RELATIONSHIP BETWEEN THE TRANSFORMATION OF ADRENALIN AND ITS EFFECT ON VASCULAR WALL CONTRACTION AND RESPIRATION

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Among the various pathways of the transformations of adrenalin in tissues, two have recently been elicited—methylation of catecholamine with the participation of O-methyltransferase and its deamination under the effect of monoamine oxidase (MAO).

The vascular wall contains both enzymes [5, 15], therefore there are grounds to assume that the transformation of catecholamines in this tissue is accompanied by monoamine oxidase and O-methyltransferase. As has become known, the state of the enzymatic apparatus participating in inactivation of catecholamines determines their incorporation into biochemical processes of tissue and cells, which in the end stipulates the magnitude and character of the physiological reaction [4]. It has been proved, for example, that adrenolytic substances inhibit the transformation of noradrenalin and thus removed its sympathomimetic effect upon stimulation of the sympathetic nerves [6-8]. The conclusion was made on the basis of these observations that the catecholamine inactivation apparatus is in the adrenergic systems. There is the assumption that MAO regulates the metabolism and supply of catecholamines in tissues, whereas O-methyltransferase mainly participates in processes of detoxication of catecholamines [9].

Proceeding from the premise concerning the relationship between the physiological effect and the transformation of catecholamines, we attempted to elicit the significance of the O-methyltransferase and monoamine oxidase pathways of transformation of adrenalin in its effect on respiration and contraction of the musculature of vessels.

EXPERIMENTAL METHOD

The experiments were carried out on an isolated strip of the aorta of rabbits according to our proposed method of simultaneous recording of respiration and contraction of the smooth muscles of vessels in a special apparatus [3].

A special feature of this method is that the isolated strip of the aorta is fastened at one end in a chamber and its second end is connected with a thread through which a mercury valve is led to a weighted (4 g) lever. The chamber is filled with Krebs-Ringer solution containing 0.01 M glucose and with oxygen, and then connected to a manometer. The contractions of the isolated muscles of the aorta were recorded by the lever on the tape of a kymograph. Absorption of oxygen was determined by the manometer. The data obtained were expressed in QO_2 (quantity of microliters of O_2 per 1 mg dry tissue weight per h) and in arbitrary units of RO_2 (quantity of microliters of O_2 per 1 mg dry weight of aorta tissue per 30 min multiplied by 100), in order to elicit the dependence between contraction and respiration.

The role of MAO in the mechanism of action of adrenalin was studied against the background of its blocking agent, iprasid, and also after the addition of catecholamine to a Krebs-Ringer solution containing 1 μm cyanide and 10 μm semicarbaside. According to the available data [10], under these conditions most enzymes are inactivated, whereas activation of MAO is retained. The role of O-methyltransferase was elicited by the addition of the blocking agent of this enzyme, pyrogallol [16, 17]. Not less than 5 animals were used in each group.

EXPERIMENTAL RESULTS

It was established in our previous investigations [1, 2], that catecholamines in small concentrations induce feeble contraction and hardly changed tissue respiration. In large doses they cause a maximal contraction of the iso-

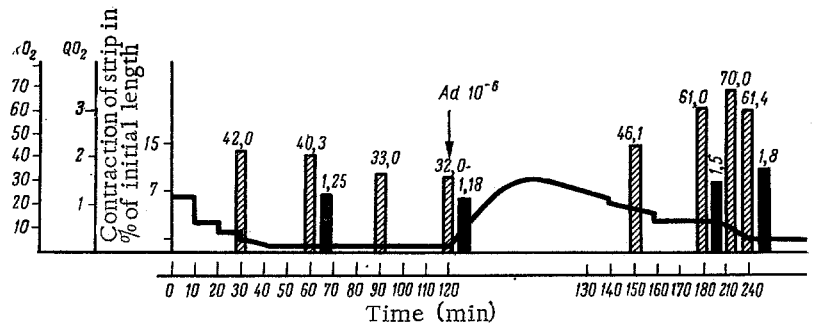

Fig. 1. Effect of adrenalin (10^{-6}) on respiration and contraction of rabbit aorta in the norm. The solid line is relaxation and contraction of strip; the light column are its absorption of oxygen in RO_2; the dark columns, in QO_2.

