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RELATION IN RAT HIND-LIMB BLOOD VESSELS BETWEEN NERVOUS VASOMOTOR TONE AND THE RESPONSE TO VASOCONSTRICTOR DRUGS

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When the nervous component of the vasomotor tone of the blood vessels of the innervated rat hind limb perfused with blood was reduced by ganglion blockade, by section of the nerves to the hind limb, or by the indirect effect of drugs, the vasoconstrictor response of the blood vessels to noradrenaline was immediately increased. This increased peripheral response was observed only when the blood perfusing the hind limb was from the same animal or from a genetically similar animal. Reduction of the nervous component of the vasomotor tone also increased the constrictor response of the hind-limb blood vessels to adrenaline, ephedrine, tyramine and 5-hydroxytryptamine, but did not increase the response to angiotensin, vasopressin or S-methyl *iso*thiourea. Pre-treatment with reserpine increased the hind-limb response to noradrenaline but reduced the response to ephedrine, tyramine and 5-hydroxytryptamine. The results suggest that, in rats, activity in the sympathetic nervous system directly influences the reactivity of peripheral blood vessels to noradrenaline and other sympathomimetic drugs.

The increase in response to vasoconstrictor drugs caused by administration of ganglion-blocking drugs to the whole animal was first observed by Page & Taylor (1947). Two explanations have been advanced to explain this increase. Moe (1948) suggested that ganglion blockade interfered with the normal cardiovascular homoeostatic mechanism, hence allowing an increased pressor response to vasoconstrictor drugs. Secondly, a peripheral action of ganglion-blocking drugs on vascular smooth muscle resulting in an increased response to vasoconstrictor drugs has been suggested (Corcoran & Page, 1947 ; Zaimis, 1955, 1956 ; Mantegazza, Tyler & Zaimis, 1958). These explanations have not been supported by more recent work. Ganglion-blocking drugs still caused an increased response to noradrenaline after destruction of the normal baroreceptor mechanisms, so that the increase was independent of alterations in circulatory homoeostasis (Bartorelli, Carpi & Cavalca, 1954 ; Maengwyn-Davies, Walz & Koppanyi, 1958). Also, no evidence was obtained for any direct peripheral action of hexamethonium on rat blood vessels when hexamethonium was added to blood perfusing a rat hind limb (Laverty, 1959 ; Blackman & Laverty, 1961). No direct action of hexamethonium on isolated vascular smooth muscle preparations could be shown (Speden, unpublished ; Lum & Rashleigh, 1961).

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Because of the lack of a satisfactory explanation of the increased response to vasoconstrictor drugs after ganglion blockade in the whole animal, the effect of ganglion blockade on the peripheral response to noradrenaline was studied. This led to a consideration of the relationship between the peripheral vasomotor tone of nervous origin and the reaction of blood vessels to various other vasoconstrictor drugs.

METHODS

In all experiments, rat hind-limb blood vessels were perfused with blood at a constant rate of flow by means of a specially designed pump (Field, de Graaf & Wallis, 1958). This pump had a steady output of 1 ml./min over a wide range of input and output pressures and over long periods of service (Field *et al.*, 1958). Constant flow rate perfusion meant that changes in the peripheral resistance of the perfused vascular bed were measured as corresponding changes in the perfusion pressure.

Albino rats (300 to 420 g) from the main Otago colony were used, except where otherwise specified. Chloralose (60 mg/kg), given through the cannulated jugular vein, was used as anaesthetic after ether induction; the trachea was cannulated in all rats.

The mode of preparation of the hind limb for perfusion and the provision of the perfusing blood in different experiments were as follows.

Single animal preparations

One rat served as both donor and recipient animal for the perfusion. Blood was taken from one femoral artery, through a cannula connected to the pump input, and pumped into the perfused hind limb through the other femoral artery, which was cannulated at the level of the inguinal ligament. In all single animal experiments, except when specified otherwise, the blood from the perfused hind limb returned to the remainder of the animal through the femoral vein (Laverty & Smirk, 1961).

Intact hind limb. The femoral artery of the perfused hind limb was isolated through a small skin incision. There was no other surgical intervention affecting that limb, all venous, nervous and collateral arterial connexions being left intact.

Isolated hind limb. In a few experiments the hind limb was isolated from the vascular system of the remainder of the animal by tying ligatures on either side of the femoral vessels and the femoral and sciatic nerves.

In most experiments the hind limb was isolated by cutting the muscles just below the level of the head of the femur (Field & Laverty, 1958). The femoral vessels and the femoral and sciatic nerves were kept intact. The femur was left to provide support for the isolated limb; no significant vascular connexion through the head of the femur could be detected. The femoral artery was tied and cannulated and the hind limb perfused by the pump.

