ROLE OF THE LUNGS IN SEROTONIN INACTIVATION

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Isolated perfused rat lungs can inactivate small quantities (5-50 ng/ml) of serotonin (ST) almost completely. An increase in the ST concentration to 200 ng/ml and lengthening of the perfusion time weaken the ST inactivation. Cooling the lungs, or preliminary perfusion of the lungs with the addition of potassium cyanide ($3 \cdot 10^{-4}$ M), monoidoacetic acid (10^{-3} M), imipramine (10^{-5} M), morphine (10^{-3} M), lysergic acid diethylamide (10^{-5} M), and cocaine (10^{-5} M) inhibit ST inactivation. The monoamine oxidase inhibitor iproniazid (10^{-4} M) does not affect the inactivation of ST by the lung tissue but increases the quantity of ST in it. It is postulated that the inactivation of ST by the lungs takes place in two stages: active uptake of ST by the lung cells from the perfusion fluid first, followed by its enzymic destruction.
KEY WORDS: lungs; serotonin; monoamine oxidase inhibitors.

Lying on the pathway of the venous blood flow the lungs carry out inactivation of various metabolites, thus preventing their entry into the arterial blood in high concentrations and thereby maintaining the constancy of the internal milieu of the organism. A contributory factor is the high concentration of various enzymes in the lung tissue: monoamine oxidase (MAO), kininase, guanase, and so on [3, 12]. The lungs can inactivate kinins and biogenic amines, including serotonin (ST). A number of investigations have been made of the role of the lungs in serotonin inactivation and these have revealed certain mechanisms of the process and its dependence on various factors (temperature, the action of pharmacological agents).

Experiments were carried out on isolated perfused albino rat lungs in order to study the intensity and mechanism of inactivation of ST by the lungs.

TABLE 1. Effect of Pharmacological Agents and of Cooling on the Inactivation of Serotonin by the Lungs

Factor acting	Concn. (M)	% of serotonin inactivation		No. of expts.
		after 5 min	after 20 min	
Control	—	86	87	15
Cooling to 15°C	—	10	10	8
Monoiodoacetic acid	10^{-3}	22	25	7
Potassium cyanide	$3 \cdot 10^{-4}$	23	24	6
Imipramine	10^{-5}	25	28	5
Iproniazid	10^{-4}	87	87	6
Morphine hydrochlor.	10^{-3}	13	10	5
LSD	10^{-5}	20	17	6
	10^{-6}	40	39	5
Cocaine hydrochlor.	10^{-5}	8	9	5
Adrenalin hydrochloride	10^{-6}	62	60	7

EXPERIMENTAL METHOD

Rats weighing 200-250 g were anesthetized with ether vapor, the chest was opened, and heparin injected into the heart (300 units). The venae cavae were ligated, one polyethylene catheter was inserted through the opened right ventricle into the pulmonary artery, and a second catheter was inserted through the aorta into the left ventricle. The lungs were perfused with oxygenated Gaddum's solution under constant pressure at 37°C at the rate of 5-7 ml/min. In some experiments, to prevent edema of the lung tissue the Gaddum's solution was made up in 10% dextran. Serotonin creatinine-sulfate (Reanal, Hungary) was used for perfusion and its concentration in the perfusion fluid determined by the biological method of Dalgliesh et al. [5] on the transverse colon of the albino rat.

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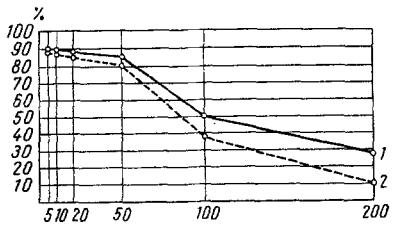

Fig. 1. Intensity of inactivation of various doses of ST by the lungs: inactivation of ST 5 min (1) and 20 min (2) after beginning of perfusion. Abscissa, ST concentration in perfusion fluid (in ng/ml); ordinate, degree of inactivation of ST, in %.

EXPERIMENTAL RESULTS AND DISCUSSION

The ST-inactivating ability of the lung tissues during administration of various doses of ST (from 5 to 200 ng/ml) was studied in the experiments of series I. Inactivation of ST was determined 5 and 20 min after the beginning of perfusion. With low concentrations of ST in the perfusion fluid (5-50 ng/ml) from 85 to 90% of the exogenous serotonin was found to be inactivated during the first 5 min (Fig. 1). With an increase in the ST concentration in the perfusion fluid, its inactivation was reduced. Lengthening the perfusion time to 20 min weakened the inactivation of large doses of ST (100-200 ng/ml), whereas the inactivation of small doses was virtually unchanged.

