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VASODILATATION IN SKELETAL MUSCLE PRODUCED BY AN APPARENT METABOLITE OF ADRENALINE

BY

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After intravenous injections into cats of adrenaline, but not of noradrenaline (Eakins & Lockett, 1961), and after infusions into rabbits of adrenaline (Roberts & Lockett, 1961) a substance appeared on chromatograms of aortic blood which resembled isoprenaline in R_F value and pharmacological activity. It was suggested (Eakins & Lockett, 1961) that this isoprenaline-like substance was a metabolite of adrenaline and that the liver was its main site of origin.

As a result of his observation that vasodilatation in skeletal muscles persisted longer after continuous intravenous infusions of adrenaline than after intra-arterial infusions, Whelan (1952) proposed that adrenaline was liberating a substance in the body which had vasodilator action.

In 1962, Glover, Greenfield & Shanks showed that in the human forearm dichloroisoprenaline blocked the vasodilator effects both of intravenously administered adrenaline and of close-arterially injected isoprenaline. They considered that their results were consistent with the hypothesis that an isoprenaline-like metabolite is formed from adrenaline and that this substance is responsible for the vasodilatation observed after the administration of adrenaline intravenously. Other interpretations of their results can be made, but in view of reports of the natural occurrence of an isoprenaline-like substance (Lockett, 1954a,b, 1957, 1959; Subrahmanyam, 1958, 1959), the idea was considered to warrant further investigation. As a first step, experiments were performed to see whether the isoprenaline-like " metabolite " would in fact cause vasodilatation in skeletal muscle for, although such an action is essential to the hypothesis, it had never been demonstrated. Also, in order to see whether the isoprenaline-like substance could arise physiologically, it was decided to make use of naturally occurring adrenaline, released as a reflex response to acute haemorrhage (Watts & Greever, 1956; Manger, Bollman, Maher & Berkson, 1957), as the parent source of the isoprenaline-like " metabolite."

METHODS

Male and female cats, 1.4 to 3.6 kg, were used. Anaesthesia was with chloralose, 7.5 ml./kg of a 1% w/v solution in 0.9% w/v saline, given by intraperitoneal injection or into a femoral venous cannula in the right hind-limb after induction of anaesthesia with ether. A tracheal cannula was inserted and heparin (1,000 U/kg) was injected intravenously.

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At 30 min after the intravenous injection of a fine suspension of harmaline (5 mg/kg) in 10 ml. of 0.9% saline, the animal was bled to death from a polyethylene catheter (" Portex " Poly 49A) introduced through the right femoral artery so that its tip lay just above the bifurcation of the aorta. The blood was collected into ice-cold centrifuge tubes and the plasma was separated without delay. The isoprenaline-like " metabolite " was isolated from protein and lipid-free extracts of plasma (Roberts, 1963b) by ascending chromatography on acid-washed papers treated with ascorbic acid (Lockett, 1957), using apparatus and materials previously described (Roberts, 1963a). Elution was conducted overnight using distilled water containing 1 mg of ascorbic acid per 100 ml. as the only eluant to reduce interference by " blank " pharmacological activity (Roberts, 1964). The eluates were compared pharmacologically with (±)-isoprenaline sulphate in parallel quantitative assays. The tests used were: (1) inhibition of the quiescent rat isolated uterus preparation contracting every 3 min in response to a fixed dose of acetylcholine, submaximal in effect; (2) depression of the carotid arterial blood pressure, measured with a Condon manometer, of rats anaesthetized with pentobarbitone sodium (Nembutal, Abbott Laboratories), 0.1 ml./100 g intraperitoneally; and (3) increase in the blood flow through the skeletal muscles of the cat hind-limb after close-arterial injection into the preparation described below.

" Resting " levels of the isoprenaline-like substance were obtained from a similar series of experiments in which aortic blood samples were again collected from cats previously treated with harmaline as above. The procedure differed from that of the haemorrhage experiments, however, in that the rate of bleeding was deliberately slowed and collection of the blood was stopped before the blood pressure had fallen by more than 20% of the original level before bleeding. In these experiments the eluates were assayed against isoprenaline on the rat blood pressure preparations only.