Separate venous return. In one series of experiments the femoral vein was also cannulated to collect the venous drainage from the isolated hind limb. The venous pressure in the hind limb was stabilized by collecting the venous blood in a reservoir to which a constant suction of 20 cm H_2O was applied. The blood in the reservoir was returned to the remainder of the animal through the cannulated jugular vein by a venous return pump (Field & Laverty, 1958).

Delay coil. In some experiments a delay coil of polyethylene tubing of 6 ml. volume was introduced between the donor femoral artery and the pump. This increased the volume of blood in the pump and tubing to over 7.5 ml. At a flow rate of 1 ml./min, blood took over 7 min to reach the isolated hind limb from the remainder of the animal; the delay was measured by the interval between giving a vasoconstrictor drug to the animal and the onset of the resultant vasoconstriction in the isolated hind limb.

Skinned hind limb. In order to separate the effects on skin blood vessels from those on muscle blood vessels, the skin was excluded from the area perfused by retracting it towards the foot;

a ligature was tied tightly around the ankle joint to prevent the perfusion of the foot and skin blood vessels. The skin was then replaced to help to maintain the hind-limb temperature.

Separate donor perfusion of an isolated hind limb

This preparation, in which the perfusing blood came from a separate rat, has been described in full by Field & Laverty (1958). Blood was taken from the carotid artery of a donor animal, perfused through the isolated hind limb of a recipient rat and returned to the donor from the femoral vein by means of a venous reservoir and pump. In this preparation, drugs given to the remainder of the recipient animal affected the hind limb through the nerves only, while drugs given to the donor animal could only affect the perfused hind-limb blood vessels through the blood stream.

After the appropriate surgery was complete, 30 min was allowed to elapse to ensure haemostasis before heparin (1,250 u.) was given and perfusion started. The blood pump and associated polyethylene tubing were filled before perfusion with heparinized blood from another rat.

All pressures were measured by small-volume mercury manometers. The hind-limb perfusion pressure was measured at the femoral artery cannula, making allowance for the resistance of the cannula; the blood pressure of the remainder of the recipient animal, and, if applicable, the blood pressure of the separate donor animal, were recorded from carotid arterial cannulae (Field & Laverty, 1958). All cannulae were glass; the pump and tubing were of polyethylene or methacrylate plastic.

The response of the hind-limb blood vessels to noradrenaline was measured as the absolute rise in perfusion pressure produced by the rapid injection of noradrenaline (bitartrate; Levophed, Winthrop; 0.01 μg free base) in 0.05 ml. saline. Identical injections of saline alone caused a rise of less than 10 mm Hg, lasting only about 5 sec, due to the sudden increase in perfusing volume. The vasoconstrictor responses to other drugs were measured by giving the appropriate dose in 0.05 ml. saline. Other vasoconstrictor drugs used were adrenaline (hydrochloride *B.P.*; 0.01 μg free base), tyramine (hydrochloride, British Drug Houses; 1 to 2 μg salt), ephedrine (hydrochloride, Hopkin & Williams, 2 μg salt), angiotensin (Ciba, 19990 *a*; 0.05 μg amide), vasopressin (Pitressin, Parke Davis; 0.2 to 1.0 m-u.), S-methyl *iso*thiourea (sulphate, British Drug Houses; 1 μg salt) and 5-hydroxytryptamine (creatinine sulphate, May & Baker; 0.02 μg salt). Ganglion-blocking drugs used were hexamethonium bromide and pentolinium tartrate (doses as weight of salt in all cases).

RESULTS

Increased peripheral response to noradrenaline after ganglion blockade

It was found in single animal perfusion experiments that the response to noradrenaline of the blood vessels of the blood-perfused rat hind limb was approximately doubled after hexamethonium had caused a fall in the hind-limb perfusion pressure by removing the nervous vasomotor tone by ganglion blockade (Table 1A). Isolating the hind limb from the circulation of the remainder of the animal, by muscle transection or with ligatures, or providing a separate venous return system from the perfused isolated hind limb by cannulation of the femoral vein did not prevent the increased response to noradrenaline (Table 1A); hence the increase in response was independent of changes in the arterial or venous pressure in the remainder of the animal.

Hind-limb dose–response curve. The response of the isolated hind-limb blood vessels to a range of noradrenaline dosages was tested before and after ganglion blockade with pentolinium tartrate (4 mg). Fig. 1, which gives the mean of five