To study the mechanism of inactivation of ST by the lungs, the lung tissue was first exposed to the action of a low temperature or of various pharmacological agents for 10-15 min, after which the lungs were perfused with Gaddum's solution containing 5-10 ng/ml ST. The use of perfusion of the lungs with Gaddum's solution cooled to 15°C to inhibit enzymic processes led to the almost complete cessation of ST inactivation (Table 1). Preliminary perfusion with the addition of monoiodoacetic acid in a concentration of 10^{-3} M and of potassium cyanide ($3 \cdot 10^{-4}$ M) was used to inhibit tissue respiration and energy-consuming processes of cell metabolism. The inactivation of ST by the lungs under these circumstances was reduced to 22-25%. ST inactivation was reduced to the same level after the addition of imipramine (10^{-5} M), a substance which has been found [15, 16] to depress the specific mechanisms of ST transport through the cell membrane, to the perfusion fluid. The addition of iproniazid (10^{-4} M), a powerful MAO inhibitor, to the perfusion fluid did not affect the rate of ST inactivation by the lung tissue, but it caused a sharp increase in the ST concentration in the lung tissue itself – up to 0.87 ± 0.11 μg/g. During perfusion with the addition of ST only, its concentration in the lung tissue was much smaller: $0\text{-}0.39 \pm 0.06$ μg/g ($P < 0.05$). The limiting stage of ST inactivation by the lungs was thus the stage of its active uptake from the perfusion fluid, for the blocking of this stage abolished ST inactivation.

The effect of some antagonists of serotonin on its inactivation also was studied. Lysergic acid diethylamide (LSD), an antagonist of the D-type, was used in concentrations of 10^{-5} M and 10^{-6} M, morphine (10^{-3} M), an antagonist of the M-type with a competitive mode of action [13, 14], and adrenalin (10^{-6} M) [1] and cocaine (10^{-5} M), with the properties of an antagonist of the M-type and, at the same time, a substance disturbing the binding of biogenic amines inside the cell [2, 11], were used for this purpose. All these substances had the property of inhibiting ST inactivation by the lungs (Table 1). Cocaine and morphine, and LSD in a concentration of 10^{-5} M, had the strongest action. With a decrease in the concentration of LSD its effect was reduced. Adrenalin, in the concentration used, reduced the ST inactivation by about 30%.

It can be concluded from these findings that the rat lungs can inactivate ST intensively. Lungs of other animals – cats, dogs, guinea pigs, rabbits [6, 7, 9, 10, 17] – have a similar property, although the lungs of guinea pigs can inactivate only about 50% of added ST. Human lungs have a similar property, for they inactivate up to 65% of exogenous ST [8].

The mechanism of uptake of ST by the lungs has not yet been adequately explained. Specific inhibitors of ST transport through the cell membrane, such as imipramine and also amitriptyline and desmethylimipramine, also inhibit ST inactivation sharply [4]. The available data point to an active process of entry of ST into lung tissue cells, requiring a supply of energy and the participation of specific carriers. However, it is not yet clear what cells perform this function – the capillary endothelium or the alveolar epithelium.

The ST that is actively absorbed by the cells later is broken down by enzymes. During perfusion of the isolated lungs with a solution containing labeled ST [4], 10% of the radioactivity is removed after 10 min as metabolites, and the remaining 90% after a further 50 min. An important role in the enzymic breakdown of ST belongs to MAO, for up to 40% of the total content of metabolites consists of 5-hydroxyindoleacetic acid. On the other hand MAO inhibitors, such as iproniazid and nialamide [17], have no significant effect on the inactivation of ST by the lungs, although their use leads to the accumulation of ST in the lung tissue. This suggests that up to a certain limit the lungs can inactivate the ST of the blood purely through its uptake by the cells, regardless of whether any ST is broken down by enzyme action or not. These findings suggest that ST is inactivated by lung tissue in two stages: the active uptake of ST from the perfusion fluid by the lung tissue cells initially, and enzymic breakdown of the absorbed ST later.

ST antagonists evidently act on the first stage of this process, inhibiting the uptake of serotonin by the cells. This inhibition is evidently connected with their action on structures responsible for transporting ST through the cell membrane (especially for antagonists of competitive type, such as LSD and morphine) or on the processes of ST deposition within the cell (cocaine). Inhibition of ST inactivation by antagonists with different mechanisms of action indicate the complex and many-sided character of this process.

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