The blood flow circuit. Cats were anaesthetized and heparinized as before and a tracheal cannula was inserted. Blood pressure was recorded by means of a mercury manometer attached to a cannula in the right common carotid artery. The skin was removed from the left leg, and the left femoral artery and vein were located close to the inguinal ligament and separated. Throughout the ensuing experiment, the skinned left-limb was bathed in liquid paraffin.

A small branch of the left femoral artery, usually the profunda femoris, was cannulated with a very short piece of heparin-filled polyethylene tubing (" Portex " Poly 46) which was sealed with a pin. Before being tied in position the polyethylene tube was pushed into the artery until its tip was level with the junction of the cannulated artery and the femoral artery.

The blood flow in the skeletal muscles of the left hind-limb was now recorded using a polyethylene catheter (" Portex " Poly 51) in the left femoral vein to feed the blood into a silver drop-tube connected to a Thorp impulse counter (C. F. Palmer, London) which was set to return to the baseline every 5 or 10 sec depending on the rate of flow. The blood was collected below the drop tube in a 3-ml. hollow-stemmed weighing boat and returned continuously and constantly through polyethylene tubing (" Portex " Poly 53B), passed under the animal's body across the warm surface of the operating table, to the left external jugular vein. Both the boat and the polyethylene tubing were initially filled with heparin solution (5 ml., 1,000 U/ml.) and the external jugular vein was cannulated before the femoral vein. Isoprenaline and the eluates containing the " metabolite " of adrenaline were administered close arterially through the polyethylene tubing in the profunda femoris artery from semi-microsyringes (0.25 ml. capacity). Drugs: (±)-isoprenaline sulphate (Burroughs Wellcome), harmaline (L. Light) and heparin (Pularin-Evans Medical) were obtained commercially.

RESULTS

pH of solutions. In some preliminary experiments small volumes of isotonic saline injected close-arterially caused an increase in venous outflow. Because the distilled water used to make this solution was acid, the effects of *p*H on blood flow in the hind-limb were investigated.

Hydrochloric acid (0.1 N) or sodium hydroxide (0.1 N) was added to 0.9% w/v saline to produce a series of solutions ranging from *p*H 2 to 10 as determined with B.D.H. narrow-range indicator papers; 0.1-ml. volumes of these solutions were injected intra-arterially;

the left hind-limbs were not skinned in these experiments. Vasodilatation, which increased linearly with increased deviation from neutrality, was observed with both acid and alkaline solutions (Fig. 1).

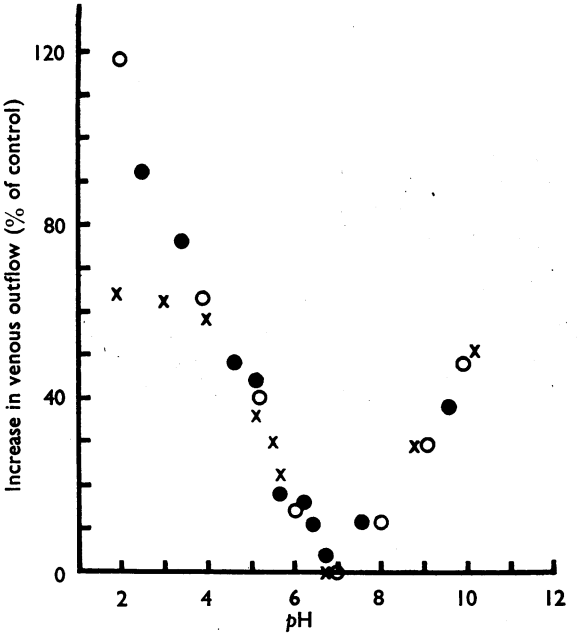

Fig. 1. The effect of *p*H (abscissa) on the increase in femoral venous outflow from the cat hind-limb (ordinate, as percentage of control). Hydrochloric acid and sodium hydroxide (0.1 ml.) were administered intra-arterially. Empty circles, female cat, 2.7 kg; filled circles, female cat, 3.2 kg; and crosses, male cat, 3.6 kg. In this last cat a 60 to 70% increase in venous outflow represented the maximum vasodilator response.