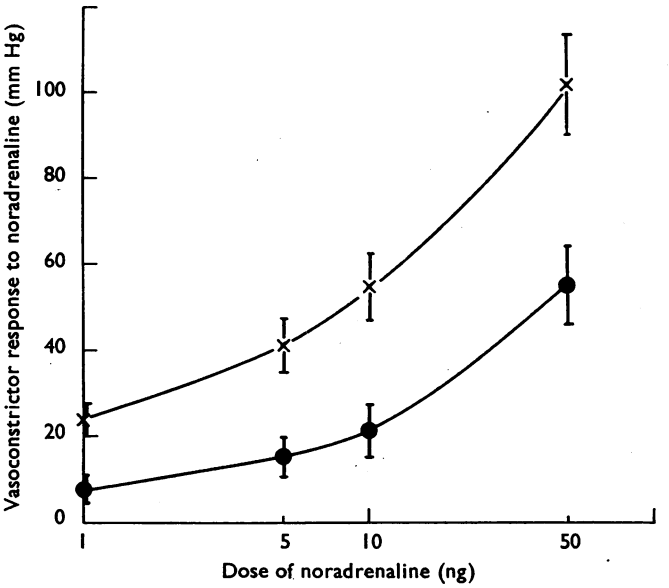

Fig. 1. Dose-response curve for the vasoconstrictor effects of noradrenaline in the blood-perfused rat hind limb. Noradrenaline dosage in nanograms (10^{-9} g). (Mean of five experiments; vertical bars give the standard error.) X——X Denervated hind limb. ●——● Innervated hind limb. The mean perfusion pressure in the innervated hind limb was 138 mm Hg; after ganglion blockade with pentolinium, the mean perfusion pressure was 76 mm Hg.

experiments, shows that the usual dose of noradrenaline (0.01 μg) did not produce a maximal response in the hind limb either before or after ganglion blockade and that the increase in response after ganglion blockade had removed the nervous vasomotor tone was observed at all dosages used. The perfused blood vessels were sensitive to small doses of noradrenaline, down to 1 ng (10^{-9} g).

Relationship between vasomotor tone and response to noradrenaline. By giving a series of small doses of hexamethonium (0.5 mg) the level of the hind-limb perfusion pressure could be altered over a wide range. The response to noradrenaline was measured at the different perfusion pressures so obtained. In 6 experiments using intact hind-limb preparations, inverse linear correlations were obtained between the noradrenaline response and the perfusion pressure at the time of the noradrenaline injection ; the correlation for all 6 experiments combined was highly significant ($r = -0.66$; $P<0.001$). Similar linear relationships were observed with the isolated hind-limb preparations (Fig. 2). Hence it appears that the peripheral reactivity to noradrenaline at any instant was related to the amount of nervous vasomotor tone as measured by the level of the perfusion pressure in the hind limb.

Experiments in the absence of a ganglion-blocking drug in the hind limb. In all the foregoing experiments the ganglion-blocking drug used to alter the peripheral resistance was also present in the blood perfusing the hind limb. To overcome this temporarily, a delay coil was inserted between the animal and the pump to provide a delay of over 7 min between the time the drug was given to the animal

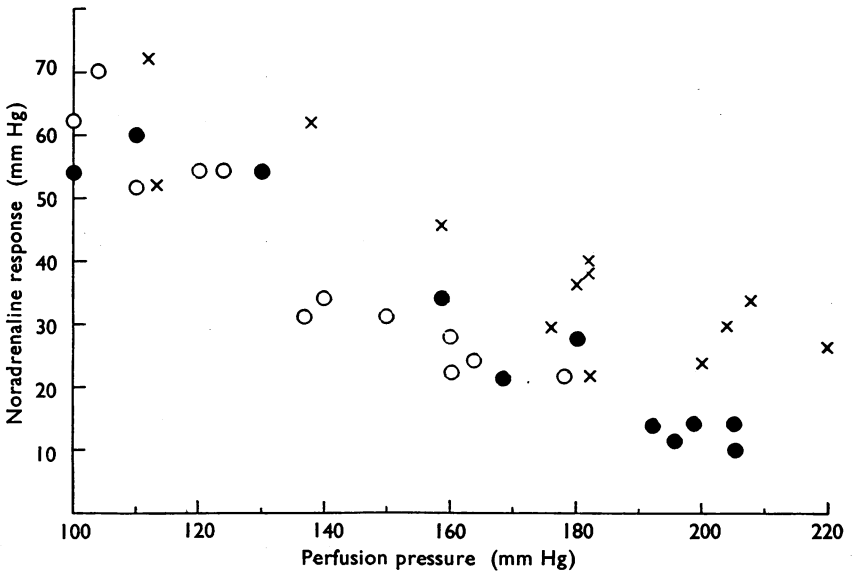

Fig. 2. Relationship between the perfusion pressure and the vasoconstrictor response to noradrenaline (0·01 μg) of the blood vessels of innervated isolated rat hind limb. The perfusion pressure was altered by ganglion blockade with small doses of hexamethonium. X Hind limb isolated by ligatures. ● Hind limb isolated surgically. ○ Isolated hind limb with separate venous return.

and the time it reached the hind limb. The response to noradrenaline was increased by ganglion blockade before the hexamethonium reached the hind limb (Table 1A).

