J. Pharm. Pharmacol., 1965, **17**, 769–781 Received May 7, 1965

The significance of an isoprenaline-like metabolite in the interpretation of the responses of blood vessels in skeletal muscle to adrenaline

D. J. ROBERTS

A depressor substance, which resembled isoprenaline in Rf value, was demonstrated after chromatography of plasma collected from chloralose-anaesthetised cats during the blood pressure responses to intravenous injections of adrenaline, but the results obtained did not substantiate the hypothesis of a functional metabolite. The amount of vasodilatation that the "metabolite" would have caused in the skeletal muscles of the hind limb could not, for example, be correlated with the increases in blood flow produced by the original intravenous doses of adrenaline. The fact that adrenaline was largely vasoconstrictor in acutely denervated hind limbs was taken to indicate that the original vasodilator effects were of reflex nervous origin. It is suggested that the afferent source of this and related reflexes involves the stimulation of chemoreceptors as opposed to mechanoreceptors and that increases in flow through denervated muscles in response to adrenaline are caused by a direct action on the walls of the blood vessels.

IT is now generally recognised that the intravenous or intra-arterial administration of small doses of adrenaline produce vasodilatation in skeletal muscles while larger doses cause vasoconstriction. A variety of explanations of these opposing biphasic actions of adrenaline have been proposed; these include different responses in separate segments of the vascular bed (Dale & Richards, 1918), stimulation of different receptors in one particular vessel (Ahlquist, 1948), "partial agonist" phenomena (Burn & Rand, 1958), the involvement of nervous reflexes (Hartman & Fraser, 1917; Duff & Swan, 1951; Gruhzit, Freyberger & Moe, 1954; Bowman, 1959a) and the liberation by adrenaline of substances in the body which have vasodilator action (Whelan, 1952), such as histamine (Staub, 1946), lactic acid (Lundholm, 1956) or isoprenaline (Cobbold, Ginsburg & Paton, 1960; Glover, Greenfield & Shanks, 1962).

A substance which resembles isoprenaline in pharmacological actions and Rf value has been demonstrated in extracts of adrenal glands (Lockett, 1954; Subrahmanyam, 1959), in extracts of cat blood collected either from the pulmonary veins after stimulation of the upper thoracic sympathetic chains (Lockett, 1957) or from the aorta following the administration of adrenaline (Eakins & Lockett, 1961), and in extracts of rabbit aortic blood collected during the intravenous infusion of adrenaline (Roberts & Lockett, 1961). Eakins & Lockett (1961) consider the substance to be formed principally in the liver as a metabolite of adrenaline. More recently, Roberts (1965a) has shown that elevation of the adrenaline content of cat blood by reflex stimulation of sympathetic nerves in response to acute terminal haemorrhage is alone sufficient to cause the appearance of this "metabolite" on chromatograms of plasma extracts,

From the Departments of Pharmacology, Chelsea College of Science and Technology, and Brighton College of Technology.

Present address: Department of Pharmacology, School of Pharmacy, Portsmouth College of Technology, Park Road, Portsmouth.

and that its isoprenaline-like nature may be extended to include vasodilatation in skeletal muscle.

It seemed possible, therefore, that the dilator effects of intravenously administered adrenaline might be due to its partial conversion to this isoprenaline-like substance; the experiments described here are a direct test of this hypothesis.

Some workers have been unable to record any vasodilatation with intra-arterially or intravenously administered adrenaline, and some confusion also exists over the effects of noradrenaline on the calibre of blood vessels (see Bowman, 1959a, for references). Because some of these discrepancies might have been due to factors such as the use of different species, different anaesthetics and different recording methods, it was decided to investigate the effects of intravenous and intra-arterial noradrenaline and adrenaline in parallel with those of isoprenaline, on blood flow through skeletal muscle as measured using the apparatus and conditions now described.

Experimental

Methods

Cats of either sex, 1·4–5·6 kg, were anaesthetised with chloralose, 7·5 ml/kg of a 1% w/v solution in 0·9% w/v saline intraperitoneally or into a femoral venous cannula, after induction with ether, and prepared to enable continuous recordings to be made of carotid arterial pressure and venous outflow from the skinned left hind limb, as previously described (Roberts, 1965a). In some experiments the left sciatic nerve was exposed high in the thigh to facilitate denervation of the muscles when required. In experiments in which the effects of intravenously administered noradrenaline, adrenaline and isoprenaline were examined, the same doses were administered before and after connecting a blood pressure stabiliser (Fig. 1) to the carotid cannula. Drugs were administered intravenously by a cannula in the right femoral vein or intra-arterially by means of the polyethylene tubing in a small branch of the left femoral artery, usually the profunda femoris (Roberts, 1965a). A semimicro syringe was used for all intra-arterial injections and solutions were adjusted to pH 6·8.

