EFFECT OF BENACTYZINE AND ADIPHENINE HYDROCHLORIDES ON THE CAPTURE AND LIBERATION OF NORADRENALIN

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UDC 615.217.34.015.45:612.452.018

Benactyzine and adiphenine hydrochlorides (20 mg/liter) reduce the percentage of capture of exogenous noradrenalin by the tissues of the rabbit and cat heart, isolated by Langendorf's method, to an equal degree (by about half). In experiments to study the effect of benactyzine and adiphenine hydrochlorides on the liberation of endogenous noradrenalin from the tissues of the isolated cat heart, only benactyzine was found to liberate stored amine into the fluid perfusing the heart, whereas its acetic analog adiphenine hydrochloride, did not liberate endogenous noradrenalin.

Previous investigations [1-4] showed that benzactyzine hydrochloride, a cholinolytic drug belonging to the group of amino-esters of substituted glycollic acid, unlike its acetic analog adiphenine hydrochloride, evokes motor excitation in rats and reduces the noradrenalin content in the brain tissues.

In view of these results it was decided to study the effect of these compounds on the capture and liberation of noradrenalin from cell depots.

EXPERIMENTAL METHOD

Experiments were carried out on cat and rabbit hearts isolated by Langendorf's method. To stabilize the catecholamines and remove the heavy metals, ascorbic acid (20 mg/liter) and EDTA (10 mg/liter) were added to the Kravkov's solution.

To investigate the action of the drugs on the capture of noradrenalin by the heart tissues, 200-300 μg/liter noradrenalin (control) was added to the nutrient fluid in one of the Marriott's vessels, while noradrenalin together with the drug for testing was added to the other (experiment). During the first 30 min the heart was perfused with solution not containing noradrenalin or the test drugs (a third Marriott's vessel), at the rate of 30-40 ml/min. At this rate of perfusion, as Swaine et al. [11, 12] and the writer's preliminary investigations showed, the heart loses endogenous noradrenalin into the perfusion fluid, so that the reserves of the stored amine are exhausted. The rate of perfusion was then reduced to 15-20 ml/min (at this rate no spontaneous liberation of noradrenalin was observed), control samples were taken, and perfusion of the heart was switched over to a fluid containing noradrenalin or noradrenalin together with the test drug. The perfusion fluid was collected in a volume of 10 ml in cylinders (to which 1 ml of 0.5 N HCl solution had first been added) 5, 10, 20, and 30 min after the change over. The noradrenalin content was determined spectrofluorometrically [5, 6]. The difference between the noradrenalin concentrations in the fluid before and after passing through the heart, expressed as a percentage, was used as the index of capture of the amine by the heart tissues.

In the experiments to study the effect of the drugs on liberation of noradrenalin from the heart tissues, the heart was perfused with nutrient solution at the rate of 15-20 ml/min. Under these conditions

(Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 72, No. 10, pp. 55-58, October, 1971. Original article submitted June 5, 1971.

TABLE 1. Effect of Benactyzine and Adiphenine Hydrochlorides on Liberation of Endogenous Noradrenalin from the Isolated Cat Heart

Experiment No.	Noradrenalin concentration (in ng/ml)			Rate of liberation of noradrenalin (in ng/min)		
	control	drug	rinsing	control	drug	rinsing
Adiphenine hydrochloride, 20 mg/liter						
1	Traces	Traces	Traces	—	—	—
2	»	»	»	—	—	—
3	»	»	»	—	—	—
4	»	»	»	—	—	—
5	0,026	0,026	»	0,403	0,403	—
Benactyzine hydrochloride, 20 mg/g						
1	0,026	0,041	Traces	0,340	0,570	—
2	0,026	0,130	0,050	0,650	2,990	1,100
3	Traces	0,028	0,023	—	0,500	0,390
4	»	0,180	Traces	—	2,880	—
5	»	0,240	»	—	3,940	—

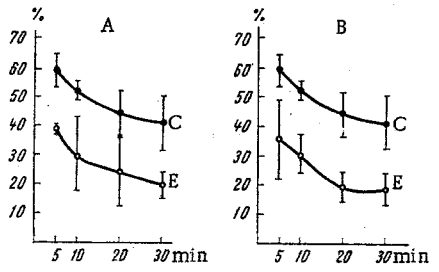

Fig. 1. Effect of benactyzine hydrochloride in a dose of 20 mg/liter (A) and adiphenine hydrochloride in a dose of 20 mg/liter (B) on capture of exogeneous noradrenalin by tissues of the isolated cat heart. C) Control; E) experiment.

the catecholamines either could not be determined in the perfusion fluid, or their liberation from the heart was extremely small. After the control samples had been taken the heart was switched over to the Marriott's vessel containing a solution of the drug to be tested, and 5-7 min later a second sample was taken. The heart was then switched over to perfusion with the first solution and a third sample was taken (elution by Kravkov's method). To increase the sensitivity of the method the volume of the samples in this series of experiments was increased to 100 ml, so that the catecholamines could be concentrated 10 times by adsorption on alumina.