Increased peripheral response to noradrenaline after decrease of peripheral resistance by other means

Other drugs. Using the single-animal isolated hind-limb preparation with the delay coil, the innervated hind-limb perfusion pressure could be lowered by means of other drugs. In 4 experiments adrenaline (1 to 1.5 μg) given intravenously to the remainder of the animal caused a raised blood pressure in the animal and a reflex lowering of the hind-limb peripheral resistance. In another experiment the noradrenaline response was measured while the perfusion pressure in the innervated hind limb was reduced by pentobarbitone (Nembutal, Abbott; 3 mg). In these 5 experiments the response to noradrenaline was increased during the reduction of the peripheral resistance (Table 1B); in all cases the response was measured before the drug reached the hind limb.

Nerve section. An increased peripheral response to noradrenaline was observed following section of the nerves to the isolated hind limb (Table 1C). In four of the 10 experiments hexamethonium (1 mg) had been given approximately 1 hr previously; the perfusion pressure had recovered before the nerves were cut. No difference due to the pre-treatment with hexamethonium was observed.

A difference was noticed between the rate of development of the increased response to noradrenaline after nerve section and that after ganglion blockade. After ganglion

TABLE 1

INCREASE OF THE PERIPHERAL RESPONSE TO NORADRENALINE OF INNERVATED HIND-LIMB BLOOD VESSELS AFTER REDUCTION OF THE PERFUSION PRESSURE IN THE BLOOD-PERFUSED RAT HIND LIMB (SINGLE ANIMAL EXPERIMENTS) (MEANS±S.E.)

Type	No. of expts.	No. of drug injections	C6 dose (mg)	Mean perfusion pressure (mm Hg)			Mean noradrenaline response (mm Hg)		
				Before fall of perfusion pressure	After fall of perfusion pressure	Mean % fall	Before fall of perfusion pressure	After fall of perfusion pressure	Mean % increase
A. *By hexamethonium* (C6)									
Intact hind limb	51	51	8	129·5±11·3	52·3±3·9	59·2±3·4	17·5±0·9	34·4± 1·6	116·5±13·3
Isolated hind limb	5	14	0·5–3	168·1± 8·8	118·2±9·2	29·6±3·4	23·6±2·0	45·6± 3·4	101·4±12·9
Separate venous return	3	10	0·5–5	170·5± 7·0	127·0±6·9	25·3±3·0	29·6±3·2	57·6± 3·9	106·2±19·1
No ganglion-blocking drug in hind limb	8	11	1–5	125·8± 8·8	88·0±3·9	28·3±3·3	28·3±2·8	46·1± 5·8	70·8±16·7
B. *By pentobarbitone or reflex effects of adrenaline*									
Isolated hind limb	5	6	—	126·3± 7·2	100·7±7·1	19·7±5·4	35·3±3·7	52·7±10·3	46·8±15·1

C. *By nerve section*

Type	No. of expts.	Mean perfusion pressure (mm Hg)			Mean noradrenaline response (mm Hg)		
		Before section	Immediately after section	1 hr after section	Before section	Immediately after section	1 hr after section
No C6 in blood	6	153·8±14·9	76·3±4·3	82·8±1·7	32·7±1·6	54·2± 7·0	62·4±6·5
With C6 in blood	4	144·8±22·1	78·5±9·0	—	34·5±5·6	49·0±10·3	—

blockade the increase in response to noradrenaline was usually complete within 15 to 20 min, whereas after the nerve section the response was still increasing after 30 to 40 min. In both cases the original fall in perfusion pressure was rapid and complete.

Perfusion of muscle blood vessels. In 5 single animal experiments using a hind limb with skin excluded from the circulation, hexamethonium and nerve section caused little or no fall in the perfusion pressure, indicating that there was only little resting vasomotor tone. However, an increase in the peripheral response to noradrenaline was observed in three experiments where hexamethonium or nerve section caused a fall in perfusion pressure, the maximum increase in noradrenaline response being 40%.

Effect of the origin of the perfusing blood on the increase in response to noradrenaline

In each of the experiments described above, only one animal was used and hence the blood circulating through the hind limb came from the remainder of the same animal. When a separate animal was used as blood donor (Field & Laverty, 1958), drugs given to the remainder of the recipient animal could not affect the hind limb by being present in the perfusing blood, but the results obtained using this preparation did not agree with those obtained using the single animal preparation (Laverty, 1960).

Donor and recipient animals from stock colony. When both donor and recipient animals were taken from the main Otago stock of albino rats, a fall in the perfusion pressure in the innervated hind limb caused either by nerve section or by ganglion blockade with hexamethonium was not followed by an increase in the peripheral response to noradrenaline (Table 2 ; Fig. 3).

