may be, in part, an indirect effect of the liberation of histamine from tissue cells.

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The influences of drugs on the uptake of 5-hydroxytryptamine by nerve-ending particles of rabbit brain stem

SIR,—Recent progress in the technique of isolation of nerve-ending particles (synaptosomes) and synaptic vesicles from brain (Whittaker, 1959; DeRobertis, Rodriguez de Lores Arnaiz & Pellegrino de Iraldi, 1962) has made it possible to investigate the detailed mechanisms of uptake, binding and release of biogenic amines at the subcellular level. Maynert & Kuriyama (1964) found that nerve-ending particles or synaptic vesicles of brain, when incubated in a medium containing noradrenaline or 5-hydroxytryptamine (5-HT), can take up these amines from the medium against the concentration gradient, and they suggested that both nerve ending particles and synaptic vesicles possessed a transport system for noradrenaline and 5-HT. Furthermore, they found that reserpine inhibited the uptake of these amines. Independently, Robinson, Anderson & Green (1965) showed that nerve ending particles and microsomes of brain can take up 5-HT and histamine *in vitro*. Little is known about the kinetics of such uptake so far.

Reserpine, cocaine, desipramine and prenylamine have been reported to inhibit the catecholamine uptake-concentrating mechanism of adrenergic neurons (Hillarp & Malmfors, 1964; Lundmar & Muscholl, 1964; Carlsson & Waldeck, 1965; Malmfors, 1965), and the present study was undertaken to investigate the influences of these drugs on the uptake of 5-HT by nerve-ending particles *in vitro*.

Male rabbits, weighing about 2·5 kg were used. Two brain stems (*ca* 5 g) were homogenized in ice-cold 0·32 M sucrose with a Teflon pestle and made up to about 50 ml. The P_1-fraction was separated by centrifuging the homogenate at 900 *g* for 10 min. This P_1-fraction was washed twice with 0·32 M sucrose and the washings were added to the supernatant fluid from the P_1 preparation. The P_2-fraction, a crude mitochondrial fraction, was prepared by centrifuging the supernatant at 11,500 *g* for 20 min. The method of subsequent sub-fractionation of the P_2-fraction was similar to that described by Gray & Whittaker (1962). The P_2-fraction was resuspended in 0·32 M sucrose (2 ml/g of original tissue) and 5 ml of this suspension was laid on the top of a discontinuous density gradient consisting of 12 ml each of 0·8 M and 1·2 M sucrose per tube, and centrifuged at 53,500 *g* for 2 hr. This resulted in the subfractions A, B and C which contained predominantly myelin, nerve ending particles and mitochondria

respectively. The top fraction, A, was removed by aspiration. B-fraction, the intermediate band between 0·8 and 1·2 M sucrose, was collected and diluted to approximately 0·32 M with respect to sucrose by the addition of two volumes of ice-cold distilled water. This fraction was centrifuged again at 35,000 *g* for 20 min to yield a particulate fraction. Electron microscopic examination showed that the B-particulate fraction contained a large number of intact nerve-ending particles slightly contaminated by free mitochondria.

For 5-HT uptake experiments, the fraction containing nerve ending particles suspended in Krebs solution of pH 7·6, was mixed with a solution of monoamine oxidase inhibitor, pheniprazine (final concentration of 5×10^{-6} M) and a solution of the drug to be tested. After incubation at 37° in air for 30 min, a solution of 5-HT in phosphate buffer of pH 7·0 was added and incubation continued for a further 30 min at 37°. In another experiment the suspension of nerve ending particles was pre-incubated with pheniprazine (5×10^{-6} M) alone for 30 min at 37°, thereafter test drugs were added to the incubation medium. After 30 min incubation at 37°, 5-HT was added and incubation continued for 30 min at 37°. The mixture was then centrifuged for 20 min at 35,000 *g* and the supernatant fluid decanted. The pellet of nerve ending particle was directly (or after washing twice with Krebs solution) subjected to 5-HT estimation. 5-HT was extracted and assayed fluorimetrically as described by Snyder, Axelrod & Zweig (1965). After the experiment the fraction containing nerve ending particles was examined by electron microscopy and most of the particles were found unchanged in structure even after incubation.

Under the conditions described, the uptake of 5-HT by the particle fraction was inhibited by all drugs tested at the concentration of 20 μg/ml (Table 1). The inhibitory effects of drugs were not reduced when the particle fraction was pre-incubated with pheniprazine alone. This result eliminates the possibility that when pheniprazine and drug were added to the medium simultaneously, the drug inhibited the entry of pheniprazine into the nerve ending particles thereby enabling any 5-HT taken up into the particles to be attacked and oxidized by intact monoamine oxidase in the cytoplasm. Among the drugs tested desipramine was found to be most potent. This would coincide with the finding that it is the most effective inhibitor of noradrenaline uptake by sympathetically innervated tissue (Iversen, 1965). However this is inconsistent with the observation that desipramine does not interfere with the uptake of 5-HT perfused into a ventricle of the brain (Palaić, Page & Khairallah, 1967). Reserpine decreased

TABLE 1. EFFECTS OF DRUGS (20 μG/ML) ON THE UPTAKE OF 5-HT AT 37° BY THE FRACTION CONTAINING NERVE ENDING PARTICLES. In expt 1 the fraction containing nerve ending particles was incubated with pheniprazine and test drug, while in expt 2 the fraction was pre-incubated with pheniprazine alone before addition of test drug.

Drug	5-HT in medium (μg base/ml)	Wash	% change from control	
			Expt 1	Expt 2
Reserpine	2	0	−37·72***(4)	
	2	2	−44·72** (8)	−47·39 (3)
Desipramine HCl	2	0	−65·55** (4)	
	2	2	−86·26* (4)	−87·79 (3)
Cocaine HCl	2	0	+12·89 (4)	
	2	2	−11·95 (4)	
	1	2	−29·86** (12)	−26·72 (8)

* $P < 0{\cdot}001$.
** $P < 0{\cdot}01$.
*** $P < 0{\cdot}05$.

5-HT uptake significantly but its effect was approximately half that of desipramine. This result might imply that there exists a reserpine-resistant uptake-concentrating mechanism in 5-HT neurons (Fuxe & Ungerstedt, 1967). In contrast to desipramine and reserpine, cocaine showed no significant effect on 5-HT uptake unless the concentration of 5-HT in the medium was decreased to 1 μg/ml.

The present findings indicate that the drugs which inhibit the uptake of catecholamine by adrenergic neurons also inhibit the uptake of 5-HT by nerve ending particles to some degree. Evidence supporting the existence of two different catecholamine uptake-concentrating mechanisms of adrenergic neurons has recently been presented (Carlsson, Hillarp & Waldeck, 1963; Hillarp & Malmfors, 1964; Malmfors, 1965; Carlsson & Waldeck, 1965; Obianwu, 1967). The first mechanism, the membrane pump, is selectively blocked by cocaine or desipramine (Hillarp & Malmfors, 1964; Lundmar & Muscholl, 1964; Carlsson & Waldeck, 1965; Malmfors, 1965), whereas the second mechanism, incorporation into a storage granule, is selectively blocked by reserpine (Hillarp & Malmfors, 1964; Carlsson & Waldeck, 1965; Malmfors, 1965). The question now arises whether similar dual mechanisms are involved in the uptake of 5-HT into 5-HT neurons, and whether these drugs act at the same site as with catecholamine uptake.

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