EXPERIMENTAL RESULTS

When the heart was perfused with noradrenalin solution in an excessively high concentration (the control experiment of series I) the percentage capture of the amine gradually diminished with time, indicating saturation of the tissues with noradrenalin (Fig. 1, top curves). When the heart was perfused with fluid containing benactyzine hydrochloride as well as noradrenalin, the percentage capture of noradrenalin by the heart tissues was reduced by almost half, although the character of the curve remained similar to that of the curve in the control experiments (Fig. 1A). A similar effect was observed during the action of adiphenine hydrochloride (Fig. 1B) which, as previous investigations [1-4] showed, unlike benactyzine, did not give rise to motor excitation in the animals, and did not reduce the noradrenalin level in the brain tissues.

It will be clear from Table 1 that spontaneous liberation of endogenous catecholamines (experiments of series II) was extremely slight (traces). Under these experimental conditions the action of benzactyzine and adiphenine hydrochlorides differed: benactyzine caused a sharp increase in liberation of catecholamines from the perfusion fluid whereas adiphenine had practically no effect on the liberation of the endogenous amines. Analysis of the fluorograms showed that catecholamines liberated from the heart tissue consisted almost entirely of noradrenalin.

The decrease in the noradrenalin level in the rat brain under the influence of benzactyzine hydrochloride and other amino-esters of diphenylglycollic acid, discovered by the present writer [2], can be attributed theoretically to the following factors: 1) inhibition of synthesis of the amine; 2) disturbance of the recapture of the amine liberated during the passage of nervous impulses along adrenergic neurons; and 3) exhaustion of the noradrenalin reserves on account of its liberation from the cell depots. The first mechanism (inhibition of synthesis of the amine) is improbable, for Spector [10] has shown that against the background of inhibition of catecholamine synthesis by the inhibitor tyrosine hydroxylase, the sympathomimetic effect of amphetamine was completely abolished. In the writer's previous investigation [3] the effect of

amphetamine, against the background of the action of glycollic acid derivatives, not only was not inhibited but, on the contrary, was potentiated.

The present investigations show that recapture of noradrenalin by the heart tissues was inhibited equally by benactyzine and adiphenine hydrochlorides. This is evidently not the decisive mechanism of the decrease in the noradrenalin content in the brain tissues.

The possibility of a third type of mechanism – the liberation of the amine from the depots – was confirmed by the results of the present investigations. The glycollic acid derivative benzactyzine hydrochloride) increased the liberation of endogenous noradrenalin from the perfusion fluid, whereas its acetic analog adiphenine hydrochloride had no effect on liberation of the amine. By the character of its action benactyzine was similar to other liberators of endogenous noradrenalin which, besides this effect, also caused motor excitation of the animals [7, 12]. The writer's previous hypothesis [2] regarding the liberation of endogenous noradrenalin in a physiologically active state by benactyzine hydrochloride, as the cause of the animal's motor excitation, was thus confirmed experimentally in the present investigation.

In the modern view, the capture of free noradrenalin by the tissues is regarded as nonspecific adsorption of catecholamines, and it is an important link in the chain of their biological inactivation [13]. Benactyzine and adiphenine hydrochloride inhibited this process equally. However, the combination of this last property in benactyzine with the ability to liberate endogenous noradrenalin evidently creates the most favorable conditions for this mediator to manifest its physiological effect.

Clearly the results obtained on the nervous structures of the heart cannot be extrapolated without some reservation to the central nervous system. Nevertheless, it can be considered that there are certain common mechanisms of capture and liberation of catecholamines in all tissues possessing a sympathetic innervation. For instance, Iversen et al. [9] established a definite similarity between the action of drugs on the capture and liberation of labelled noradrenalin both in the heart tissues and in the brain tissues of animals.

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