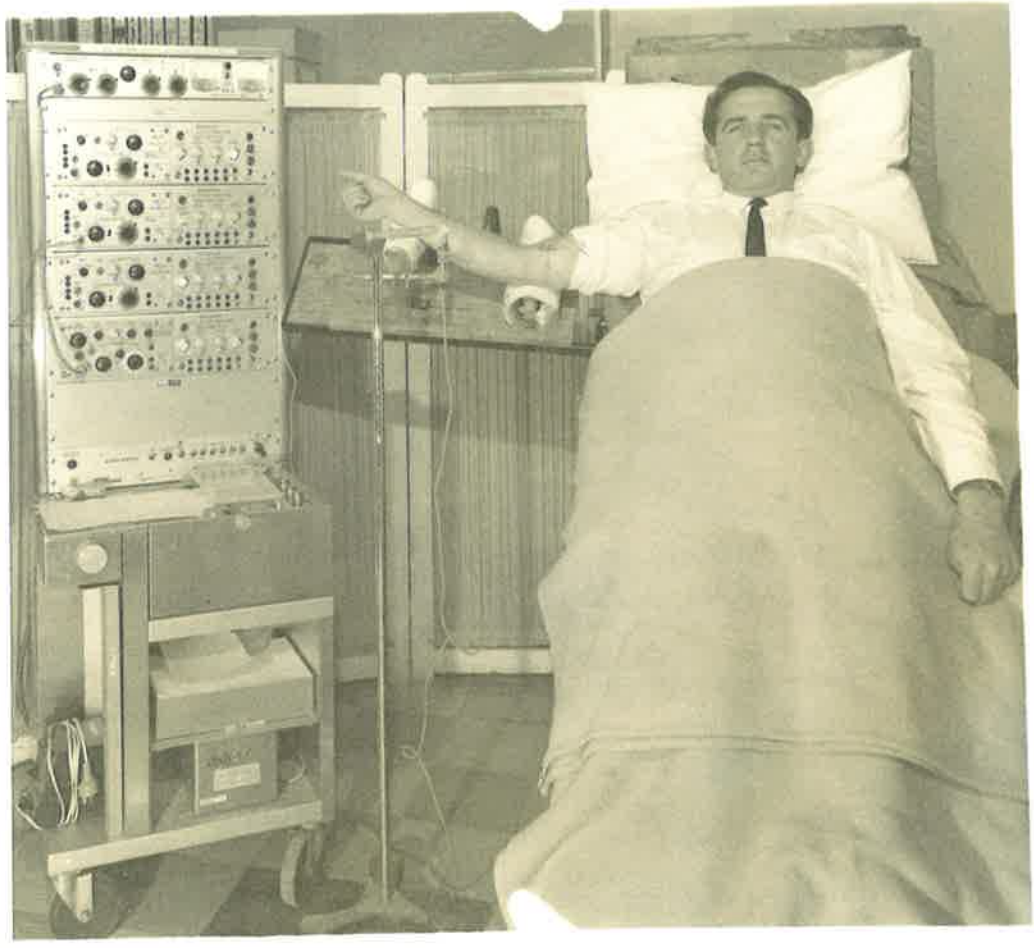


Fig. Method 9 Measurement of systemic arterial blood pressure using a Statham transducer attached to a needle in the brachial artery and recorded on a Grass polygraph. The subject's postural reflexes are being tested in this experiment.

flow unrelated to drug action. In studies on the hand, this is particularly important as the resting level of flow fluctuates considerably.

Intra-arterial infusions and intra-arterial pressure recording: Intra-arterial infusions were administered through a 22 or 23 gauge, short-bevel (buttless) needle, 3.1 cm long, which was connected to a constant infusion device by a 30 cm length of Sterivac No. 1 polyethylene tubing.

The needles were inserted percutaneously under local anaesthesia (2% lignocaine) and then threaded centrifugally or centripetally down the lumen of the artery. They were then secured in place, using adhesive tape, and remained in-situ for the rest of the experiment (Fig. Method 8).

When the need to measure arterial blood pressure arose, this was achieved by inserting either a 19 gauge, buttless needle centripetally or centrifugally into the artery, or by using a polyethylene catheter (Intramedic P.E.90, Clay Adams). The catheters were inserted by a modified Seldinger technique (Seldinger, 1953), and heparinized saline was "flushed" through the intra-arterial leads, in between pressure measurements, to prevent clotting. The transducer used to monitor pressure was a Statham model P23 DC, and the output from this was recorded on a Grass Polygraph (Model 5D) (Fig. Method 9).

Most infusions were at the rate of 2.0 ml per minute. Saline 0.9% (w/v) was infused during the control periods and also used as a vehicle for the drugs.

Intravenous infusions: These were administered through a polyethylene catheter (Intracath No. 17 or 19 gauge, Bardic) which was inserted centripetally into an antecubital vein under local anaesthesia (2% Lignocaine). The infusion apparatus and connecting tubing were identical to that used in the intra-arterial infusions.

Expression of results:

Blood flow was expressed in terms of ml blood flow per 100 ml hand or forearm tissue per minute and plotted either as instantaneous flow values or as minute averages.

Percentage changes in hand blood flow with intra-arterial infusions were determined from the average values for the two minutes before drug infusion and for the last two minutes of the infusion period, by which time the response to the drug had become stable. The small doses used did not cause systemic effects and hence the blood flow on the uninfused side was regarded as a control. In calculating the percentage changes in blood flow, allowance was made for spontaneous variations in the flow by assuming that in the absence of the infusion the two sides would have maintained the same relationship as in the pre-infusion period (Duff, 1952). This correction could not be used for those experiments where the

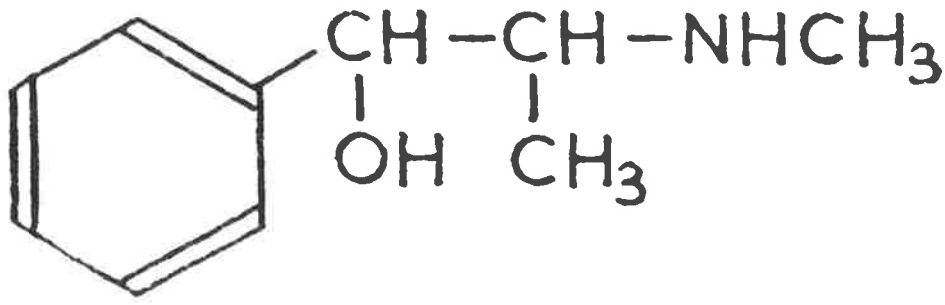
sympathetic nervous transmission to the infused side had been interrupted in any way.

Arterial pressure was expressed in the conventional terms of systolic, diastolic and mean pressures, the latter being given by the equation:

Mean Pressure = Diastolic + 1/3 (Systolic - Diastolic).

The values for mean pressure were also used in the calculation of resistance changes and derived from the ratio:

$$\text{Resistance (in arbitrary units)} = \frac{\text{Mean Blood Pressure (mm Hg)}}{\text{Blood flow (ml per 100 ml of hand or forearm per min)}}$$



Ephedrine

Fig. 3-1

CHAPTER 3

The action of ephedrine on forearm blood vessels in man.

The evidence presented in the previous chapters indicates that in the rabbit and in man the sympathetic nerve endings in the peripheral arteries are located at the medio-adventitial junction of the vessel. Various pharmacological studies on the rabbit arteries have shown that the sympathetic nerves influence the effect of vaso-active agents on the vascular smooth muscle, and this influence can be modified by drugs, such as cocaine, which are known to interfere with noradrenaline uptake into sympathetic nerve endings.

In the living human, evidence of the physiological role of the sympathetic nerves can be obtained by studying the effects of pharmacological agents on normal vascular beds and those previously denervated by sympathectomy. One of the drugs so studied is ephedrine (Fig. 3-1) because of its wide use in therapeutics.

It has been found that the constrictor action of intra-arterial ephedrine on the hand blood vessels gradually diminishes following surgical sympathectomy to become absent six weeks after operation, and is absent in patients with spontaneous idiopathic autonomic degeneration in which the nerves to the hand are involved (Parks, Sandison, Skinner and Whelan, 1961).

In addition to its vasoconstrictor action, parenterally

administered ephedrine has been shown to have vasodilator properties. Starr, Gamble, Margolies, Donal, Joseph and Eagle (1937) found a decrease in total peripheral resistance after subcutaneous administration and deduced that vasodilatation of some vascular beds had occurred. They observed an increase of the blood pressure, but attributed it to an increased cardiac output. Forearm blood flow was observed by Allen (1948) to increase following intramuscular injections, and Cohn (1965) reported a fall in forearm vascular resistance during intravenous infusions and sometimes with large intra-arterial infusions. Cohn (1965) also found that adrenergic blockade with phentolamine abolished the constrictor action of ephedrine intra-arterially, but reversal to a dilator effect was not observed.

In the present study, the mechanisms of the vasoconstrictor and vasodilator actions of ephedrine on the forearm vessels in man have been further investigated. The vasoconstrictor action has been demonstrated to be almost entirely indirect through release of transmitter from the sympathetic nerve endings, and the vasodilator action has been shown to be due to direct stimulation of β-adrenergic receptors.

MATERIALS AND METHODS

The subjects were volunteer medical students. The laboratory temperature was maintained at 22-25°C and the subject rested recumbent on a couch for at least 30 min before observations began, during which time recording apparatus was applied and infusion needles inserted.

A number of sympathectomized subjects were also studied. Two of these had undergone surgical cervical sympathectomy for mild Raynaud's phenomenon and two suffered from idiopathic spontaneous autonomic degeneration. All of them showed complete loss of sympathetic innervation to the vessels of the hand, as determined by the absence of reflex constriction in response to painful or cold stimuli (Cooper, Fewings, Hodge and Whelan, 1963) and by failure to show vasodilatation or sweating with indirect heating. Denervation of the forearm muscle vessels was demonstrated by a reduced or absent vasodilator response to mental arithmetic (Blair, Glover, Greenfield and Roddie, 1959).

Blood flow through both forearms was recorded three or four times a minute by the technique of venous occlusion plethysmography, using water-filled, temperature-controlled plethysmographs similar to those described by Greenfield (1954).

Drugs were introduced into the brachial artery in the

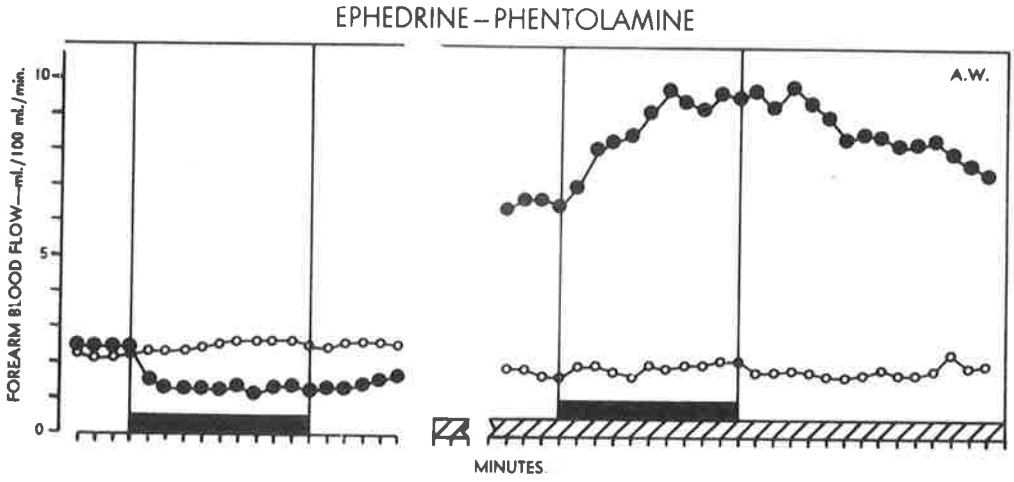


Fig. 3-2 The response of the blood flow through the forearm of a normal subject to infusion of ephedrine 50 µg/min for 10 min (black rectangle) into the brachial artery of one side (●) before (left) and during (right) infusion of phentolamine 50 µg/min (hatched rectangle). The break in the hatched area represents an interval of 17 min. O, blood flow through the opposite control forearm. Each point represents the mean of the three or four flow values recorded during the preceding minute.

cubital fossa of one arm through a 22-gauge, short-bevel needle inserted under local anaesthesia and connected through a 30 cm length of polyethylene tubing to a mechanically-driven syringe which delivered 2 ml of solution per min. Saline (0.9%, w/v) was infused during control periods and was used as a vehicle for the drugs.

The drugs used were ephedrine hydrochloride (Elliotts and Australian Drug Pty Ltd), isoprenaline hydrochloride (Isuprel, Winthrop), phentolamine (Regitine, Ciba) and propranolol hydrochloride (Inderal, I.C.I.). The doses are expressed as weights of the salts.

RESULTS

The predominant action of ephedrine when given intra-arterially on the circulation of the normal forearm was to produce a decrease in flow. During blockade of α-adrenergic receptors by administration of phentolamine into the brachial artery, however, ephedrine caused a marked vasodilatation. Fig. 3-2 illustrates 1 of 4 such experiments carried out on four normal subjects. The left-hand curve represents the response of the forearm blood flow to the administration of 50 μg/min ephedrine for 10 min into the brachial artery before α-receptor blockade. When this response had been

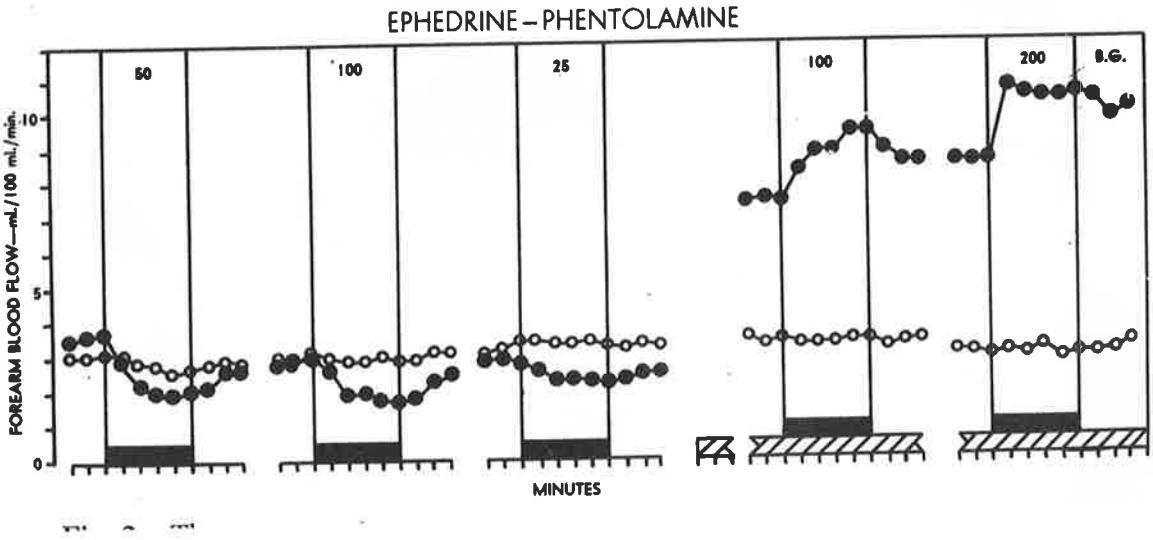


Fig. 3-3 The response of the forearm blood flow to ephedrine 25, 50 and 100 µg/min intra-arterially (black rectangles) before, and 100 and 200 µg/min during continuous infusion of phentolamine (50 µg/min - hatched area). ● , treated forearm; O , control forearm. The breaks in the hatched area represent intervals of 23 and 15 min. The numbers at the top of the frame indicate the doses of ephedrine in µg/min.

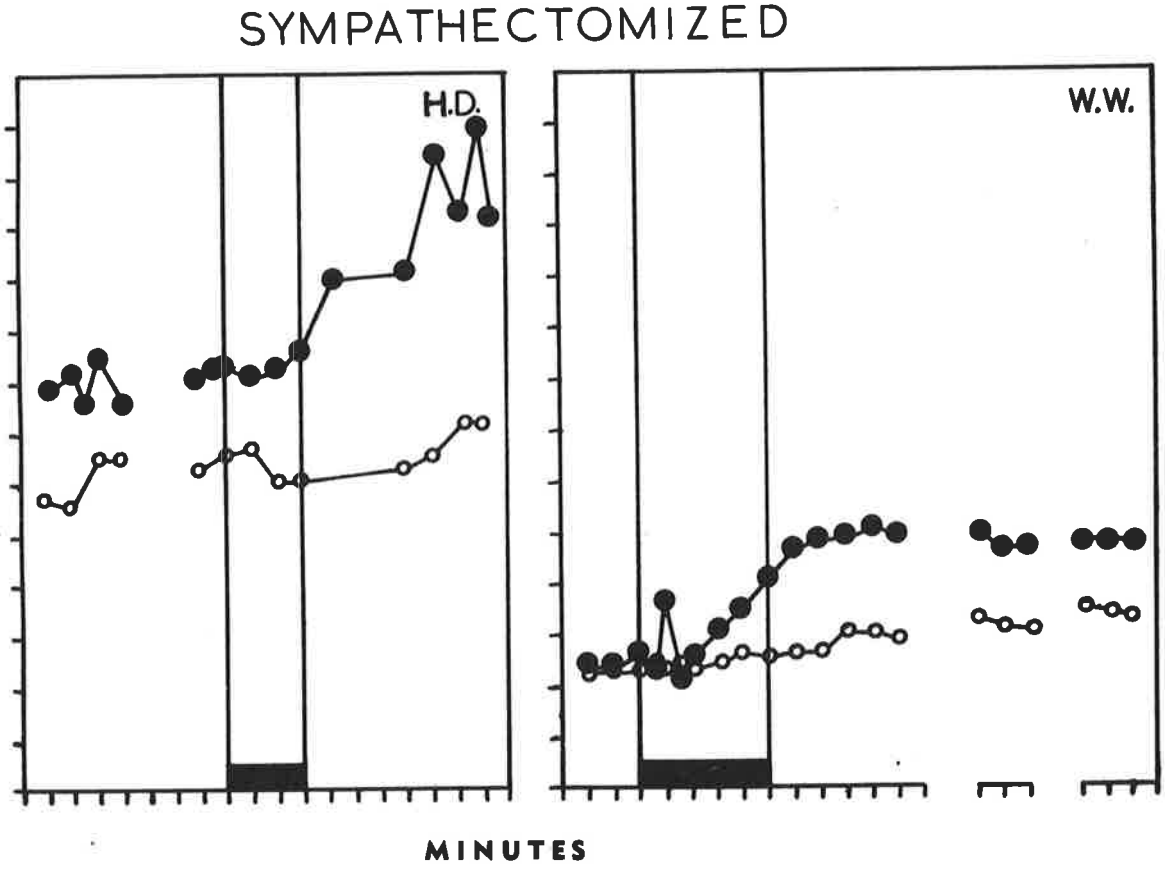


Fig. 3-4 The response of the forearm blood flow to intra-arterial ephedrine (black rectangles) in the sympathectomized forearms of two patients. In patient H.D. the dose of ephedrine was 1 mg/min for 3 min; in W.W. 500 µg/min for 5 min. ●, treated forearm; O, control forearm. The breaks in the abscissa of the right hand frame represent intervals of 6 min.

recorded an infusion of phentolamine 50 µg/min was commenced. After 17 min the infusion of ephedrine (50 µg/min) was repeated, the phentolamine continuing throughout. The blood flow promptly increased and continued to rise during the first 6 min of the 10-min infusion, after which it remained fairly constant, falling back over 15-20 min to the previous phentolamine-induced level after the ephedrine infusion ceased. The flow through the opposite control forearm remained constant throughout. Similar results were obtained in the three other subjects.

In five further subjects the doses of the infusions of ephedrine during phentolamine administration were increased in an attempt to compensate for dilution caused by the increased level of flow induced by the phentolamine. The results of one of these experiments is illustrated in Fig. 3-3. Before phentolamine was given, ephedrine (25, 50 and 100 µg/min) reduced the forearm blood flow. During phentolamine administration the flow rose to about double the previous level, and ephedrine infusions 100 and 200 µg/min were then given. A further vasodilatation was produced with each infusion. Similar results were obtained in the other four subjects.

The vessels of the sympathetically denervated forearm responded to intra-arterial administration of ephedrine by dilatation, and the responses in the two patients surgically sympathectomized for mild Raynaud's disease are shown in Fig. 3-4. The dilatation

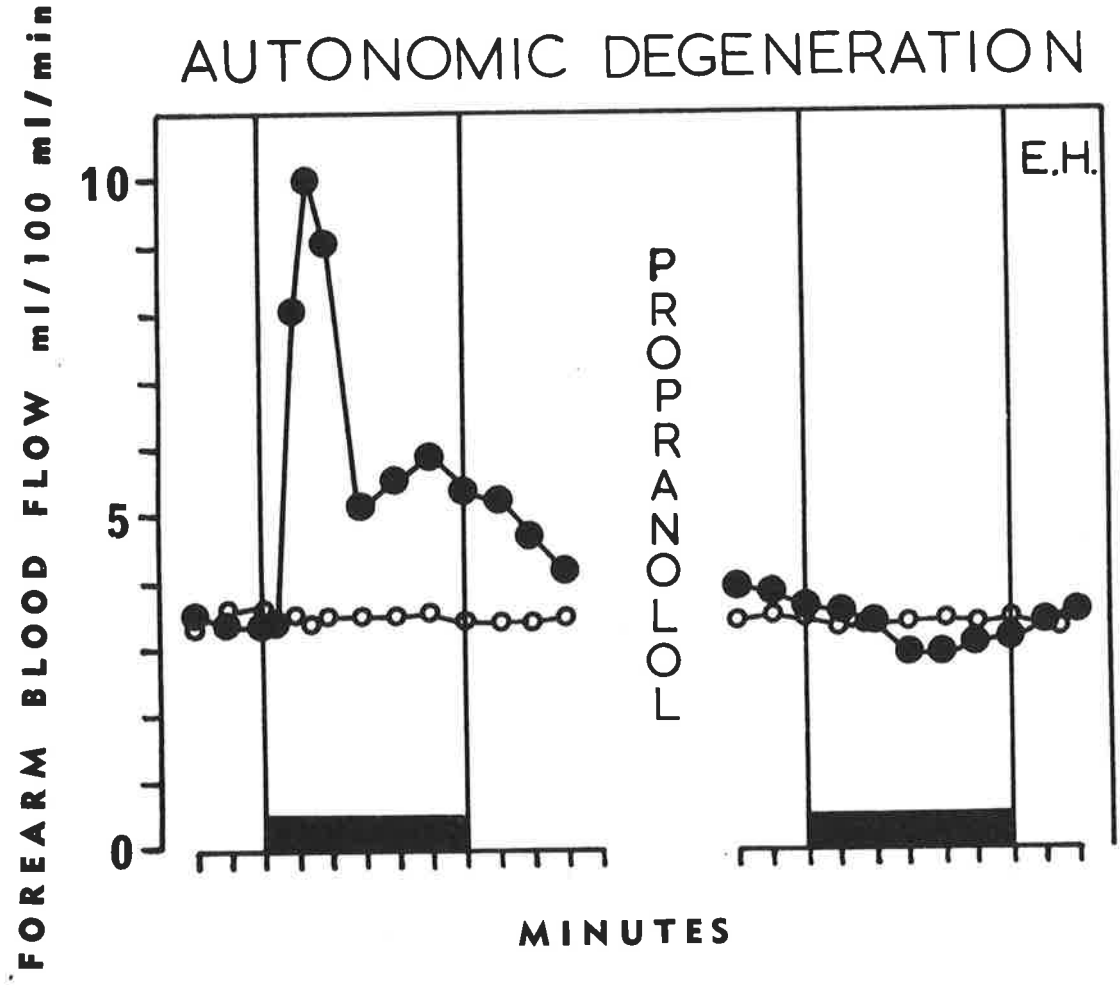


Fig. 3-5 The response of the forearm blood flow to intra-arterial ephedrine (500 µg/min - black rectangles) in a patient suffering from spontaneous idiopathic autonomic degeneration before (left) and after (right) administration of propranolol (100 µg/min for 8 min). ●, treated forearm; ○, control forearm.

