EFFECT OF DESYMPATHIZATION AND CATECHOLAMINES ON METABOLISM IN THE AURICULAR ARTERY OF RABBITS

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The effect of blocking the sympathetic innervation of the auricular artery on the activity of the following oxidoreductases in the vessel wall was investigated by quantitative histochemical methods in rabbits: glucose-6-phosphate (G6PD), lactate (LD), isocitrate (ICD), and succinate (SD) dehydrogenases. Blocking sympathetic influences leads to a sharp fall in the activity of all these enzymes. Adrenalin and noradrenalin differ in their effect on dehydrogenase activity. Experiments with perfusion of the intact vessels showed that adrenalin increases LD activity but does not affect ICD and G6PD activity. Noradrenalin, on the other hand, increases both LD and G6PD activity. In the case of the desympathized vessel the action of noradrenalin depends on its site of application and it acts from the intima or adventitia.
KEY WORDS: artery; catecholamines; desympathization; enzyme activity.

Regulatory influences of the sympathetic nervous system on the blood vessels are not confined to the control of their contractile activity but extend also to metabolism [2, 3, 12, 15]. The vasomotor function of the sympathetic nerves has now been investigated relatively completely, but information on the sympathetic regulation of metabolic processes in the blood vessels is very scanty and, in some cases, contradictory [6]. The problem of the effect of catecholamines on metabolism in the vessel wall has also been inadequately studied [5, 9, 10, 13, 16]. Biochemical investigations have been carried out chiefly on vessels of elastic type, and using mainly adrenalin (A), not noradrenalin (NA), the true mediator of vasomotor nerves. Very little evidence is available on the effects of blocking the sympathetic innervation on metabolism in blood vessels [14].

In the investigation described below the effect of desympathization on metabolism in the vessel wall and the effect of exogenous catecholamines (A and NA) on intact and desympathized vessels were studied. Noradrenalin reached the vessel either from the intima or from the adventitia. The choice of these two pathways of entry of NA into the vessel was based on the specific localization of the terminal adrenergic plexus on the medioadventitial boundary, on the surface of the muscular coat, and data showing differences in the contractile response of the artery to NA depending on whether it enters the vessel from without or within [8, 11].

EXPERIMENTAL METHOD

Experiments were carried out on the central auricular artery of the rabbit, the vessel of muscular type with a well-developed adrenergic innervation. The indicator of metabolic processes in the vessel wall was the activity, measured under standard conditions, of certain oxidoreductases of carbohydrate metabolism: glucose-6-phosphate (G6PD), lactate (LD), isocitrate (ICD), and succinate (SD) dehydrogenases. Activity was determined quantitatively by a micromethod, a modification of Altmann's histochemical method [4]. Freshly frozen cryostat sections through the artery up to 100 μ thick, with a total weight of 0.2-0.3 mg, were transferred to fluoroplast [Teflon] disks, weighed on torsion scales with a scale division of 0.002 mg, and kept for 30 min in constant-temperature (37°C) cuvettes with 3 ml of incubation medium [1]. Enzyme activity was measured from the quantity of formazan formed on reduction of nitro-BT, which was extracted from the tissue with