Essentially similar results to these were obtained in the dog hind-limb by Kester, Richardson & Green (1952) and Deal & Green (1954) using buffered solutions although, using unbuffered solutions, as in my experiments, they were unable to influence blood flow by varying *p*H. The observation of Kester *et al.* (1952) that the blood vessels in skin were dilated with acid but constricted with alkali, whereas those of muscle were dilated at both low and high *p*H, provides an explanation of the observation that the slope of the *p*H/response curve was steeper on the acid side than on the alkaline side when blood flow was measured in the whole limb (Fig. 1).

In order to minimize the effects of *p*H all solutions and eluates were adjusted to the same acid *p*H (as near neutrality as was possible) before bioassay on the cat blood flow preparation, and the " metabolite " was compared with isoprenaline at identical dose volumes.

Isoprenaline-like effects of the " metabolite." Similar results, in terms of the isoprenaline-like activity of the " metabolite," were obtained on each of the three preparations used for biological assay (Table 1). The responses to both isoprenaline and the " metabolite " were also qualitatively identical (Fig. 2). The " metabolite " of adrenaline, like isoprenaline, caused only an increase in blood flow through the hind-limb when injected

intra-arterially. By contrast, minimal effective doses of adrenaline administered by the same route in the same animals caused only a decrease in flow. As the dose of adrenaline was increased, vasodilator activity was, however, demonstrable but only as small increases in blood flow superimposed upon vasoconstriction (Fig. 2,*c*).

In four of the five experiments the plasma separated from the blood collected during haemorrhage was divided into 10- to 15-ml. aliquots and treated individually until the final