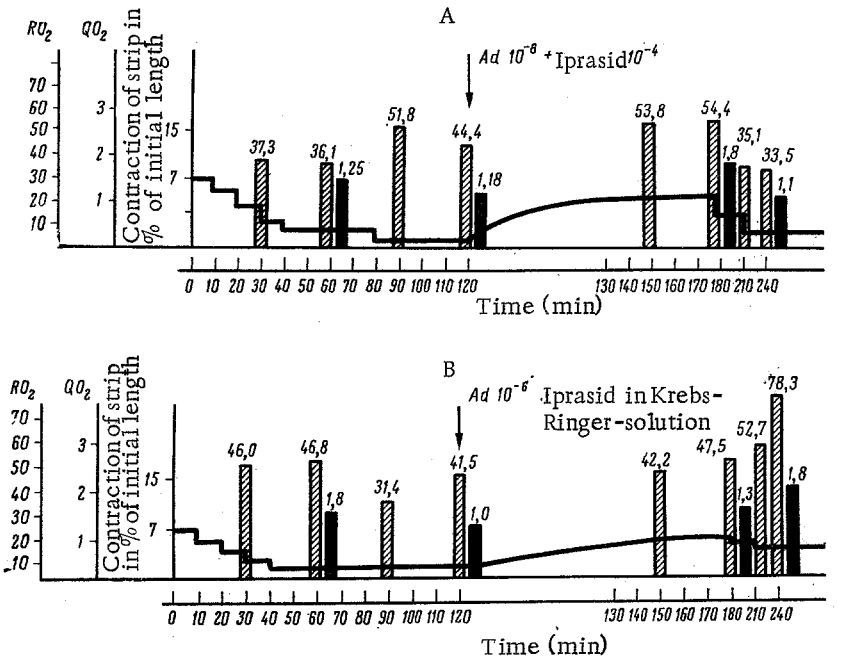

Fig. 2. Reaction of respiration and contraction of the smooth muscles of the aorta to the effect of adrenalin in the presence of iprasid (10^{-4}). A) Iprasid was added to the chamber simultaneously with the use of adrenalin; B) 2 h before using adrenalin. Designations are the same as in Fig. 1.

lated aorta of rabbit and enhance its absorption of oxygen (Fig. 1). The biochemical principles of these phenomena have not been explained. It is usually considered that deamination occurs slowly; methylation of catecholamine with subsequent deamination of its methylated product is more rapid. Our observations have shown that the inhibitor of O-methyltransferase (pyrogallol) in Krebs-Ringer solution and in the presence of oxygen is rapidly oxidized, therefore,it is not possible to determine the magnitude of oxygen absorption by tissues. The contraction of the strip under these conditions is greater in comparison with control magnitudes, which coincides with the data of other investigators [12, 13, 17] obtained in vivo. The enhancement and prolongation of the pressor effect and an increase in the contractile reaction of the aorta muscles to adrenalin in the presence of pyrogallol can be explained by the fact that pyrogallol, while blocking O-methyltransferase, protects adrenalin against inactivation.

The experiments with the MAO inhibitor (iprasid) when the latter was added simultaneously with adrenalin, yielded the following results. Iprasid (10^{-4}) lowered to a very small degree the contractile reaction of the strip. For 1 h after the administration of adrenalin, respiration increased, just as under the effect of adrenalin in the control (see Fig. 1); on continuing the observation up to 2 h it was possible to elicit weakening of respiration (QO_2 under the effect of adrenalin alone was 1.8 ±0.12, whereas with the joint administration of adrenalin and iprasid it was

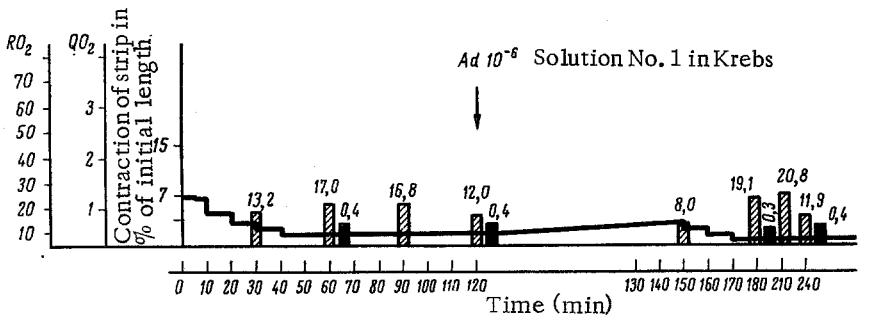

Fig. 3. Contraction and respiration of isolated aorta of rabbits under the effect of adrenalin with exclusion of metal-containing enzymes by cyanide. Designations are the same as in Fig. 1.

