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Structural specificity for inhibition of [^{14}C]-5-hydroxytryptamine uptake by cerebral slices

A. CARLSSON

Department of Pharmacology, University of Göteborg, Göteborg, Sweden

Cerebral slices were prepared from mice pretreated with reserpine and nialamide. The slices were incubated for 40 min at 37° in a Krebs-Henseleit solution equilibrated with 5% carbon dioxide in oxygen and containing [^{14}C]-5-hydroxytryptamine. An uptake, dependent on energy metabolism and temperature, was observed. The uptake was blocked by tricyclic antidepressants, the order of activity being chlorimipramine > imipramine > desipramine. For 50% inhibition 0·03 μg/ml of chlorimipramine was required, when added to the suspension medium, or 1 mg/kg, when injected intraperitoneally 20 min beforehand. Comparison with earlier experiments using different experimental techniques appears to justify the conclusion that the evidence obtained represents the uptake mechanism of cerebral 5-hydroxytryptamine neurons.

In earlier experiments we have investigated the reserpine-resistant "membrane pumps" by means of which monoamine-carrying neurons take up and concentrate amines from the extracellular fluid. To identify the neurons under investigation we have concentrated on the influence of membrane pump blockade on the endogenous monoamines–noradrenaline, dopamine, and 5-hydroxytryptamine (5-HT). This has necessitated the use of indirect methods like the blockade of the monoamine-displacing action of certain synthetic analogues, and the release of monoamines after membrane pump blockade (Carlsson, Corrodi, & others, 1969a, b; Carlsson & Lindqvist, 1969; Carlsson, Jonason & Lindqvist, 1969; Carlsson, Jonason & others, 1969).

Using these techniques clear-cut differences in the structural requirements for inhibition of the membrane pumps of the different monoamine carrying neurons of the brain have been detected. In the present preliminary report this problem is further examined using *in vitro* uptake of ^{14}C-5-HT by cerebral slices.

EXPERIMENTAL

White female mice (NMRI), 17–25 g, were injected intraperitoneally with reserpine, 25 mg/kg, 17 h before death. After 14·5 h the animals were placed at an ambient temperature of +30°. Nialamide (250 mg/kg i.p.) was given 40 min before decapitation of the mice. In some experiments test drugs were injected 20 min before death.

Brain hemispheres (except the corpus striatum) were dissected in a petri dish filled with ice and then quickly rinsed twice with Krebs-Henseleit solution (equilibrated with 5% carbon dioxide in oxygen at room temperature, 23–25°) to remove blood on the surface. Each hemisphere was divided into 0·8 mm slices by means of a miniature "household egg slicer": the tissue was supported in a shallow depression whilst a grid of longitudinal wires (0·05 mm diameter) was pressed through it.

The slices of 3 hemispheres from 3 different brains (about 200 mg) were transferred into a glass tube containing 10 ml Krebs-Henseleit solution equilibrated with 5% carbon dioxide in oxygen and with the test drug added to the medium. The interval between killing an animal and the introduction of its second sliced hemisphere into the

Krebs-Henseleit solution was about 90 s. Preincubation was made at 37° for 10–25 min. In some experiments 5% carbon dioxide in nitrogen was used and the glucose in the Krebs-Henseleit solution was omitted.

After the preincubation, 10 ng of 5-HT-2-^{14}C (18 mCi/mmol) were added and the incubation was continued for additional 40 min. The incubation was terminated by transferring slices and incubation medium to plastic centrifuge tubes and the slices were immediately spun down in a refrigerated centrifuge at 14 000 *g* for 5 min. The supernatant was decanted and the slices were washed for 10 min by shaking in 10 ml of 0·9% saline at +4°. After centrifugation and decantation the slices were extracted twice in 5 + 4 ml 0·4N perchloric acid containing totally 0·2 ml of 10% EDTA (disodium ethylenediamine tetra-acetate) and 0·1 ml of 2% ascorbic acid.