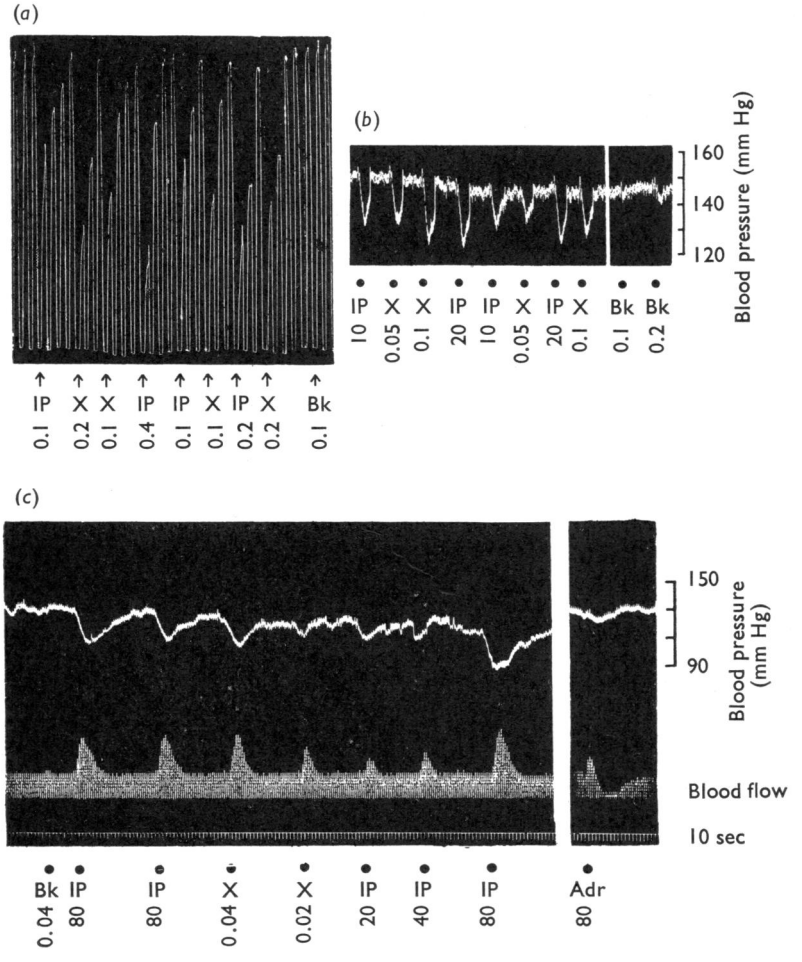

Fig. 2. Comparison of the isoprenaline-like " metabolite " (×) obtained from cat plasma with (±)-isoprenaline sulphate (IP) in parallel biological assays. (*a*), Record of a rat isolated uterus preparation contracting every 3 min in response to 0.5 μg of acetylcholine acting for 30 sec; at the arrows, inhibitory drugs were applied 30 sec before the next addition of acetylcholine. (*b*), Arterial blood pressure of a rat anaesthetized with pentobarbitone sodium dosed intravenously at 5-min intervals. (*c*), Arterial blood pressure and venous outflow from the skeletal muscle of the hind-limb of a cat anaesthetized with chloralose; responses are to intra-arterial administration. Doses of isoprenaline sulphate are in ng. Doses of × and blank (Bk) are: on the rat uterus, ml. of a 1 in 1,000 dilution; on the rat blood pressure, ml. of a 1 in 10 dilution; and, on the cat preparation, ml. of the appropriate eluates. Extreme right of (*c*), 80 ng of (−)-adrenaline acid tartrate (Adr).

stage when the individual solutions in ethanol saturated with sodium chloride were bulked, evaporated to dryness and re-extracted with a mixture of acetone and ethanol before application to the chromatography paper. In the remaining experiment the plasma was bulked before extraction began and was treated as one sample. The quantity of isoprenaline-like substance found in this experiment (Table 1, cat No. 5) was much smaller than that found in the other experiments and estimation on the cat blood flow preparation was not possible. The extract was strongly alkaline (*p*H 9).

TABLE 1

CONCENTRATIONS OF "ISOPRENALINE-LIKE SUBSTANCE" SEPARATED FROM CAT PLASMA FOLLOWING TOTAL HAEMORRHAGE AFTER INTRAVENOUS INJECTION OF HARMALINE (5 MG/KG)

Concentrations are in μg of (−)-isoprenaline base per 100 ml. of plasma. * Blank activity on cat blood flow in Expt. 3 was equivalent to approximately 0·18 μg of (−)-isoprenaline base per 100 ml. of plasma

Expt. No.	Sex	Weight (kg)	Plasma volume (ml.)	Test preparation		
				Rat blood pressure	Rat uterus	Cat blood flow
1	♀	2·8	60	1·67	1·63	1·77
2	♀	3·1	72	0·94	0·95	1·08
3	♀	1·4	42	0·33	0·29	0·58*
4	♂	2·9	66	0·82	0·99	0·85
5	♂	3·6	66	0·09	0·08	—

Only small amounts of isoprenaline-like substances were detected after chromatography of the plasma samples collected under "resting" conditions (see Methods). In terms of (−)-isoprenaline base (anhydrous) the actual levels obtained from three experiments were 0.09, 0.13 and 0.15 μg/100 ml. of plasma respectively.

Blank activity. Control eluates were prepared from strips cut from the chromatograms at R_F values either greater than that of isoprenaline or intermediate between those of adrenaline and isoprenaline. It was possible to demonstrate pharmacological activity in all of these eluates, and this activity (fall in the mean arterial blood pressure of the rat, inhibition of acetylcholine-induced contractions of the isolated rat uterus and vasodilatation in the cat hind-limb) was qualitatively similar to that of isoprenaline. The possibility therefore existed that the isoprenaline-like activity of the "metabolite" was due to an artifact of the chromatographic technique, but for the following reasons this was considered to be unlikely.

When the eluates prepared from areas of paper above the isoprenaline R_F values were diluted to the same extent as the "metabolite" eluates were before bioassay, their activities on the rat blood pressure and rat uterus preparations were reduced to negligible proportions. Although similar dilution of eluates prepared from areas of paper of R_F value intermediate between those of adrenaline and isoprenaline did not, on occasion, render the eluate pharmacologically inactive, dose volumes equated with isoprenaline on rat blood pressure preparations proved to be some three- to five-times more potent than isoprenaline in inhibiting acetylcholine-induced contractions of the rat uterus.

