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THE LOCAL METABOLIC ACTION OF ADRENALINE ON SKELETAL MUSCLE IN MAN

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The relation between the vascular and the metabolic effects of adrenaline has recently been emphasized by Lundholm (1956), who has advanced the proposition that the dilator action of adrenaline on the vessels of skeletal muscle is indirect and mediated by release of lactic acid. Although it is well known that adrenaline promotes lactic acid formation in animal muscle (Griffith, 1951; Lundholm, 1956), evidence on this point in the human is lacking. Several investigators have suggested that adrenaline may release lactic acid from human skeletal muscle (Barcroft & Cobbold, 1956) but Hildes, Purser & Sherlock (1949) were unable to demonstrate a direct metabolic action of adrenaline on the muscles of the lower limb in man. They could find no increase in the lactic acid level of the femoral venous blood and no reduction in glycogen content of biopsy specimens from gastrocnemius muscle following infusions of adrenaline into the femoral artery.

Using adrenergic blocking agents, de la Lande & Whelan (1959) have demonstrated a potent dilator action of adrenaline when administered locally in the forearm, and the findings are consistent with the view that a metabolite released from muscle might be responsible for this effect.

The possibility of a direct local metabolic effect of adrenaline on human muscle has been re-examined in the upper limb where more precise correspondence of infused tissue and sampling site can be ensured than in the leg. Changes in the levels of a number of metabolites have been found in the venous effluent, which indicate a direct glycogenolytic action of adrenaline on human skeletal muscle.

METHODS

The subjects were ourselves, our colleagues and volunteer medical students. The laboratory was maintained at a temperature of 23–24° C and the subject rested on a couch for at least 30 min before observations began.

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A polythene catheter ('Intracath', Bard) was inserted through a wide-bore needle into an antecubital vein of one arm and passed down into a deep vein carrying blood from forearm muscle (Roddie, Shepherd & Whelan, 1956) and maintained free from clotting by the occasional injection of 0·1 ml. heparin in NaCl 0·9 g/100 ml. (25 i.u./ml.). In a few experiments a catheter was also inserted into a superficial vein draining blood from the skin of the forearm. The oxygen saturation of samples of blood withdrawn through the catheter before, during and after a period of rhythmic exercise lasting one minute served to check that the catheter was inserted into a vein draining muscle. The oxygen saturation of the samples was measured by the spectrophotometric method described by Roddie, Shepherd & Whelan (1957). The deep-muscle blood saturation showed a sharp fall during exercise and rose above the resting value after exercise ceased.

A 23 gauge, short-bevel needle was next inserted into the brachial artery at the elbow through an area of skin anaesthetized by 2 % lignocaine, and was connected by a length of polythene tubing to a constant-infusion apparatus which delivered NaCl 0·9 g/100 ml., containing ascorbic acid (1:50,000), at a rate of 1 ml./min.

A water-filled venous occlusion plethysmograph at 35° C was then applied to the forearm and blood-flow recordings begun, the circulation through the hand being arrested by a pneumatic cuff at the wrist inflated to 200 mm Hg.

After a control period of 10–15 min, during which 3 or 4 flow measurements were made each minute, an infusion of adrenaline in ascorbic-acid saline, in a dose of 0·05 μg/min was given into the brachial artery for a period of 10 min, flow recordings being continued throughout. This infusion demonstrated that the forearm was behaving in a characteristic fashion to adrenaline (Whelan, 1952).

Thirty minutes was allowed to elapse for recovery from this adrenaline infusion. The plethysmograph remained in position throughout the experiments, to maintain the temperature of the forearm segment. Samples of muscle venous blood were taken through the catheter into nylon syringes, for estimation of the levels of blood lactic acid, blood pyruvic acid, blood phosphate and plasma potassium. Two or three such groups of resting samples were taken with an interval of 5 min, and then an infusion of adrenaline (0·05 μg/min) was given for 10 min and sampling continued at various intervals during the infusion and in the 10 min after it was discontinued. Flow measurements were not made during this infusion, as it was desired to avoid venous congestion in the sampling periods, but the wrist cuff was inflated throughout to 200 mm Hg to exclude the hand circulation.

After a further interval of 10–15 min a third infusion of adrenaline made up in 0·9 % NaCl containing Evans Blue dye (T 1824) (0·5 mg/min), or an infusion of dye alone, was given into the brachial artery and samples of the muscle blood withdrawn through the catheter before and during the infusion. These samples were centrifuged and the plasma examined for dye content.