In another series of experiments (Table 2), hexamethonium (3 to 5 mg) had been given to the donor animal before reducing the vasomotor tone. The presence of

TABLE 2

THE EFFECT OF REDUCING THE PERFUSION PRESSURE BY HEXAMETHONIUM OR NERVE SECTION ON THE RESPONSE TO NORADRENALINE OF INNERVATED RAT HIND LIMBS PERFUSED WITH BLOOD FROM SEPARATE DONOR ANIMALS (MEANS±S.E.)

* Hexamethonium given also to the separate donor animal prior to reducing the perfusion pressure

No. of expts.	Method of reducing the perfusion pressure	No. of falls in perfusion pressure	Mean % fall in perfusion pressure	Mean noradrenaline response (mm Hg)	
				Before fall in perfusion pressure	After fall in perfusion pressure
Donor and recipient animals from stock colony					
8	Hexamethonium	13	24·1±3·3	49·8±3·6	49·9±2·5
12	Hexamethonium*	19	34·0±2·8	46·7±2·7	46·7±3·7
11	Nerve section	11	39·6±4·7	35·0±2·8	34·9±3·3
Donor and recipient animals from highly inbred colony					
11	Hexamethonium	13	41·1±3·3	36·8±2·6	67·8±4·9
10	Nerve section	10	49·0±4·6	32·4±2·5	60·9±7·8

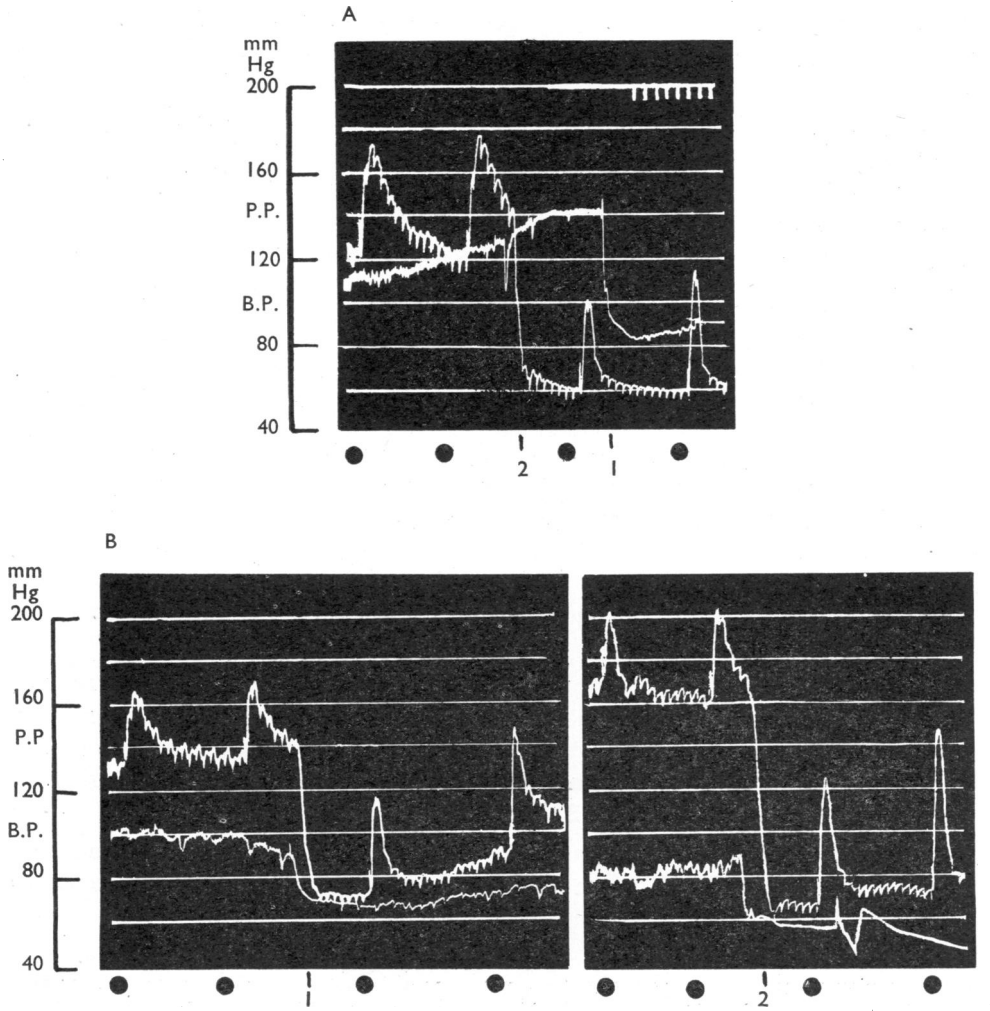

Fig. 3. The effect of the source of the perfusing blood on the vasoconstrictor response to noradrenaline of the blood vessels of the isolated innervated hind limb before and after removal of the nervous vasomotor tone in the blood vessels. Expt. A: The isolated hind limb of a stock recipient rat was perfused with blood from a separate stock donor rat. Nerve section caused no increase in the peripheral response to noradrenaline. Expt. B: The isolated hind limb of an inbred rat was perfused with blood from a genetically similar separate donor rat. Reduction in the hind-limb perfusion pressure by hexamethonium or nerve section was followed by an immediate increase in the peripheral response to noradrenaline. ● Noradrenaline (0·01 μg) into the blood entering the hind limb. (1) Hexamethonium (2 mg) given to the recipient animal. (2) Nerves connecting the hind limb to the remainder of the recipient animal cut. B.P. marks the blood pressure of the recipient rat. P.P. marks the pressure in the perfused hind limb. Time marker in min.