For the second part of the investigation arterial blood samples were required and these were collected by an additional polyethylene catheter ('Portex' Poly 49A) introduced through the right femoral artery so that its tip lay just beyond the inguinal ligament. In these experiments, after setting up the blood flow circuit, the following procedure was adopted.

Stage 1. Blood samples were collected from the right femoral artery at rest and after a minimum of two, and a maximum of five, doses of intravenously administered adrenaline (0·5–25 μg). In each instance the plasma was separated without delay and protein and lipid-free extracts were prepared for chromatography (Roberts, 1963b).

Stage 2. The blood cells from Stage 1 were mixed with aqueous 0·9% w/v sodium chloride and infused back into the cat to re-establish the blood volume.

Stage 3. The blood pressure stabiliser (Fig. 1) was connected to the carotid artery and the effects of intravenous injections of adrenaline

(0·5–25μg) were measured on the blood flow through the left hind limb of the same preparation.

Stage 4. Adrenaline and isoprenaline (0·001–1·0 μg) were injected intra-arterially by way of the profunda femoris and the blood flow responses in the hind limb were similarly recorded. Log dose response curves were then constructed for each of these two amines.

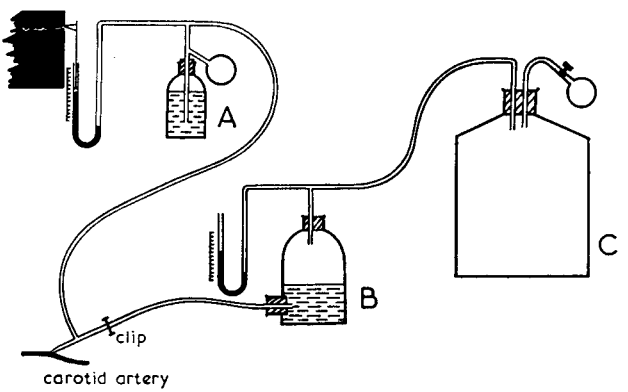

FIG. 1. Diagram of the blood pressure stabiliser. The cat's blood pressure is first measured on the mercury manometer connected to A with the clip on the tubing leading to B closed. The pressure in B, as measured on its attached manometer, is then raised to equal the blood pressure of the cat using the pump connected to C. The clip is then released and the blood pressure is effectively stabilised. A and B contain 0·9% w/v sodium chloride solution; C contains air.

Stage 5. The adrenaline and any isoprenaline-like metabolite present in the plasma extracts of Stage 1 were separated chromatographically on acid-washed papers treated with ascorbic acid (Lockett, 1957) by development in phenol containing 15% v/v 0·1 N hydrochloric acid using apparatus and materials previously described (Roberts, 1963a). Elution was conducted overnight using distilled water containing 1 mg ascorbic acid per 100 ml only as the eluant to reduce interference by 'blank' pharmacological activity (Roberts, 1964a). The eluates were then assayed on rat blood pressure preparations in terms of (–)-isoprenaline activity (pentobarbitone sodium anaesthesia) and (–)-adrenaline activity (the animals being pithed and treated with cocaine, 5 mg/kg, and pronethalol, 5 mg/kg, intravenously) respectively. A 50% recovery was assumed (Roberts, 1963b) and the assay results were adjusted accordingly.

The doses refer to the quantity of catecholamine calculated as base, but the amines were administered as salts. With the (–)-adrenaline acid tartrate and (–)-noradrenaline acid tartrate this involved simple calculations of molecular weights. In the case of (±)-isoprenaline sulphate the calculated amount of base was further divided by 2 to convert all doses in terms of (–)-isoprenaline base. The errors which must result from attributing the activity of (±)-isoprenaline solely to the laevorotatory component are not large since this isomer is several hundred times more

potent than the *dextro* form (Lands, Ludueña & Tuller, 1954). Quantitative measurements of absolute vasodilator and vasoconstrictor activities were calculated as previously described (Roberts, 1965b).

Drugs used

(−)-Adrenaline acid tartrate, (±)-isoprenaline sulphate, cocaine hydrochloride (Burroughs Wellcome & Co.), (−)-noradrenaline acid tartrate (L. Light & Co.), pronethalol (Alderlin, I.C.I. Ltd.), heparin (Pularin, Evans Medical Ltd.) and pentobarbitone sodium (Nembutal, Abbott Laboratories) were obtained commercially.

Results

Intravenously administered noradrenaline, adrenaline and isoprenaline

The influence of any one dose of any one amine on blood pressure and venous outflow varied from animal to animal but the effects of each amine were qualitatively similar in 4 out of 6 experiments. In these the resting mean arterial pressure was between 110 and 180 mm Hg and the results obtained were essentially similar to those described by Bowman (1959a). Typical records are shown in Fig. 2.