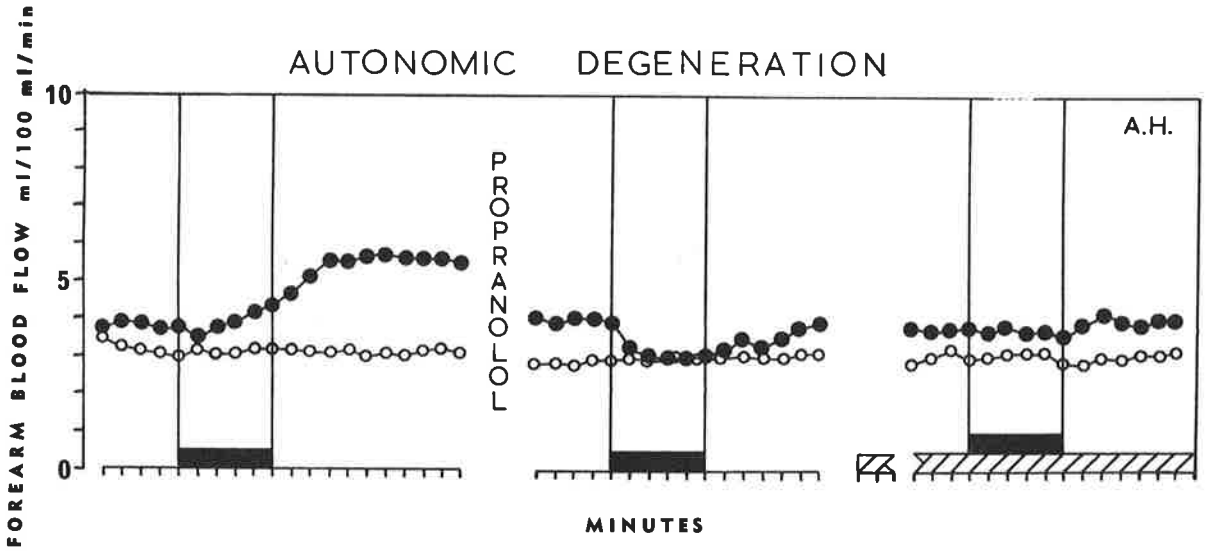


Fig. 3-6 The response of the blood flow through the denervated forearm of a patient suffering from autonomic degeneration to ephedrine (500 µg/min for 5 min - black rectangles) before and after β-receptor blockade with propranolol (100 µg/min for 8 min) and α-receptor blockade with phentolamine (50 µg/min - hatched rectangle). ●, treated forearm; ○, control forearm.

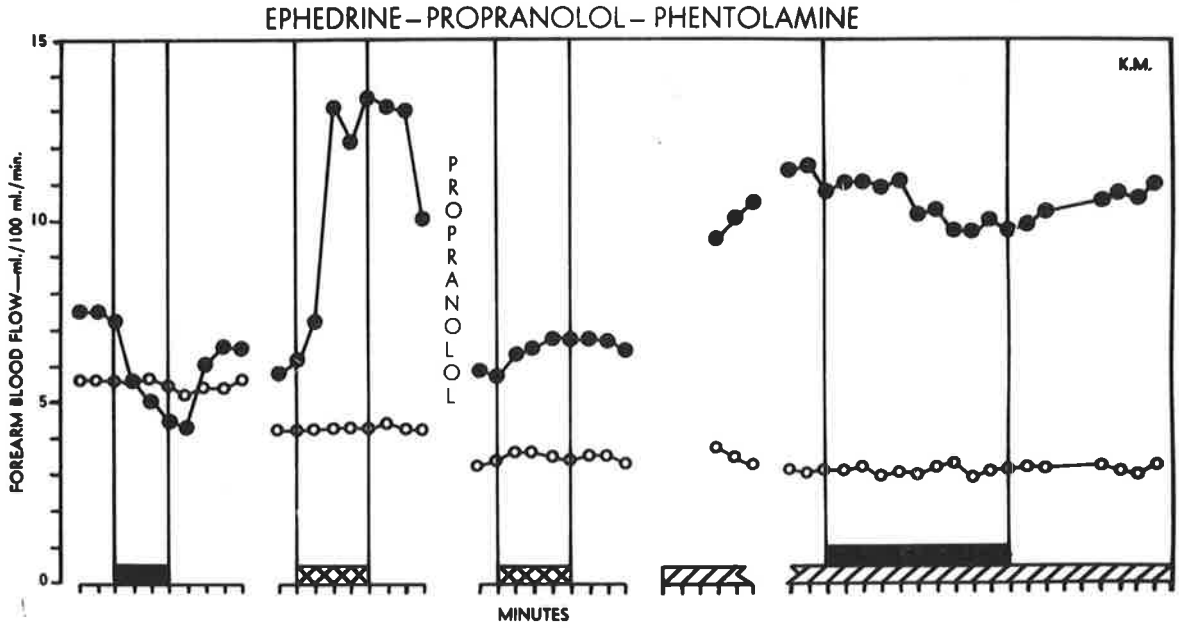


Fig. 3-7 The response of the blood flow through the forearm of a normal subject to ephedrine (50 µg/min - black rectangles) and to isopropylnoradrenaline (0.05 µg/min - cross-hatched rectangle) before and after propranolol (100 µg/min for 8 min) and phentolamine (50 µg/min - hatched rectangle). ●, treated forearm; ○, control forearm. The break in the hatched rectangle represents an interval of 9 min.

was slower in onset than in normal phentolamine-treated forearms, and in W.W. a brief constrictor phase succeeded an initial transient dilatation and preceded the gradual development of a long-lasting dilatation.

Dilator responses were also obtained with intra-arterial ephedrine in the two patients suffering from spontaneous idiopathic autonomic degeneration. One of these is shown on the left of Fig. 3-5.

In this patient, after the above response had been recorded, the β-receptor blocking agent propranolol was given into the brachial artery in a dose of 100 μg/min for 8 min. This dose had been shown in other subjects to be effective in blocking the response of the forearm vessels to the β-stimulating drug isoprenaline after a period of 20 min (Fig. 3-7). When ephedrine infusion was repeated, a modest, but distinct, vasoconstriction replaced the previous dilatation.

Fig. 3-6 shows the responses of the forearm blood flow to intra-arterial ephedrine before and after β- and α-receptor blockade in the second patient suffering from autonomic degeneration. Administration of propranolol converted the dilator action to a vasoconstriction, as in the previous patient (Fig. 3-5). When the α-receptor blocking agent phentolamine was given, the constrictor effect was abolished.

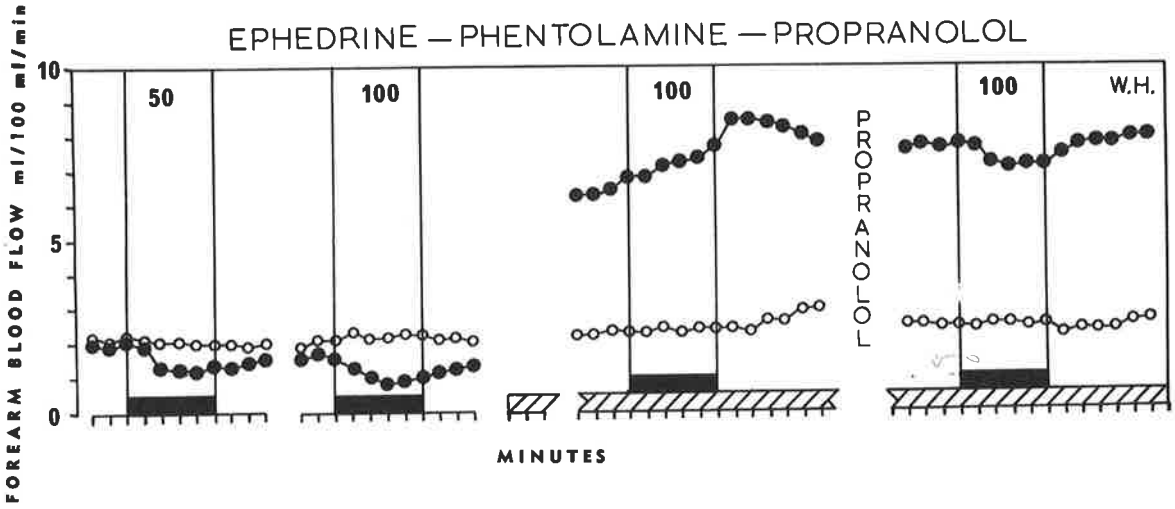


Fig. 3-8 The response of the blood flow through a normal forearm to intra-arterial ephedrine (black rectangles) before and during administration of phentolamine (50 µg/min - hatched rectangle) and after administration of propranolol (100 µg/min for 8 min). The numerals at the top of the frame indicate the doses of ephedrine in µg/min. ●, treated forearm; ○, control forearm. The breaks in the hatched rectangle indicate intervals of 13 and 22 min, respectively.

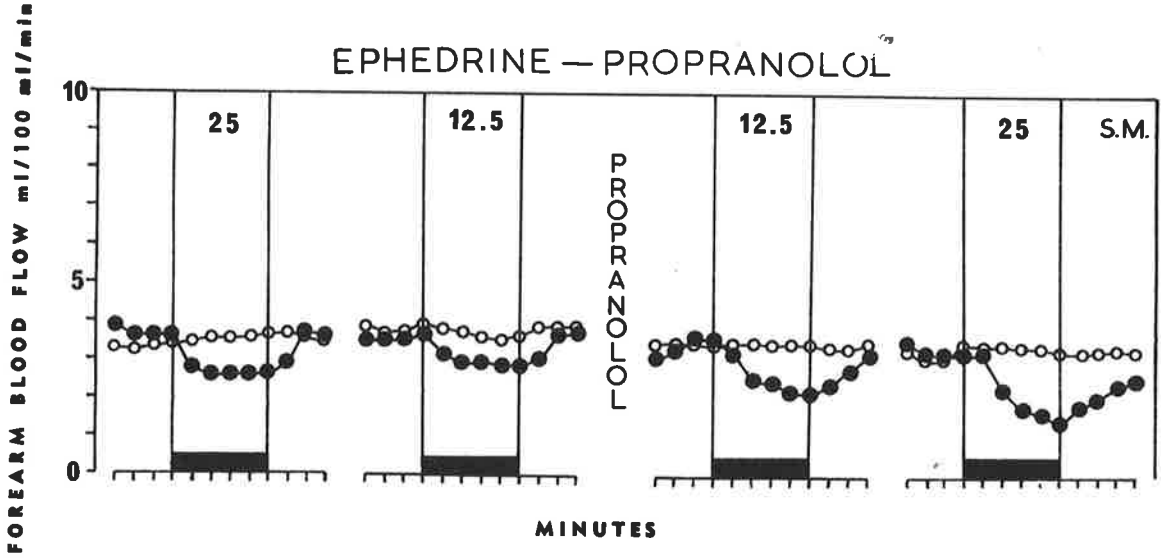


Fig. 3-9 The effect of propranolol intra-arterially (100 µg/min for 8 min) on the responses of the forearm blood flow of one subject to intra-arterial ephedrine, the doses of which in µg/min are indicated by the numbers at the top of the frame. ●, treated forearm; o, control forearm.

Similar results were obtained on normal subjects who were given both α- and β-receptor blocking agents. In Fig. 3-7 are illustrated the responses to ephedrine and to isoprenaline before and after administration, first of propranolol and then of phentolamine. Propranolol almost abolished the dilator response to a large dose of isoprenaline (0.05 µg/min). In the presence of phentolamine, ephedrine now failed to produce vasodilatation and a modest constriction resulted. In 3 experiments the order of adrenergic blockade was reversed and the results of 1 of these is illustrated in Fig. 3-8. The vasoconstrictor responses to infusion of 50 and 100 µg/min of ephedrine were first recorded. Phentolamine administration (50 µg/min) was then commenced, and, after 13 min, ephedrine (100 µg/min) was repeated. A vasodilatation ensued. After an interval of 22 min, during which the phentolamine infusion was maintained and propranolol (800 µg in 8 min) was administered, a third infusion of ephedrine (100 µg/min) was given. This, now, produced a constrictor response. Similar responses were seen in the two other subjects.

The effect of propranolol administration alone on the response of the forearm vessels to ephedrine was examined in four subjects. The constrictor response was always greater after propranolol than before. The data from 1 of these experiments are shown in Fig. 3-9 and the pooled data for all subjects illustrated

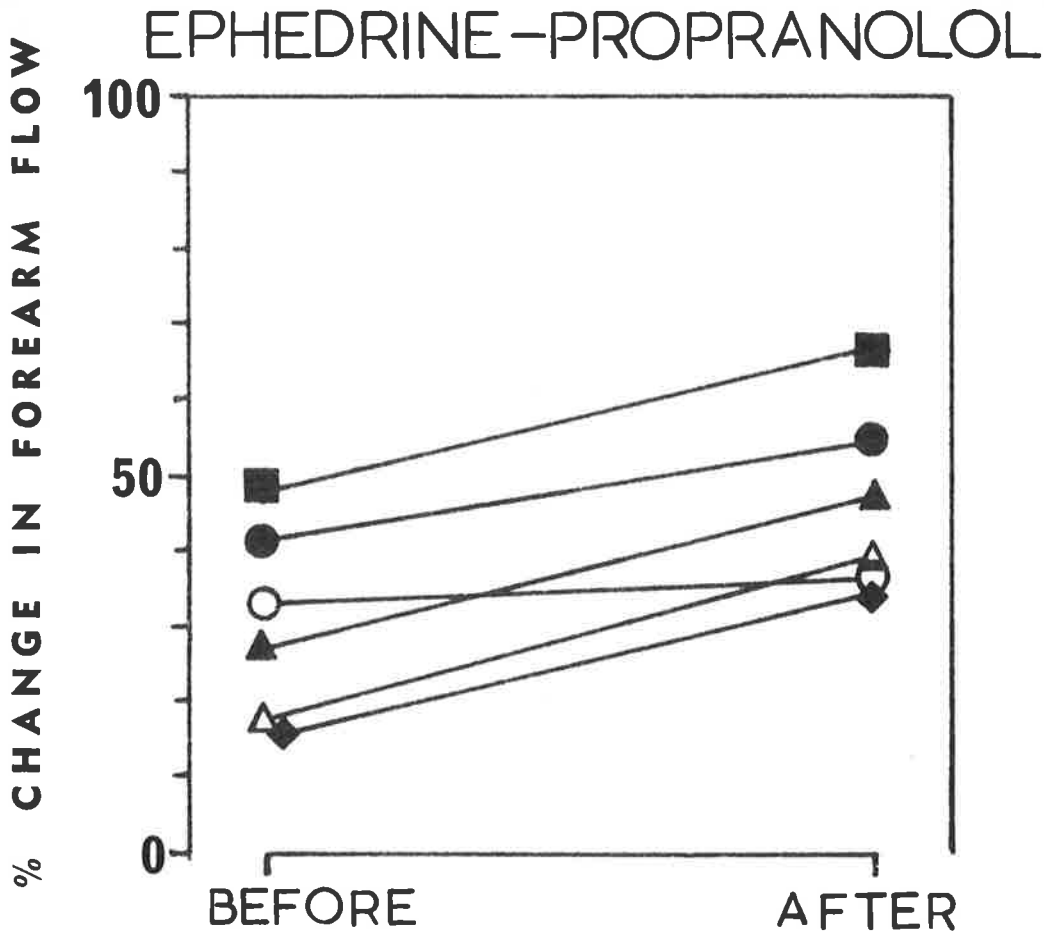


Fig. 3-10 Pooled data from 6 infusions in 4 subjects of ephedrine intra-arterially before and after administration of propranolol (100 µg/min for 8 min). The fall in blood flow with each infusion was determined by expressing the mean of the flow values during the last 2 min of a 5 min infusion of ephedrine as a percentage of the mean flow value during the last 2 min of the immediately preceding control period.

● A.W. ephedrine 50 µg/min.
△ S.M. ephedrine 12.5 µg/min.
○ C.M. ephedrine 50 µg/min.
▲ S.M. ephedrine 25 µg/min.
♦ C.M. ephedrine 25 µg/min.
■ H.T. ephedrine 12.5 µg/min.

in Fig. 3-10.

DISCUSSION

The results of the foregoing experiments demonstrate that ephedrine given by local arterial infusion has two actions on the smooth muscle of the vessels of the forearm in man. The predominant action is vasoconstriction which is dependent on the integrity of the sympathetic nerves and is presumably due to the release by ephedrine of a constrictor substance from the nerve endings. Phentolamine introduced into the vessels results in blockade of the α-adrenergic receptors on the smooth muscle and abolishes this constrictor effect of ephedrine. It does not prevent release of transmitter from the nerve ends (Swaine, 1963).

Administration of phentolamine not only prevents the constriction of the vessels in response to ephedrine, but also unmasks a marked and prolonged vasodilatation. This vasodilatation is due to a direct action of ephedrine on the vessels. It is not a consequence of a dilator property of released transmitter, since it is seen in sympathectomized and autonomically degenerated limbs in which no sympathetic nerve ends are present. This dilator action of ephedrine can be attributed to a direct effect on the β-receptors

of the vessels, since it is abolished by administration of the β-blocking drug propranolol into the sympathectomized or phentolamine-treated limb.

The increase in forearm blood flow produced by the administration of phentolamine is due, in part, to blockade of α-adrenergic receptors, with consequent loss of the resting sympathetic vascular tone, and, in part, to a direct dilator action of phentolamine on the vessels (Taylor, Sutherland, Mackenzie, Staunton and Donald, 1965). As a consequence of the elevated flow level, the concentration of ephedrine reaching the forearm vessels during intra-arterial administration would be less when phentolamine was being infused than during the previous control infusion of ephedrine. Since the rate of forearm flow was increased two or threefold by phentolamine, in a number of experiments an attempt was made to correct for the dilution effect of increased flow by increasing the doses of ephedrine given in the infusions during phentolamine administration (Fig. 3-3). A further vasodilatation was always produced. Thus, the dilator action of ephedrine after phentolamine blockade did not appear to be related to the change in initial level of forearm flow.

When the limb of the normal subject was treated by both phentolamine and by propranolol, a small, but distinct, vasoconstriction was produced by ephedrine. The mechanism of this

effect is not clear. It is possible that the administration of propranolol after phentolamine may have had the effect of antagonizing the α-blocking activity of the latter, and transmitter released from the nerve ends was then able to act on receptors, as has been suggested by Prichard and Ross (1966).

Another explanation might be that the phentolamine had not been fully effective in blocking the α-receptors, and the partial constrictor action of the transmitter released by ephedrine became apparent when its dilator component was blocked.

However, the fact that a constrictor effect of ephedrine was seen in the two autonomically degenerated limbs following β-receptor blockade points to a direct effect of ephedrine, unless it be postulated that a few sympathetic fibres remained on which ephedrine could act. As far as could be determined clinically or by physiological tests, the denervation of the forearms was complete in these patients. In the patient A.H. the constrictor effect of ephedrine after β-blockade was abolished by administration of phentolamine, indicating an action on α-adrenergic receptors.

It seems clear, therefore, that ephedrine given by local arterial injection has two, and possibly three, effects on the forearm vessels. The response in the normal limb, however, represents a balance between the vasoconstrictor and vasodilator actions, since, when the dilator component is abolished by

administration of propranolol, an enhanced vasoconstriction ensues.

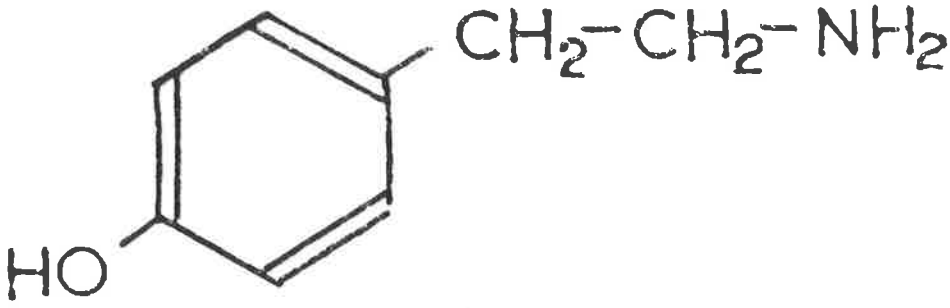
SUMMARY

1. Ephedrine hydrochloride given into the brachial artery has been shown to have two distinct actions on the vessels of the human forearm.

2. The predominant effect is a vasoconstriction which is mediated by release of a constrictor substance from the sympathetic nerve ends acting on α-adrenergic receptors of the vascular smooth muscle.

3. The second effect is a vasodilatation which is unmasked when the α-receptors are blocked by phentolamine or when the sympathetic nerves are absent. This effect is due to a direct action of ephedrine on β-adrenergic receptors.

4. A mild vasoconstriction is seen following blockade of both α- and β-receptors and in the β-blocked sympathectomized limb. The mechanism of this effect is uncertain.



Tyramine

Fig. 4-1

CHAPTER 4

The mechanism of action of tyramine on the blood vessels of the forearm in man.

Tyramine (Fig. 4-1) was selected for this study because it was believed to cause vasoconstriction solely by the release of neurotransmitter substance from sympathetic nerve endings in vessel walls without any direct action on vascular smooth muscle such as was found with ephedrine.

In 1926, Tainter showed that tyramine acted as a circulatory stimulant in rabbits, cats and dogs. The stimulation involved the heart and blood vessels, and occurred independently of the vasomotor centres, sympathetic ganglia and the adrenal glands. The pressor response that resulted from the infusion of tyramine resulted partly from the constriction of the peripheral vessels and partly from a direct action on cardiac muscle. The vasoconstriction that occurred in the cat was attributed to stimulation of the sympathetic nerve endings and in the dog to direct stimulation of vascular smooth muscle. Further experimentation, using a variety of isolated organs, suggested that direct smooth muscle stimulation was the predominant action of tyramine.

In 1958, Burn and Rand found that tyramine lost its pressor action in a cat which had been pretreated with reserpine

and no longer caused contraction of the nictitating membrane or the spleen. The action of tyramine could be restored in such an animal by infusion of noradrenaline into the blood stream. These observations suggested to them that tyramine acted by releasing noradrenaline or adrenaline from stores in the artery wall.

Farmer (1966) studied the central artery of the rabbit ear, using the technique described by de la Lande and Rand (1965), and reported that tyramine had both direct and indirect constrictor actions. The indirect action was abolished by sympathectomy and reserpine pretreatment, while the direct action remained unaffected by these procedures.

In human subjects, Cohn (1965) showed that tyramine infused into the brachial artery usually caused constriction of forearm vessels, but with large doses (more than 80 µg/min) vasodilatation sometimes occurred. In explanation of these responses Cohn offered a number of possibilities: (a) that tyramine causes release of adrenaline and dopamine, as well as noradrenaline, from storage sites in the adrenergic neurones, and these could cause forearm vasodilatation; (b) that tyramine might exert a direct sympathomimetic action without the intervention of noradrenaline; and (c) that tyramine might have both direct and indirect actions.

In the present study, the effects of local intra-arterial infusions of noradrenaline and tyramine on the forearm blood vessels

have been studied before and during blockade of the alpha- and beta-adrenergic receptors in an endeavour to establish the mechanism of action of tyramine on the peripheral blood vessels in man.

METHODS

The subjects for the experiments were normal volunteer medical students and one patient (W.W.) who had undergone bilateral cervical sympathectomy 6 years previously for mild Raynaud's phenomenon in the hands. The vessels of the forearm of this patient responded in a normal fashion to drugs which act directly on vascular smooth muscle (Parks, Sandison, Skinner and Whelan, 1961; Parks, Skinner and Whelan, 1961), but did not respond to sympathetic stimuli such as application of ice to the face, deep inspiration or mental arithmetic (Cooper, Fewings, Hodge and Whelan, 1963; Blair, Glover, Greenfield and Roddie, 1959).