hexamethonium in the perfusing blood had no effect on the perfusion pressure or on the reactivity of the perfused hind limb to noradrenaline (Laverty, 1959 ; Laverty & Smirk, 1961). As may be seen from Table 2, it also had no influence on the lack of change in the response to noradrenaline after reducing the nervous vasomotor tone.

Alterations in the peripheral resistance in the innervated hind limb by carotid occlusion and administration of adrenaline or carbon dioxide to the remainder of the recipient animal caused no appreciable change in the reactivity to noradrenaline.

Highly inbred donor and recipient animals. Because of the contradiction between the above results and those obtained with the single animal preparations, identical experiments were made using highly inbred animals as separate donors and recipients. These animals were either litter mates of a strain closely inbred for 7 or more generations, or were members of a strain which had been inbred for 14 or more generations for use in tissue transplantation experiments. These genetically similar animals when used in identical experiments as donor and recipient rats gave a similar reaction on reduction of nervous vasomotor tone to that observed with the single animal preparations (Table 2 ; Fig. 3).

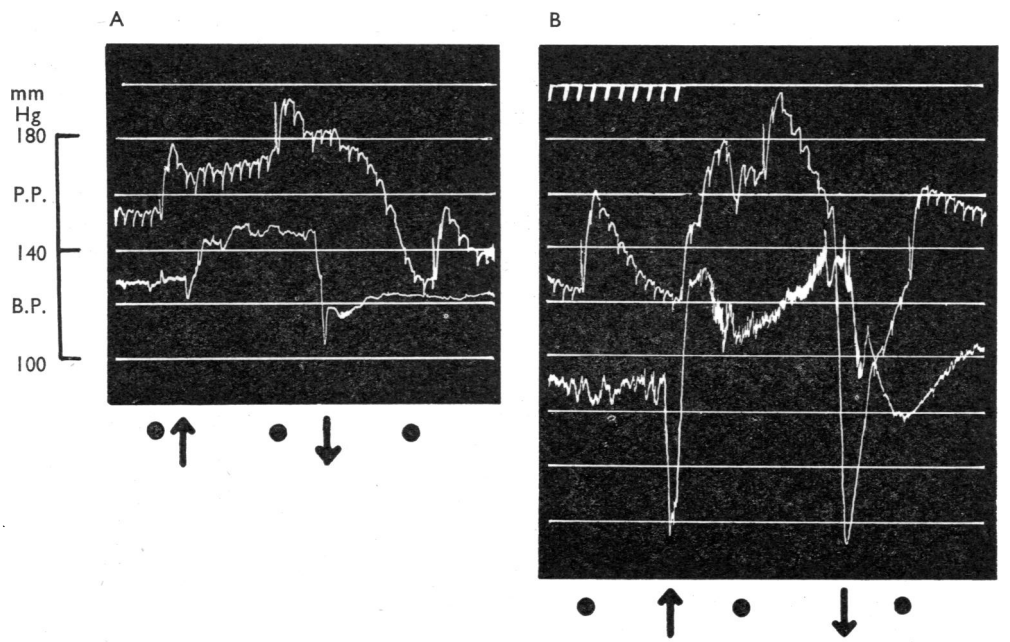

Fig. 4. Effect on the vasoconstrictor response to noradrenaline of increasing the peripheral nervous vasomotor tone in the blood vessels of the isolated innervated rat hind limb. The hind limb of the recipient rat was perfused with blood from a genetically similar separate donor rat. A: Tone increased by clamping the carotid artery of the recipient rat (the other artery was already cannulated). B: Tone increased by administration of carbon dioxide to the recipient animal. ● Noradrenaline (0·01 μg) into the blood entering the hind limb. The arrows mark the duration of the stimulus. B.P. marks the blood pressure of the recipient rat. P.P. marks the pressure in the perfused hind limb. Time marker in min.

Reduction in hind-limb resistance by administration of adrenaline or pentobarbitone instead of hexamethonium to the remainder of the recipient animal caused an increased peripheral response to noradrenaline in three experiments.

In seven experiments, raising the peripheral resistance in the innervated hind limb by carotid occlusion or by carbon dioxide administration to the recipient animal caused a decrease in the peripheral response to noradrenaline (Fig. 4).