Small doses of adrenaline and noradrenaline (0·1–2·5 μg/kg) caused only increases in venous outflow, the responses increasing with increase in dose. After larger doses the increases in flow were followed by decreases in flow which became more prominent as the doses increased; the initial increases in flow then became progressively less. Whereas, weight for weight, noradrenaline produced a greater pressor response than adrenaline, the increases in blood flow were smaller.

When the blood pressure stabiliser was connected to the carotid artery, the increases in blood flow produced by intravenously administered adrenaline were reduced both in extent and duration and those produced by intravenously administered noradrenaline were abolished. In both cases the secondary decreases in flow now appeared at lower dose levels and increased with increase in dose. Intravenously administered isoprenaline caused decreases in blood flow through the skeletal muscles which were shown by means of the blood pressure stabiliser to be passive effects caused by the fall in blood pressure; when changes in blood pressure were prevented, isoprenaline caused only an increase in flow from the blood vessels of the hind limbs.

The responses to the amines were further modified by cutting the sciatic nerve high in the thigh. After this acute denervation of the skeletal muscles, and when the blood pressure had been stabilised, intravenously administered adrenaline caused a reduction in blood flow; this effect of adrenaline increased with increase in dose. When large doses (2·5 μg/kg and above) of adrenaline were injected, small increases in flow became apparent superimposed upon, and occuring 20–30 sec after, the onset of the decreases in flow (Fig. 3, upper record). Intravenous noradrenaline also decreased the venous outflow after acute

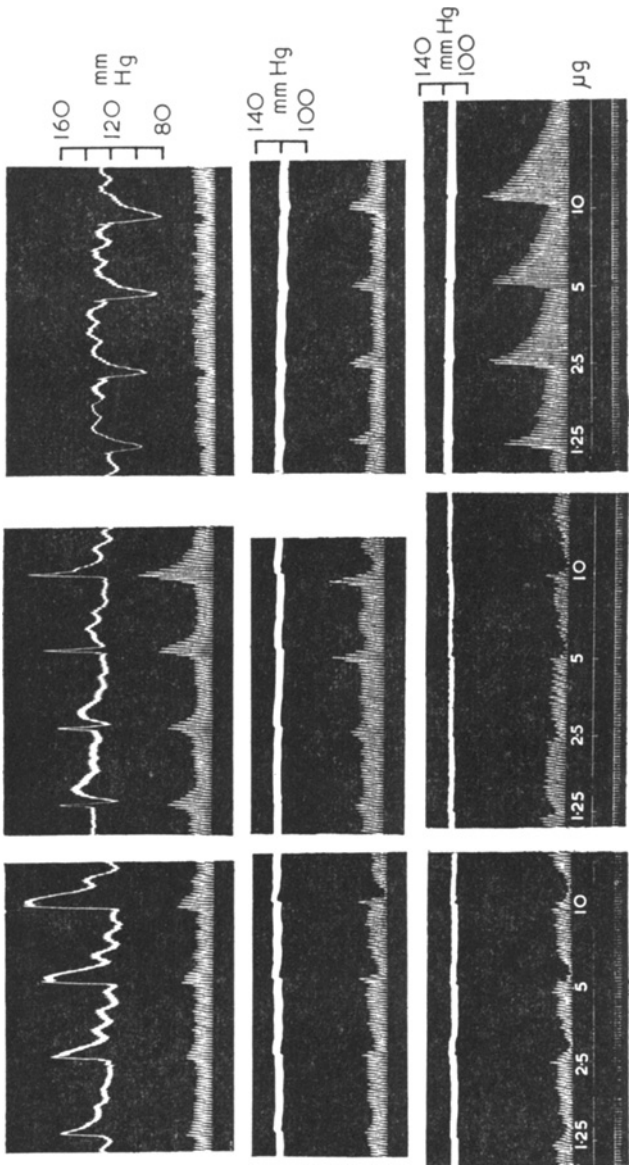

FIG. 2. Female cat, 3.3kg. The effect of stabilising the blood pressure and of acute denervation on the vascular responses in hind limb skeletal muscles to intravenously administered noradrenaline, adrenaline and isoprenaline. Upper records: innervated muscles; middle records: innervated muscles, blood pressure stabilised; lower records: acutely denervated muscles, blood pressure stabilised. On each record the upper trace is carotid arterial blood pressure and the lower trace is venous outflow. Time trace, 10 sec.

denervation, the reduction for any given dose being greater than that obtained in the innervated muscle. Increases in flow superimposed upon the vasoconstriction were only seen occasionally. In contrast, intravenously administered isoprenaline, after sectioning of the sciatic nerve, produced on most occasions a much greater increase in venous outflow than that obtained from the innervated muscle, the increase being most marked at dose levels which produced maximal vasodilatation before denervation. In some experiments the effects of pronethalol (5 mg/kg) on the vasodilator responses to the catecholamines in denervated limbs were investigated. Under these conditions increases in flow other than those occurring passively as a result of increases in blood pressure were absent.

