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ACCUMULATION OF TRITIATED 5-HYDROXYTRYPTAMINE IN BRAIN SLICES

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Recent histochemical studies have demonstrated the presence of 5-hydroxytryptaminergic neurons in brain (1). Like the nerve cells that contain catecholamines, the 5-hydroxytryptamine (5-HT) neurons are situated mainly in the lower brain stem.

The mechanism of the intraneuronal storage of 5-HT seems to resemble closely that of catecholamines. For instance tissues are depleted of their 5-HT and catecholamines by reserpine, probably by inhibition of the binding of the amines in the storage granules (2,3). Another catecholamineaccumulating mechanism, which is located in the neuron membranes, is responsible for the rapid uptake of catecholamines by the neurons (4,5). The 5-hydroxytryptaminergic neurons might have a similar transfer mechanism for 5-HT in the membranes. In order to examine this possibility we have recorded the uptake and accumulation of tritiated 5-HT in slices of mouse brain.

Methods

The accumulation of tritiated 5-HT (5-hydroxytryptamine-^{3}H creatinine sulphate, generally labelled, 5.65 C per mmole, The Radiochemical Centre, Amersham, England) in the brain slices was recorded by the method used previously for determining the uptake of catecholamines in brain slices (6,7).

The incubation mixture consisted of about 100 mg of brain

slices, 0.2 nmole of 5-HT-^{3}H, 2 ml of Krebs-Henseleits solution, pH 7.4, and, when present, the inhibitor to be examined. The incubation was performed at 37°C in a metabolic shaker with an atmosphere of 93.5 per cent oxygen and 6.5 per cent carbon dioxide. After the incubation the slices were dried on a filter paper, weighed and homogenized in 1 ml of ethanol. The radioactivity in the centrifuged ethanol extract was recorded in a liquid scintillation system (Packard TriCarb). The liquid used consisted of 0.4 per cent of 3.4-diphenyloxazole (PPO) and 0.01 per cent of β-bis-[2-(phenyloxazolyl)]benzene (POPOP) in toluene containing 10 per cent of ethanol. The amount of 5-HT taken up by the tissue was expressed in nanomoles per gramme of tissue. No attempt was made to separate 5-HT from its metabolites.

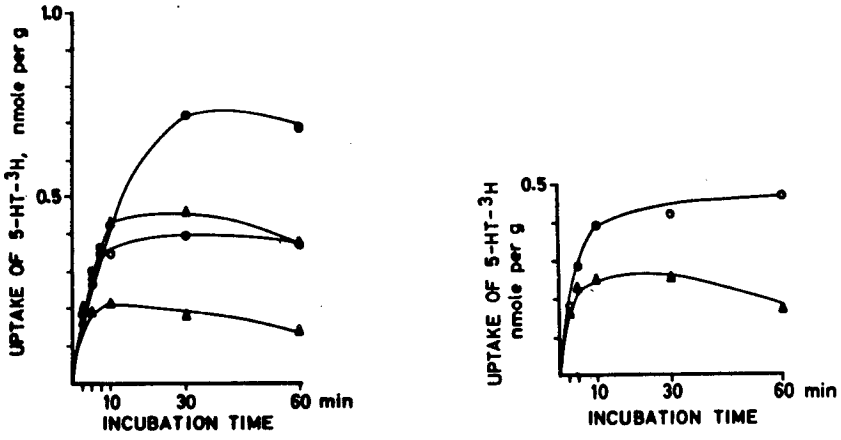
The uptake of tritiated noradrenaline was performed as previously described (6,7).

The tissue slices were taken from brain stem of mouse. The cerebral cortex, the olfactory lobes, the cerebellum and the medulla were removed and the remainder was sliced.

Results

There was a large accumulation of 5-HT in the brain slices, especially when the monoamine oxidase (MAO) was inhibited by pretreatment of the animals with pheniprazine (Fig. 1A). The initial rate of the uptake was, however, not increased by this MAO inhibitor. The amount of 5-HT found in the tissue was reduced by administration of reserpine 18 hours before the experiment (Fig. 1A). The same inhibitory effect was observed in mice given pheniprazine after the administration of reserpine. The accumulation of 5-HT was also reduced when the reserpine was added *in vitro* (Fig. 1B).