1.1 ±0.14) (Fig. 2A). These data can be interpreted from the point of view of the existence of a competitive mechanism, i.e., assuming that adrenalin and iprasid react with one of the active centers of the receptor. This is all the more probable since there are indications of a structural similarity between monoamine oxidase and the adrenergic system [11].

The results of another group of experiments, in which iprasid (10^{-4}) was added 2 h before the introduction of adrenalin (10^{-6}), agree with the supposition expressed; the uptake of oxygen under the effect of iprasid hardly changes for 2 h, remaining at the level of the control magnitudes (Fig. 2B). After the addition, of adrenalin, oxygen consumption for 1 h remains within the control magnitudes (QO_2 = 1.3 ±0.3) and only by the second h is there a tendency toward an increase in oxygen consumption. Iprasid (10^{-4}) with a prolonged exposure lowers the contractile reaction of the smooth muscles of the vessels to adrenalin (contraction in percent of initial length in the control was 8.2 ±0.9, and in the experiment 5.0 ±0.6). The data on the inhibition of the contractile reaction of the smooth muscles of the vessles are of interest since it is known that iprasid is used as a vasodilator, whereas a mechanism of action has still not been explained.

The results of the two groups of experiments gave us certain grounds to assume that enhancement of respiration and the maximal contraction can be partially associated with monoamine oxidase transformation of adrenalin However, it is difficult to preclude that here long exposure of the strip in the iprasid solution does not lead to inhibition of certain enzymes of the tri- and dicarboxylic acid cycle. Therefore we investigated simultaneously the magnitude of the contraction of the isolated aorta muscle and its oxygen consumption after using cyanide to exclude metal-containing enzymes from metabolism. During blocking of the metal-containing enzymes, the contractile reaction of the muscle strip was inhibited, and its respiration appreciably dropped (QO_2 in the experiment was 0.4 ±0.1, in the control 1.25 ±0.1)* and remained at this level for almost the entire experiment (Fig. 3). Hence, it follows that the maximal contractile reaction of the strip to adrenalin and the enhancement of respiration are to a considerable extent determined by the metal-containing enzymes, the blocking of which by cyanide prevents stimulation of respiration and contraction in response to the use of amine.

Thus, blocking of the O-methyltransferase pathway of the transformation leads to an increase in the contraction of the isolated muscle.

Blocking of monoamine oxidase by iprasid (with prolonged exposure) leads to a decrease in the contraction of the isolated aorta muscle and less stimulation of respiration with the addition of adrenalin. In this case iprasid was added 2 h before the use of catecholamine, then stimulation of respiration induced by adrenalin was inhibited for 1 h. If iprasid was added simultaneously with adrenalin, then, as in the control experiments, respiration was enhanced for 1 h and by the second h dropped to values recorded in the experiments with the use of adrenalin. Usually under the effect of adrenalin respiration is enhanced by the second h more than during the first h.

The exclusion of metal-containing enzymes from metabolism by using cyanide while retaining the activity of

* The average value of QO_2 in the control was obtained by statistically processing the data of all experimental groups.

MAO leads to complete blocking of contraction in response to the effect of adrenalin. Respiration of the vascular strip is inhibited and does not change under the effect of catecholamine.

Consequently, the contractile reaction of the smooth muscles of vessels to adrenalin and stimulation of respiration of the vascular wall by its greater doses can partially depend on O-methyltransferase and monoamine oxidase pathways of adrenalin transformation. A major role in the contractile reaction and respiration of the smooth muscles of vessels is played by metal-containing enzymes which are inactivated by cyanide.

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