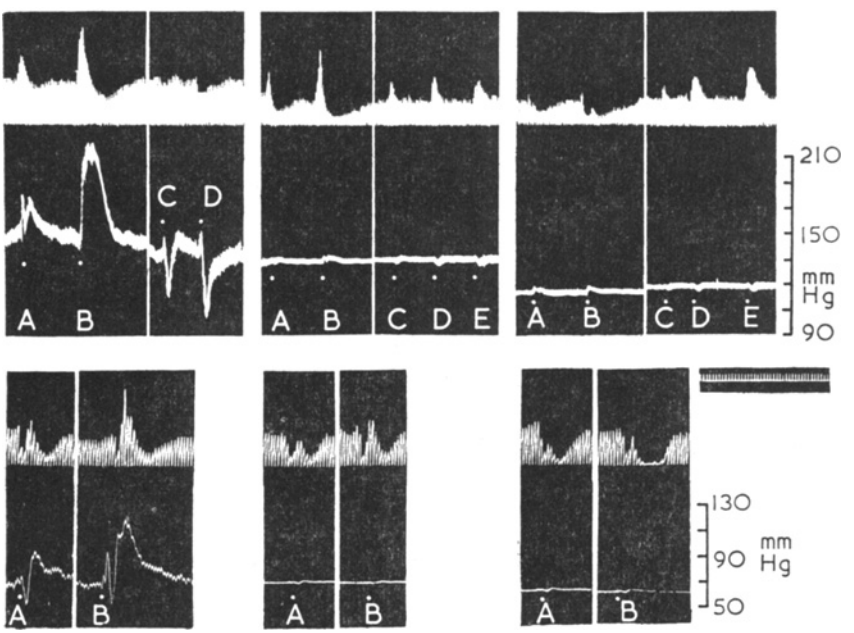

FIG. 3. Upper records, male cat 2kg; lower records, male cat 2·9kg. The effects of intravenously administered adrenaline (A, 1; B, 5 μg) and isoprenaline (C, 0·5; D, 2·5; E, 5 μg) on the blood flow through the skeletal muscles of the cat hind limb. Left-hand records: innervated muscles; centre records: innervated muscles, blood pressure stabilised; right-hand records: acutely denervated muscles, blood pressure stabilised. On each record: upper tracings, venous outflow and lower tracings, arterial blood pressure. Time trace, 10 sec.

In two experiments the resting blood pressure was low (70 mm Hg). In one of these no increase in blood flow, other than passive increases as the blood pressure rose, were observed following the administration of either noradrenaline or adrenaline. The vasoconstrictions that were observed instead increased with increase in dose and were intensified by acute denervation. The responses in the other experiment differed from the above in that increases in blood flow were observed in response

to adrenaline injected intravenously, but only as increases superimposed upon vasoconstriction. These increases were reduced by stabilising the blood pressure but persisted even after acute denervation (Fig. 3, lower record). In both experiments noradrenaline was more potent than adrenaline in decreasing the blood flow from the hind-limb and the responses to isoprenaline were essentially similar to those already described.

In all cases, the cutting of the sciatic nerve resulted in an initial increase in flow which subsequently subsided (30 sec to 5 min) to reach the initial presectional resting level.

INTRA-ARTERIALLY ADMINISTERED NORADRENALINE, ADRENALINE AND ISOPRENALINE

The resting systemic mean arterial pressure lay between 110 and 180 mm Hg in all experiments in which the effects of intra-arterially administered noradrenaline, adrenaline and isoprenaline were studied.