Three ml aliquots of the supernatants of incubation medium, saline and extract were each taken and mixed with 3 ml Insta-Gel. Radioactivity was measured by liquid scintillation (Packard Tri Carb). Quenching was checked by internal standards. The sum of ^{14}C-5-HT recovered from the 3 supernatants was approximately 100% of the amount added to the incubation medium. The extract activity values, corrected for background, were used for calculating ^{14}C-5-HT uptake.

RESULTS

When added to the incubation medium, chlorimipramine caused a pronounced inhibition of ^{14}C-5-HT uptake. At maximum inhibition the uptake was only about 20% of the control value (Fig. 1). This remaining chlorimipramine-resistant uptake probably represents a true uptake mechanism and not an unspecific adsorption phenomenon. This assumption is supported by the fact that the ^{14}C-5-HT taken up had evidently not been removed by the washing procedure routinely employed.

The concentration of chlorimipramine required for 50% inhibition (EC50) was 0·027 μg/ml, corresponding to 10^{-7} M. Imipramine was some 5 times weaker than chlorimipramine but 10 times stronger than desipramine as an inhibitor of 5-HT uptake.

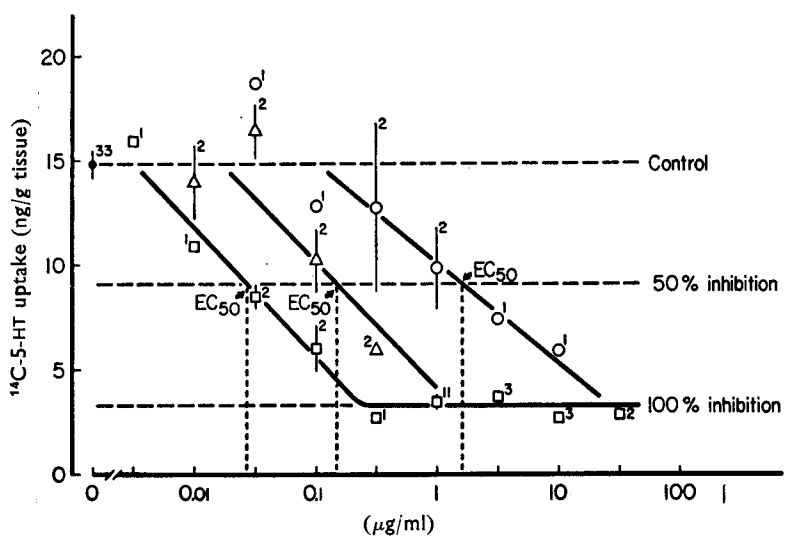

FIG. 1. Effect of tricyclic antidepressant drugs on [^{14}C]-5-hydroxytryptamine uptake by mouse cerebral slices. Addition of inhibitors *in vitro*. For estimation of EC50 the 100% inhibition level is obtained from the chlorimipramine data as shown in the figure. ○—○, desipramine (EC50 = 1·6 μg/ml); △—△, imipramine (EC50 = 0·15 μg/ml); □—□, chlorimipramine (EC50 = 0·27 μg/ml).

Fig. 2 shows the results obtained when the inhibitors had been administered intraperitoneally 20 min before killing the animals and preparing the cerebral slices for the *in vitro* uptake experiments. Again, chlorimipramine proved more active than imipramine, whereas desipramine was devoid of activity in the doses employed.

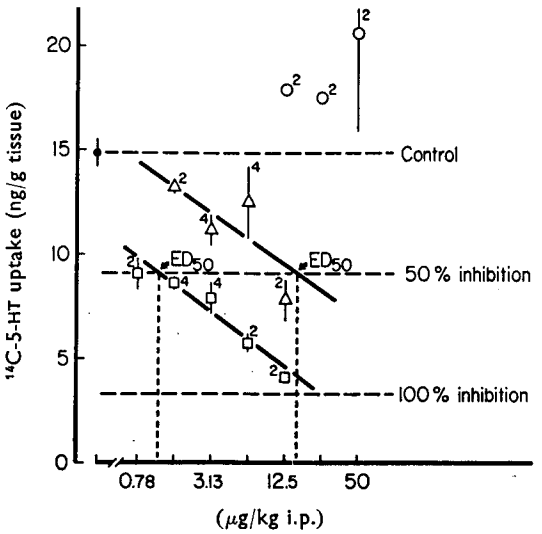

FIG. 2. Effect of tricyclic antidepressant drugs on [^{14}C]-5-hydroxytryptamine uptake by mouse cerebral slices. Intraperitoneal injection of inhibitors. The 100% inhibition level is obtained from the chlorimipramine *in vitro* data. ○—○, desipramine; △—△, imipramine (ED50 = 15·6 mg/kg); □—□, chlorimipramine (ED50 = 1·1 mg/kg).

