THE UPTAKE OF CATECHOLAMINES BY SUBCELLULAR GRANULES IN THE BRAIN STEM

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It is well known that reserpine causes a marked decrease in the catecholamine content of the brain, heart, adrenal medulla etc. Spector *et al.* (1) reported that oxypertine (Win 18501-2; 1-(5,6-Dimethoxy-2-methyl-3 indol)-4-phenyl piperazine) depleted the norepinephrine content of the brain without causing any change in the serotonin level. They also found a close relationship between the norepinephrine content and the behavioral change produced by the administration of oxypertine. According to Matsuoka (2), norepinephrine seemed to be more important for brain function than dopamine, because a marked decrease in the norepinephrine content was observed on administration of oxypertine, while the dopamine level of rabbit brain was unchanged. In recent years, many studies have been reported on the accumulation of catecholamines in the subcellular granules in brain tissue *in vitro* (3-7).

In the present paper, the uptake of ^{14}C -labelled catecholamines, dopamine and norepinephrine, by subcellular granules of the brain stem *in vitro* is reported.

MATERIALS AND METHODS

Preparation of the brain stem subcellular granules

Six to eight male guinea pigs, weighing about 150 g were used for one experiment. Guinea pigs were killed by decapitation and their stems were removed immediately into chilled 10 volume of 0.25 M sucrose solution, and then homogenized by Potter type glass homogenizer. The homogenate was centrifuged at a rate of $800 \times g$ for 10 minutes to discard cell debris. The supernatant was recentrifuged at a rate of $10,000 \times g$ for 30 minutes to obtain the sediment of subcellular granules. The granules which were suspended in 6 to 8 ml of 0.25 M sucrose solution were used for the experiments.

Chemical agents

Reserpine phosphate was offered from Ciba Pharmaceutical Product (Summit N.J., U.S.A.). Iproniazid and oxypertine (Win 18501-2; 1-(5,6 Dimethoxy-2-methyl-3 indol)-4-phenyl piperazine), were offered from Sterling Drug Inc. Oxypertine was dissolved in

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acetic acid and neutralized by potassium hydroxide. The same concentration of acetate solution in the absence of oxypertine had no effects on the experiments. Radioactive 7- ^{14}C labelled dopamine and norepinephrine were obtained from New England Atomic Energy Corp.

The uptake of ^{14}C -labelled catecholamines by subcellular granules

Each incubation mixture consisted of 0.8 ml of the granules suspension, 0.02 M potassium phosphate buffer, pH 7.0, 0.18μ moles of 7- ^{14}C labelled dopamine (30×10^4 c.p.m.) or 7- ^{14}C norepinephrine (73×10^4 c.p.m.), and various concentrations of the effectors in 0.25 M sucrose solution. The total volume was 2 ml. Incubation was carried out at 30°C or 0°C in air. After incubation, 3 ml of chilled 0.25 M sucrose containing 0.02 M potassium phosphate buffer, pH 7.0 was added immediately, and centrifuged at 0°C . The sediments were washed twice with the same bufferized sucrose solution. The washed sediments were resuspended in 5 ml of 0.25 M sucrose solution, and then 1.25 ml of 2 N perchloric acid was added to extract catecholamines. In order to complete the extraction, the samples were allowed to stand over night at 0°C . After centrifugation, 0.3 ml of the supernatant was transferred into a vial to estimate the radioactivity by Packard liquid scintillation counter. The efficiency of the counter was about 60 per cent.

RESULTS AND DISCUSSION

As shown in Fig. 1, both dopamine and norepinephrine were taken up by the

FIG. 1. The uptake of catecholamines by the large granules of the brain stem of the guinea pig.

Incubation was carried out with a suspension of large granules of brain stem in 0.25 M sucrose containing, 0.02 M potassium phosphate buffer, pH 7.0, and 9×10^{-5} M NE- ^{14}C (73×10^4 c.p.m.), or DA- ^{14}C (30×10^4 c.p.m.).

The numbers in brackets indicate the absolute amount of labelled CA.

TABLE 1. Effects of various drugs on the uptake of catecholamine by the large granules of the brain stem.