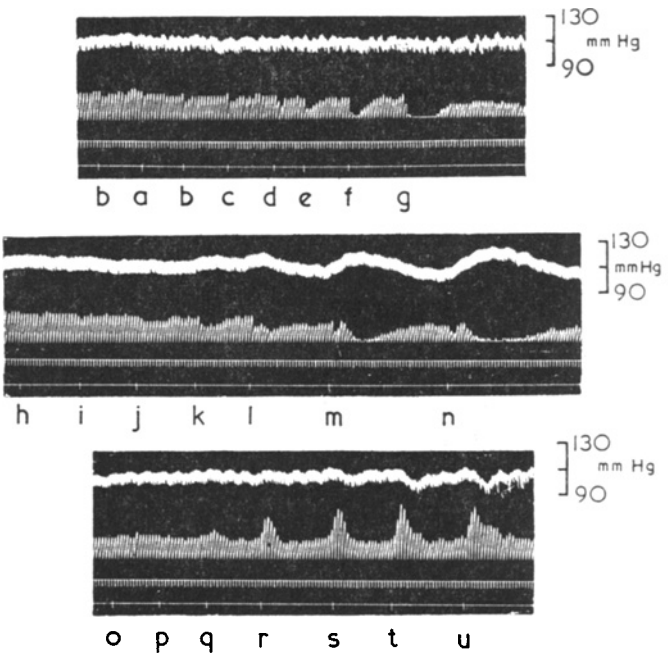

FIG. 4. Female cat, 3kg. The effect of intra-arterially administered noradrenaline (upper record), adrenaline (middle record) and isoprenaline (lower record) on the venous outflow from the innervated skeletal muscles of the cat hind limb. In each case traces show from above downwards: arterial blood pressure, venous outflow, 10 sec interval marker and injection of drug. At a, b, c, d, e, f and g, 0·001, 0·005, 0·01, 0·025, 0·05, 0·1 and 0·5 μg noradrenaline. At h, i, j, k, l, m and n, 0·001, 0·005, 0·01, 0·05, 0·1, 0·5 and 1 μg adrenaline. At o, p, q, r, s, t and u, 0·0005, 0·001, 0·005, 0·01, 0·05, 0·1 and 0·5 μg isoprenaline.

Minimal effective intra-arterial doses (0·001–0·01 μg) of adrenaline produced only a short lasting decrease in flow. This response increased as the dose was increased, but after larger doses (0·05–0·5 μg) a

short-lasting increase in flow sometimes occurred 20–30 sec after the onset of the reduction in venous outflow. On these occasions the overall picture was one of a constant increase in flow superimposed upon an increasing reduction in flow as the dose was raised (Fig. 4, middle record).

Minimal effective intra-arterial doses of noradrenaline (0·001–0·01 μg) caused only a decrease in blood flow from the skeletal muscles (Fig. 4 upper record) and this response increased with increase in dose (0·01–1·0 μg). In general, noradrenaline produced a greater reduction in venous outflow than that produced by adrenaline.

Minimal effective intra-arterial doses of isoprenaline (0·001–0·01 μg) caused only an increase in the venous outflow from the hind limb. This response increased with increase in the dose to reach a maximum at a dose of 0·1 μg and a further increase in dose merely prolonged the effect (Fig. 4, lower record). The increases in flow were abolished after treatment of the cats with pronethalol (5 mg/kg).

After acute denervation, the decreases in flow caused by intra-arterial doses of noradrenaline were slightly greater than, and the increases in flow caused by doses of isoprenaline slightly less than, those obtained in the innervated muscle.

Both the decreases and the superimposed increase in flow obtained in response to intra-arterial adrenaline were greater and longer lasting in the acutely denervated than in the innervated limb. In no experiment was the increase in flow abolished by acute denervation; by contrast, no superimposed increases in flow were demonstrable in cats treated with pronethalol (5 mg/kg).

Significance of the isoprenaline-like substance

The changes in blood flow resulting from the different intravenous doses of adrenaline were measured from the traces obtained during stage 3 of the experimental procedure described on page 770. From stages 1 and 5 the amounts of isoprenaline-like "metabolite" and adrenaline likely to be reaching the hind limb as a result of these different intravenous doses were calculated, and from the intra-arterial log dose response curves of Stage 4 the changes in blood flow that these amounts of isoprenaline-like "metabolite" and adrenaline would have caused were estimated. Sufficient information was now available to investigate whether or not the amount of isoprenaline-like "metabolite" reaching the hind limb following the intravenous injection of any one dose of adrenaline could account for the vasodilator effect of such a dose.

Unfortunately, the variations in the responses to any one intravenous dose of adrenaline in the different experiments were enormous and on occasions, particularly at the higher dose levels, very poor recoveries of amine were encountered following chromatography of the plasma extracts. The same clear general trends were apparent in all experiments, however, and these are accepted. These trends are well exemplified by the means of the observations as expressed in Table 1 and Fig. 5.

In 7 out of 10 experiments intravenously administered adrenaline caused increases in venous outflow which increased with increase in

dose to reach a maximum (5–10 μg); thereafter increase in dose resulted in smaller overall increases in flow as vasoconstriction became predominant. In the remaining three experiments minimal effective doses of intravenously administered adrenaline produced only vasoconstriction which increased in intensity with increase in dose. These experiments have been omitted from the calculations of means and standard errors expressed in Table 1 and Fig. 5.