The experiments were carried out at laboratory temperatures ranging from 23° to 26°C, the subjects lying recumbent on a couch for at least 30 min before observations were made, during which time recording apparatus was applied and the infusion needle inserted.

Forearm blood flow was measured by venous occlusion plethysmography, using water-filled plethysmographs maintained at a

temperature of 34°-35°C (Greenfield, 1954), three or four records of flow being obtained each minute.

Intra-arterial infusions were given into the brachial artery at the elbow of one side through a 22 or 23 gauge needle connected by a length of polyethylene tubing to a mechanically driven syringe which delivered 2 ml of solution per min. Saline (0.9%, w/v) was infused during control periods and was also used as a vehicle for the drugs. The doses of the drugs were such that they did not produce systemic effects, making it possible to use the opposite uninfused limb as a control.

Percentage changes in forearm flow produced by tyramine and noradrenaline were determined from the averaged flow values during the 2 min before the drug infusion and the last 2 min of the infusion period, by which time the responses to the drugs had become stable. Allowance was made for spontaneous variations in flow unrelated to drug action by assuming that in the absence of the drug infusion the infused and the control sides would have maintained the same relationship to each other as in the pre-infusion period (Duff, 1952).

The drugs used were tyramine hydrochloride (Koch-Light Laboratories Ltd), phentolamine methanesulphonate (Regitine, Ciba), propranolol hydrochloride (Inderal, I.C.I.) and noradrenaline bitartrate monohydrate (Levophed, Winthrop). The doses of

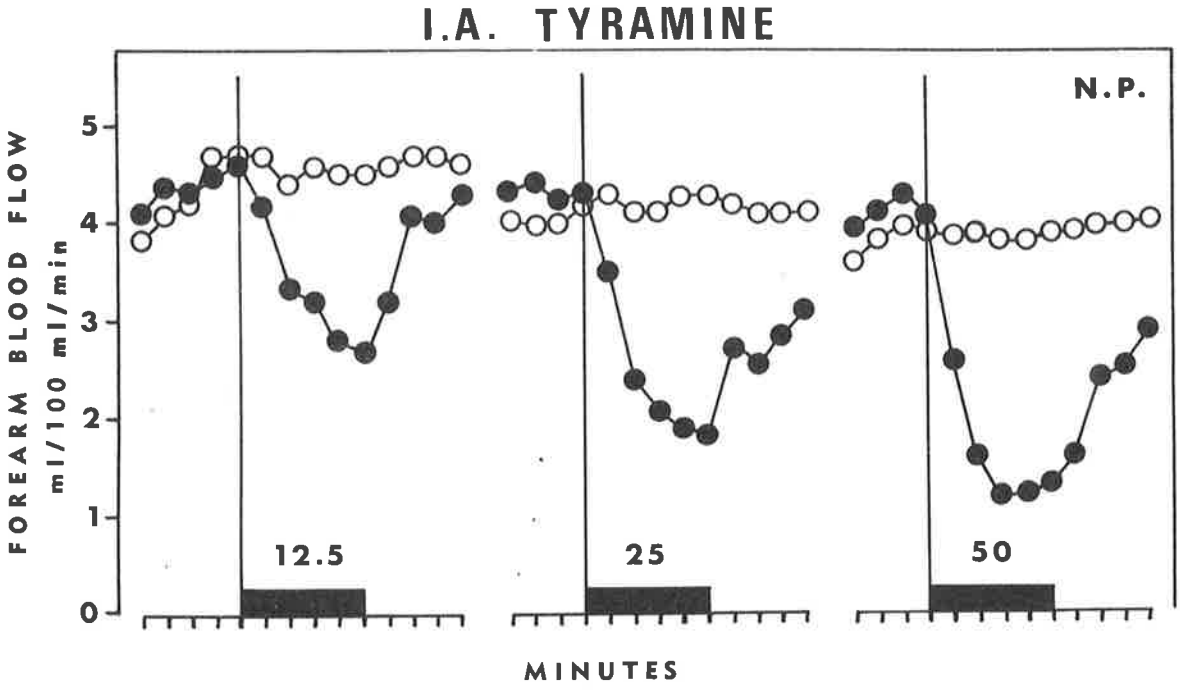


Fig. 4-2 Effect on the blood flow through the forearm of three doses of tyramine infused into the brachial artery in a normal subject. ●, infused side; O, control side. The 5 min periods of drug infusion are indicated by the black rectangles, above which are shown the doses of tyramine in μg/min.

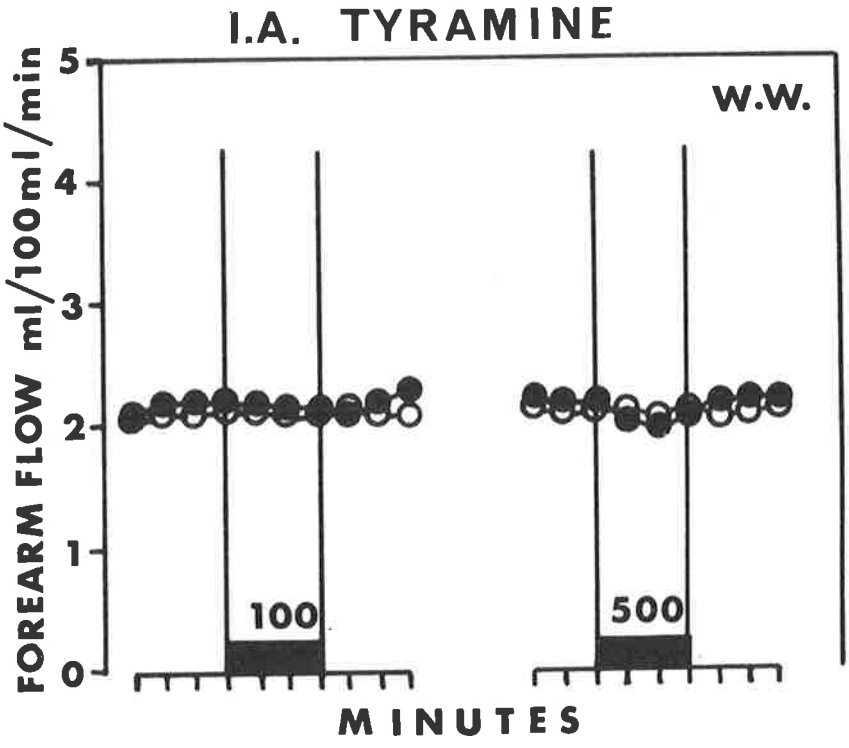


Fig. 4-3 Effect on the forearm blood flow of intra-arterial infusions of tyramine (100 and 500 μg/min) in a surgically sympathectomized limb. ●, infused side; ○, control side. The periods of drug infusion are indicated by the black rectangles.

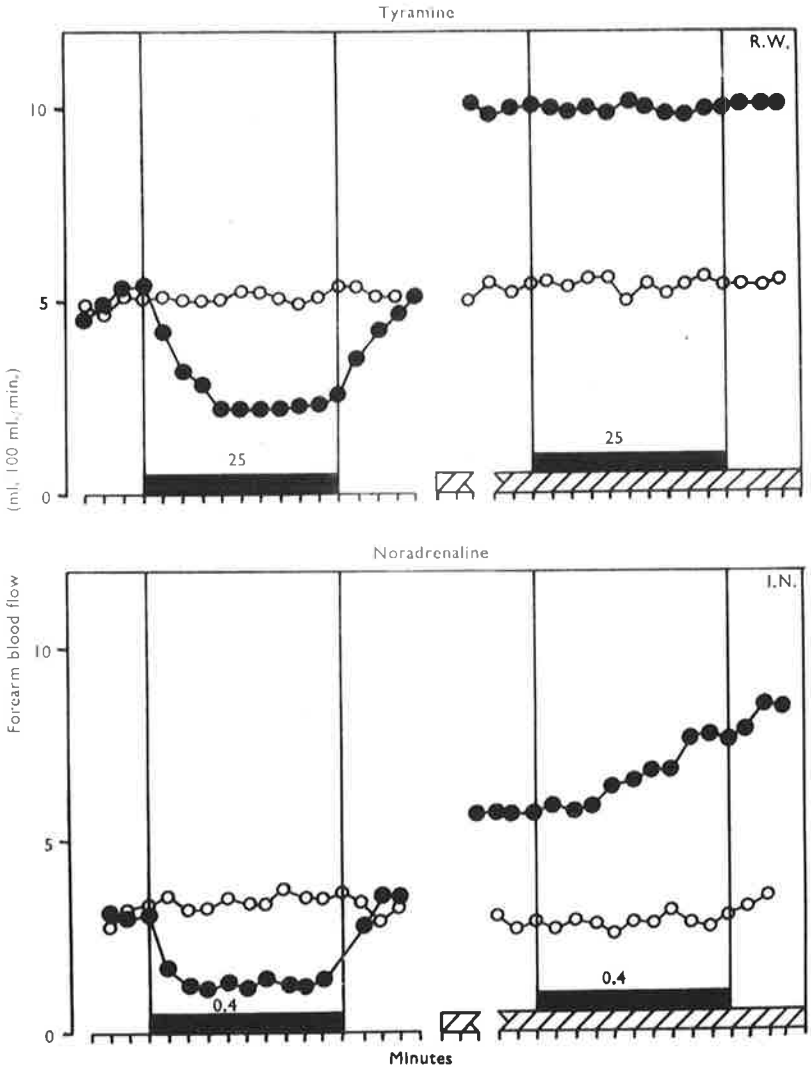


Fig. 4-4 Effect on the forearm blood flow of intra-arterial infusions of tyramine (upper frame) and noradrenaline (lower frame) before (left) and during (right) intra-arterial infusion of phentolamine (50 µg/min). ●, infused side; ○, control side. The infusions of tyramine (25 µg/min) and of noradrenaline (0.4 µg/min) are indicated by the black rectangles and the infusions of phentolamine by the hatched rectangles, the interruptions in which represent time intervals of 12 min (.R.W.) and 23 min (I.N.).

noradrenaline are expressed as weights of the base and those of the other drugs as weights of their salts. Ascorbic acid (1:50,000) was added to the noradrenaline solutions.

RESULTS

The constrictor action of tyramine on the vessels of the forearm of one subject is illustrated in Fig. 4-2. Doses of 12.5, 25 and 50 µg/min infused into the brachial artery of one side for 5 min caused a fall in blood flow which increased in magnitude with increasing doses. There was no effect on the vessels of the opposite control forearm.

This action of tyramine was absent in the sympathetically denervated forearm of the patient (W.W.) even when very large doses were used (Fig. 4-3). It was also abolished by alpha-receptor blockade. Fig. 4-4 shows the changes in forearm blood flow in one subject during intra-arterial infusion of tyramine (25 µg/min) and noradrenaline (0.4 µg/min), in another subject before (left of figure) and during blockade of the alpha-receptors with phentolamine (50 µg/min, right of figure). Before alpha-receptor blockade the tyramine and noradrenaline infusions produced approximately equal degrees of reduction of forearm flow. During administration of

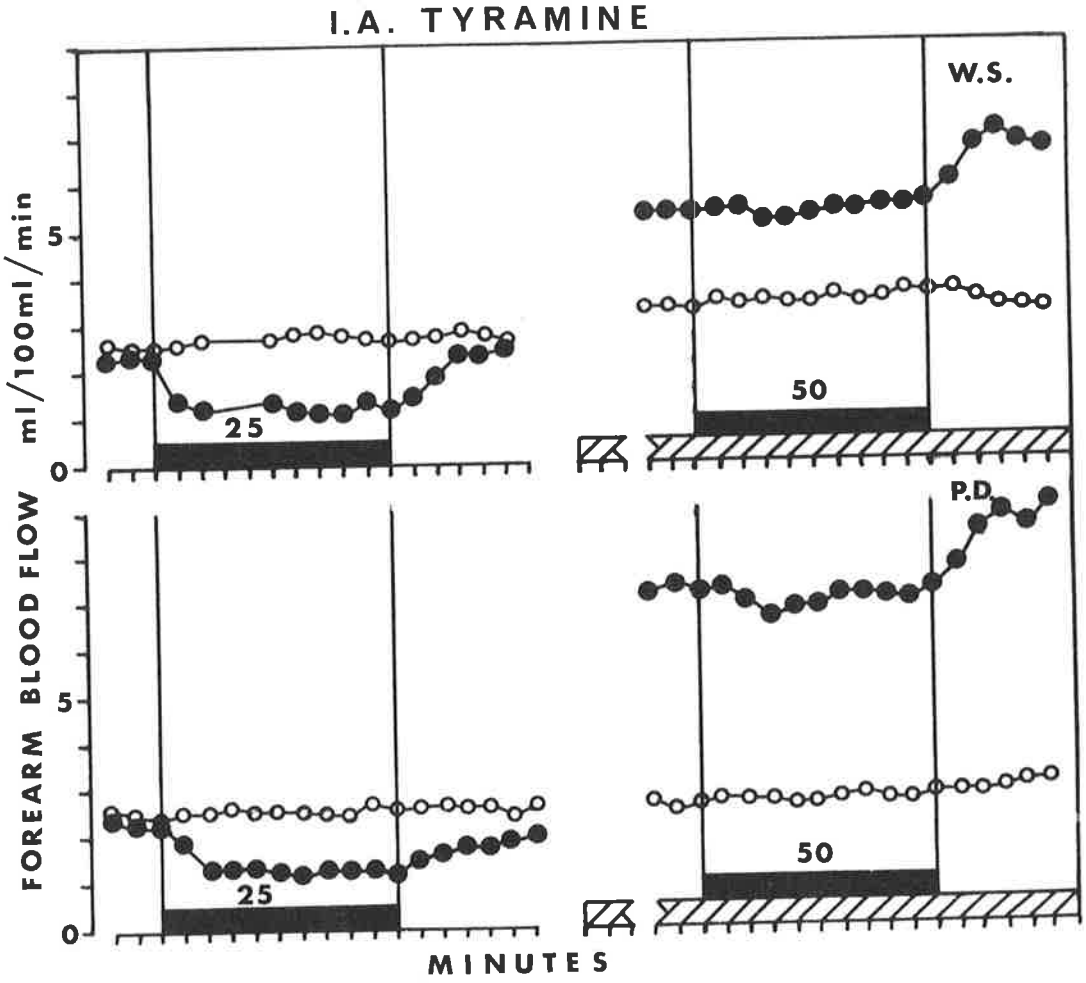


Fig. 4-5 Effect on the forearm blood flow in two subjects of intra-arterial infusions of tyramine (black rectangles) before (left) and during (right) intra-arterial infusion of phentolamine (50 µg/min, hatched rectangles). ● , infused side; o , control side. The figures above the black rectangles indicate the doses of tyramine in µg/min. The interruptions in the hatched rectangles represent time intervals of 17 min (W.S.) and 12 min (P.D.).

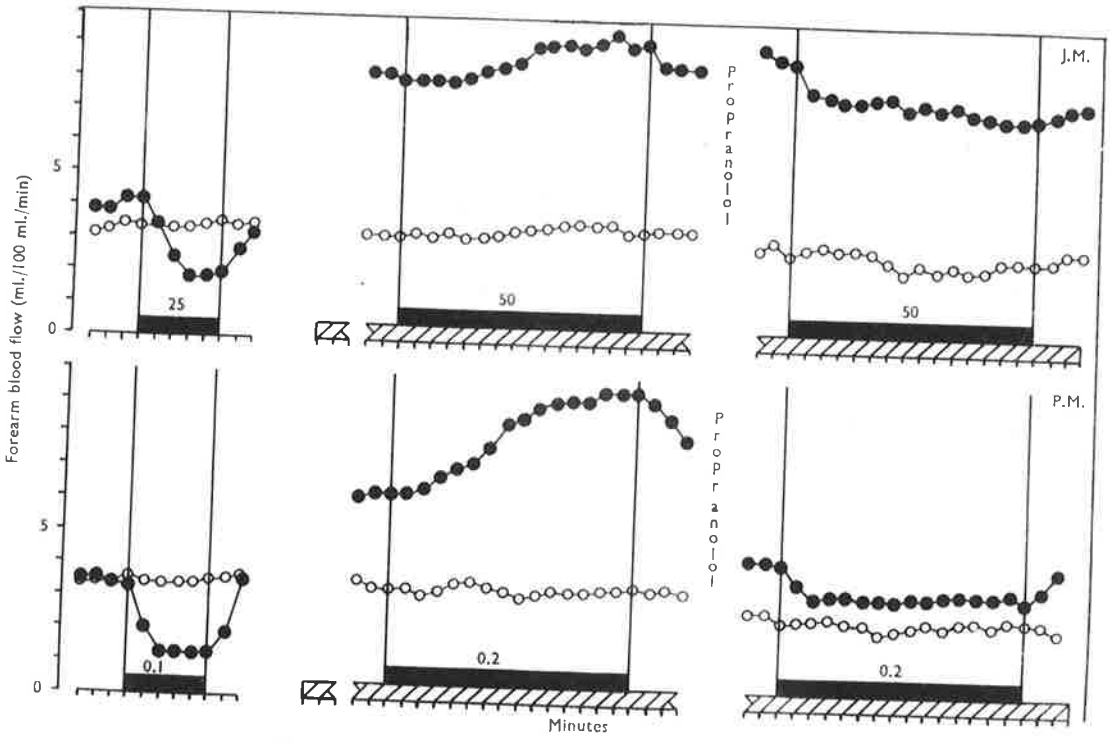


Fig. 4-6 Effect on the forearm blood flow of intra-arterial infusions of tyramine (upper frames) and of noradrenaline (lower frames) before (left) and during intra-arterial administration of phentolamine (50 µg/min, middle) and during phentolamine administration after intra-arterial propranolol (100 µg/min for 8 min, right). The infusions of tyramine and noradrenaline are represented by the black rectangles and the figures above each indicate the doses in µg/min. The interruptions in the hatched areas represent time intervals of 10 min and 32 min (J.M.) and 21 min and 23 min (P.M.).

phentolamine, tyramine was without effect, whereas a vasodilatation was caused by noradrenaline. Similar results were obtained in two other subjects, using 5 and 10 min infusions of the drugs. In two further subjects, however, an increase in forearm blood flow occurred after 10 min tyramine infusions had ceased and while the phentolamine infusion was continued (Fig. 4-5).

In view of the delayed dilator responses seen in these two experiments, more prolonged infusions of 15 min duration were carried out in four subjects during phentolamine infusion. A vasodilatation was seen after the onset of the tyramine infusion, taking 6, 6, 9 and 12 min, respectively, to develop. In two of the subjects the beta-receptor blocking agent propranolol was administered after the above response had been recorded and tyramine now caused a fall in flow. The response obtained with tyramine on one of these subjects is illustrated in Fig. 4-6 and is compared with the similar pattern of response seen with noradrenaline.

In the experiments illustrated in Fig. 4-5, the same dose of drug was given before and during the phentolamine infusion. In all subsequent experiments, however, an attempt was made to compensate for the increase in blood flow produced by phentolamine by appropriately increasing the doses of tyramine which were given during the phentolamine blockade so that comparable concentrations of the drug arrived at the vessels of the forearm before and during

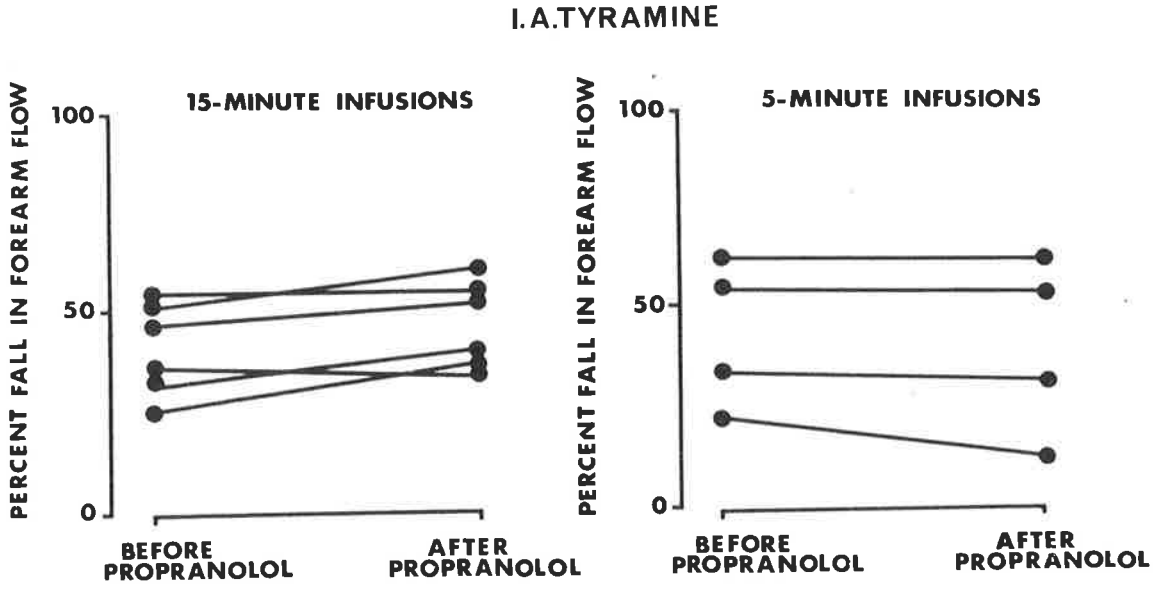


Fig. 4-7 Fall in forearm blood flow during the last 2 min of intra-arterial infusion of tyramine expressed as the percentage fall from the pre-infusion level before and after intra-arterial administration of propranolol (100 µg/min for 8 min). The left hand frame includes data from six 15 min infusions of tyramine each in a different subject and the right hand frame from four 5 min infusions each on a different subject.

the phentolamine infusions.

The effect of propranolol alone on the response of the forearm vessels to tyramine was studied in ten experiments. When the responses were observed over periods of 5 min (four experiments), no potentiation of the constrictor action of tyramine was observed after beta-blockade. With 15 min infusions, however, in four out of six experiments a potentiation of 6, 12, 9 and 6%, respectively, was obtained, while in two there was no increase in the constrictor response. The pooled data from all these experiments are shown in Fig. 4-7.

Time courses of the responses:

The length of the polyethylene connection between the infusion syringe and the needle in the artery was constant in all experiments (35 cm), and the time taken for the drug solution to reach the needle at an infusion rate of 2 ml/min was 6-9 sec. The time of onset of the infusion of drug into the vessels could thus be determined within a second or two.

The onset of the response of the vessels was taken to be that time at which the first flow measurement after the beginning of the infusion was either greater or less than the control level of flow. When doses of the two drugs producing similar constrictor effects were compared, the responses to noradrenaline were more rapid in onset than those to tyramine.