Table 1 summarizes some preliminary results with other inhibitors of ^{14}C-5-HT uptake. Among the most potent "inhibitors" found thus far 5-HT itself has a comparably high affinity for the membrane pump. 5-HT is equally or closely followed by chlorimipramine and dexbrompheniramine. Other fairly potent inhibitors are dexchlorpheniramine, H75/12 (4-methyl-α-ethyl-*m*-tyramine), tryptamine and its *N*-dimethyl and α-ethyl derivatives. Ouabain was active in a relatively low concentration ($3 \cdot 10^{-6}$M).

When glucose was omitted from the suspension medium and oxygen was replaced by nitrogen, ^{14}C-5-HT uptake dropped to approximately the same level as after a high concentration of chlorimipramine.

Table 1. *The inhibition of* ^{14}C-5-HT *uptake in cerebral slices by drugs.* Shown are approximate concentrations necessary for 50% inhibition.

	μg/ml	M
5-hydroxytryptamine	0·02	10^{-7}
Tryptamine HCl	0·06	3×10^{-7}
NN-Dimethyltryptamine	0·06	3×10^{-7}
α-Ethyltryptamine acetate	0·06	2×10^{-7}
H75/12 HCl	0·10	5×10^{-7}
p-Chloromethamphetamine HCl	0·03	10^{-7}
Chlorimipramine HCl	0·03	10^{-7}
Imipramine HCl	0·15	5×10^{-7}
Desipramine HCl	1·6	5×10^{-6}
Dexbrompheniramine maleate	0·07	2×10^{-7}
Dexchlorpheniramine maleate	0·20	5×10^{-7}
Ouabain octahydrate	2	3×10^{-6}
Iodoacetic acid	20	10^{-4}

At an incubation temperature of 0° the uptake of ^{14}C-5-HT was approximately the same as after addition of chlorimipramine in a high concentration at 37°.

DISCUSSION

Our experiments indicate a relatively high structural specificity of the 5-HT uptake mechanism of mouse cerebral slices. The observations are in fairly close agreement with our earlier experiments using indirect methods for measuring inhibition of the membrane pump of cerebral 5-HT neurons. Thus the order of potency chlorimipramine > imipramine > desipramine is the same for all the various techniques we have used so far for studying 5-HT uptake. This order is reversed for the membrane pump of central noradrenaline neurons.

The ED50 of chlorimipramine found in the present investigation after intraperitoneal injection was 1 mg/kg. In our earlier study using H75/12 the ED50 observed was 7 mg/kg (Carlsson & others, 1969a). This discrepancy may be apparent rather than real. In the H 75/12 test the inhibitor probably has to compete for the uptake sites with H 75/12 which has a relatively strong affinity for these sites, as indicated by the present data. This should result in a right-hand shift of the dose-response curve, compared to the present conditions where little or no competition will occur. Moreover, a very close agreement between the two sets of data cannot be expected for another reason; in the 4-h test with H 75/12 the duration of action of the inhibitor may be a more important factor than in the present experiments.

Part of the present experiments confirms earlier work on ^{14}C-5-HT uptake by cerebral slices (Blackburn, French & Merrills 1967, Ross & Renyi 1967, 1969). For example, Ross & Renyi found imipramine and desipramine to inhibit 5-HT uptake in approximately the same concentrations as in the present investigation. On the other hand about 10 times higher concentrations of *NN*-dimethyltryptamine were required for 50% inhibition in their investigation compared to ours. The most likely explanation of this difference appears to be that our animals, but not theirs, had been pretreated with reserpine and nialamide.

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