TABLE 1. THE CONCENTRATIONS OF ADRENALINE AND ISOPRENALINE-LIKE SUBSTANCE REACHING THE HIND LIMB AS A RESULT OF INTRAVENOUS INJECTIONS OF ADRENALINE; THE RESPECTIVE VASOCONSTRICTOR AND VASODILATOR EFFECTS THAT THESE AMOUNTS OF AMINE WOULD HAVE CAUSED IN THE SKELETAL MUSCLE OF THE HIND LIMB; THE RESULTANT MEAN VASCULAR EFFECT OF THE ADRENALINE AND THE ISOPRENALINE-LIKE SUBSTANCE REACHING THE HIND LIMB AND THE VASODILATION PRODUCED BY THE ORIGINAL INTRAVENOUS DOSE OF ADRENALINE. (Figures quoted are means ± standard errors)

Adrenaline i.v.(μg)	Conc.(μg) of amine reaching hind limb		Adrenaline† (constriction)	'Isoprenaline†' (dilatation)	Mean effect† + = dilatn − = constr.	Dilatn† i.v. adrenaline
	Adrenaline	'Isoprenaline'				
None (8)*	0·005 ±0·0009	0·003 ±0·0005				
0·5 (4)	0·01 ±0·002	0·004 ±0·0007	10·25 ± 3·15	13·25 ±6·95	+ 3 ±4·38	3·25 ±0·95
1 (4)	0·034 ±0·003	0·013 ±0·003	22·5 ± 5·85	30·75 ±6·75	+ 8·25 ±3·32	5·25 ±1·33
2·5 (3)	0·04 ±0·12	0·018 ±0·002	29 ±10·69	40·67 ±5·46	+11·67 ±8·47	15·33 ±4·06
5 (6)	0·106 ±0·021	0·057 ±0·009	48·83 ±10·64	66·5 ±15·99	+17·77 ±8·51	32·71 ±5·79
10 (4)	0·255 ±0·056	0·114 ±0·034	78·25 ±32·87	75·75 ±27·07	− 2·5 ±7·84	115·5 ±28·5
25 (5)	0·433 ±0·061	0·143 ±0·081	127·8 ±25·34	82·4 ±22·28	−45·4 ±8·47	120·4 ±44·5

*No. of observations.
†The figures expressing vasodilatation and vasoconstriction are those obtained using the equation described by Roberts (1956b) multiplied by 100.

In all experiments the eluates prepared from strips taken at the isoprenaline Rf value had depressor activity on rat blood pressure preparations and with few exceptions the isoprenaline-like activity found became progressively greater as the intravenous dose of adrenaline was increased. The amounts of isoprenaline-like "metabolite" found were sufficient to have caused vasodilatation in the cat hind limb, but these calculated increases in blood flow produced by the "metabolite" could seldom be correlated with the vasodilatation produced by the "parent" doses of intravenously administered adrenaline. Furthermore, the calculated effects of the amounts of adrenaline reaching the hind limb (1–4% of the amount of adrenaline injected intravenously) were always vasoconstrictor, and since both the isoprenaline-like "metabolite" and this adrenaline must be considered to be reaching the hind limb together, the true expected response must be the mean of the two calculated effects. At dose levels of less than 5 μg of adrenaline intravenously these means represented increases in blood flow, but at higher dose levels the calculated vasoconstriction produced by the adrenaline reaching the hind limb counteracted and predominated over the calculated vasodilatation produced by the "metabolite". At no dose level could the calculated mean effect of the "metabolite" and the adrenaline reaching the hind limb be correlated with the effect of the intravenously administered adrenaline, although

at the lower dose levels an apparent correlation results from statistical treatment of all the observations (Fig. 5).

Blank eluates were not prepared during this series of experiments but the low resting levels of catecholamine (Table 1) indicated that activity other than that due to catecholamine was negligible.

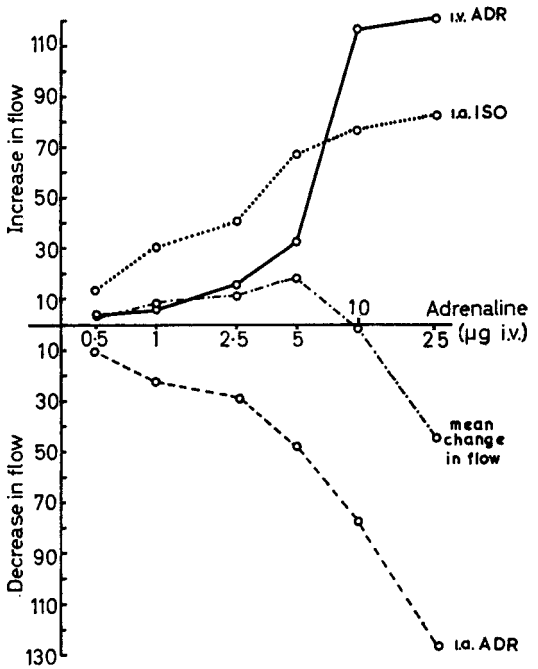

FIG. 5. The significance of the isoprenaline-like "metabolite" in the vasodilator responses to intravenously administered adrenaline. i.a. ISO and i.a. ADR respectively represent the changes in blood flow that would have been obtained from the amounts of "isoprenaline-like substance" and adrenaline reaching the hind limb following the intravenous administration of different doses of adrenaline. The difference between i.a. ISO (vasodilatation) and i.a. ADR (vasoconstriction) is the mean change in flow. The increases in blood flow caused by the original doses of intravenously administered adrenaline (measured with the blood pressure stabilised) are represented by i.v. ADR. The points plotted at each dose level are the means of several observations; the number of observations and the standard errors of these means are given in Table 1.