In a number of experiments the infusion of adrenaline during which blood flow was measured, and the forearm exercise test for position of the catheter, were both carried out at the end of the experiment, and the venous sampling during adrenaline infusion was made before any muscle activity or previous adrenaline infusion. Similar results were obtained in both patterns of experiment. In a few of the experiments samples of brachial artery blood were taken in the preliminary resting period and immediately after the infusion of adrenaline ceased. Arterial samples were not taken during the infusion period, as this would have necessitated interruption of the infusion.

Blood lactic acid was measured by the method of Barker & Summerson (1941), phosphate by the method of Fiske & SubbaRow (1925), pyruvic acid by the method of Friedemann & Haugen (1943) and plasma potassium by flame photometry. For the phosphate estimations blood samples were weighed for greater accuracy and values were reproducible to within 0·1 mg/100 ml.

RESULTS

Figure 1 shows the concentrations of lactic acid in the venous effluent from the forearm muscles before, during and after infusions of adrenaline into the brachial artery in six different subjects. In every case there was a gradual rise during the infusion to a peak level which ranged from 36 to 173% above the resting level. In two cases the highest value was not obtained until some minutes after the infusion ceased. The concentration of lactic acid returned only slowly to the resting level and was still elevated 10–20 min after the end of the infusion.

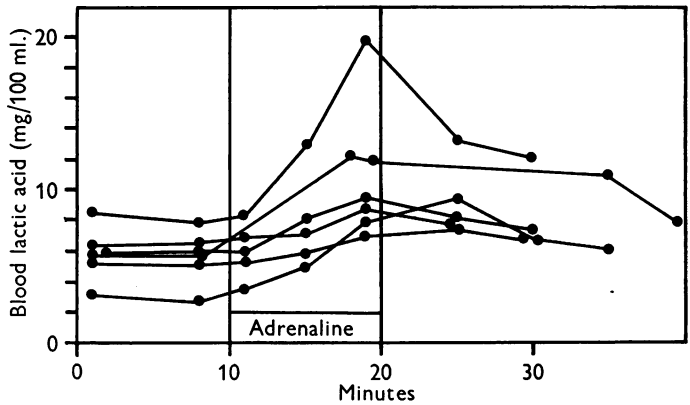

Fig. 1. Concentrations of lactic acid in the venous blood returning from the forearm muscles in six different subjects before, during and after infusion of adrenaline into the brachial artery. Adrenaline (0·05 μg/min) was infused between the two vertical lines.

The changes observed in the concentrations of the other metabolites in venous blood during adrenaline infusion were as follows: the concentration of pyruvic acid rose (3 experiments) while that of phosphate fell (5 experiments). Potassium showed a transient rise, corresponding in time to the period of transient vasodilatation, and then fell below the initial resting level for the remainder of the infusion (7 experiments). The results of a typical experiment illustrating the above changes, together with changes in forearm blood flow, are shown in Fig. 2. The 'after-dilatation' (Whelan, 1952) was seen following most infusions and blood flow only returned to the pre-infusion resting level after 10–20 min during which the metabolites also returned towards resting levels.

In Table 1 all the data on all the subjects are shown. The resting value (*A*) is the mean of the control values taken during the 10 min period before the adrenaline infusion began. The value (*B*) is that obtained when the change from resting was at its greatest, which in most cases was during

the last 3 min of the adrenaline infusion, but in a few was in the first 5 min after the infusion ceased. In every case the pattern of change in the metabolites measured was the same as that in TR, illustrated in Fig. 1, differences being quantitative only. The arterial concentrations were not