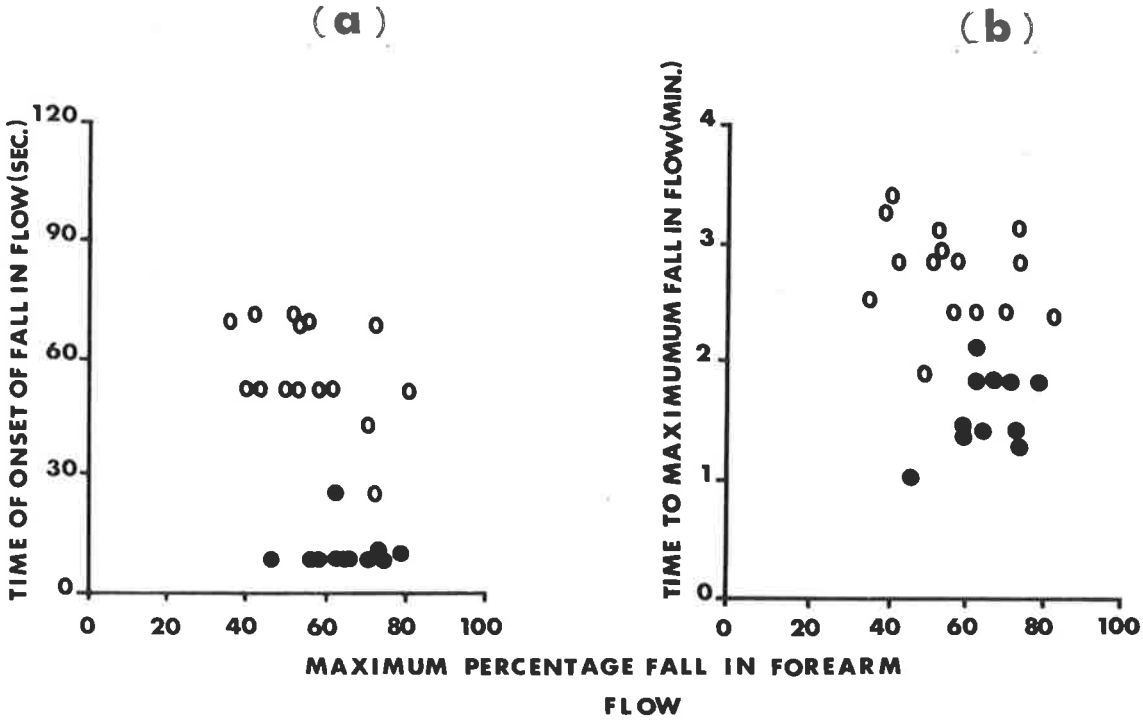


Fig. 4-8 Time of onset of the reduction in blood flow (left) and the time taken for maximum flow reduction to develop (right) with infusions of tyramine (O) and of noradrenaline (●) plotted against the maximum fall in blood flow attained during the infusions, expressed as per cent fall from the pre-infusion level of flow corrected for any spontaneous changes in blood flow by reference to the opposite control side.

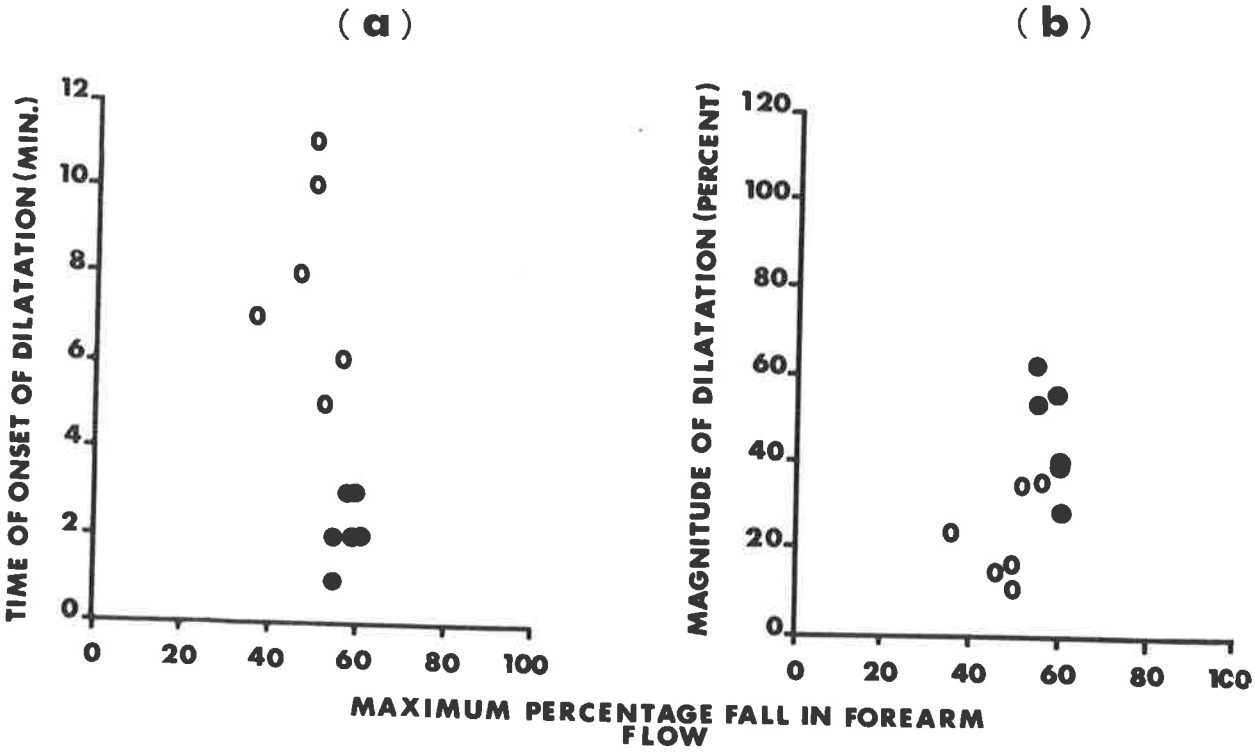


Fig. 4-9 Time of onset (left) and magnitude (right) of the increase in forearm flow above the control level produced by infusions of tyramine (O) and noradrenaline (●) following alpha-receptor blockade by phentolamine, plotted against the maximum fall in blood flow attained during infusions of the drugs before phentolamine administration.

Fig. 4-8a shows the pooled data from fifteen infusions of tyramine and eleven infusions of noradrenaline on sixteen subjects, in doses which had approximately equal constrictor effects on the forearm vessels, the time of onset of the constrictor effect being plotted against the maximum fall in flow attained during the infusion period, expressed as a percentage fall from the pre-infusion level of flow. For a given degree of vasoconstrictor response, noradrenaline had a more rapid onset of effect than did tyramine (means: noradrenaline 10 sec; tyramine 55 sec). The time taken for the maximum constrictor effect to be attained was also much shorter with noradrenaline (mean: 1 min 47 sec) than with tyramine (2 min 59 sec) (Fig. 4-8b).

A similar time relationship was seen between the onset of the dilator actions of the two drugs when given during administration of phentolamine (Fig. 4-9a). The time of onset of the dilator response to noradrenaline was 2 min 10 sec, while that to tyramine was 7 min 50 sec (means of six infusions in six subjects with each drug). With doses of noradrenaline and tyramine which had comparable vasoconstrictor actions (Fig. 4-9b and Fig. 4-6) the magnitude of the dilator response was less with tyramine.

DISCUSSION

The fact that tyramine is without effect on the sympathetically denervated forearm vessels indicates that in the doses used the drug has no significant direct action on the vessels and implies that its effect is mediated solely by the release of a substance from the nerve endings.

The abolition of the constrictor action of tyramine by alpha-receptor blockade with phentolamine and the unmasking of a dilatation which is caused by beta-receptor stimulation indicates that the released substance has properties similar to those of adrenaline and noradrenaline. The beta-receptor stimulating action of adrenaline on forearm vessels in the presence of alpha-receptor blockade was demonstrated by de la Lande and Whelan (1959) and Allwood and Ginsburg (1961). That of both adrenaline and noradrenaline was inferred by Lowe and Robinson (1964) from the enhancement of the constrictor response to these drugs after beta-receptor blockade with pronethalol. Further evidence of the beta-stimulating action of noradrenaline was provided by Glover and Hutchison (1965) from the potentiation of the effect of noradrenaline by the beta-receptor antagonist propranolol and also supported by the observation by Brick, Hutchison and Roddie (1967) of a dilatation of the forearm vessels with noradrenaline in the

presence of phentolamine. The results of the present experiments confirm their findings and extend them by showing that a beta-receptor stimulating action of noradrenaline can be elicited with small doses of the two drugs.

When beta-receptor blockade was introduced following alpha-receptor blockade, the dilator responses to both noradrenaline and tyramine were abolished, and a small vasoconstriction occurred (Fig. 4-6). The mechanism of this residual constrictor response is not clear. A similar effect was observed in the action of ephedrine on forearm vessels. It is possible that the administration of propranolol after phentolamine may have had the effect of antagonizing the alpha-blocking activity of the latter, and the infused noradrenaline and the transmitter released by tyramine were then able to act on receptors (Prichard and Ross, 1966). Another explanation might be that the phentolamine had not been fully effective in blocking the alpha-receptors, and the partial constrictor action of noradrenaline and of tyramine became apparent when their dilator effects were blocked.

The vasoconstrictor and the vasodilator actions of tyramine differed from those of noradrenaline in that there was a greater delay between the beginning of the infusions and the onset of the responses of the vessels. Such a difference in time course between the effect of a catecholamine released by tyramine from

nerve endings and that of catecholamines injected intra-arterially may be accounted for in a number of ways.

It was shown in an earlier chapter that the sympathetic nerve endings in peripheral human arteries lie between the adventitia and the media of the vessels, and this observation is supported by the findings of Falck and Rorsman (1963) and Waterson (1967). Tyramine introduced into the arterial blood stream would need to penetrate through the intima and the media before reaching the nerve stores, which could account for the delayed onset of effect compared to infused noradrenaline.

Once released by tyramine, the transmitter substance would, in addition to being subject to degradation by monoamine oxidase, be also readily accessible for re-uptake by the store. Catecholamine introduced intra-arterially, on the other hand, would reach the smooth muscle receptors more rapidly and uptake by the nerve stores would occur only after it had exerted its effect on the muscle (de la Lande, Frewin and Waterson, 1967). These factors could account for the beta-stimulating action of tyramine being less readily demonstrable with infusions which lasted less than 5 min.

When a dose of tyramine was given which matched in its constrictor effect a given dose of noradrenaline (and hence might be presumed to achieve a similar quantity of transmitter at the

smooth muscle), the dilator effect of this dose, adjusted for increased flow after alpha-receptor blockade, was less marked than that of noradrenaline (Fig. 4-6). There are a number of possible explanations for this difference. The location of the beta-receptors in relation to the lumen of the vessel might be of relevance. If these lay closer to the intimal surface of the smooth muscle coat than to the adventitia, they would be more readily accessible to infused noradrenaline arriving in the blood stream than to the substance released from the nerve plexus between the adventitia and the muscle coats.

Another factor capable of influencing the time course and magnitude of the dilatation induced by tyramine compared with that of noradrenaline is a blocking action of phentolamine on the transport of tyramine into the storage sites. Phentolamine has been shown to have a weak inhibitory effect on noradrenaline uptake by these sites (Iversen, 1967) and it is likely that it has the same effect on tyramine, which probably has a similar uptake mechanism. Such inhibition would reduce the rate and amount of transmitter released after phentolamine treatment, while the amount of infused noradrenaline accumulating at the receptor sites would be increased.

If the substance released from the sympathetic nerve endings by tyramine can be presumed to be the natural transmitter, the demonstration that it has both alpha and beta stimulating

properties implies that it could be noradrenaline, adrenaline, or a similar sympathomimetic substance. This conclusion is at variance with that of Brick, Hutchison and Roddie (1966), who compared the responses of the phentolamine-treated forearm vessels to noradrenaline with the reflex activity of the sympathetic nerves to the vessels induced by exposure of the legs and lower trunk to negative pressure. Noradrenaline caused an increase in flow, but reflex nerve activity did not, implying that the transmitter was not noradrenaline. The present observation of a weaker and slower beta-stimulating effect of transmitter released by tyramine when compared with infused noradrenaline may offer an explanation for these findings. The reflex activity induced by the application of negative pressure may have been transient, and the transmitter released in the presence of phentolamine may have been insufficient in amount to affect the beta-receptors or to reach them if these are situated at a more distant site from the point of release than the alpha-receptors. The failure of Brick *et al.* (1966) to demonstrate a potentiation by propranolol of the constrictor effect of nerve activity might be accounted for in the same way, because in the present investigation no potentiation was observed with infusions of tyramine which lasted less than 5 min, and with 15 min infusions it was present in only four of six subjects and was small in degree.

The relative distribution and densities of alpha and beta

receptors in the smooth muscle of blood vessels are not known, but the results of the present investigation suggest that such relationships might influence vascular responses to locally released and to circulating catecholamines.

SUMMARY

1. The constrictor action of tyramine on the blood vessels of the human forearm is dependent on the presence of the sympathetic nerves.

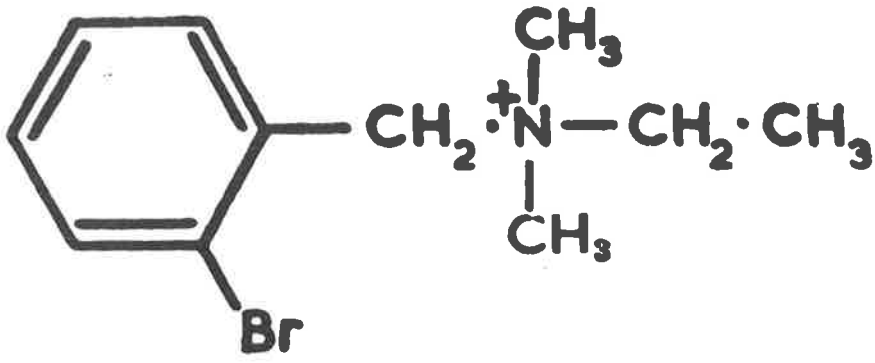
2. The constrictor substance liberated from these nerve endings by tyramine has both alpha-receptor and beta-receptor stimulating properties.

3. The alpha-receptor action of tyramine is its predominant effect, whereas the beta-receptor activity, which is modest, is only seen after blockade of the alpha-receptors with phentolamine.

4. Comparison of the times of onset of the constrictor and dilator responses, using doses of tyramine and noradrenaline which produced constrictor effects of comparable magnitude, demonstrated that responses to tyramine always appeared later than those to noradrenaline and its dilator effects were of lesser magnitude.

5. It is suggested that the differences between the times of

onset of the actions of tyramine and noradrenaline might be the result of the fact that infused noradrenaline exerts its action directly on the vascular smooth muscle coat, whereas tyramine is required to penetrate to the nerve plexus and release transmitter which may be subject to re-uptake and degradation before reaching its site of action. The actions of the two substances might also be influenced by the relative distributions of the alpha and beta receptors in the vascular smooth muscle.



BRETYLIUM

Fig. 5-1

CHAPTER 5

Modification of the vasoconstrictor action of sympathomimetic agents by bretylium tosylate and tranylcypromine in man.

The evidence presented in the preceding chapters indicates that both ephedrine and tyramine cause vasoconstriction by the release of sympathetic neurotransmitter from the termination of the post-ganglionic fibres. Several therapeutic hypotensive agents, such as bretylium (Fig. 5-1), guanethidine and debrisoquin, act principally by preventing the release of this neurotransmitter (Exley, 1960; Abrams, 1969). Thus, pretreatment of the peripheral vessels with one of them, e.g. bretylium, theoretically could attenuate or abolish the vasoconstrictor action of sympathomimetic agents, such as tyramine, methylamphetamine or ephedrine, which act by releasing sympathetic neurotransmitter. On the other hand, McCoubrey (1962), and Giachetti and Shore (1967) have reported that bretylium has antimonoamine oxidase activity, and this could have the opposite effect, viz. potentiation of the vasoconstrictor action of such indirectly acting sympathomimetic agents.

This chapter examines the responses of the hand blood vessels to tyramine, ephedrine and methylamphetamine alone, in the presence of bretylium and after treatment with the monoamine oxidase inhibiting drug tranylcypromine.

METHODS

The subjects for these experiments were normal volunteer medical students.

The experiments were carried out at laboratory temperatures ranging from 24° to 28°C, the subjects lying recumbent on a couch for at least 30 min before the observations were made, during which time the recording apparatus was applied and the infusion needle inserted.

Hand blood flow was measured by venous occlusion plethysmography, using water-filled plethysmographs maintained at a temperature of 32° - 33°C (Greenfield, 1954), three or four records of flow being obtained each minute.

Intra-arterial drug infusions of 4 to 5 min duration were given into the brachial artery at the elbow of one side through a 22-gauge needle connected by a length of polyethylene tubing to a mechanically driven syringe which delivered 2 ml of solution per min. Saline (0.9%, w/v) was infused during the control periods and also used as a vehicle for the drugs. The doses of drugs were such that they did not produce systemic effects, making it possible to use the opposite uninfused limb as a control.

Percentage changes in hand flow produced by the sympathomimetic agents were determined from the averaged flow values during

the 2 min before the drug infusion and the last 2 min of the infusion period, by which time the responses to the drugs had become stable. Allowance was made for spontaneous variations in the flow unrelated to drug action by assuming that in the absence of each drug infusion the infused and the control sides would have maintained the same relationship to each other as in the pre-infusion period (Duff, 1952).

The drugs used were tyramine hydrochloride (Koch-Light Laboratories Ltd), ephedrine hydrochloride (David G. Bull Laboratory Pty Ltd), methylamphetamine hydrochloride (Methedrine, Burroughs Wellcome), bretylium tosylate (Darenthin, Burroughs Wellcome), tranylcypromine sulphate (Smith, Kline and French) and noradrenaline bitartrate monohydrate (Levophed, Winthrop). The doses of drugs are expressed as weights of their salts, except in the case of noradrenaline in which the weight of the base is used. Ascorbic acid (1:50,000) was added to the noradrenaline solutions.

The constrictor responses of the hand blood vessels to intra-arterial infusions of tyramine (50 or 75 µg/min), methylamphetamine (10 or 20 µg/min) and ephedrine (25 or 50 µg/min) were compared in five experiments with each amine 15-20 min before and 30-50 min after the administration of bretylium tosylate (4 mg/min for 5 min) and of tranylcypromine (50 µg/min for 5 min). The dose of sympathomimetic agent chosen for each subject was that expected to produce a fall in hand blood flow within the range of 20-50%.

Bretylium tosylate caused an initial constriction of the hand vessels in most of the experiments. This persisted for about 5 minutes after the infusion ceased. The hand blood flow then gradually rose within the next 10 minutes to or slightly above the previous resting level (Cooper, Fewings, Hodge and Whelan, 1963).

To test for the sympathetic blockade of the hand blood vessels caused by the intra-arterial infusion of bretylium, ice was applied to the neck of the subject and the resulting vasoconstriction in both hands recorded. This procedure is a potent sympathetic stimulus and usually produces intense vasoconstriction in both hands (Cooper *et al.*, 1963). When the effects of bretylium were fully developed, the hand vessels on the treated side no longer constricted when ice was applied to the neck, while on the untreated side vasoconstriction of the same magnitude as before was seen. Approximately 30 min were required in most of the experiments before blockade of the hand blood vessels was fully effective, and the second infusion of each sympathomimetic was given 35-45 min after bretylium administration.

Tranylcypromine caused an initial constriction of the hand vessels which persisted throughout the infusion period. The blood flow returned to about the previous resting level approximately 15 min after the infusion ceased. A second infusion of each sympathomimetic agent was given 30-50 min after the tranylcypromine

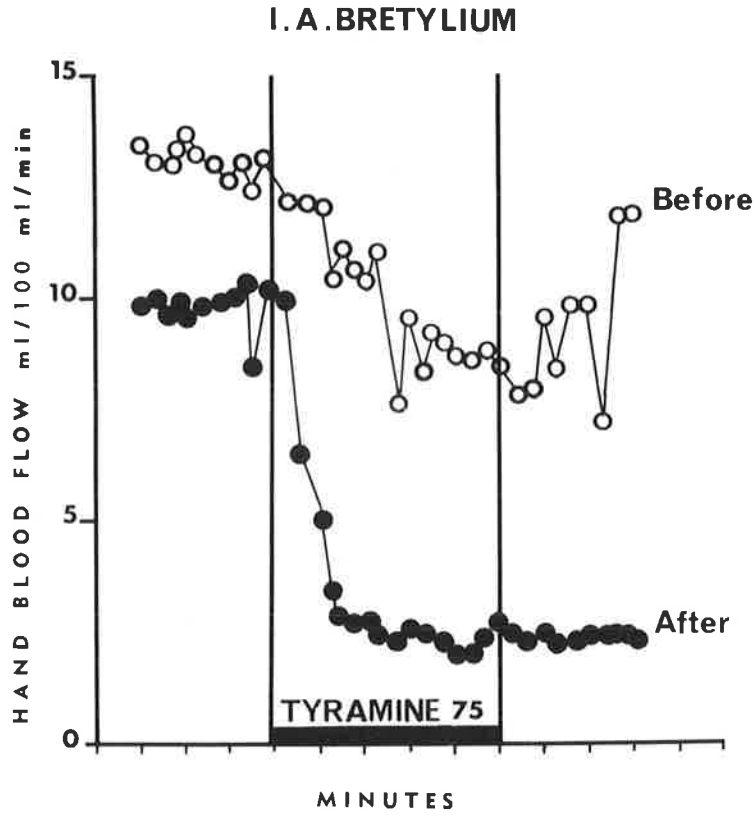


Fig. 5-2 Constrictor response of the hand blood vessels to intra-arterial infusion of tyramine (75 µg/min, black rectangle) 19 min before (○) and 40 min after (●) intra-arterial administration of bretylium tosylate (4 mg/min for 5 min).

infusion, corresponding in time with those following bretylium.

The effect of tranylcypromine on hand vessel sensitivity was determined in five experiments in which noradrenaline (50 ng/min for 5 min) was given 10 min before and at 10 min intervals after tranylcypromine (50 µg/min for 5 min). The second and subsequent infusions of noradrenaline were given at a time when the blood flow had returned to the previous resting level. To conform with the time sequence which was observed in the experiments with the sympathomimetic amines, the percentage fall in flow caused by the noradrenaline infusion given 30-40 min after tranylcypromine was the one used in the calculations.

RESULTS

The response of the blood flow through the hand to tyramine (75 µg/min for 5 min) before, and then after, bretylium tosylate (4 mg/min for 5 min) in one subject is shown in Fig. 5-2, both drugs being given by infusion into the brachial artery. The degree and duration of the constrictor response to tyramine was markedly enhanced after bretylium administration. Similar results were obtained in each of four other subjects, and Fig. 5-3A shows the falls in hand blood flow produced by tyramine in all five

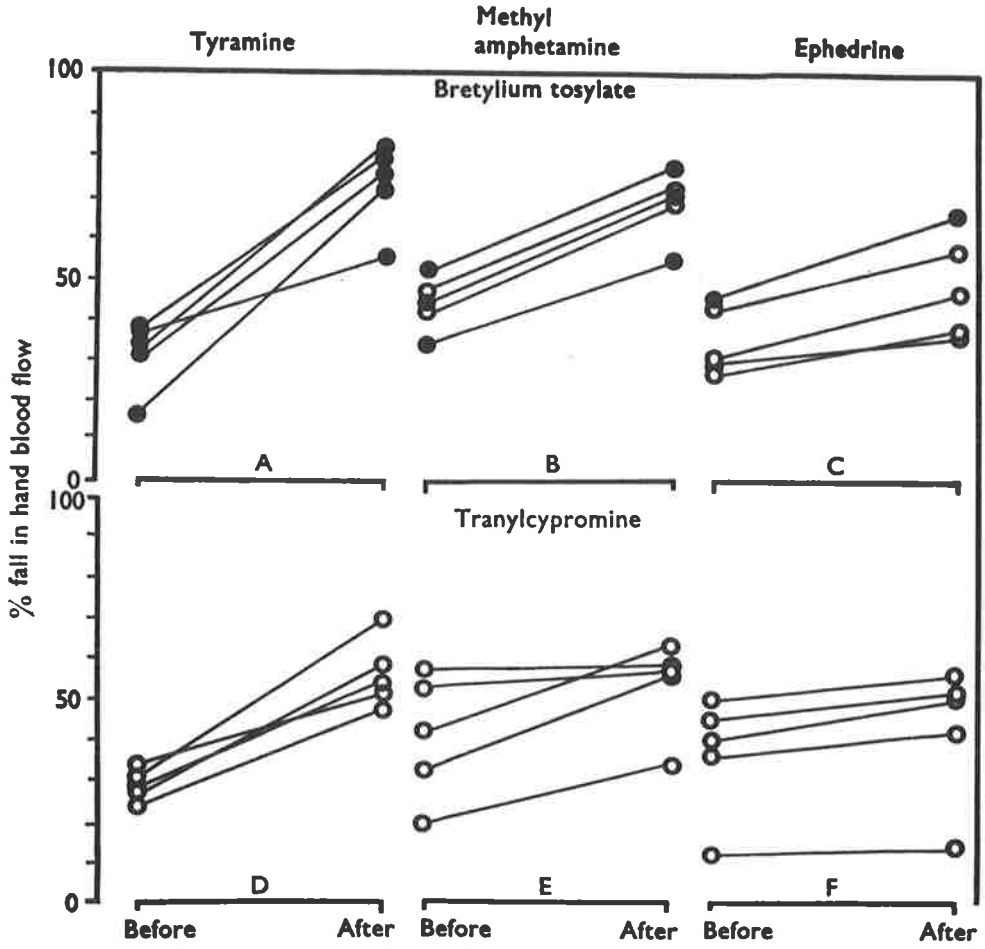


Fig. 5-3 Per cent fall in hand blood flow in response to tyramine (O , 50 µg/min; ● , 75 µg/min), methylamphetamine (O ,10 µg/min; ● , 20 µg/min) and ephedrine (O , 25 µg/min; ● ,50 µg/min) before and after bretylium tosylate (4 mg/min for 5 min) (A, B and C, respectively) and before and after tranylcypromine (50 µg/min for 5 min) (D, E and F, respectively). Five experiments, each on a different subject, were carried out with each sympathomimetic, the infusions being given 15-20 min before and 30-50 min after the administration of bretylium or tranylcypromine.

subjects expressed as percentage fall from the resting level of flow. The symbols to the left of the figure represent the values for percentage fall in flow caused by tyramine before treatment of the hand blood vessels with bretylium, and those to the right of the figure those after treatment. The enhancement of the vasoconstrictor action of tyramine averaged 41.4% following bretylium, an increase which was statistically significant ($0.0005<P<0.0025$).