Discussion

The experiments described are an attempt to test directly the proposal (Cobbold & others, 1960; Glover & others, 1962) that the vasodilator effect of intravenously administered adrenaline may be due to the formation of an isoprenaline-like metabolite (Eakins & Lockett, 1961).

Intravenous injections of adrenaline in the cat do give rise to the appearance of a depressor substance at the isoprenaline Rf value on chromatograms of extracts of femoral arterial blood (Table 1) and this substance, similarly formed from the adrenaline released during haemorrhage

(Roberts, 1965a), equates fairly well with isoprenaline in assays on the blood pressure, the rat uterus and the cat hind limb blood flow. Also, the concentrations of this depressor substance reaching the hind limb usually increase with increase in the intravenous dose of adrenaline and are sufficient to cause vasodilatation. The concentrations of adrenaline reaching the hind limb are, by contrast, vasoconstrictor and at intravenous dose levels in excess of 5 μg the decreases in flow are sufficient to counteract the increases in flow due to the "metabolite" and the resultant mean effect is vasoconstriction. On the other hand, a mean effect of vasodilatation is arrived at when the lower doses, perhaps more representative of expected 'physiological' levels, are considered.

The results of other experiments indicate that most of the vasodilator effect of intravenously administered adrenaline is not mediated via the formation of an isoprenaline-like vasodilator metabolite but, in confirmation of the work of Bowman (1959a), via the nerves. Thus, in the innervated preparation with the blood pressure stabilised, intravenous doses of adrenaline produce a greater degree of vasodilatation than similar doses of isoprenaline. Also, doses of intravenously administered adrenaline causing increases in blood flow in the innervated limb result in marked decreases in flow after the sciatic nerve has been cut. Under the same conditions, the vasodilator response to intravenously administered isoprenaline is potentiated. Similarly, the results of the three experiments in which intravenously administered adrenaline caused uncomplicated vasoconstriction do not support the idea of a functional metabolite, because the intra-arterial dose-response curves and the concentrations of adrenaline and isoprenaline-like substances found in these experiments were no different from those found in the experiments where adrenaline was vasodilator.

In contrast, the increases in flow that were sometimes seen superimposed upon vasoconstriction when adrenaline was given intravenously to animals in which the blood pressure had been stabilised (Fig. 3), must be independent of nervous reflexes as they also occurred in denervated preparations. The observation that similar responses are often obtained after intra-arterial administration (Fig. 4) is compatible with a direct vasodilator action of adrenaline (Bowman, 1959a) being responsible for the superimposed increases in flow, especially as such an action has been demonstrated both in the denervated (Dale & Richards, 1918) and the isolated (Dale & Richards, 1927) perfused hind legs of cats. In this respect it is significant that the intra-arterial dose levels of adrenaline required to cause the transient vasodilatation, correspond to the concentrations of adrenaline that would be reaching the hind limb as a result of the intravenous dose levels required to produce the same effect. The absence of the superimposed increases in flow in the presence of pronethalol is also consistent with a direct vasodilator action.

Since both intra-arterial and intravenous injections of noradrenaline cause more intense vasoconstriction, but fewer superimposed increases in flow, than adrenaline, reactive hyperaemia in response to the reduction in blood flow cannot be the cause of the increases. Similarly, the fact that

the transient vasodilatations were seen at all following noradrenaline administration is inconsistent with the idea of an isoprenaline-like metabolite being responsible, because noradrenaline does not give rise to such a substance (Eakins & Lockett, 1961).

In none of the experiments described did a permanent hyperaemia result from cutting the sciatic nerve, suggesting that the vasomotor tone in the muscles may have been low (Bowman, 1959a). This does not detract in any way from the significance of the results obtained showing the importance of intact nervous connections in the vasodilator response to intravenously administered adrenaline. An "isoprenaline-like substance" is demonstrable on paper chromatograms of plasma extracts, and since it cannot be considered to be the cause of the vasodilator effects of adrenaline, its function might be to antagonise any local constrictor action of adrenaline so leaving the vasodilator reflex free to exert its full effect. Alternatively, it may be an artifact and the observation that adrenaline in the presence of hydrochloric acid can form multiple spots, one of which has an Rf value similar to that of isoprenaline (Roberts, 1964b), indicates a possible way by which the use of hydrochloric acid during extraction and chromatography of plasma samples containing adrenaline might give rise to such an artifact.