Condition		Norepinephrine (10^3 c.p.m.)	
		30°C	0°C
Control		34.9±5.5 (24)	13.0±2.5 (19)
Reserpine	10 ⁻⁵ M	30.6±6.1 (14) (3)	12.9±2.3 (3)
	10 ⁻⁴ M	22.5±7.0 (35) (2)	13.0±2.0 (2)
Win 18501-2	10 ⁻⁵ M	18.9±4.1 (44) (4)	11.4±0.6 (4)
	10 ⁻⁴ M	17.3±3.4 (50) (4)	10.8±1.8 (4)
Iproniazid	10 ⁻⁴ M	26.7±2.7 (23) (4)	14.0±1.3 (3)
	10 ⁻³ M	23.5±3.1 (33) (2)	14.7±3.8 (2)
ATP	2×10 ⁻⁴ M	30.5±7.8 (12) (7)	14.0±4.6 (6)
	2×10 ⁻³ M	31.4±7.4 (10) (3)	13.1±2.4 (3)
Iproniazid 5×10 ⁻⁴ M** + Reserpine 10 ⁻⁴ M		9.3±0.2 (73) (2)	

Condition		Dopamine (10^3 c.p.m.)	
		30°C	0°C
Control		22.5±5.6 (14)	5.9±1.0 (11)
Reserpine	10 ⁻⁵ M	24.7 (10)* (1)	5.3 (1)
	10 ⁻⁴ M	15.3±3.0 (30) (4)	5.3 (1)
Win 18501-2	10 ⁻⁵ M	20.8±3.2 (7) (2)	5.0±1.3 (2)
	10 ⁻⁴ M	21.3±2.5 (5) (2)	6.4±1.9 (2)

Incubation was carried out with 0.25 M sucrose suspension of large granules in guinea pig brain stem (2 ml sucrose suspension equivalent to each gram of tissue wet weight), 0.02 M potassium phosphate buffer, pH 7.0 and 9×10^{-5} M norepinephrine-¹⁴C (73×10^4 c.p.m.) or dopamine-¹⁴C (30×10^4 c.p.m.) for 30 minutes. Each value was expressed as a mean value and deviation of the experiments. The parenthesized number indicates number of experiments and the square bracketed number indicates per cent decrease to the control.

* per cent increase.

** preincubated with iproniazid for 10 minutes at 30°C.

granules at 30°C, but no uptake was observed at 0°C. Much more dopamine was taken up by the granules than norepinephrine. Table 1 shows the effects of various drugs on the uptake of catecholamines by the subcellular granules. A very low concentration of oxypertine blocked the uptake of radioactive norepinephrine, while dopamine uptake was not influenced even by addition of 10⁻⁴ M oxypertine to the medium. Reserpine, at a concentration of 10⁻⁴ M, inhibited both dopamine and norepinephrine uptake by the granules. These results are similar to the data obtained on the catecholamine level *in vivo*, and suggest that the norepinephrine depleting effect of oxypertine might be due to inhibition of norepinephrine uptake by the storage granules. Oxypertine might be a useful tool for investigating the mechanism of norepinephrine storage in the granules.

Contrary of the findings with adrenal medullary particles (8), adenosine triphosphate was found to exhibit a slight inhibitory effect on the uptake of catecholamines in the present work. Iproniazid inhibited the uptake to some extent. However, a paradoxical difference between *in vivo* and *in vitro* experiments was observed on pretreatment with iproniazid and subsequent administration of reserpine. *In vivo* pretreatment with iproniazid did not cause depletion of the storage granules of catecholamines, following administration of reserpine

(9). But *in vitro*, preincubation of the granules with iproniazid potentiated the inhibitory effect of reserpine on the uptake of dopamine and norepinephrine. If iproniazid does

not influence the release of catecholamine (10), when it is administered, it might be changed in the body and affect the storage granules in such a way as to prevent the release of catecholamine. However, it was observed by both biochemical and histochemical methods that the monoamine oxidase activity of the brain stem of reserpine treated animals increased remarkably (11). Recently, Shore *et al.* (12) reported that, *in vivo*, the release of metaraminol from brain tissue was enhanced by reserpine injection even after pretreatment with iproniazid. These findings strongly suggest that iproniazid and reserpine do not act on the same site in the granules but that the effect of iproniazid is to cause accumulation of catecholamines in the tissue associated with the inhibition of monoamine oxidase activity.

SUMMARY

1. The uptake of ^{14}C -labelled dopamine and norepinephrine by the subcellular granules in brain stem was investigated.
2. The uptake was observed when the granules were incubated at 30°C and not occurred at 0°C . Much more dopamine was taken up than norepinephrine by the granules.
3. Reserpine inhibited the uptake of both dopamine and norepinephrine, but oxy-pertine (Win 18501-2) inhibited only the uptake of norepinephrine at a very low concentration. Iproniazid and adenosine triphosphate influenced the uptake of norepinephrine to some extent.
4. Preincubation of the granules with iproniazid rather potentiated the inhibitory action of reserpine on the uptake of norepinephrine.

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