Figure 5-3B shows the results from five experiments on five subjects with methylamphetamine, the doses used being 20 µg/min on three occasions and 10 µg/min on two occasions. After treatment with bretylium the enhancement of the constrictor response of the hand blood vessels to methylamphetamine was not as well marked as with tyramine, being on average 24.6%, a significant difference ($P<0.0005$).

Figure 5-3C shows the results obtained from five experiments on five subjects in which ephedrine was given at infusion rates of 25 µg/min (on four occasions) and 50 µg/min (on one occasion). A significant enhancement of the constrictor action to ephedrine was seen after bretylium treatment, averaging 12.16% ($0.0025<P<0.005$), which was smaller than that seen in the case of the other two sympathomimetic agents.

The lower three frames of Fig. 5-3 show the effects of the monoamine oxidase inhibitor, tranylcypromine, on the percentage

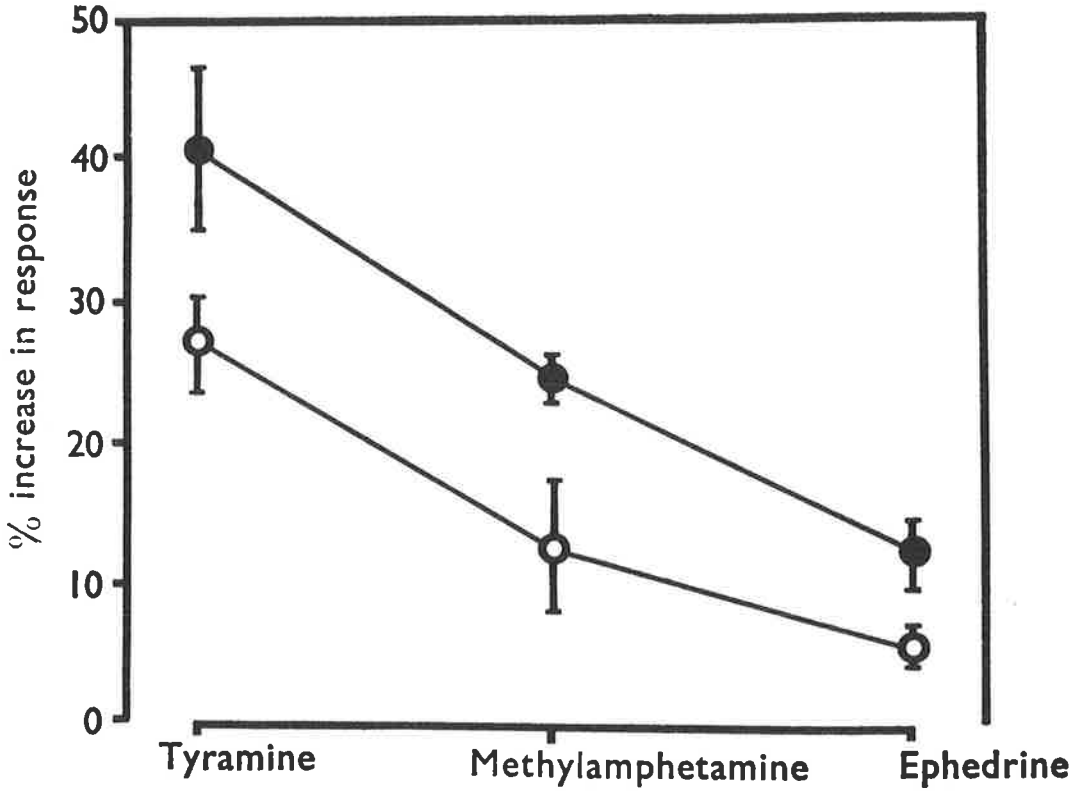


Fig. 5-4 Data in Fig. 5-3 averaged and expressed as % increase in response of the hand vessels to tyramine, methylamphetamine and ephedrine after bretylium (●) and after tranylcypromine (○). The vertical lines through each symbol represent one standard error on either side of the mean.

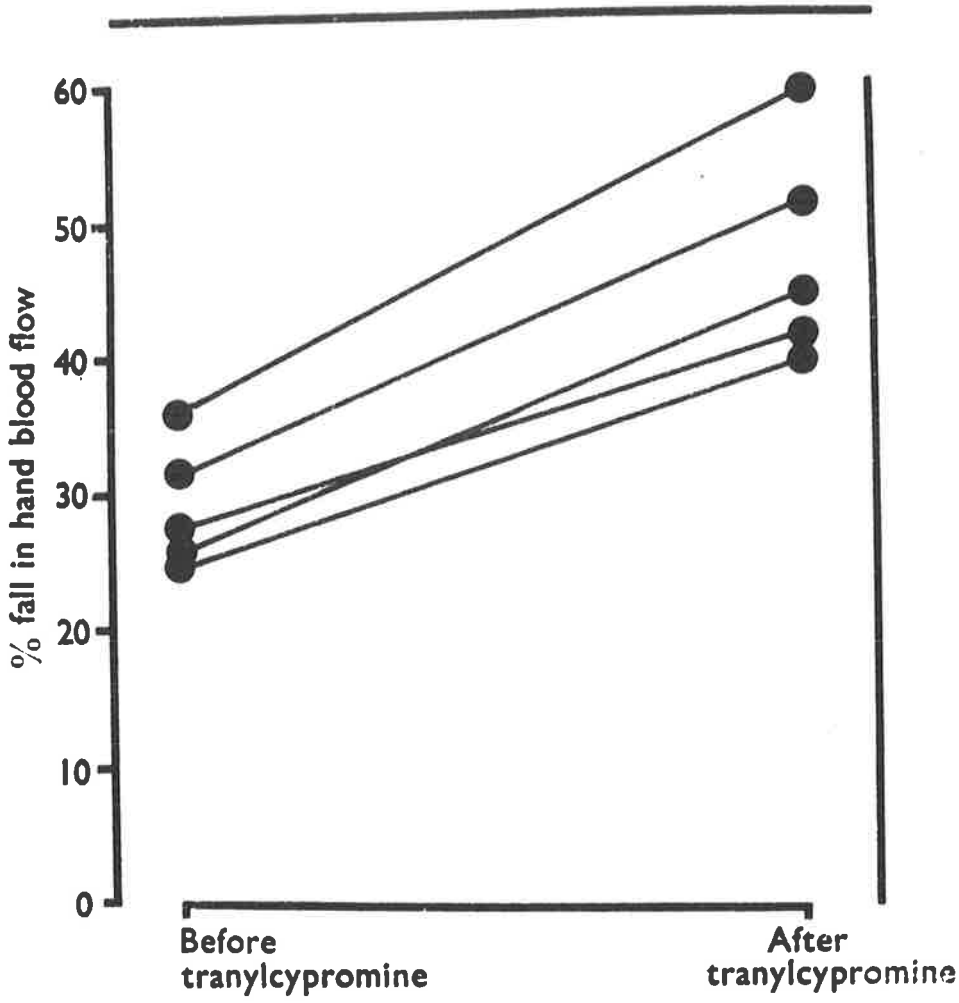


Fig. 5-5 Per cent fall in hand blood flow in each of five subjects in response to noradrenaline (50 ng/min) before and after tranylcypromine (50 µg/min for 5 min).

falls in hand blood flow produced by tyramine, methylamphetamine and ephedrine, respectively. Figure 5-3D shows the results from five experiments on five subjects with tyramine (50 µg/min). In the presence of tranylcypromine the enhancement of the constrictor response of the hand blood vessels to tyramine averaged 27.5%, this being a significant increase ($0.0005<P<0.0025$).

Figure 5-3E shows the results from five experiments on five subjects with methylamphetamine (10 µg/min), and Fig. 5-3F those from five experiments on five subjects with ephedrine (25 µg/min). Tranylcypromine enhanced the constrictor response to methylamphetamine by 12.9% ($0.0125<P<0.025$), while the constrictor response to ephedrine was increased by an average of 5.9% ($0.0025<P<0.005$).

The means of the increases in percentage fall in hand blood flow caused by tyramine, methylamphetamine and ephedrine in the presence of bretylium and tranylcypromine are shown in Fig. 5-4. The trend in the enhancement of the constrictor responses to the sympathomimetic amines caused by both the hypotensive agent and the monoamine oxidase inhibitor is similar.

Effect of tranylcypromine on the response to noradrenaline:

Five experiments were carried out in which noradrenaline (50 ng/min for 5 min) was given intra-arterially and the constrictor effect on the hand vessels recorded (Fig. 5-5). Tranylcypromine (50 µg/min for 5 min) was then infused over a 5 min period and later

the noradrenaline infusion repeated. In every case the response to noradrenaline was enhanced by the prior administration of tranylcypromine, the mean % increase being 16.3% which was statistically significant ($0.0005<P<0.0025$).

Control experiments:

Three experiments were carried out in which repeated infusions of the same dose of tyramine were made at intervals of 10 min, saline (0.9% NaCl) at a rate of 2 ml/min being continuously infused between the periods of drug administration. The constrictor responses were very reproducible and did not vary from the mean in each case by more than 6% (average 2.5%, 2.3% and 2.2%, respectively).

In four experiments the vehicles used as the solvents for bretylium and for tranylcypromine were diluted with saline, as in the case of the drug solutions, and infused for 5 min at 2 ml/min. These had no effect on the blood flow through the hand, on the reflex constrictor response to ice on the neck, nor on the magnitude of the hand vessel responses to tyramine, methylamphetamine or ephedrine. The latter responses were constrictions of 33.0%, 52.1% and 41.1% before tranylcypromine vehicle, and 36.1%, 57.2% and 43.0%, respectively, after tranylcypromine. In the case of the vehicle for bretylium the corresponding values were: before 45.2%, 53.7% and 56.6%, and after 39.2%, 55.4% and 54.4%.

DISCUSSION

In the present study the striking feature is that in the presence of bretylium the constrictor effect of tyramine and methylamphetamine, and to a lesser extent of ephedrine, on the hand blood vessels is enhanced. The effect of bretylium alone on these vessels was to block reflexly induced sympathetic activity, vasoconstriction no longer being produced in the bretylium-treated hand when ice was applied to the subject's neck.

This finding is in keeping with that of Burn and Rand (1960) who observed that the action of tyramine in constricting the nictitating membrane of the spinal cat was more prolonged after administration of bretylium at a time when the response to sympathetic nerve stimulation was abolished. Hucković (1960) found a similar effect with tyramine after bretylium in the perfused rabbit ear and the isolated atrium, but the response to amphetamine on the latter was not potentiated.

Wilson and Long (1960) demonstrated that when bretylium was administered to hypertensive patients on amphetamine therapy for weight reduction, no hypotensive response to the drug could be obtained. This effect was attributed to antagonism of the hypotensive action of bretylium by amphetamine. In view of the results of the present investigation, it might also be related to

the potentiating action of bretylium on the peripheral vascular action of the amine.

The mechanism of enhancement of tyramine's constrictor response by bretylium is not clear. It is unlikely to be related to continuous release of noradrenaline from the nerve endings because at the time that the tyramine and other sympathomimetic amines were given the constrictor effect of bretylium had worn off and the flow had returned to, or slightly above, the resting level. At this time, also, it has been shown that there is no change in sensitivity of the vessels to infused noradrenaline (Cooper *et al.*, 1963).

The enhancement may be related to the monoamine oxidase inhibiting property which bretylium is said to possess. There is recent evidence to support such a facet of the action of bretylium (Giachetti and Shore, 1967). The effect of such an action would be twofold: (a) to protect tyramine from degradation by monoamine oxidases as it passes into the noradrenaline store, and (b) to protect the noradrenaline that is released by tyramine from inactivation by the enzyme within the nerve ending.

These two effects, taken together, would mean that a greater amount of noradrenaline would be released from the storage sites by the sympathomimetic agent in the presence of bretylium. This conclusion has also been reached by Pettinger and Oates (1968)

from the results of studies which showed that reduction of the metabolism of tyramine within the nerve ending is a major mechanism in the supersensitivity to this amine during monoamine oxidase inhibition.

While reduction in breakdown as a result of monoamine oxidase inhibition might account, at least in part, for the potentiation of tyramine's action, such a mechanism would be unlikely to apply in the case of the other sympathomimetics, methylamphetamine and ephedrine, which are not substrates for the enzyme. In the case of these drugs the potentiation must be accounted for in other ways, and it has been suggested that potentiation of their action by monoamine oxidase inhibitors may be due to inhibition of intraneuronal breakdown of transmitter providing an enhancement of the store available for release (Pettinger and Oates, 1968).

Rand and Trinker (1968) have presented evidence to show that monoamine oxidase inhibitors potentiate the pressor responses of indirectly acting sympathomimetic amines, not by interfering with the metabolism of endogenous noradrenaline, but by retarding the binding or breakdown of these amines within the liver microsomal enzyme system. In the present experiments, however, bretylium and tranylcypromine potentiated the constrictor action of tyramine, methylamphetamine and ephedrine on the blood vessels of the hand when the drugs were given by local arterial injection, and this

effect cannot be attributed to any action on the liver. If the potentiating effect of bretylium is due to monoamine oxidase inhibition, this must be a local action at the peripheral nerve endings or vessel wall.

It is not clear why the sympathetic nerves, after bretylium treatment, are capable of releasing transmitter in response to the indirectly acting sympathomimetic amines and yet do not do so in response to reflex activation. It may be that nerve impulses and the amines release transmitter either by different release mechanisms or from separate stores within the nerve ending. A selective blocking action of bretylium on the nervously activated store or mechanism, coupled with monoamine oxidase inhibiting action on the amine activated store or mechanism, could account for the observed effect.

The potentiation of the response of the hand vessels to the sympathomimetic amines by tranylcypromine could, as in the case of bretylium, be attributed to its monoamine oxidase inhibiting property. However, unlike bretylium, tranylcypromine also increases the sensitivity of the vessels to noradrenaline, and this could contribute to its potentiating effect on the action of the amines.

The parallelism in the pattern of enhancement of the constrictor response of the hand blood vessels to tyramine, methylamphetamine and ephedrine in the presence of both bretylium and

tranylcypromine suggests that monoamine oxidase inhibition may be a common factor in their potentiating actions.

SUMMARY

1. The vasoconstrictor actions of tyramine, methylamphetamine and ephedrine on the blood vessels of the human hand have been found to be potentiated by administration intra-arterially of the adrenergic neurone blocking agent, bretylium tosylate.

2. One mechanism suggested for the enhancement of vasoconstriction is that bretylium possesses monoamine oxidase inhibiting activity, which, in the case of tyramine, is protective both to the sympathomimetic agent and the intraneuronal transmitter which it releases. In the case of methylamphetamine and ephedrine, which are not substrates for the enzyme, protection of the intraneuronal transmitter alone might occur, accounting for the lesser degree of potentiation of the effect of these amines by bretylium.

3. Comparison of the influences of bretylium and the monoamine oxidase inhibitor, tranylcypromine, on the vasoconstrictor action of the sympathomimetic agents shows a similar pattern of enhancement in the presence of both these drugs.

4. Tranylcypromine caused enhancement of the response of the

hand vessels to noradrenaline, and this action could contribute to its potentiation of the effect of the sympathomimetic amines.

5. For a monoamine oxidase inhibiting action of bretylium to be effective in potentiating the constrictor actions of the sympathomimetic agents on the hand blood vessels at a time when reflex sympathetic activity is blocked it is necessary to postulate that these drugs and reflex nerve activity act either on different compartments of the transmitter store or by different release mechanisms.

CHAPTER 6

The mechanism of action of magnesium sulphate on the forearm blood vessels in man.

Magnesium sulphate has been used intravenously in the treatment of eclampsia in pregnancy to reduce blood pressure, but its method of action is not clear.

Meltzer and Auer (1906) reported a fall in blood pressure with intravenous administration of magnesium salt in rabbits and attributed the response to a depressant action of this substance on the vasomotor centre. Smith, Winkler and Hoff (1939) showed that the continuous intravenous administration of magnesium sulphate at low doses in the anaesthetised dog and cat produced an increase in heart rate which gave way to a bradycardia as the serum concentration of the magnesium ion increased. They noted a depression of intracardiac conduction. In a subsequent paper, Winkler, Hoff and Smith (1940) reported that injections of magnesium salt in the dog produced a fall in blood pressure associated with cutaneous vasodilatation when the serum concentration of the ion reached 3-4 mg %, and cardiac block and arrest when the concentration of magnesium was above 20 mg %.

In human subjects, Bernstein and Simkins (1939) used magnesium sulphate to measure the circulation time, and noted that,

after receiving an intravenous injection of the drug, their subjects all experienced a transient sensation of heat, first in the pharynx, then progressively in the face, one or both hands, the perineum, and finally in the feet. Haury (1939) gave intravenous injections of magnesium sulphate and observed an increase in the volume of the foot which he attributed to vasodilatation.

Browne (1964) has used intramuscular or intravenous magnesium sulphate in the treatment of peripheral arterial disease, and suggests that the beneficial results appear to be due mainly to a prolonged vasodilator action which the substance possesses.

Despite the evidence that magnesium sulphate has a vasodilator action, the mechanism of this effect seems obscure. Mayes (1950) suggested that the drug paralysed nerve-muscle connections in the walls of arterioles, and thereby relaxed these vessels and reduced blood pressure. This inference as to a possible action of magnesium sulphate on vasoconstrictor nerves led to the present study. In an attempt to clarify its vasodilator action, the drug was given by close arterial infusion and its effects on the blood vessels of the forearm and hand recorded, in the presence and absence of a number of vasoactive blocking agents.

METHODS

The subjects were normal volunteer medical students and one patient (W.J.A.) who had undergone a unilateral cervical sympathectomy four months previously for Raynaud's phenomenon in the hand. The forearm vessels of this patient showed a minimal response to the sympathetic stimulus of mental arithmetic (Blair, Glover, Greenfield and Roddie, 1959), and only a small constriction of these vessels was seen when a large dose of the indirectly acting sympathomimetic agent tyramine was infused intra-arterially into the part. It was shown in an earlier chapter of this thesis that the constrictor action of tyramine in man is dependent on the presence of sympathetic nerves, and the small constrictor response of the patient's forearm blood vessels to the drug therefore indicated that only a few vasomotor fibres remained. The results of physiological tests indicated that the hand vessels were completely sympathectomised.

The experiments were carried out at laboratory temperatures ranging from 21 to 28°C, the subjects lying recumbent on a couch at least 30 min before observations were made, during which time the recording apparatus was applied and the infusion needle inserted.

Forearm or hand blood flow was measured by venous occlusion plethysmography, using water-filled plethysmographs

(Greenfield, 1954) maintained at a temperature of 34-35°C for the forearm and 32-33°C for the hand. Three or four records of flow were obtained each minute.

Intra-arterial infusions were given into the brachial artery at the elbow of one side through a 22 gauge needle inserted centrifugally into the artery. The needle was connected by a length of polyethylene tubing to a mechanically-driven syringe which delivered 2 ml of solution per min. Saline (0.9% w/v sodium chloride) was infused during the control periods and also used as a vehicle for the drugs. The doses of drugs administered were such that systemic effects were not produced, making it possible to use the opposite uninfused limb as a control.

Per cent changes in forearm flow produced by drug infusions were calculated from the averaged flow values during the two minutes before the infusion and the last two minutes of the infusion period, by which time the responses to the drugs had become stable. Allowance was made for spontaneous variations in flow unrelated to drug action by assuming that in the absence of the drug infusion, the infused and the control sides would have maintained the same relationship to each other as in the pre-infusion period (Duff, 1952). This correction was not used in the experiment where sympathetic nervous transmission to the infused side had been interrupted.

Superficial (skin) and deep (muscle) oxygen saturations

were determined using the method described by Roddie, Shepherd and Whelan (1956, 1957b).

Polyethylene catheters (Intracath, No. 17, Bardic) were inserted centrifugally into a deep and superficial vein in the cubital fossa and, following the inflation of a wrist cuff to 200 mm Hg to exclude hand flow, sampling of the venous blood from skin and muscle commenced. The oxygen saturation of each sample was rapidly determined by estimating the percentage of light transmission through each sample with a Unicam SP 1400 prism absorptiometer using a wavelength of 660 mμ and a fixed cuvette. The assumption was made that during the test procedure the metabolic activity, and therefore the oxygen requirement of the tissue, remained constant, and the changes in the oxygen saturation of the returning venous blood reflected changes in blood flow through the tissue. A rise in the oxygen saturation therefore indicated an increase in blood flow and a decrease in oxygen saturation a fall in flow in the vessels concerned.

The drugs used were magnesium sulphate ($MgSO4.\ 7H_2O$), calcium gluconate (Farmer Hill Pty Ltd), histamine acid phosphate (David G. Bull Laboratory Pty Ltd), mepyramine maleate (Anthisan, M. & B.), propranolol hydrochloride (Inderal, I.C.I.), isoprenaline hydrochloride (Isuprel, Winthrop), acetylcholine chloride (Acetylcholin, Roche), and hyoscine hydrobromide (David G. Bull

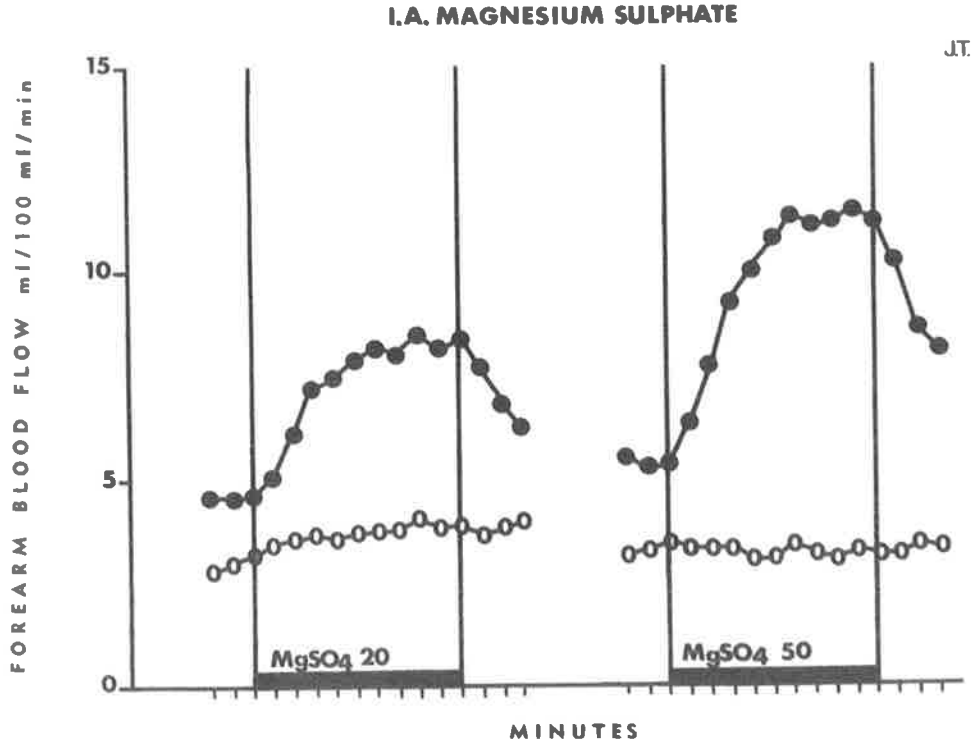


Fig. 6-1 The effect on blood flow through the forearm of two doses of magnesium sulphate infused into the brachial artery in a normal subject. ● , infused side; O , control side. The ten minute periods of drug infusion are indicated by the black rectangles, above which are shown the doses of magnesium sulphate in mg/min.