Cocaine, which has been shown to inhibit the uptake of nor-



A B

FIG. 1

Time curve of the uptake of 5-HT-^{3}H in slices of brain stem of the mouse and the effect of pheniprazine and reserpine on this uptake.
A. Untreated animals ○, reserpine, 5 mg/kg i.p. △, pheniprazine hydrochloride, 10 mg/kg i.p. ●, reserpine 4 hours before pheniprazine ▲. The injections were given the day before the experiments.
B. Control slices ○, reserpine, 0.03 µg/ml △. The plotted values are means of three determinations.

adrenaline and dopamine in brain slices (7), also decreased the uptake of 5-HT (Fig. 2) and the concentration range of the inhibitory effect (EC_{50} = $3 \cdot 10^{-6}$M) was about the same as that found for the uptake of the catecholamines. As the dose-response curve shows, almost half the uptake of 5-HT was resistant to cocaine.

Ouabain decreased significantly the uptake of 5-HT present in a concentration known to inhibit active transfer mechanisms in cell membranes (8) (Table 1).

Desipramine and (±)-amphetamine, both potent inhibitors of the uptake of noradrenaline (5,9), excerted a weaker inhibitory effect on the uptake of 5-HT than of noradrenaline (unpublished

TABLE 1

Inhibition of the Accumulation of Tritiated 5-Hydroxy-tryptamine in Slices of Brain Stem of Mouse.

	Conc. M	Decrease in uptake of 5-HT (nmole per g)
Ouabain	$3 \cdot 10^{-5}$	0.064
Desipramine	$3 \cdot 10^{-5}$	0.137
	$7 \cdot 10^{-6}$	0.079
(±)-Amphetamine	$5 \cdot 10^{-5}$	0.074
	$1 \cdot 10^{-5}$	0.010
(-)-Noradrenaline	$6 \cdot 10^{-5}$	0.038
	$6 \cdot 10^{-6}$	0.014
Dopamine	$5 \cdot 10^{-5}$	0.104
	$5 \cdot 10^{-6}$	0.037
Tryptamine	$5 \cdot 10^{-5}$	0.164
	$1 \cdot 10^{-5}$	0.062
	$2 \cdot 10^{-6}$	0.007
N,N-Diethyltryptamine	$5 \cdot 10^{-5}$	0.171
	$5 \cdot 10^{-6}$	0.072
	$5 \cdot 10^{-7}$	0.035
Bufotenine	$3 \cdot 10^{-5}$	0.162
	$3 \cdot 10^{-6}$	0.113
	$3 \cdot 10^{-7}$	0.043
LSD 25	$3 \cdot 10^{-6}$	0.031
Mescaline	$4 \cdot 10^{-5}$	0.040

The slices were pre-incubated with the compound for 5 min. and incubated for further 5 min. with 10^{-7}M 5-HT-^{3}H. The amount of 5-HT in the control slices was 0.290±0.003 nmole per g (n = 27). The values are means of three determinations.

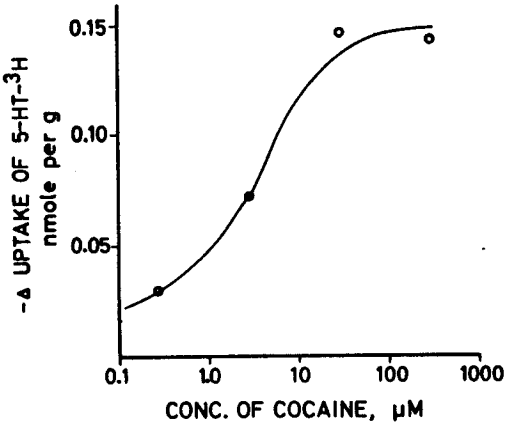


FIG. 2

Inhibitory effect of cocaine on the uptake of 5-HT-^{3}H in slices of brain stem of mouse.
The experimental conditions were as described in the footnote to table 1. The amount of 5-HT-^{3}H in the control slices were 0.290 ± 0.003 nmole per g.

observations). The effect of noradrenaline and dopamine was also quite weak. The uptake of tritiated noradrenaline was inhibited by 5-HT and tryptamine, but quite high concentrations were necessary (Table 2).

Several tryptamine derivatives were examined for their inhibitory effect on the uptake of 5-HT (Table 1). The most potent of these compounds was bufotenine; at the other end of the scale is LSD 25, which was almost without effect either *in vitro* or *in vivo* (10 mg per kg i.p. one hour before the experiment).