Secondary to the main investigations, the experiments described yielded information about the mechanism of the nervous reflexes influencing the blood flow responses to the catecholamines. In preparations where blood pressure changes are prevented by the use of the stabiliser, the vasodilatation caused by intravenously administered adrenaline is abolished by acute denervation while that caused by similar administration of isoprenaline is potentiated. With the blood pressure stabilised the reflex vasodilatation and reflex vasoconstriction required to explain the observations cannot originate from elevation or reduction of the pressure in the carotid sinuses. Similarly, since the inotropic cardiac action of isoprenaline is greater than that of adrenaline (Lands & Howard, 1952) any abolition of a reflex vasodilatation mediated via an inotropic cardiac effect (Gruhzit & others, 1954) would be expected to reduce the vasodilator response to isoprenaline and not potentiate it. This interpretation is, of course, subject to the limiting sensitivity of the apparatus used to produce and measure a stabilised blood pressure.

Bowman (1959b) has suggested that it is stimulation of chemoreceptors rather than mechanoreceptors that provides the afferent sources of the reflexes and the experiments of Taylor & Page (1951) have indicated that such receptors could exist in the cephalic circulation. To explain the effects of acute denervation described in this present investigation, however, two types of chemoreceptor are required. The first type must respond to adrenaline and to noradrenaline causing reflex vasodilatation, while the second type must respond to isoprenaline and cause reflex vasoconstriction. While it is tempting to classify these receptors as α-adrenotropic and β-adrenotropic respectively (Ahlquist, 1948) there is as yet no evidence for the existence of cephalic chemoreceptors responding to isoprenaline with a reflex vasoconstriction.

References

Ahlquist, R. P. (1948). *Amer. J. Physiol.*, **153**, 586–600.
Bowman, W. C. (1959a). *J. Pharm. Pharmacol.*, **11**, 104–119 and references there cited.
Bowman, W. C. (1959b). *Ibid.*, **11**, 143–149.
Burn, J. H. & Rand, M. J. (1958). *Brit. J. Pharmacol.*, **13**, 471–479.
Cobbold, A. F., Ginsburg, J. & Paton, A. (1960). *J. Physiol.*, **151**, 539–550.
Dale, H. H. & Richards, A. N. (1918). *Ibid.*, **52**, 110–165.
Dale, H. H. & Richards, A. N. (1927). *Ibid.*, **63**, 201–210.
Duff, R. S. & Swan, H. J. C. (1951). *Ibid.*, **112**, 41 P.
Eakins, K. E. & Lockett, Mary F. (1961). *Brit. J. Pharmacol.*, **16**, 108–115.
Glover, W. E., Greenfield, A. D. M. & Shanks, R. G. (1962). *Ibid.*, **19**, 235–244.
Gruhzit, C. C., Freyberger, W. A. & Moe, G. K. (1954). *J. Pharmacol.*, **112**, 138–150.
Hartman, F. A. & Fraser, Lois M. (1917). *Amer. J. Physiol.*, **44**, 353–368.
Lands, A. M. & Howard, J. W. (1952). *Ibid.*, **106**, 56–76.
Lands, A. M., Ludueña, F. P. & Tullar, B. F. (1954). *Ibid.*, **111**, 469–474.
Lockett, Mary F. (1954). *Brit. J. Pharmacol.*, **9**, 498–505.
Lockett, Mary F. (1957). *Ibid.*, **12**, 86–96.
Lundholm, L. (1956). *Acta physiol. scand.*, **39**, Suppl. 133, 1–52.
Roberts, D. J. & Lockett, Mary F. (1961). *J. Pharm. Pharmacol.*, **13**, 631–633.
Roberts, D. J. (1963a). *Ibid.*, **15**, 532–537.
Roberts, D. J. (1963b). *Ibid.*, **15**, 579–583.
Roberts, D. J. (1964a). *Ibid.*, **16**, 313–322.
Roberts, D. J. (1964b). *Ibid.*, **16**, 549–556.
Roberts, D. J. (1965a). *Brit. J. Pharmacol.*, **24**, 735–741.
Roberts, D. J. (1965b). *J. Pharm. Pharmacol.*, **17**, 245–246.
Staub, H. (1946). *Helv. physiol. acta*, **4**, 539–550.
Subrahmanyan, S. (1959). *Indian J. med. Sci.*, **13**, 305–310.
Taylor, R. D. & Page, I. H. (1951). *Circulation*, **4**, 563–575.
Whelan, R. F. (1952). *J. Physiol.*, **118**, 575–587.