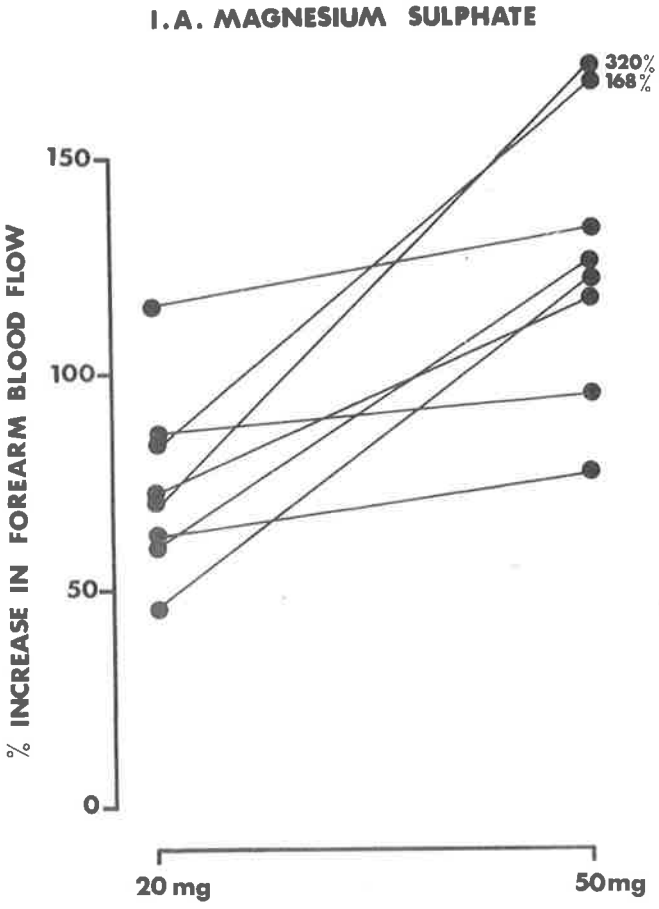


Fig. 6-2 Forearm blood flow during the last 2 min of an intra-arterial infusion of magnesium sulphate expressed as the percentage increase from the pre-infusion level. The values to the left of the figure are the percentage increases in flow caused by an infusion of 20 mg/min, and those to the right of the figure the percentage increases in flow produced by infusion of 50 mg/min of magnesium sulphate.

Laboratory Pty Ltd). The doses of drugs are expressed as weights of their salts.

RESULTS

The dilator action of magnesium sulphate on the vessels of the forearm of one subject is illustrated in Fig. 6-1. Doses of 20 and 50 mg/min infused into the brachial artery of one side for 10 min caused a rise in blood flow which was greater with the larger dose. There was no effect on the vessels of the opposite forearm. The effect of magnesium sulphate on the hand blood vessels was to produce a dilatation of this vascular bed though the dose of the salt required was much greater than that needed to produce dilatation in the forearm. These large doses of magnesium sulphate which had to be infused to produce an increase in flow in the hand invariably produced dilatation of the hand vessels on the opposite side, indicating that the drug was exerting a systemic effect. The subsequent studies were therefore confined to the forearm blood vessels.

The pooled data from eight experiments with intra-arterial magnesium sulphate on eight different subjects is illustrated in Fig. 6-2. In all studies the infusion of magnesium sulphate caused an increase in forearm blood flow. In five subjects the increase

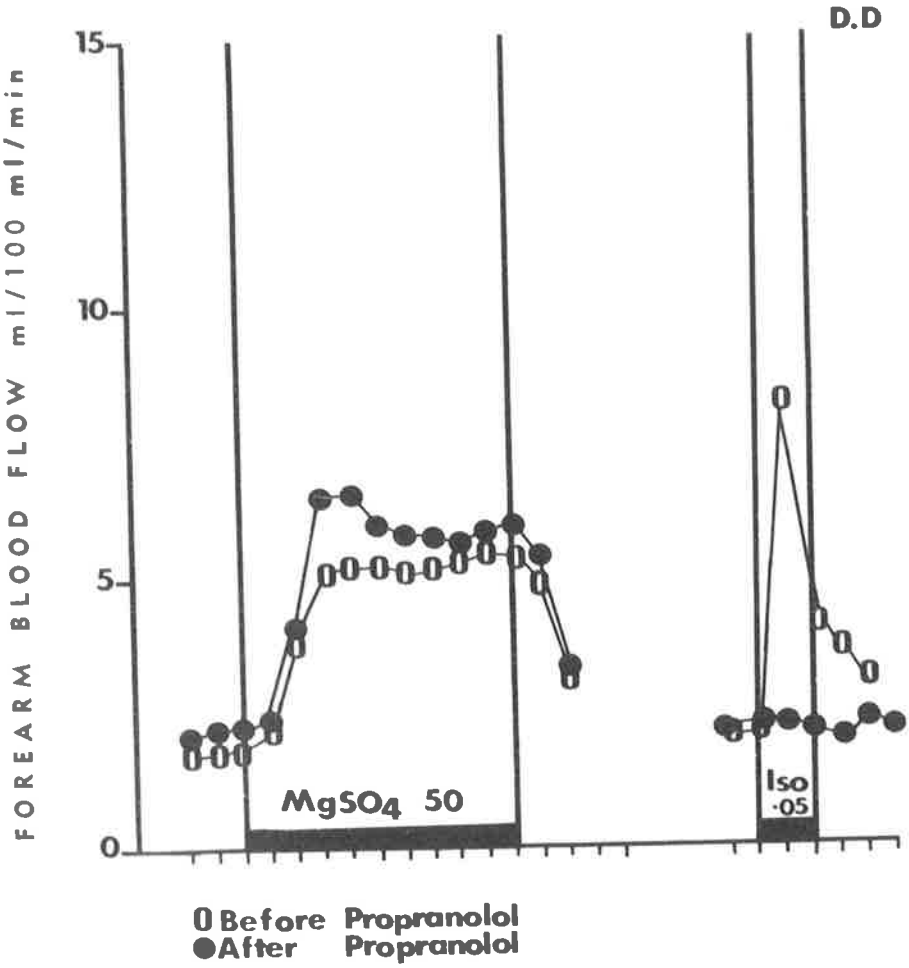


Fig. 6-3 The effect of propranolol intra-arterially (100 µg/min for 8 min) on the responses of the forearm blood flow of one subject to intra-arterial magensium sulphate, the dose of which in mg/min is indicated by the number above the black rectangle. ● , treated forearm; O , untreated forearm. The frame to the right of the figure is the response of the forearm vessels to isoprenaline (.05 µg/min) O , before; ● , after propranolol.

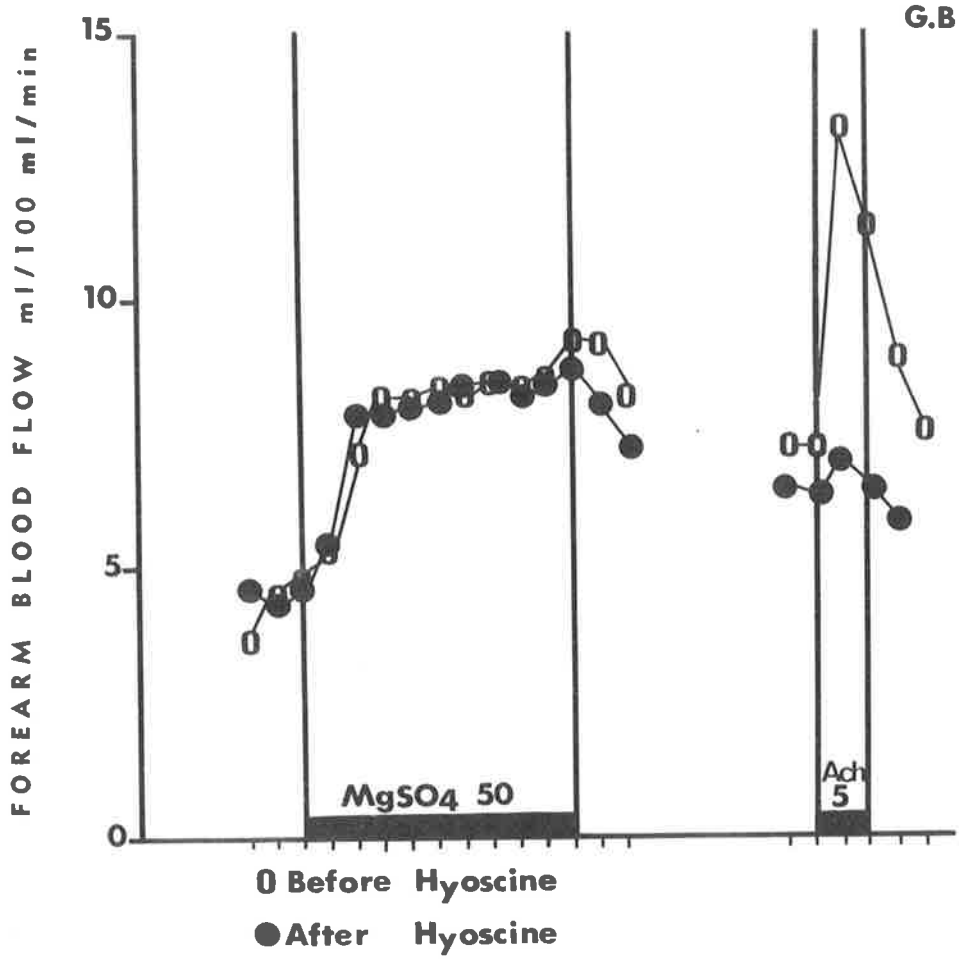


Fig. 6-4 The effect of hyoscine intra-arterially (0.1 mg/min for 4 min) on the response of forearm blood flow of one subject to magnesium sulphate (50 mg/min). The frame to the right of the figure is the effect of acetylcholine (5 µg/min) on forearm blood flow, O , before and ● , after hyoscine.

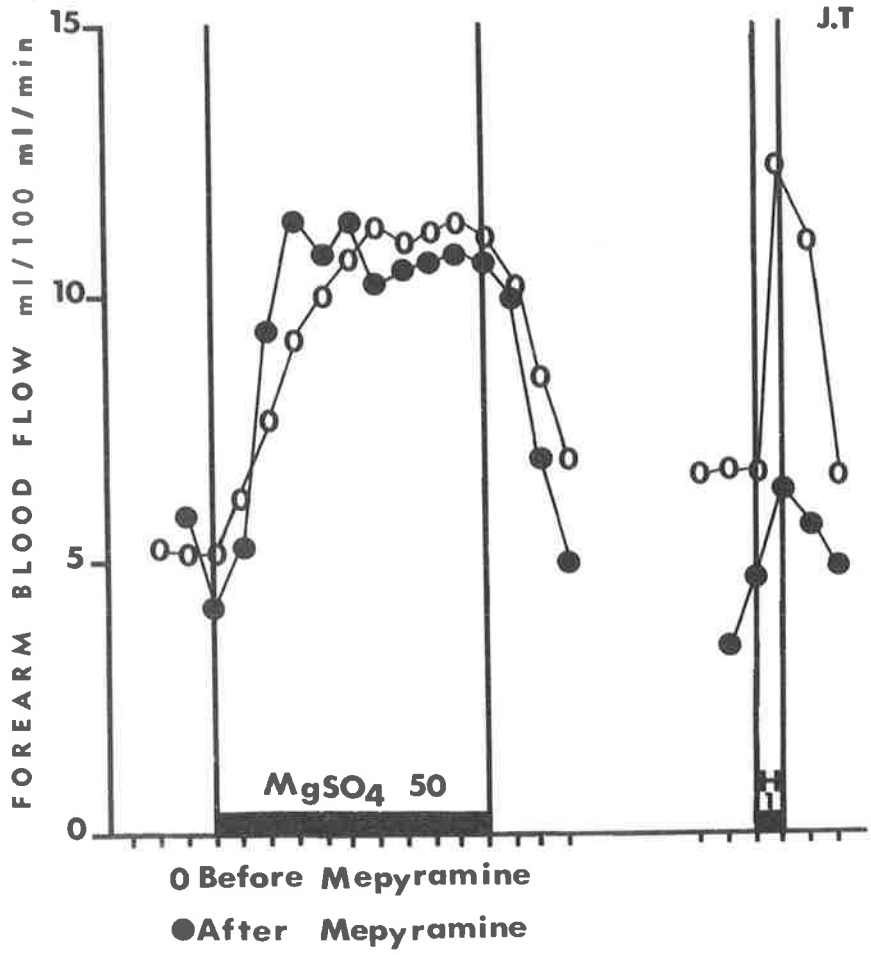


Fig. 6-5 The effect of mepyramine intra-arterially (2.5 mg/min for 10 min) on the response of forearm blood flow of one subject to magnesium sulphate (50 mg/min). The frame to the right of the figure is the effect of histamine (1 µg/min) on forearm blood flow, O , before and ● , after mepyramine.

was greater during infusion at 50 mg/min than at 20 mg/min. In three the increase at 50 mg/min was not much greater than that produced by the dose of 20 mg/min.

The effect of the β-receptor antagonist propranolol on the forearm vascular response to magnesium sulphate was studied in two experiments, the results of one of which are shown in Fig. 6-3. The left hand frame shows that the vasodilator effect of magnesium sulphate was not blocked by propranolol. The frame on the right shows that the same dose of propranolol blocked the vasodilator effect of isoprenaline. A similar result was obtained in the other subject.

The left hand frame of Fig. 6-4 portrays the fact that hyoscine did not modify the dilator effect of magnesium sulphate on the forearm vessels. To the right of the figure is shown the abolition of acetylcholine's dilator action by the same dose of hyoscine (0.1 mg/min) administered intra-arterially for 4 min. This experiment was performed on another subject and a similar result obtained.

The results in Fig. 6-5 (left frame) are from one of two experiments which showed that the antihistamine drug mepyramine did not diminish magnesium sulphate's dilator action on the forearm blood vessels. To the right of the figure is portrayed the dilator effect of histamine on this vascular bed and the attenuation of this

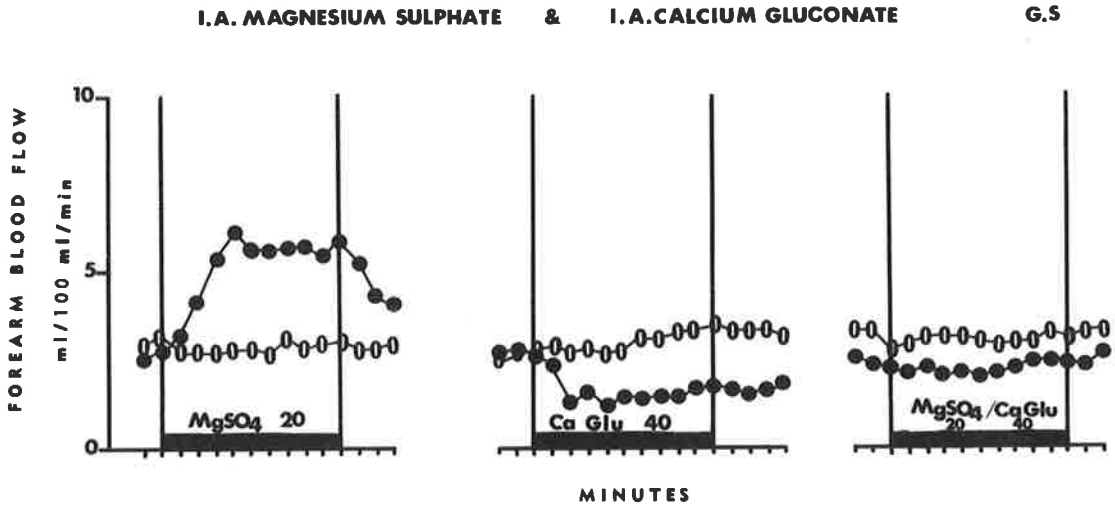


Fig. 6-6 The response of the blood flow through a normal forearm to intra-arterial magnesium sulphate, 20 mg/min (L frame), calcium gluconate, 40 mg/min (middle frame), and a mixture of magnesium sulphate 20 mg/min and calcium gluconate 40 mg/min (R frame). ● , infused side; ○ , control side.

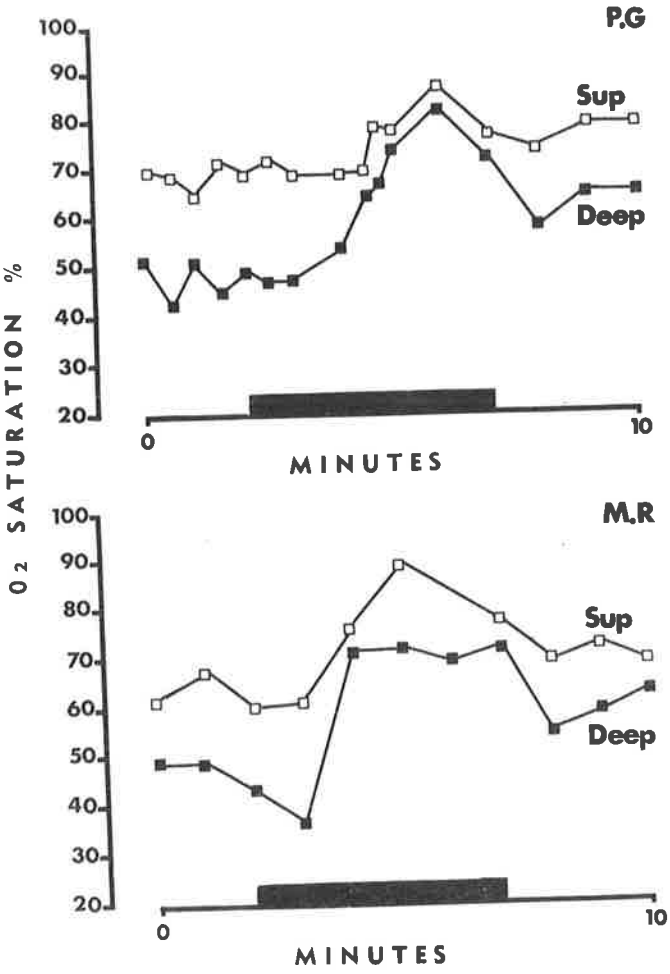


Fig. 6-7 The effect of an intra-arterial infusion of magnesium sulphate (50 mg/min) into the brachial artery on superficial and deep venous oxygen saturation of the forearm of two subjects.

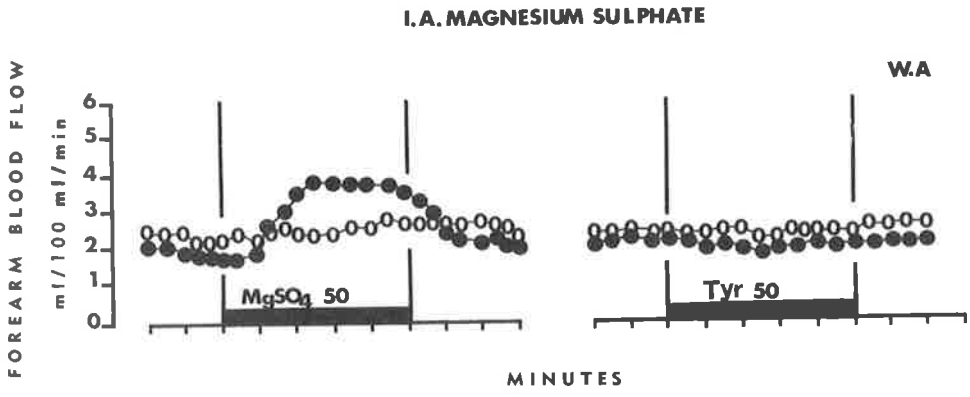


Fig. 6-8 The effect of intra-arterial magnesium sulphate (50 mg/min) on blood flow in a sympathectomised forearm (L frame) and that of intra-arterial tyramine (50 µg/min) on the same vascular bed (R frame).

effect by mepyramine.

Figure 6-6 shows the results of one of two experiments performed to test the response of the forearm blood vessels to a mixture of magnesium sulphate and calcium gluconate infused together into the brachial artery in equimolar doses. The first frame illustrates the dilator response to an infusion of 20 mg of magnesium sulphate per min, while the middle frame shows the constriction produced by an infusion of 40 mg of calcium gluconate per min. The third frame demonstrates the response of the forearm blood flow to infusing the calcium salt and magnesium salt together. The resultant effect was a small fall in the flow. Similar results were obtained in the second experiment.

In Figure 6-7 is shown the results of two experiments performed to study the effect of magnesium sulphate on the circulation through the skin and the muscle in the human forearm. A dose of 50 mg/min increased the oxygen saturation of the effluent venous blood draining both the muscle and skin, indicating that the vasodilatation occurred in both of these vascular beds.

Figure 6-8 portrays the effect of magnesium sulphate on the blood vessels of a surgically sympathectomised forearm. The frame to the right of the figure shows the effect of tyramine (an indirectly acting sympathomimetic amine) infused intra-arterially in a dose of 50 µg/min. There was a very small fall in flow,

indicating almost complete sympathectomy. Despite the sympathetic denervation, magnesium sulphate produced a definite dilatation in the forearm of the usual order.

DISCUSSION

The results of the foregoing experiments demonstrate that magnesium sulphate has a direct local vasodilator action on the blood vessels of the forearm in man. The vessels of both the skin and the muscle are affected. The response is not modified by administration of propranolol, hyoscine, or mepyramine, indicating that the dilator action is not due to stimulation of β-receptors, to a cholinergic mechanism, or to release of histamine. The local direct action on the blood vessels is further confirmed by the presence of a vasodilator action in the sympathectically denervated forearm.

These results extend the findings of Overbeck, Daugherty and Haddy (1969) who reported that infusions of magnesium sulphate into the brachial artery caused an increase in forearm blood flow in man. On the basis of previous findings (Overbeck, Molnar and Haddy, 1961) of an absence of a dilator action of the sulphate ion, they suggested that it was the magnesium ion that was responsible for

the dilatation. They considered magnesium to be "an endogenous vasodilator similar to bradykinin, histamine and acetylcholine."

Haddy (1960) has shown that infusions of sodium and magnesium salts into the brachial artery of the dog, at rates which raised the serum cation concentrations in the leg without significantly affecting concentrations in the body, caused vasodilatation, whereas calcium salts caused vasoconstriction. In our study, the dilator action of magnesium sulphate was abolished when an equimolar dose of calcium gluconate was infused simultaneously into the brachial artery. When the calcium gluconate was administered by itself, it produced a considerable fall in forearm flow, and it would appear that the constrictor effect of this compound on the forearm vessels counteracted the dilator action of the magnesium salt when the two were infused together. However, the precise mechanism of this antagonistic action is not clear.

SUMMARY

1. Magnesium sulphate, given into the brachial artery, has been shown to have a dilator action on the blood vessels of the human forearm.

2. The dilatation affects the vessels of both skin and muscle

and is not due to stimulation of the β-receptors to a cholinergic mechanism or to the release of histamine.

