EFFECT OF NEUROTROPIC AGENTS ON INTENSITY OF ^{3}IHI-DOPA UPTAKE INTO MAST CELLS OF LABORATORY ANIMALS

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Catecholamines are currently attfacting the attention of research workers for they are biologically active substances with an extremely wide range of action.

It has been shown [1-6] that catecholamines at the periphery play the role both of neurotransmitter and of tissue hormone, implying the wide involvement of catecholamines in the regulation of vital functions not only under normal but also under pathological conditions.

That is why interest has not declined not only in peripheral sources of catecholamines, but also in substances which diminish or enhance the action of catecholamines [4, 8, 9].

There is evidence that neurotropic agents affect the catecholamine content in the mast cells (MC) of lymphoid organs [4].

However, the few studies which have already been undertaken are insufficient to allow any reliable judgement regarding the role of MC in catecholamine metabolism.

The aim of this investigation was to study the effect of neurotropic agents disturbing the general level of catecholamines in the body on the intensity of incorporation into MC of the specific catecholamine precursor ^{3}IHI-dioxyphenylalanine (DOPA).

EXPERIMENTAL METHOD

Experiments were carzied out on 180 laboratory animals of both sexes (Wistar rats weighing 180-200 g and CC57W mice weighing 48-53 g), the traditional objects used to study the MC system. It is also generally accepted that autoradiography is a sufficiently adequate and highly sensitive method for demonstrating catecholamines in cells with the aid of their radioisotope precursors. The animals were divided into three groups with 60 in each group. Group 1 (control, 30 rats and 30 mice) received an intraperitoneal injection of 0.2 ml physiological saline, followed 30 min later by the labeled precursoz ^{3}IHI-DOPA ("Amersham," England), specific radioactivity 19.8 mCi/mmole in 0.1 ml physiological saline (dose rate 8 μCi/g body weight). Animals of the control and experimental groups received an injection of the indicator after 30 min and 1, 6, 24, 48, and 72 h.

Pharmacologic effects on catecholamine metabolism in vivo weze produced by injection of reserpine and noradrenalin, creating artificial conditions for blocking and activating the adrenergic system. The doses of the drugs given, the intervals between injections, and subsequent sacrifice were chosen on the basis of data in the literature [4, 8, 9].

Animals of group 2 (experimental) received an intraperitoneal injection of 0.1% naradrenalin solution in a dose of 0.5-9.25 mg/kg body weight, after which ^{3}IHI-DOPA was injected.

Animals of group 3 received injections of resezpine (Rausedil, England) in a dose of 5 mg/kg body weight, followed by injection of ^{3}IHI-DOPA. All the animals were killed after injection of the indicator by rapid decapitation at the same time of day, allowing for the circadian rhythms of biogenic amines [6]. The material was fixed in Carnoy's fluid. Several populations of MC, living in different tissue microenvironments (skin, tongue, heart, mesentery, liver, thymus, small intestine, adrenal gland) were studied. The average thickness of the section was 10 μ. The section was coated with "Ilford" emulsion and exposed for 18 days at 4°C. After development in D-19 amidol developer, the autoradiographs weze stained with 0.1% toluidine blue solution

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TABLE 1. Effect of Noradrenalin and Reserpine on Labeling Index of MC (in percent) in Organs of Laboratory Animals after Exposure for 3 Days to ^{3}IHI-DOPA

Species of animals	Number of labeled MC					
	skin	tongue	heart	thymus	mesentery	small intestine
CC57W mice						
Group 1	2,7±1,2	2,5±1,7	0,8±1,1	0,5±1,3	7,3±2,1	0,7±1,5
Group 2	17,3±2,0*	17,1±1,8*	3,1±1,7*	1,3±1,1*	26,3±4,3*	1,1±2,4
Group 3	0,5±1,1*	0,5±1,6*	0,1±1,2*	0*	0,9±1,7*	0*
Wistar rats						
Group 1	0,7±2,6	0,8±2,6	0,6±1,9	6,3±4,3	10,3±5,6	0,6±1,3
Group 2	6,3±4,3*	4,7±1,7*	1,8±4,3*	18,6±1,2*	27,6±1,7*	1,1±0,5
Group 3	0,3±1,1*	0,3±1,7*	0,1±0,5*	0,5±1,6*	0,8±1,7*	0*

Legend. *$p < 0.05$ compared with control.