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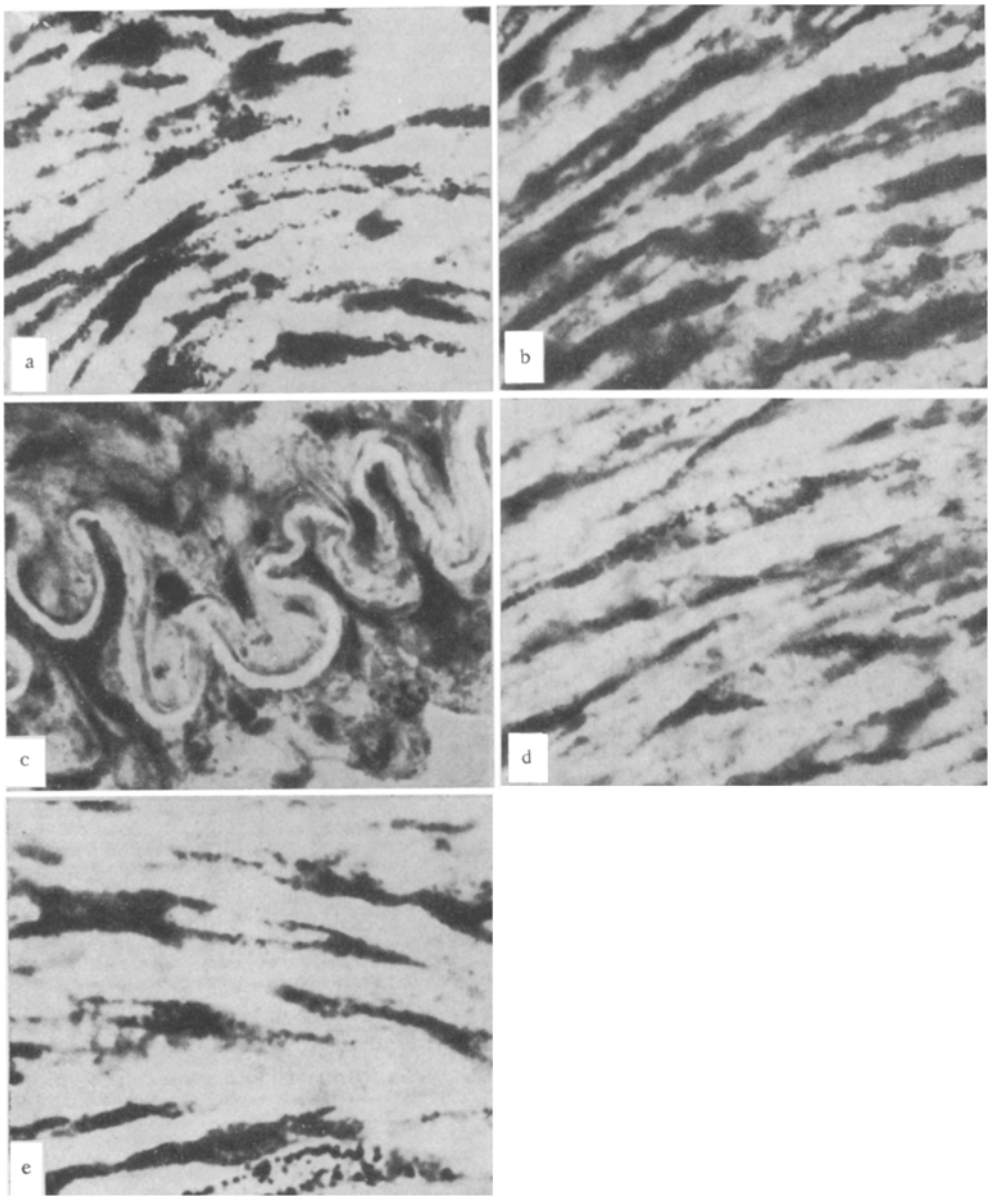

Fig. 1. Localization of formazan, the histochemical reaction product, in auricular artery of rabbit. a) Intact artery: deposition of formazan in smooth muscle cells (reaction for SD; ocular 10, objective 20); b) ICD activity in endothelial cells of intima of intact vessel (ocular 10, objective 90); c) strong reaction for LD in muscular coat of intact artery (ocular 10, objective 20); d) reduction in amount of precipitate in smooth muscle cells of desympathized artery compared with intact (ocular 10, objective 20); e) increase in G6PD activity in smooth muscle coat under influence of noradrenalin (ocular 10, objective 20).

alkaline dimethylformamide (pH 11.0). The extract, in a volume of 0.12 ml, was subjected to colorimetry on the MKFK-1 microphotocolorimeter and the activity of the enzyme was expressed in μg formazan/mg wet weight of tissue. Parallel with the quantitative determination, the location of the reaction product was identified histochemically.

Desympathized arteries were used in the experiments 4-5 weeks after unilateral removal of the superior cervical ganglion.

In the experiments with catecholamines the isolated auricular artery was bathed externally and internally with Krebs' solution; in some cases NA or A was added to the perfusion fluid, in other cases to the solu-

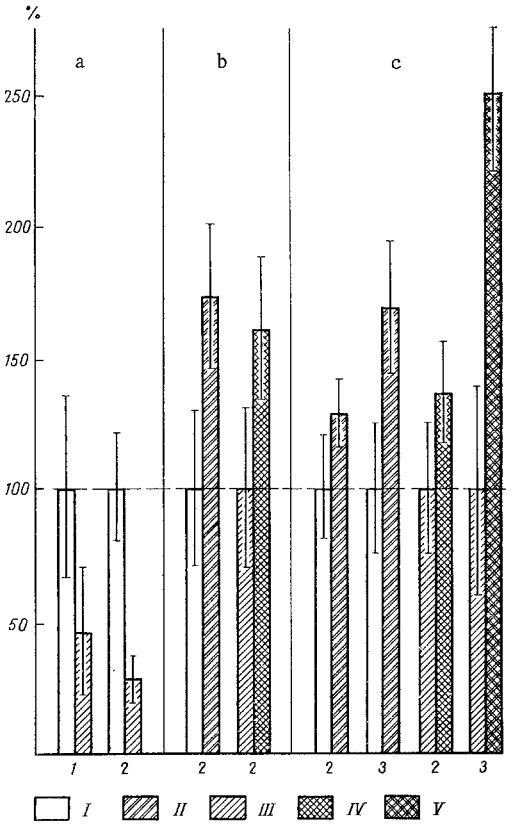

Fig. 2. Change in dehydrogenase activity (M ± mt) in auricular artery of rabbit: a) desympathization; b) action of A ($5.5 \cdot 10^{-7}$ M); c) action of NA ($5.9 \cdot 10^{-7}$ M). 1) ICD; 2) LD; 3) G6PD I) Intact artery; II) intact artery (perfusion); III) desympathized artery; IV) desympathized artery (perfusion); V) desympathized artery (application). Ordinate, change in hydrogenase activity, % of control.

tion bathing the vessel externally. The concentration of NA and A in the solution was $1 \cdot 10^{-7}$ g/ml ($5.9 \cdot 10^{-7}$ and $5.5 \cdot 10^{-7}$ M, respectively). The duration of perfusion and incubation was 20 min. Administration of the catecholamines after removal of the vessel was preceded by a period of 40 min to establish equilibrium between the vessel and medium, during which the vessel was kept in Krebs' solution. Results were obtained with 7-11 animals.