No obvious differences in blood groups could be detected by simple direct cross-matching of the cells and plasma of bloods from different stock and highly inbred albino rats.

Peripheral reactivity to other vasoconstrictor drugs

Sympathomimetic drugs. The vasoconstrictor responses of the blood vessels of the isolated innervated hind limb to adrenaline, tyramine and ephedrine were measured in single animal experiments before and after reduction of the hind-limb perfusion pressure by hexamethonium or nerve section (Table 3). The responses to these drugs were increased by a similar proportion as those to noradrenaline.

Other vasoconstrictor drugs. The responses of the blood vessels of the isolated innervated hind limb to angiotensin (0.05 μg), vasopressin (0.2 to 1.0 m-u.) and S-methyl *iso*thiourea (1 μg) were also measured before and after hexamethonium or nerve section (Table 3). The responses to these drugs were not increased after the fall in the hind-limb perfusion pressure.

TABLE 3

THE EFFECT OF REDUCING THE PERFUSION PRESSURE BY HEXAMETHONIUM OR NERVE SECTION ON THE REACTIVITY OF PERFUSED INNERVATED HIND-LIMB BLOOD VESSELS TO OTHER VASOCONSTRICTOR DRUGS (SINGLE ANIMAL EXPERIMENTS)

Drugs	Dose	No. of expts.	No. of falls in perfusion pressure	Mean % fall in perfusion pressure	Mean drug response (mm Hg)	
					Before fall in perfusion pressure	After fall in perfusion pressure
Sympathomimetic drugs						
Adrenaline	0·01 μg	6	9	43·1±3·8	20·6±5·1	58·1±6·8
Tyramine	1 μg	6	9	33·3±4·7	13·0±1·4	28·4±3·6
	2 μg	2	5	27·0±8·1	32·6±1·8	44·8±5·0
Ephedrine	2 μg	5	5	36·6±6·3	16·8±4·2	35·6±4·1
Other vasoconstrictor drugs						
Angiotensin	0·05 μg	6	9	30·0±4·8	43·9±4·2	36·0±4·5
Vasopressin	1·0 m-u.	3	5	40·0±4·3	47·4±4·1	47·2±6·1
	0·5 m-u.	1	1	36	29	30
S-methyl *iso*-thiourea	1 μg	7	9	45·7±2·6	25·6±2·1	33·1±4·9
5-Hydroxy-tryptamine	0·02 μg	6	11	36·7±4·2	41·5±8·1	80·4±8·8
Comparison of noradrenaline, angiotensin and vasopressin in the same hind limbs						
Noradrenaline	0·01 μg	10	10	52·6±2·8	24·9±3·1	41·2±5·1
Angiotensin	0·05 μg	10	10	52·4±2·7	23·0±5·2	13·3±3·1
Vasopressin	0·2 m-u.	10	10	51·9±2·8	32·8±9·2	29·2±11·0

In a further 10 single animal experiments the responses to angiotensin, noradrenaline and vasopressin were each measured in ten hind limbs before and after nerve section (Table 3). In these hind limbs, in which the response to noradrenaline was increased by the reduction of the hind-limb perfusion pressure, the responses to angiotensin and vasopressin were unaltered or even reduced.

The response of the hind-limb blood vessels to 5-hydroxytryptamine (0.02 μg) was also measured before and after hexamethonium or nerve section. A marked increase in the response to this drug followed the reduction in the peripheral resistance.

Effect of reserpine on peripheral response. In four experiments, rats which had been pre-treated with reserpine (3 mg/kg daily) on each of the preceding two days were prepared as single-animal isolated hind-limb experiments. By comparison with control rats, the vasomotor tone was small and the responses to tyramine, ephedrine and 5-hydroxytryptamine were reduced, whereas the response to noradrenaline was increased.

The mean responses were, for tyramine (2 μg) 8 mm Hg, ephedrine (2 μg) 8 mm Hg, 5-hydroxytryptamine (0.02 μg) 15 mm Hg, and for noradrenaline (0.01 μg) 50 mm Hg. These responses were obtained at a mean perfusion pressure in the innervated hind limb of 75 mm Hg; the perfusion pressure fell to 69 mm Hg following nerve section, a mean percentage fall of only 8%. Thus pre-treatment with reserpine reduced the vasoconstrictor response to 5-hydroxytryptamine as well as to the sympathomimetic drugs, tyramine and ephedrine (Burn & Rand, 1958).

DISCUSSION

There have been two recent studies on the peripheral effects of vasoconstrictor drugs following alterations in the peripheral resistance. Zaimis and co-workers (Zaimis, 1956; Mantegazza, Tyler & Zaimis, 1958) observed in cats an increased peripheral response to adrenaline and noradrenaline after administration of ganglion-blocking drugs, which they attributed to a direct action of the ganglion-blocking drug on the sensitivity of the peripheral blood vessels to adrenaline and noradrenaline. Folkow & Öberg (1959) showed that reduction of the nervous component of the vascular tone in a perfused cat hind limb caused an increased peripheral response to noradrenaline and angiotensin, which they thought to be due to a change in the ratio between wall thickness and lumen of the perfused blood vessels. The present study suggests that these explanations do not apply to the rat.