TABLE 2. Intensity of Incorporation of ^{3}IHI-DOPA (specific radioactivity 19.8 Ci/mmole) into MC ($x \pm X$)

Species of animals	Mean number of grains of silver per unit area for different MC populations					
	skin	tongue	mesentery	small intestine	heart	thymus
Wistar rats						
Group 1	2,0±0,5	3,2±0,1	4,4±0,9	1,7±0,5	1,5±0,7	1,5±0,5
Group 2	8,1±0,5	7,8±0,4	10±0,5	5,5±0,3	4,0±0,3	3,5±0,5
Group 3	0,7±0,69	1,0±0,1	2,0±0,3	2,0±0,1	1,5±0,5	0,5±0,3
CC57W mice						
Group 1	2,7±0,3	3,6±0,1	4,6±0,7	1,9±0,25	1,7±0,3	1,7±0,5
Group 2	7,6±0,5	7,9±0,1	12,1±0,4	5,7±0,5	4,3±0,5	3,8±0,1
Group 3	0,8±0,1	1,5±0,7	2,1±0,4	0,8±0,5	0,5±0,3	0,7±0,5

Legend. In all cases $p < 0.05$ compared with control.

at pH 5.6. The number of labeled MC (labeling index, in percent) and the intensity of labeling (number of grains of silver per cell) were counted in each preparation. The area in which the label was counted measured 100 μ^2. The numerical results were subjected to statistical analysis on the EC-1045 computer, using VMDR-3 and 7-D pzograms. Preliminary experiments showed that injection of noradrenalin and reserpine caused degranulation of MC to different degrees.

EXPERIMENTAL RESULTS

The experiments showed that incorporation of ^{3}IHI-DOPA into MC of mice and rats takes place at different rates and in different intensities. Analysis of the distribution of ^{3}IHI-DOPA shows that the isotope was taken up more intensively by MC of the tongue, skin, and mesentery of the mice than by cells of the heart and small intestine. MC selectively accumulate ^{3}IHI-DOPA during the first hours after exposure of the animal to the isotope, and reach maximal uptake after 24 h, after which the intensity of labeling falls, due to elimination of the indicator from the body. Incorporation of ^{3}IHI-DOPA into MC of rat tissues was rather different in character. The label was found 1 h after injection of ^{3}IHI-DOPA in MC of the mesentery and thymus, while the remaining rat organs had only single (1-3 grains of silver) autographs. Between 6 and 48 h after the beginning of the investigation, virtually no incorporation of ^{3}IHI-DOPA took place in MC of the rat skin, heart, liver, and small intestine, evidence of the slow course of catecholamine metabolism. These results justify the conclusion that the intensity of incorporation of ^{3}IHI-DOPA into MC of the rat mesentery differs in the rate of catecholamine turnover, for the peak of incorporation into MC of the mesentery occurred sooner than into MC of the other tissues. This particular feature of distribution of the ^{3}IHI-DOPA label into MC of the rat mesentery is connected with the higher level of functional activity of the cells and of catecholamine metabolism taking place in them.

When the catecholamine levels of the animals were disturbed experimentally, the intensity of incorporation of ^{3}IHI-DOPA into MC of the organs, both of rats and mice, was substantially altered. Injection of noradrenalin into the animals led to a marked increase both in the labeling index and in the intensity of labeling of MC (Tables 1 and 2). An induced rise of the catecholamine level led to saturation of MC with exogenous precursor ^{3}IHI-DOPA.

Definite patterns of intensity of incorporation were found in young, maturing, and mature MC. Young MC were characterized by the following features: diameter 5 μ, few granules, weak metachromasia, absence of label. Maturing MC had a diameter of 7 μ, a small number of granules, marked metachromasia, and single autographs (grains of silver). Mature MC had a diameter of 8-12 μ, were packed with granules, showed clear metachromasia, and had many grains of silver. In some MC there were solitary grains of silver, but in others the label occupied the whole cell or was concentrated in its peripheral zone. After 6 h of exposure of the animal to ^{3}IHI-DOPA, values of the labeling index and intensity of label increased and remained high for the next 24 h, after which they fell statistically significantly compared with the control. In experiments in which noradrenalin was injected, autographs were found in MC at all levels of differentiation, between 6 and 24 h of to ^{3}IHI-DOPA. Changes in the intensity of incorporation of ^{3}IHI-DOPA into MC also were observed in animals receiving reserpine. Reserpine is known [3, 6-8] to exhaust catecholamine reserves in the tissues and to block their synthesis.

Under these conditions there was a sharp decrease in the labeling index and in the intensity of labeling of MC.

This indicates that the intensity of catecholamine metabolism in MC of the organs of the rats and mice was significantly lower than in the control.

Thus when the time course of incorporation of ^{3}IHI-DOPA into MC of different organs of rats and mice is compared, it will be noted that in both species of animals the catecholamine level in the body is directly dependent on the intensity of incorporation of the precursor and also on the duration of exposure to it.

The results of this investigation are in agreement with those of luminescence studies in the literature [4, 10], demonstrating the effect of neurotropic agents on catecholamine concentrations in MC. We showed by autoradiography that MC can concentrate the exogenous catecholamine precursor ^{3}IHI-DOPA, the intensity of incorporation of which is directly dependent on the body levels of catecholamines, induced or blocked pharmacologically.

This may be indirect confirmation of the involvement of MC in catecholamine metabolism.

By using neurotropic drugs, differential changes can be brought about in the functional state of MC and in their catecholamine levels.

The results suggest that components essential for catecholamine metabolism are present in MC of the organs and tissues of fats and mice.

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