EXPERIMENTAL RESULTS

The histochemical investigation of the intact rabbit auricular artery revealed activity of all four dehydrogenases tested. Formazan, formed as a result of the reaction for SD, was detected only in the muscular coat of the vessel; the reaction, moreover, was stronger in the smooth-muscle cells of the outer layers. In the muscle cells themselves it was deposited mainly in the perinuclear space, in the region of the mitochondria, groups of which in the form of long bands were largest at the ends of the nuclei (Fig. 1b). Unlike SD activity, ICD activity was formed not only in the smooth muscle cells, but also in the endothelium of the intima (Fig. 1c). In all three layers of the vessel G6PD and LD activity was discovered (Fig. 1d).

The changes obserbed after desympathization of the auricular artery were expressed as a general decline in enzyme activity (Fig. 1e). Quantitative analysis showed that activity of LD was highest in the intact vessel and activity of SD was lowest. Blocking sympathetic influence led to a reduction of SD and ICD activity by 40-50% and LD activity by 27% (Fig. 2a).

During perfusion of the intact vessel a difference was found in the effects of A and NA on enzyme activity. Whereas A increased only LD activity considerably (by 65%), NA increased not only LD activity (by 26%), but also G6PD activity (by 70%) (Fig. 2b, c).

In experiments on the desympathized artery the action of NA on enzyme activity depended on whether it was introduced inside the vessel or applied to it externally. During perfusion of the vessel an increase in LD activity was observed (by 36%), and when applied externally to the vessel NA increased the activity of G6PD only (by 140%) (Fig. 2c).

The results of the histochemical investigation coincided with those of quantitative analysis: Deposition of formazan formed during the reaction for LD and G6PD in preparations of the intact and desympathized arteries increased after the action of the catecholamines (Fig. 1e).

The results confirm the existence of regulatory influences of the sympathetic nervous system on metabolism in the vessel wall. The rabbit auricular artery receives a powerful adrenergic innervation, and blocking that innervation leads to a reduction in enzyme activity in the vessel. Exogenous catecholamines have the opposite action, although the fact should be noted that when NA was introduced into the desympathized vessel it did not completely restore the picture observed in the intact vessel: Desympathization led to a decrease in the activity of all dehydrogenases tested, whereas NA affected only LD and G6PD activity. The reason for this difference could be either that desympathization was carried out under chronic experimental conditions and perfusion with NA under acute conditions, or the possible existence of some other factor than NA in the vasomotor nerves which influences the nutrition of the vessel wall [7].

The differences in the response of the artery to A and NA are interesting. Besides LD activity, NA increased G6PD activity; in the desympathized artery the latter effect depended on whether the NA penetrated into the vessel through the intima or the adventitia. It is difficult to explain this fact at present and further investigations are necessary.

LITERATURE CITED

1. A. G. E. Pearse, Histochemistry [Russian translation], Moscow (1962), p. 844.
2. Yu. V. Postnov and N. G. Kovaleva, Arkh. Pat., No. 4, 60 (1969).
3. C. W. Adams, Vascular Histochemistry, London (1967).
4. F. P. Altmann, Histochemie, 17, 319 (1969).
5. A. Beviz et al., Acta Physiol. Scand., 62, 109 (1964).
6. A. Burton, Physiol. Rev., 42, Suppl. 5, 191 (1962).
7. J. H. Chamley et al., Cell Tissue Res., 161, 497 (1975).
8. J. S. de la Lande et al., Circulat. Res., 7, 41 (1970).
9. S. Greenberg et al., Fed. Proc., 32, 2772 (1973).
10. J. Himms-Hagen, Pharmacol. Rev., 19, 367 (1967).
11. W. P. Keatinge, Acta Cardiol. (Brussels), Suppl. 19, 5 (1974).
12. A. Lehninger, in: The Arterial Wall (ed. by A. Lansing), Williams and Wilkins, Baltimore (1959), p. 220.
13. L. Lundholm et al., Pharmacol. Rev., 18, 255 (1966).
14. V. Martinescu et al., J. Cardiovasc. Surg., 9, 54 (1968).
15. A. P. Somlyo et al., Pharmacol. Rev., 22, 249 (1970).
16. C. Sutherland et al., Fed. Proc., 32, 3148 (1973).