3. The vasodilator action of magnesium sulphate on the forearm does not appear to be influenced by the sympathetic nerves, but is abolished by the simultaneous infusion of calcium gluconate into the brachial artery.

CHAPTER 7

The syndrome of progressive autonomic nervous system degeneration.

In an earlier chapter of this thesis reference has been made to the use of two patients suffering from autonomic nervous system degeneration in the studies on ephedrine. Both subjects had loss of sympathetic control to their forearm blood vessels as well as a general disorder of cardiovascular autonomic function. The degenerative process in the autonomic nervous system also affected such structures as the sweat glands, the reproductive tract and the gastro-intestinal system. A third patient with autonomic degeneration, who participated in a study on vascular sensitivity to noradrenaline, also had abnormalities in the reactions of her pupils to light and accommodation and loss of knee jerks. The latter signs, considered together, are referred to as the Holmes-Adie syndrome (Brain, 1962).

The association of the Holmes-Adie syndrome and autonomic nervous system degeneration with cardiovascular involvement has been reported previously by Croll and Duthie (1935) and Bonnin, Skinner and Whelan (1961). Reports of the Holmes-Adie syndrome with coexisting sudomotor denervation, but without cardiovascular involvement, have been made by Ross (1958) and Petajan, Danforth,

D'Allesio and Lucas (1965). Frick (1966), in a review of the treatment of postural hypotension with 9-alpha fluorohydrocortisone, describes three patients with postural hypotension caused by autonomic nervous system degeneration, one of whom had pupillary responses which appeared to be of the Holmes-Adie type, although this was not commented upon specifically.

This chapter describes the clinical, physiological and pathological findings in a further case of autonomic nervous system degeneration with associated Holmes-Adie syndrome and peripheral neuropathy, and the findings presented serve to highlight the most important role which the autonomic nervous system has in maintaining cardiovascular homeostasis.

CLINICAL RECORD

A housewife (P.B.), aged 40 years, presented at the hospital after a syncopal attack which had proceeded to unrouseable coma. On recovery, she gave a history of progressive weight loss of 14.1 Kg over the preceding six years, paraesthesiae in the hands and feet, and episodic syncope, usually associated with postural changes from the supine to the erect position. Her appetite had been poor for some time, and she had had occasional bouts of diarrhoea.

On examination elsewhere, one year before this admission to hospital, she was found to have generalized wasting, non-reactive pupils and absent tendon reflexes in the legs. She was also found to have mild hip-flexor and peroneal weakness, and bilateral sensory impairment over the distal halves of all fingers and below the malleoli. The only abnormal laboratory finding at this time was a raised cerebrospinal fluid protein level of 200 and 280 mg per 100 ml on two consecutive occasions. No physiological tests were performed at this stage.

On this present admission, she was apyrexial, wasted and stuporous, although she recovered consciousness within 24 hours. Nevertheless, she continued to have mild syncopal attacks, her blood pressure on one such occasion being 90/60 mm Hg. Her pupils did not react to light and only sluggishly to accommodation. The reflexes in her lower limbs were absent, and the plantar responses were flexor. There was loss of power, wasting and distal loss of cutaneous and proprioceptive sensation in the lower limbs, although the upper limbs were normal in this regard. Her heart rate was 88 beats per minute and her supine blood pressure was 130/80 mm Hg. All other systems were clinically normal.

The results from a number of laboratory investigations, including urine analysis, haematological studies and serum biochemistry, were within normal limits.

Lumbar puncture indicated normal fluid dynamics, although the protein was again elevated to 165 mg per 100 ml. A Wassermann reaction on the cerebrospinal fluid was negative. The plasma electrophoretogram showed low albumin and slightly elevated $alpha_2$-globulin levels. The erythrocyte sedimentation rate was 40 mm in the first hour. Absence of lacrimal secretions was confirmed by Schirmer's test. Rectal biopsy on two occasions showed no evidence of amyloid infiltration.

Two consecutive estimations of the 24-hour urinary excretion of 3-methoxy 4-hydroxy mandelic acid gave results of 1.0 and 1.4 mg, which are at the lower limit of the normal range for the method used by the hospital laboratory.

PHYSIOLOGICAL STUDIES

METHODS

During all investigations the patient, or the normal volunteer students who acted as controls, lay supine on a couch in a temperature-controlled laboratory. The laboratory temperature was maintained at 23 to 26°C., except during the indirect heating test for the assessment of sweating distribution when it was raised to 31°C.

Intra-arterial infusions were administered from a motor-driven syringe delivering a volume of 2 ml per minute through a short-bevel, 3.5 cm, 21-gauge needle inserted centripetally under local anaesthesia (2% solution of lignocaine) into the brachial artery at the elbow. Saline (0.9%, w/v) was given throughout the control periods and was also used as a vehicle for the drugs.

Hand or forearm blood flow was measured by venous occlusion plethysmography, using water-filled, temperature-controlled plethysmographs (Greenfield, 1954) with the water temperature maintained at 32°C and 34°C, respectively, for the hand and forearm.

Intravenous infusions were administered through a polythene catheter (Intracath, Bardic) inserted into an antecubital vein.

Blood pressure was recorded with a model 5D Grass polygraph and a Statham P23 DC transducer attached to a polythene catheter (Intramedic PE 90, Clay-Adams) inserted centripetally into the brachial artery at the elbow, using a modified Seldinger technique (Seldinger, 1953). Heart rate was read directly from the blood pressure tracing and forearm or hand vascular resistance calculated from the values for mean blood pressure and blood flow (General Methods).

In performing the Adler-Scheie test (Adler and Scheie, 1940), which is specific for the Holmes-Adie pupil, two drops of a 2.5% solution of methacholine chloride were instilled into the

conjunctival sacs of both eyes at intervals of two minutes, a total of six drops being used in each eye. Pupil size was measured at intervals of 5 minutes, using a Wesley keratometer.

Expression of results:

Percentage changes in hand blood flow with intra-arterial infusions of angiotensin and noradrenaline were determined from the average values for the two minutes before drug infusion and for the last two minutes of the infusion period, by which time the responses to both drugs had become stable. Allowance was made for spontaneous variations in the flow by assuming that in the absence of the infusion the two sides would have maintained the same relationship as in the pre-infusion period (Duff, 1952).

Drugs:

The drugs used were noradrenaline bitartrate monohydrate ("Levophed", Winthrop), angiotensin II (val^5-hypertensin II-asp-β-amide, "Hypertensin", Ciba) and methacholine chloride ("Mecholyl", Sharp and Dohme). Doses of noradrenaline are expressed as weights of the base and those of angiotensin as weights of the salt. Ascorbic acid (1:50,000) was added to the noradrenaline solutions.

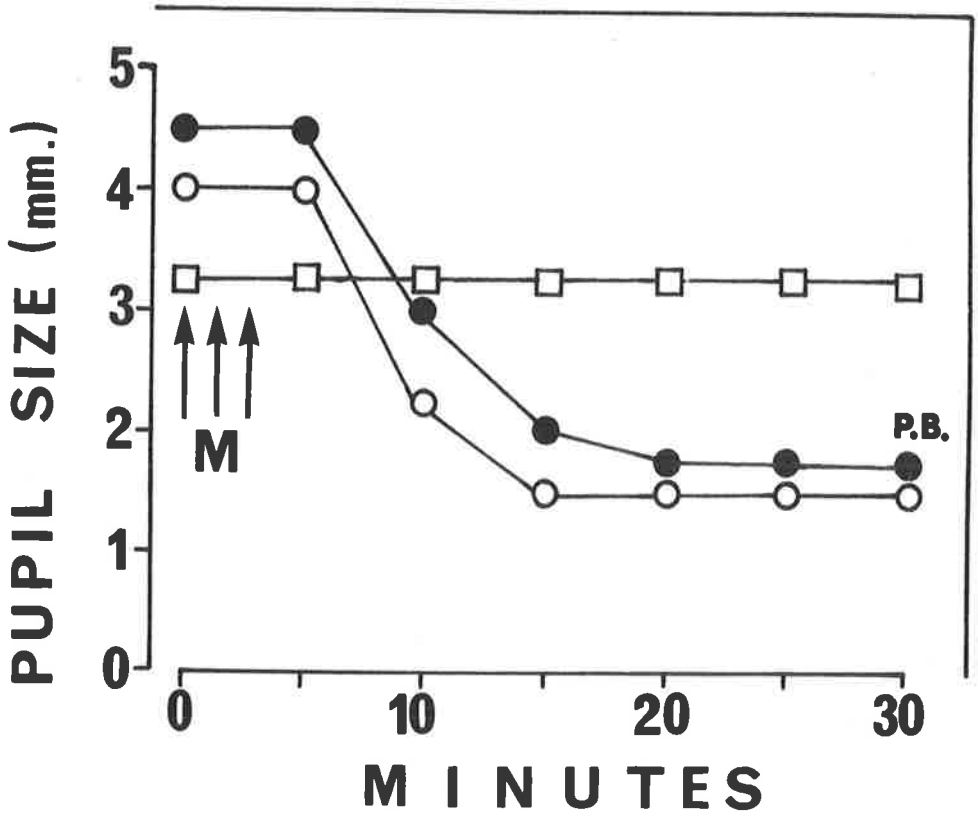


Fig. 7-1 The effect of conjunctival instillation of a 2.5% solution of methacholine chloride (M, two drops at each arrow) on the pupillary diameter in patient P.B. (● , left pupil; ○ , right pupil), and in a normal medical student (□ , left pupil).

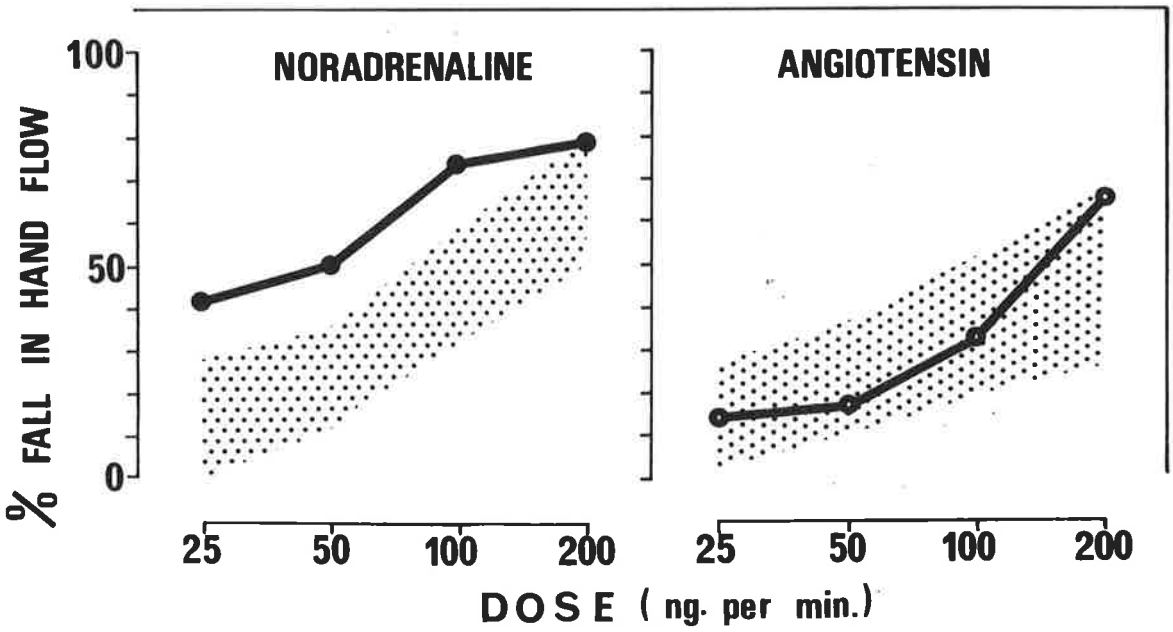


Fig. 7-2 The hatched areas in both frames include one standard deviation about the mean responses for each of the four doses of noradrenaline (left-hand frame) and angiotensin (right-hand frame) administered by intra-arterial infusion to five normal subjects. The superimposed dose-response curves (●—●) were constructed from values for the percentage falls in hand blood flow in response to the intra-arterial infusion of noradrenaline and angiotensin in the same doses into patient P.B.

RESULTS

Adler-Scheie test:

Figure 7-1 shows the pupillary responses of patient P.B. to methacholine chloride compared with those of a normal medical student. The patient showed a pupillary constriction of equal magnitude in both eyes which reached a maximum after 20 minutes and did not return to the pre-test diameter until approximately 14 hours later. The normal student showed no response to this concentration of the drug.

This increased reactivity of the patient's pupils to a cholinergic agent suggests that there was partial or complete parasympathetic denervation, and is in agreement with previous findings in this condition (Bonnin *et al.*, 1961). The specificity of this test for the Holmes-Adie pupil was demonstrated by Adler and Scheie (1940), who showed that neither the normal nor the Argyll-Robertson pupil was constricted by this particular concentration of methacholine chloride.

Intra-arterial infusions:

Figure 7-2 shows the percentage falls in hand blood flow resulting from the intra-arterial infusion of 25, 50, 100 and 200 ng per minute for four minutes of both angiotensin and noradrenaline. The hatched areas in the figure include one standard deviation

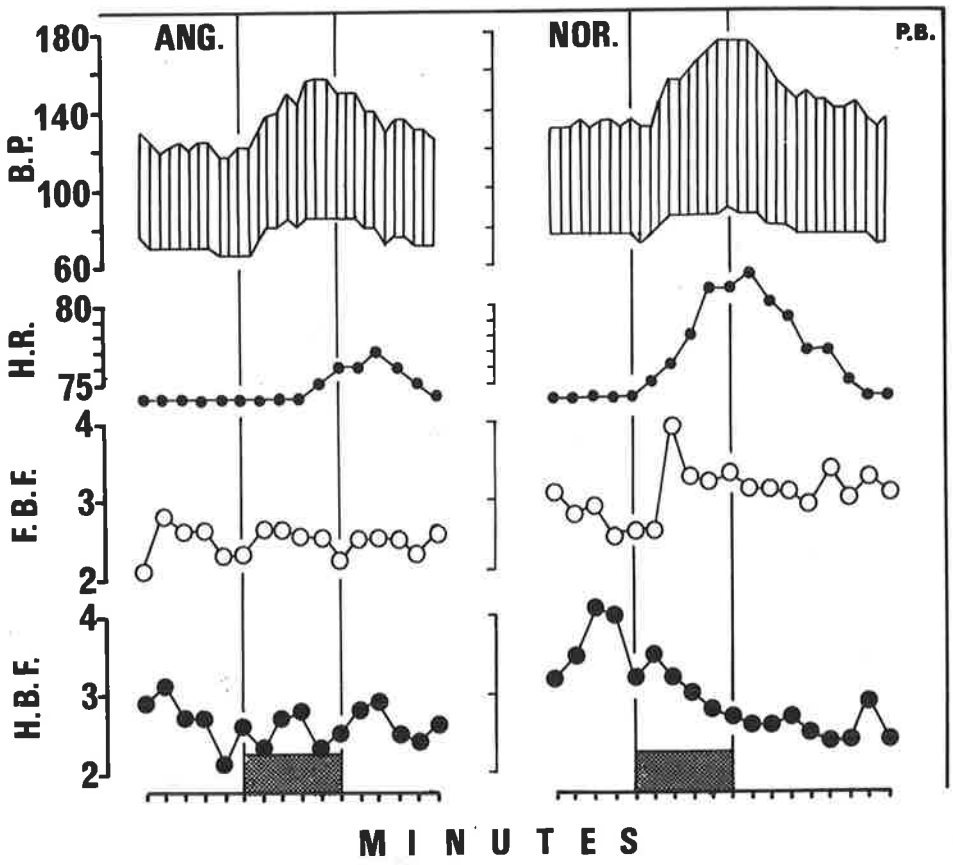


Fig. 7-3 The response of arterial blood pressure, heart rate, and forearm (O) and hand (●) blood flow during the intravenous infusion of angiotensin (0.25 µg per minute, left-hand frame) and noradrenaline (2.0 µg per minute, right-hand frame).

about the mean responses obtained from five normal subjects for the respective dose of each drug.

The patient's responses to angiotensin were within normal limits, whereas there was an increase in the responsiveness to noradrenaline at all dose levels.

Intravenous infusions:

Figure 7-3 shows the changes in arterial blood pressure, heart rate, and hand and forearm blood flow during intravenous infusions of angiotensin (0.25 µg per minute for five minutes, left-hand frame) and noradrenaline (2.0 µg per minute for five minutes, right-hand frame).

With both drugs there was an increase in heart rate during the infusion period. Although the changes in heart rate observed during angiotensin infusions in this condition have been inconstant (Scroop, Walsh and Whelan, 1965), the degree of pressure rise seen in this patient with noradrenaline would normally result in reflex bradycardia, and its replacement by marked tachycardia may reflect the direct, positive chronotropic action of noradrenaline.

Hand and forearm blood flow changed little during the intravenous infusion of angiotensin, although calculated resistance increased. With noradrenaline, however, forearm blood flow increased so that there was no appreciable change in resistance,

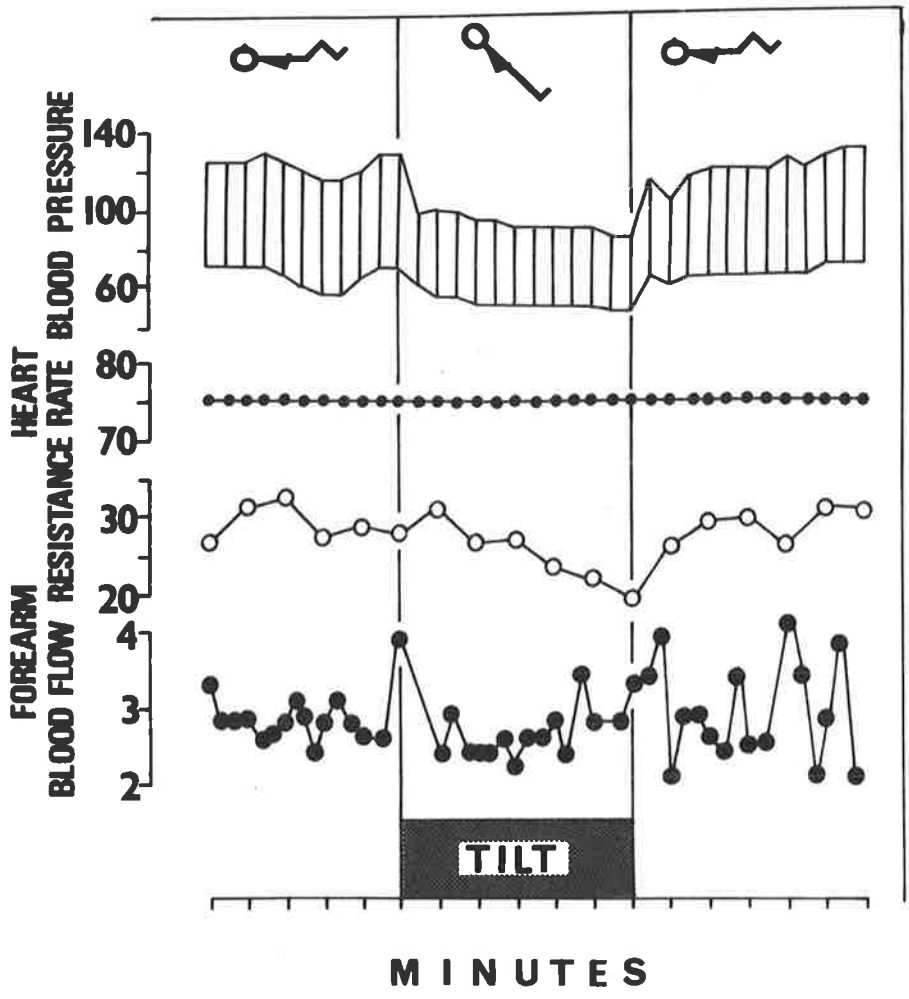


Fig. 7-4 The response of arterial blood pressure, heart rate, forearm blood flow (●) and forearm vascular resistance (O) during postural change from the supine to a semivertical position (45°).

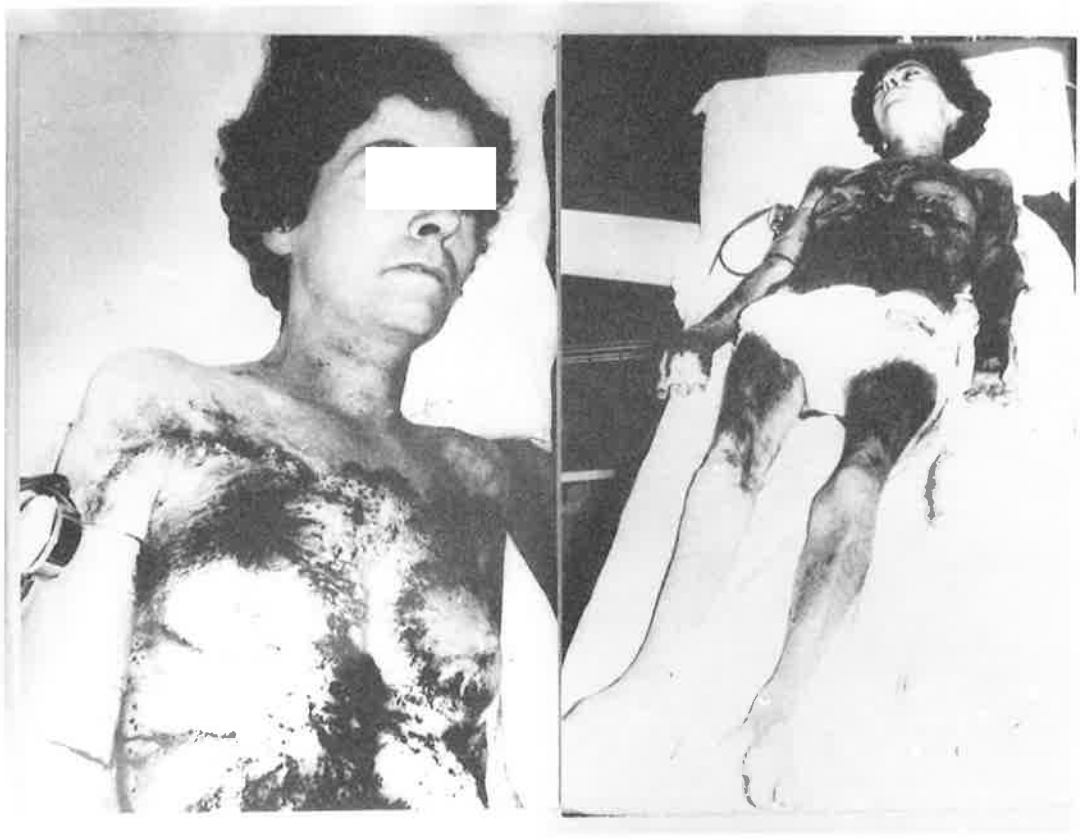


Fig. 7-5 Demarcation by Quinizarin Compound powder of the sweating distribution after indirect body heating.

and a long-lasting fall in hand blood flow occurred with an increase in resistance.

Noradrenaline produced a 21% increase in mean blood pressure and angiotensin a 29% increase. Both these responses are between two and three times greater than those seen in normal subjects (Scroop *et al.*, 1965). The enhanced pressor response to the intravenous infusion of angiotensin and noradrenaline may be due to an absence of the normal reflex baro-receptor adjustments, or, at least in the case of noradrenaline, to an increased responsiveness of the blood vessels themselves to the direct action of the drug.

Postural reflexes:

Figure 7-4 shows the changes in arterial blood pressure, heart rate, forearm blood flow and calculated resistance during tilting from the horizontal to a feet-down position (45°). After six minutes in the tilted position, the mean blood pressure had fallen by 25 mm Hg from a resting value of 85 mm Hg. The normal compensatory tachycardia was not seen, and forearm resistance actually decreased, possibly due to a local myogenic response (Bayliss, 1902).