Earlier work in this laboratory (Laverty, 1959; Blackman & Laverty, 1961) failed to show any marked increase in the reactivity to noradrenaline of rat blood vessels when hexamethonium was added to the perfusing blood. The present results show that hexamethonium in the perfusing blood is neither necessary nor sufficient to cause the increased response to noradrenaline (Tables 1 and 2), especially since an increased response to noradrenaline may be obtained when ganglion-blocking drugs are not used at all (Table 1B, C). Hence the increased response to noradrenaline after ganglion blockade is not due solely to a peripheral action of the ganglion-blocking drug.

The present experiments relate the reactivity of the hind-limb blood vessels to noradrenaline and other sympathomimetic drugs to the level of the perfusion pressure in the innervated hind limb. However, the relationship with perfusion pressure was not observed in all experiments in which the perfusion pressure was changed (Table 2), nor was it observed with all vasoconstrictor drugs (Table 3). This suggests that the peripheral response to vasoconstrictor drugs does not depend solely on the level of the hind-limb perfusion pressure and so does not depend on peripheral haemodynamic changes such as that suggested by Folkow & Öberg (1959).

A possible explanation of the present observations is that the peripheral response to noradrenaline and other sympathomimetic drugs depends on the amount of vasomotor tone of nervous origin in the peripheral blood vessels, rather than on the actual perfusion pressure or peripheral resistance in the hind limb. In these experiments, changes in the perfusion pressure induced by ganglion blockade or nerve section were directly related to changes in the amount of vasomotor tone of nervous origin in the peripheral blood vessels. The effect of this nervous tone on reactivity of the blood vessels to noradrenaline and other sympathomimetic drugs is explicable in terms of receptor saturation. In the innervated hind limb the noradrenaline receptors would be nearly fully occupied by the endogenous noradrenaline which is released by the sympathetic nervous system and which gives rise to the nervous vasomotor tone of the blood vessels. Addition of exogenous noradrenaline could then give rise to only a small response. On reduction of the nervous tone of the blood vessels by ganglion blockade or nerve section, the endogenous noradrenaline release would be reduced and so a greater number of receptors would be available for combination with exogenous noradrenaline. The gradual increase in the peripheral response to added noradrenaline with time after reduction of nervous activity may reflect a gradual increase in the number of available receptors.

The peripheral response to 5-hydroxytryptamine was increased by reduction of the nervous component of the vasomotor tone to a similar degree as sympathomimetic drugs. It has been suggested (Trendelenburg, 1960; Beleslin & Varagić, 1960) that 5-hydroxytryptamine may act through an adrenergic mechanism; the present results using reserpinized rats are not contrary to this view, but more work on this point is necessary.

The increase in reactivity in these experiments is probably not related to the marked increase in sensitivity observed after chronic nerve section (Cannon & Rosenblueth, 1949; Burn & Rand, 1959), since in this work the increase in response developed much more rapidly, being complete within 30 min after the removal of the nervous vasomotor tone.

The present results help to explain the observed differences in the potentiation of different vasoconstrictor drugs after ganglion blockade in the whole animal (Haas & Goldblatt, 1959). Sympathomimetic drugs which have a markedly greater peripheral response after ganglion blockade were also potentiated more markedly in the whole animal; the response to other drugs such as angiotensin and S-methyl isothiourea which do not have an increased peripheral response were not greatly increased by ganglion blockade in the whole animal. However, in the whole animal,

other factors such as blockade of homoeostatic reflexes and effects on cardiac output must still be considered. For instance, the response to angiotensin in the whole animal was increased by ganglion blockade whereas the peripheral response was, if anything, reduced; the response to 5-hydroxytryptamine is usually depressor in the whole rat, whereas in the hind limb it is vasoconstrictor.

Another point of interest is the difference in the reaction of the hind limb to reduction in vasomotor tone when the limb is perfused with blood from a different donor animal. Even though the separate donor animal was from the same stock colony and of the same apparent blood group as the recipient animal, an increased peripheral response to noradrenaline on reduction of vasomotor tone was not observed. When, however, under identical experimental conditions the animal supplying the blood was the same as, or very closely similar genetically to, the animal supplying the hind limb, the reduction of vasomotor tone caused an increased response to noradrenaline. The lack of increased response must be due to some reaction of the hind-limb blood vessels when perfused continuously with the blood of animals not closely inbred. Difficulties due to blood incompatibilities between dogs have already been reported (Maher, Watkins, Broadbent & Bollman, 1958; Bliss, Johns & Burgen, 1959).

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