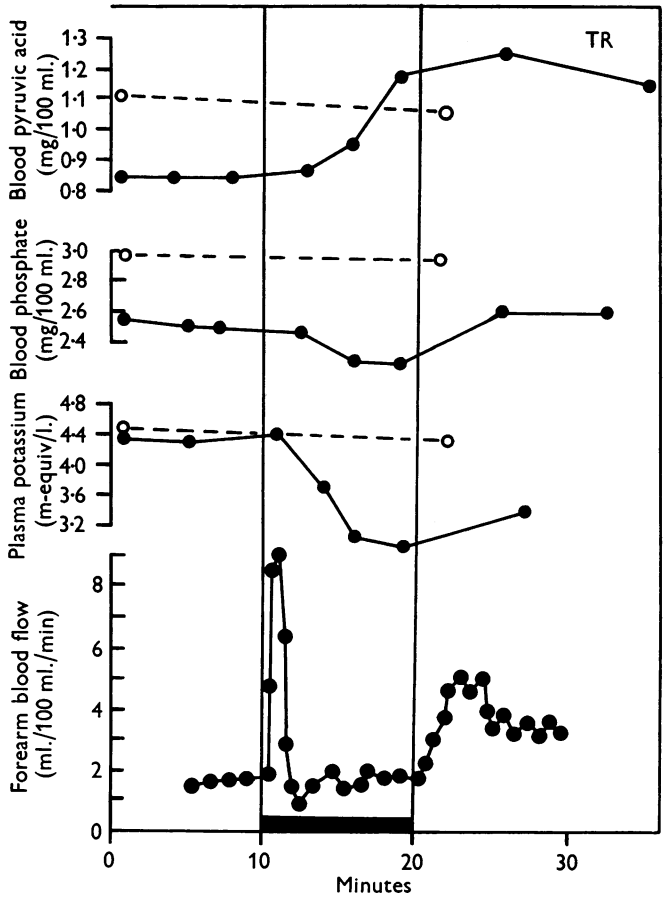

Fig. 2. The changes in the venous effluent from the forearm muscles in blood pyruvic acid, blood phosphate, plasma potassium and forearm blood flow during infusion of adrenaline (0·05 μg/min) into the brachial artery in subject TR. O---O = values for brachial arterial blood for each of the constituents mentioned. The infusion of adrenaline is indicated by the black rectangle.

measured in every experiment but sufficient determinations were made to indicate that a slight positive arteriovenous difference existed in pyruvic acid, phosphate and potassium in the initial 'resting' period. At the height of the changes caused by adrenaline the venous levels of lactic acid and pyruvic acid rose above the corresponding arterial levels, indicating an increased output of these constituents from the muscles. In the case of

potassium and phosphate a positive uptake occurred, since the arterio-venous differences increased during the adrenaline infusion.

In one experiment (ISD(3)) in which blood samples from a superficial vein draining the skin of the forearm were taken at the same time as samples from a deep-muscle vein during intra-arterial adrenaline, no changes occurred in the lactic, pyruvic or potassium levels of the superficial

TABLE 1. Effect of intra-arterial infusions of adrenaline (0·05 μg/min) on venous blood levels of metabolic products from forearm muscle and skin

	Lactic acid (mg/100 ml.)		Potassium (m-equiv/l.)		Pyruvic acid (mg/100 ml.)		Phosphate (mg/100 ml.)	
Subject	*A*	*B*	*A*	*B*	*A*	*B*	*A*	*B*
			Muscle blood samples					
AH	6·0	9·7	—	—	—	—	—	—
ISD (1)	2·9	8·0	—	—	—	—	—	—
JG	8·2	19·9	—	—	—	—	—	—
MD	5·3	7·2	—	—	—	—	—	—
AGS	6·3	9·2	3·8	3·1	—	—	—	—
JM	5·6	12·0	3·8	2·7	—	—	2·5	1·3
ISD (2)	—	—	3·9	3·4	—	—	—	—
RS	—	—	3·9	3·4	0·42	0·92	3·1	3·0
TR	—	—	4·4	3·0	0·85	1·26	2·5	2·3
BP	—	—	3·6	3·0	—	—	2·6	2·4
ISD (3)	—	—	4·9	3·7	0·71	1·04	3·3	3·1
			Skin blood samples					
ISD (3)	10·5	11·1	4·4	4·5	—	—	3·3	3·3
PH	13·6	13·9	4·1	4·0	1·70	1·35	—	—
LRM	—	—	4·1	4·2	1·32	1·22	2·5	2·5

A, mean of 2 samples during pre-infusion period; *B*, value obtained during the last 3 min of adrenaline infusion or within a few minutes thereafter, when the change from *A* was at its maximum.

blood while the usual changes in these metabolites were observed in the muscle blood. In two experiments superficial blood samples only were taken, and no changes in metabolite levels were produced by adrenaline infusions. In each experiment Evans Blue dye was detected in the returning venous blood on subsequent infusion of adrenaline solution containing the dye.

DISCUSSION

The changes in the levels of lactic acid, potassium, pyruvic acid and phosphate in the venous effluent from the forearm muscles during intra-arterial infusions of adrenaline are indicative of a direct effect of adrenaline on the metabolism of human skeletal muscle. The finding is directly contrary to the observations of Hildes *et al.* (1949), who could detect no changes in metabolite concentrations in the femoral venous blood during infusions of adrenaline into the femoral artery in the groin nor any reduction in glycogen content of biopsy tissue from gastrocnemius muscle.