Indirect heating:

Figure 7-5 shows the sweating distribution in this patient following indirect heating. The room temperature was elevated to

31°C and the patient kept in this environment for 45 minutes. The substance used to demarcate the areas of sweating was Quinizarin Compound powder (Burroughs Wellcome) which changes from a blue-grey to a dark blue-violet colour on contact with moisture, the anhidrotic areas remaining unchanged (Guttman, 1947).

Sweating was absent from the clavicular level upwards, on the breasts, palms of both hands, and on the lower limbs from the knees downwards, indicating partial or complete sympathetic denervation of the sweat glands in these areas.

Miscellaneous tests of autonomic function:

The application of ice to the neck usually results in a sympathetically mediated reflex fall in hand blood flow (Cooper, Fewings, Hodge and Whelan, 1963). The response in this patient was less than normal, suggesting partial denervation of this vascular bed.

In normal subjects a stressful situation, invoked, for example, by mental arithmetic, usually causes an increase in the forearm muscle blood flow due to activation of the cholinergic vasodilator fibres to muscle vessels (Blair, Glover, Greenfield and Roddie, 1959). However, the forearm blood flow in this patient remained unchanged during several periods of mental arithmetic.

CLINICAL COURSE

The results of the physiological tests supported the diagnosis of autonomic nervous system degeneration, and suggested that the degenerative change was widespread and, although incomplete in certain areas, was probably responsible for many of the patient's symptoms and signs. The various laboratory tests failed to disclose a cause for the degeneration (Wagner, 1959), and symptomatic treatment was begun with 9-alpha fluorohydrocortisone (0.1 to 0.3 mg per day) and digitalis in an attempt to combat her postural hypotension.

On this regime her postural hypotension improved, but over a period of six months her general condition deteriorated and she eventually died.

AUTOPSY FINDINGS

The immediate cause of death was pneumonia with abscess formation, and apart from general wasting of adipose and muscle tissue, no other abnormality was apparent on macroscopic examination. Because of the clinical observations and the results from the physiological studies, a specific histological examination

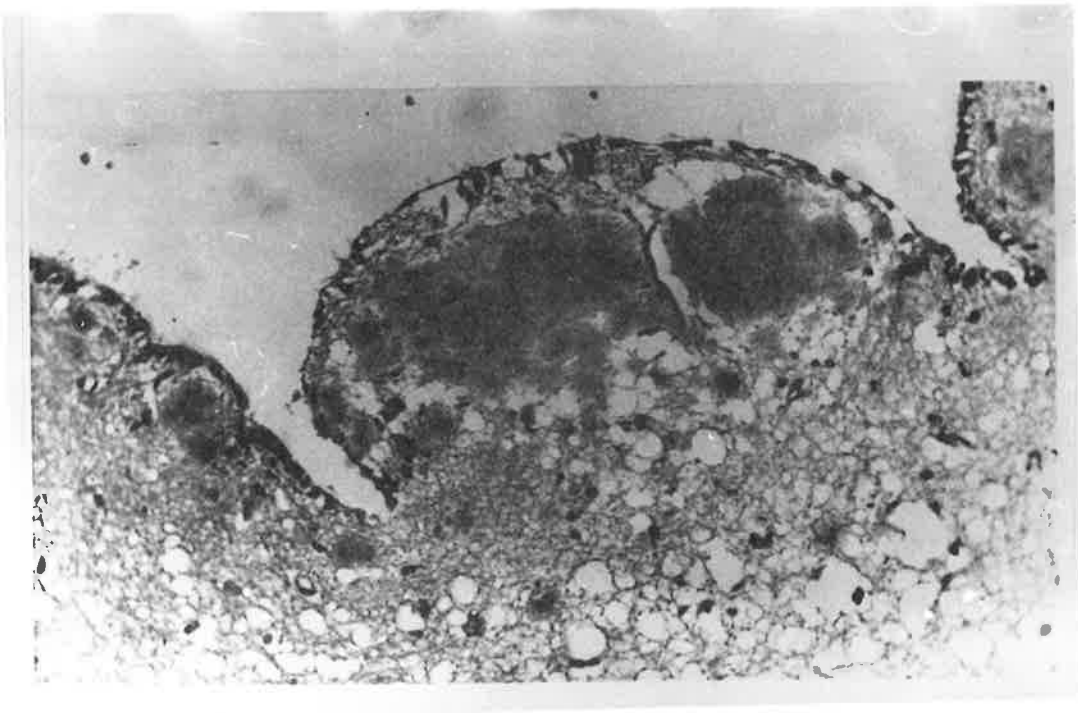


Fig. 7-6 Subependymal deposits of amyloid material in the third ventricle. (Haematoxylin and eosin, x 120).

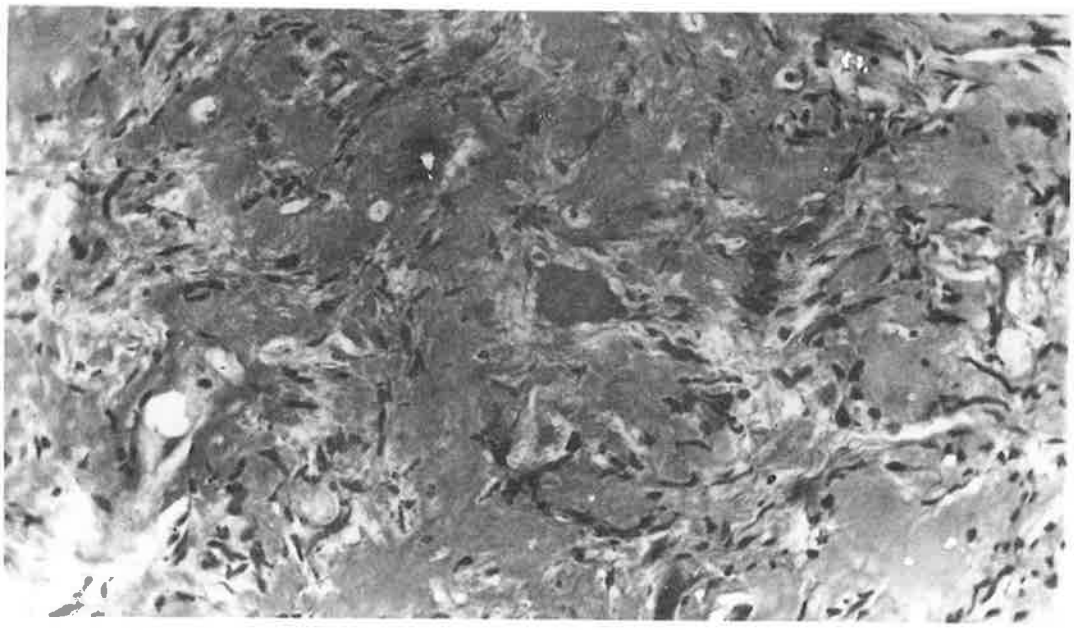


Fig. 7-7 Amorphous amyloid deposits diffusely scattered in a sympathetic ganglion. A solitary ganglion cell can be seen. (Haematoxylin and eosin, x 120).

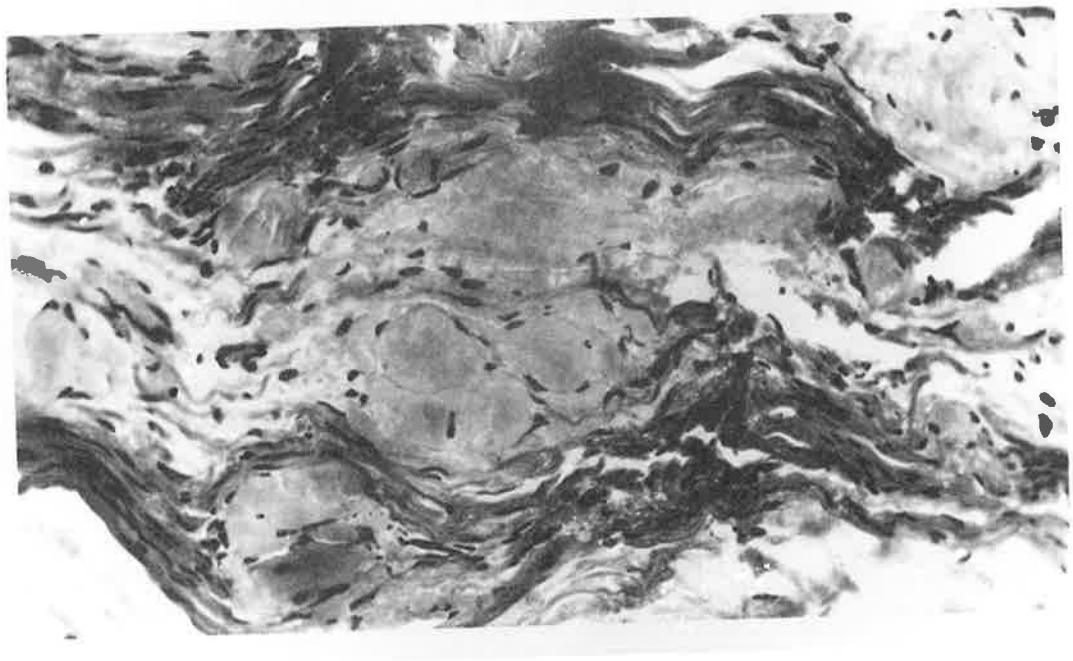


Fig. 7-8 Longitudinal section of a femoral nerve showing discrete masses of amyloid within and between the nerve fibres. (Haematoxylin and eosin, x 120).

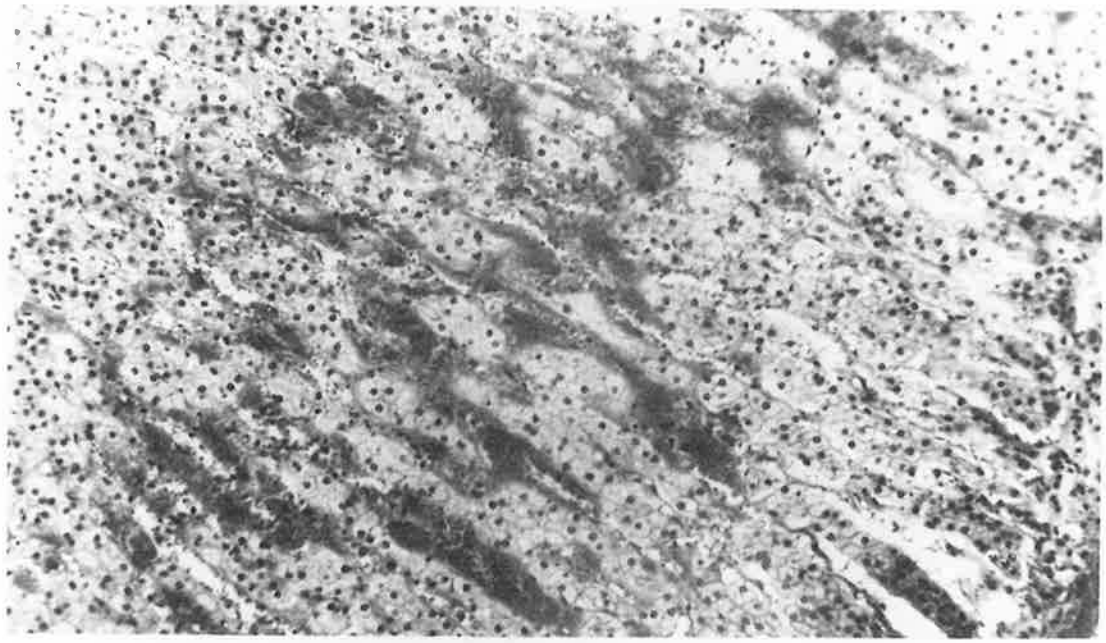


Fig. 7-9 Dense confluent peri-collagenous pattern of amyloid deposition in adrenal cortex. (Haematoxylin and eosin, x 60).

was made of the thoracic sympathetic ganglia, selected muscle groups and peripheral nerves, in addition to the routine organ studies.

Haematoxylin and eosin stained sections showed an amorphous, pink, hyaline material in almost all sections, mostly associated with the stromal elements and forming discrete masses in the subependymal, subpial and perivascular areas of the brain and spinal cord (Figure 7-6), in the sympathetic ganglia (Figure 7-7), peripheral nerves (Figure 7-8), and the thyroid and adrenal glands (Figure 7-9). It was also present amongst the cardiac muscle fibres, resulting in marked compression and degeneration, especially in the region of the sino-atrial node.

The use of crystal violet and congo red stains showed that the amorphous substance described above was amyloid, giving positive reaction, in most of the involved tissues. The apple-green birefringence method advocated by Heller, Missmahl, Sohar and Gafni (1964), with its ability to differentiate the fine pericollagenous amyloid fibres, enabled amyloid tissue to be detected in the alimentary rete mucosa and in the endomysium of muscle bundles, as well as in the small nerves and blood vessels of all tissues examined.

DISCUSSION

An increased pressor response to the intravenous infusion of noradrenaline has been reported previously in patients with idiopathic autonomic nervous system degeneration (Johnson, Lee, Oppenheimer and Spalding, 1966) and also in patients with familial dysautonomia (Smith and Dancis, 1964). Abolition of the normal baroreceptor reflex adjustments to changes in arterial blood pressure and an increase in vascular reactivity to noradrenaline (Trendelenburg, 1963) have both been suggested as possible mechanisms to explain the hypersensitivity. In our patient the hand vessels exhibited an enhanced constrictor response during intra-arterial infusions of noradrenaline but not during those of angiotensin. However, there was an increased pressor response during intravenous infusions of both drugs, suggesting a common mechanism such as impairment of the baroreceptor reflexes.

The absence of bradycardia during the pressor infusion of noradrenaline and the absence of tachycardia during tilting despite marked hypotension suggested that the patient's cardiac autonomic innervation was lacking. Furthermore, the plasma levels of catecholamines in these patients have been shown to alter little with cardiovascular stimuli such as postural changes (Hickler, Wells, Tyler and Hamlin, 1959). This combination of absent or

reduced inotropic and chronotropic actions of the catecholamines will impair cardiovascular performance, and therefore digitalis can be of some therapeutic importance in these cases, particularly if combined with expansion of the circulating blood volume with mineralocorticoids.

The most striking feature to emerge from the autopsy findings in this case was the involvement by amyloid degeneration of the sympathetic ganglia, peripheral somatic nerves, heart, and endocrine and alimentary systems, and on this basis the clinico-pathological correlation in this case is easily established.

Disabling orthostatic hypotension due to sympathetic insufficiency, in the absence of drug treatment, is not common, and may be due to a central, preganglionic or postganglionic lesion. Johnson *et al.* (1966) reviewed the condition and presented two cases in which there was neuronal loss from the intermedio-lateral grey columns of the thoraco-lumbar segments of the spinal cord (that is, sympathetic preganglionic cells of origin). The intermedio-lateral columns in the present case were not obviously depleted. Navasquez and Treble (1938) and Rukavina, Block, Jackson, Falls, Carey and Curtis (1956) presented cases of generalized amyloidosis with nervous system involvement, and Andrade (1952) described 12 pedigrees with 74 cases of a peculiar neuropathy with pupillary changes in which two cases at post-mortem examination showed

widespread amyloid deposits similar to those of the present case. Wagner (1959) and von Sallmann (1960) presented cases of orthostatic hypotension in patients with primary amyloidosis. Gafni, Sohar and Heller (1964), in discussing inherited amyloidosis, described three types of presentation: nephropathic with renal failure, cardiopathic with intractable cardiac failure, and neuropathic with progressive polyneuritis, gastro-intestinal disturbances and wasting.

The clinical presentation of our patient would seem to agree with the neuropathic type of Gafni *et al.* (1964), and the observed cardiac and renal insufficiency was probably related to the length of survival.

SUMMARY

1. The case history is reported of a woman aged 40 years who presented with weight loss, postural hypotension and paraesthesiae in the hands and feet. The diagnosis of widespread autonomic nervous system degeneration with associated peripheral neuropathy and Holmes-Adie syndrome was made from the results of a series of clinical and physiological tests.

2. Laboratory investigation failed to disclose a cause for

the degenerative changes, and symptomatic treatment with 9-alpha fluorohydrocortisone and digitalis was commenced in an effort to combat the patient's postural hypotension. On this regime there was transient improvement in her clinical state, but over a period of six months her general condition gradually deteriorated and she eventually died.

3. At autopsy, widespread amyloid infiltration of the sympathetic ganglia, peripheral nerves, heart and alimentary canal was found, and this appears to have been the pathological basis for the clinical and physiological findings.

G E N E R A L S U M M A R Y

The introductory chapter of this thesis is a historical survey of the sympathetic nervous system. Up to the beginning of the nineteenth century the advances in knowledge concerned chiefly the anatomy of this important autonomic component. From that time onwards, however, the physiological aspects of sympathetic nerve function became more clearly understood, and in 1946 the true nature of the sympathetic neurotransmitter was first described. The advent of such techniques as fluorescence histochemistry, electron microscopy and refined assay methods have further advanced our understanding of the anatomy and physiology of the sympathetic nerves.

Section 1 of this thesis describes studies performed on isolated blood vessels. The material presented in Chapter 1 relates to the influence of sympathetic innervation on the sensitivity of the central artery of the rabbit ear to noradrenaline. Preliminary histochemical studies on this vessel showed the sympathetic nerve stores to be located at its medio-adventitial junction.

When the artery was perfused with Kreb's bicarbonate solution and its sensitivity to noradrenaline tested, either by adding the drug to the solution bathing the outside of the vessel (extraluminal application), or by injecting or perfusing the drug through its lumen (intraluminal application), it was found that noradrenaline applied intraluminally produced a much greater

constrictor effect than noradrenaline applied extraluminally. It was also found that the drug cocaine greatly enhanced the sensitivity of the vessel to noradrenaline applied extraluminally, but not to noradrenaline applied intraluminally, and, further, that the effects of denervation resembled those of cocaine. Cocaine also enhanced the constrictor responses of the artery to electrical stimulation, but this enhancement was much less than that on extraluminal noradrenaline. It was concluded that the position of the noradrenaline stores at the medio-adventitial border of the artery contributed to the low sensitivity of the vessel to extraluminal noradrenaline. Cocaine and denervation produced their potentiating effects by eliminating the uptake of noradrenaline into these storage sites.

With the knowledge that the position of the sympathetic nerve stores in an artery was related to the responsiveness of the vessel to noradrenaline, human peripheral vessels were examined, using a fluorescent histochemical technique, to study the distribution of their sympathetic nerve supply. The results of this study are presented in Chapter 2. Specific catecholamine fluorescence was observed at the medio-adventitial junction in human digital and gingival vessels obtained from young adults, infants and children, but not in the vessels obtained from the older adults. These findings suggested a relationship between the

age of the subject and the quantity of transmitter present in the sympathetic nerve endings or in the density of the nerve network in the vessel.

Section 2 of this thesis presents a series of studies performed on the upper limbs of man, measuring blood flow, using venous occlusion plethysmography. The local effects of various vasoactive agents were assessed by infusion into the brachial artery. Specialised techniques, such as forearm superficial and deep venous blood oxygen saturation estimations, and the monitoring of blood pressure using transducers, were also used in some of these studies.

Chapter 3 (the first chapter of Section 2) deals with the action of ephedrine hydrochloride on human forearm vessels. The predominant effect of this substance was to produce vasoconstriction, mediated by the release from the sympathetic nerve endings of a constrictor agent which acted on the α-adrenergic receptors of vascular smooth muscle. A secondary effect produced by ephedrine was vasodilatation which was seen when the α-receptors were blocked by phentolamine or when the sympathetic nerves to the blood vessels were absent due to a direct action of ephedrine on β-adrenergic receptors. Ephedrine also produced mild vasoconstriction of the forearm vessels following blockade of both the α- and β- receptors in the normal limb and also in the β-blocked sympathectomised limb. The nature of this effect was uncertain.

The mechanism of action of tyramine on human forearm vessels is described in Chapter 4. It was found that the constrictor action of this drug was dependent on the presence of the sympathetic nerves and that the vasoconstrictor substance liberated by tyramine from the sympathetic nerve endings possessed both α-receptor and β-receptor stimulating properties. The α-receptor action of tyramine was the predominant effect, whereas the β-receptor activity was seen only after blockade of the α-receptors with phentolamine.

A comparison of the times of onset of the constrictor and dilator responses to tyramine and noradrenaline was made using doses of the drugs which produced constrictor effects of comparable magnitude. This comparison showed that the responses to tyramine always appeared later than those to noradrenaline and also that the dilator effects of tyramine were less marked. It is suggested that the differences between the times of onset of the actions of tyramine and noradrenaline might be due to the fact that infused noradrenaline exerts its action directly on the vascular smooth muscle, whereas tyramine must penetrate to the nerve plexus and release transmitter which may be subject to re-uptake and degradation before reaching its site of action. It is also suggested that the action of the two substances might be influenced by the relative distributions of the α- and β- receptors in vascular smooth muscle.

Chapter 5 deals with the modifying effect which the hypotensive agent bretylium tosylate and the monoamine oxidase inhibitor tranylcypromine have on the vasoconstrictor actions of tyramine, methylamphetamine and ephedrine. This study was performed on the hand blood vessels, and bretylium was found to potentiate the constrictor actions of all these sympathomimetic agents but to different degrees. Tyramine was potentiated most, then methyl-amphetamine, and least of all ephedrine. It is suggested that one of the mechanisms responsible for this enhancement of vasoconstrictor action is mediated through the monoamine oxidase inhibiting property of bretylium. The effect of this action in the case of tyramine would be to protect both this sympathomimetic agent and the intra-neuronal transmitter which it releases. In the case of the other two sympathomimetics, which are not substrates of the monoamine oxidase enzyme system, protection of intra-neuronal transmitter alone was thought to occur, accounting for a lesser degree of potentiation of these amines.

To test the hypothesis that monoamine oxidase inhibition contributed significantly to enhancing the constrictor effects of the amines on the hand blood vessels, the study was repeated using the monoamine oxidase inhibitor tranylcypromine. The latter produced a pattern of enhancement similar to that seen in the presence of bretylium. However, tranylcypromine was also found to

enhance the responsiveness of the hand vessels to intra-arterially infused noradrenaline, and this could have contributed to the overall effect.

Chapter 6 examines the effect of magnesium sulphate on the blood vessels of the human forearm. The salt was infused into the brachial artery and caused a dilatation of the blood vessels of both skin and muscle which was not mediated through β-receptor stimulation, a cholinergic mechanism or the release of histamine. Magnesium sulphate also produced vasodilatation of the usual magnitude in the sympathectomised forearm, suggesting that the effect was due to the direct action of the salt on vascular smooth muscle. However, the simultaneous administration of calcium gluconate into the brachial artery abolished the dilatation. The mechanism of this antagonistic effect is not clear. It was found that calcium gluconate administered alone caused constriction of the forearm vessels, and, in the light of this finding, it is suggested that the constrictor action of one salt was counteracting the dilator effect of the other when the two were infused together.

The last chapter of this thesis describes the clinical, physiological and pathological findings in a patient with autonomic nervous system degeneration. The patient - a woman aged 40 years - presented with postural hypotension, weight loss, and paraesthesiae in the hands and feet, and, in addition to widespread autonomic

degeneration, was found to have an associated peripheral neuropathy and the Holmes-Adie syndrome. Initial laboratory investigation failed to disclose a cause for the degenerative changes, and symptomatic treatment with 9-alpha fluorohydrocortisone and digitalis was commenced in an effort to combat her postural hypotension. On this regime there was transient improvement in her clinical state, but over a period of six months her condition gradually deteriorated and she eventually died. At autopsy, widespread amyloid infiltration of the sympathetic ganglia, peripheral nerves, heart and alimentary canal was found, and this appeared to have been the pathological basis for the clinical and physiological findings. The case is presented to highlight the important role which the autonomic nervous system plays in the maintenance of cardiovascular homeostasis.

The theme of this thesis is therefore the functional significance of sympathetic nerve endings in blood vessels. The relationship between the location of these structures and the vascular effects of some sympathomimetic agents has been examined, and the symptomatology, investigation and autopsy findings in a case of autonomic nervous system degeneration presented to emphasise their vital role in normal cardiovascular function.

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