Discussion

The results obtained in this investigation show that as regards the response of 5-HT to inhibitors the accumulation of this amine in brain slices was in some respects like that of catecholamines. However, there were also several differences. For instance

TABLE 2

Inhibition of the Uptake of Tritiated Noradrenaline in Slices of Brain Stem of Mouse.

	Conc. M	Decrease in uptake of noradrenaline (nmole per g)
5-Hydroxytryptamine	$2 \cdot 10^{-5}$	0.052
	$2 \cdot 10^{-6}$	0.032
Tryptamine	$5 \cdot 10^{-5}$	0.095
	$5 \cdot 10^{-6}$	0.056

The experimental conditions were the same as described in the footnote to table 1 except that noradrenaline-7-^{3}H was used instead of 5-HT. The amount of noradrenaline accumulated in the control slices was 0.158 ± 0.003 nmole per g (n = 3). The values are means of three determinations.

though desipramine is a much weaker inhibitor of the uptake of 5-HT than of noradrenaline ($EC_{50} = 3 \cdot 10^{-8}$M, unpublished observation), its activity was about the same as has been found for the uptake of dopamine in slices of corpus striatum of rabbit ($EC_{50} = 6 \cdot 10^{-5}$M, unpublished observation). (±)-Amphetamine, on the other hand, was a much weaker inhibitor of the uptake of 5-HT than of dopamine ($EC_{50} = 1 \cdot 10^{-6}$M, unpublished observation). Noradrenaline and dopamine had also quite weak inhibitory effect on the uptake of 5-HT. Although it cannot be excluded that the uptake of 5-HT may take place partly in adrenergic neurons, the results indicate that the uptake of this amine by the brain tissue differed in its mechanism from the uptake of the catecholamines, and it is possible that in the former case the specific 5-hydroxytryptaminergic neurons demonstrated by Dahlström and Fuxe (1) are responsible.

The inhibitory effect of ouabain points to a membrane transfer mechanism for the uptake of 5-HT, since this compound inhibits several transfer reactions in cell membranes (8). Cocaine is thought to inhibit the uptake of noradrenaline in adrenergic neurons at the site of the cell membranes (4,5). Although we could not reliably differentiate between the two amineaccumulating mechanisms it seems probable that the inhibitory action of cocaine is similar in type for 5-HT and catecholamines.

The reduction of the accumulation of 5-HT by reserpine may be due to destruction of the intraneuronal binding action. The amine is then metabolized by MAO, and the 5-hydroxyindoleacetic acid formed diffuses to the incubation medium in the same way as for the corresponding acid metabolite of noradrenaline (10). That the inhibition of MAO greatly increased the amount of 5-HT accumulated in the tissue indicates that the capacity of the intraneuronal binding of 5-HT is less than that of the uptake.

If 5-HT is assumed to act as a neurotransmitter the uptake of 5-HT in the neurons may be a factor in its physiologic inactivation just as has been proposed for the uptake of the catecholamines (4,5). The view that MAO is localized intraneuronally (11) is supported by the observed large increase in the amount of 5-HT accumulated in the slices on inhibition of this enzyme, especially with prolonged incubation. There is at present no information on any other enzyme metabolizing 5-HT outside the neurons. The action of this amine as a neurotransmitter may thus possibly be potentiated by inhibitors of the uptake of 5-HT through the neuron membrane. The inhibition effected by tryptamine derivatives may account for the hallucinogenic effect of this class of compounds (12). The psychotomimetic action of LSD 25 and mescaline cannot, however, apparently be caused by inhibition of the uptake

of 5-HT.

Summary

Slices of brain stem of the mouse accumulated tritiated 5-hydroxytryptamine (5-HT) when incubated with low concentrations of this amine. This accumulation was greatly increased by the monoamine oxidase inhibitor pheniprazine, especially with prolonged incubation. The amount of 5-HT accumulated in the slices was reduced by reserpine, ouabain, cocaine, desipramine, amphetamine, catecholamines and tryptamine derivatives, but LSD 25 and mescaline had almost no such effect.

It would seem that the accumulation of 5-HT occurs mainly in specific 5-hydroxytryptaminergic neurons, and it is effected by two amine-accumulating processes: (1) a transfer mechanism in the neuron membrane, which is inhibited by ouabain and cocaine and (2) an intraneuronal binding mechanism, which is inhibited by reserpine.

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