The indication that adrenaline was in fact perfusing the limb was the blanching of the skin noted during the infusion. There was no evidence, however, to suggest that adrenaline was perfusing the calf muscles, or, if it were, that the femoral vein samples were in fact coming from the same area of the calf, nor that the biopsies were taken from an area perfused by adrenaline or drained by the femoral vein.

All these factors are particularly important in the case of the lower limb, where the site of injection of adrenaline and of sampling lay at a considerable distance from the muscle concerned. The opportunities for localization of an arterially infused solution into an area of the thigh, for example, are considerable, as is evidenced by flushing confined to distinct areas usually on the lateral or medial aspect of the thigh during femoral intra-arterial infusions of vasodilator drugs (Marshall & Whelan, 1956).

In the present series of experiments the upper limb was used and the site of arterial infusion was much closer to the muscle group under study. Localization of the infused drug in a small area, however, can still occur in the upper limb. That it did not do so in the experiments described is demonstrated, first, by the fact that the characteristic blood-flow changes always occurred in the forearm segment immediately distal to the needle and, secondly, by the fact that dye injected along with adrenaline into the artery was recovered from the blood sampled by the catheter. That a catheter inserted as described into a deep forearm vein samples mainly muscle venous blood has been shown by Roddie *et al.* (1956), and was checked in each experiment by the changes in venous oxygen saturation accompanying exercise of the forearm muscles.

No correction of the values obtained for lactic acid and other substances assayed was made for changes in blood flow accompanying the adrenaline infusions, since with the dose chosen (0·05 μg/min) the blood flow through the forearm segment during the greater part of the infusion was little different from the resting value. Griffith, Lockwood & Loomis (1946) were able to demonstrate increases in venous blood lactic acid on intra-arterial infusion of adrenaline in the perfused limb of the cat only if the dose did not produce marked vasoconstriction in the limb. Adrenaline given by this route into the human arm is known to produce vasoconstriction of the vessels of the hand, which is mainly skin (Barcroft & Swan, 1953). Evidence about the effect of intra-arterial adrenaline on skin and muscle vessels in the human forearm is lacking. If the forearm skin vessels were constricted in the present experiments, the fact that the total forearm blood flow showed little or no change may indicate that the muscle blood flow was increased. Such an increase would tend to dilute the concentration of any metabolite released from the muscles and thus lead to an underestimate of the amount released, but it would also have the effect of causing

an apparent rise in the concentration of any substance with a positive 'resting' arteriovenous difference by tending to move its venous concentration towards the arterial level. It could not, however, account for a rise above the arterial level, such as occurred with lactic and pyruvic acids, nor for a fall in venous concentration, as occurred in the case of potassium and inorganic phosphate. The finding that no changes occurred in the metabolite levels of blood returning from a superficial vein draining skin, at a time when there were changes in the muscle blood levels, indicates that the metabolic action of adrenaline is confined to the muscle and also that the changes, for example, in plasma potassium could not be accounted for by any action of adrenaline in causing shifts of this electrolyte between the plasma and the red cells.

In the case of lactic and pyruvic acids the findings are in accord with other evidence that adrenaline promotes an increase in glycogenolytic activity in muscle (Ellis, 1956, 1959). The changes in potassium and phosphate may also be linked with glycogenolysis, but direct biochemical evidence on this point is lacking. An ability of adrenaline to depress potassium and phosphate loss from skeletal muscle *in vitro* and to promote potassium influx in smooth muscle and uptake of phosphate into muscle *in vivo* has been reported by a number of investigators (Ellis, 1959). Hence the metabolic effects of adrenaline which have been observed in the human in the present experiments are consistent with the observations on other species *in vivo* and on isolated tissues *in vitro*.

The present finding that the 'after-dilatation' seen in the forearm following cessation of intra-arterial infusion of adrenaline subsides over a period of 10–20 min, and is paralleled by the return of the metabolite levels towards their respective base-line values suggests that the two phenomena are intimately related. Lundholm (1956) has proposed on the basis of animal studies that the glycogenolytic action of adrenaline may be related to its dilator effect on vascular smooth muscle, but whether lactic acid, or indeed any of the products of the metabolic action of adrenaline, participates in the vascular responses in the human forearm remains to be decided.

SUMMARY

Infusions of adrenaline into the brachial artery in man cause an increase in glycogenolytic activity in the forearm muscles which is manifested by an increase in lactic and pyruvic acid and a fall in potassium and phosphate levels in the effluent venous blood.

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