The vasodilator activity of the blank eluates was, on all but one occasion (Expt. 3), abolished by neutralization, whereas after similar neutralization the "metabolite" eluates still increased blood flow through the cat hind-limb; solutions of low *p*H which caused

considerable vasodilatation in the blood flow preparation were relatively inactive in tests on the rat blood pressure and rat uterus.

The only eluates that were both qualitatively and quantitatively " isoprenaline-like," therefore, were those prepared at the isoprenaline R_F value.

DISCUSSION

The isoprenaline-like activity (Fig. 2) demonstrated after chromatography of plasma separated from blood collected during massive haemorrhage was found in concentrations (Table 1) in excess of the mean resting concentrations of 0.12 μg/100 ml. of plasma (this paper) and of 0.27 μg/100 ml. of plasma (Eakins & Lockett, 1961) for cats anaesthetized with chloralose and treated with harmaline as a monoamine oxidase inhibitor. Eakins & Lockett (1961) were able to increase the arterial plasma concentrations of the isoprenaline-like substance by intravenous injections of adrenaline and it seems likely therefore that adrenaline, released as a reflex response to haemorrhage, is also the source of the depressor and vasodilator substance obtained in my experiments. The identity of this " metabolite " remains a problem, however, as it is difficult to envisage any metabolic route, other than demethylation with subsequent isopropylation, by which adrenaline could be converted to isoprenaline, and Eakins & Lockett (1961) were unable to increase the plasma concentrations of the isoprenaline-like substance by intravenous injections of noradrenaline (demethylated adrenaline). On the other hand, the fact that quantitative isoprenaline-like activity is only demonstrable at the isoprenaline R_F value makes it equally difficult at present to envisage any alternative structure for the " metabolite." Whatever its structure, however, it now seems certain that the isoprenaline-like substance only appears in significant amounts on paper chromatograms of plasma extracts when the adrenaline concentration of the blood has been raised. As the amount of adrenaline released into the circulation as a result of haemorrhage depends upon the extent of stimulation of sympathetic reflexes which will not be constant in different experiments, the amount of isoprenaline-like substance found will also vary (Table 1). In Expt. 5, however, only trace amounts of the " metabolite " were found and either reflex activity was not elicited by haemorrhage or the " metabolite " was destroyed during the extraction. The bulking of the plasma before extraction in this experiment resulted in less hydrochloric acid being used per ml. of plasma than in the other experiments and the alkalinity of the final extract would appear to be due to insufficient acid being present to counteract the high *p*H of the plasma resulting from the use of harmaline. Catechol amines are unstable under alkaline conditions.

The present experiments have demonstrated that the isoprenaline-like substance has a pure dilator action on the blood vessels of voluntary muscle and if, in fact, it is formed in the liver as a metabolite of adrenaline, as suggested by Eakins & Lockett (1961), then the observations of many workers that the vasodilatation produced by intravenously administered adrenaline is very much greater than that which can be produced by any dose given intra-arterially may be explained.

SUMMARY

1. It has been proposed that an isoprenaline-like metabolite of adrenaline is responsible for the vasodilatation observed in skeletal muscle after the intravenous injection of adrenaline. The vascular activity of this metabolite has been investigated as its vasodilator activity, required by the above hypothesis, had not been demonstrated.

2. Cats, anaesthetized with chloralose and treated with harmaline, were rapidly bled from the lower abdominal aorta to increase blood adrenaline levels by reflex sympathetic activity, and any isoprenaline-like substance was isolated from protein- and lipid-free extracts of plasma by ascending paper chromatography in a phenol-hydrochloric acid mixture.

3. Eluates corresponding to isoprenaline in R_F value caused vasodilatation in skeletal muscles when injected intra-arterially into cats in which femoral venous outflow was continuously recorded.

4. Parallel quantitative assays distinguished between the activity of the metabolite and that of control eluates and acid solutions.

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