The influences of drugs on the uptake of 5-hydroxytryptamine by synaptic vesicles of rabbit brain stem

SIR,—Previous work in our laboratory has shown that reserpine, desipramine and cocaine, when incubated in a medium containing nerve ending particles, 5-hydroxytryptamine (5-HT) and the monoamine oxidase inhibitor, pheniprazine, exerted an inhibitory effect on the uptake of 5-HT by these nerve ending particles (Segawa & Kuruma, 1968). In adrenergic neurons it has been demonstrated that desipramine and cocaine selectively blocked the amine transport through the neuronal membrane while reserpine selectively blocked incorporation into the storage granule (Hillarp & Malmfors, 1964; Carlsson & Waldeck, 1965; Malmfors, 1965). Whether these drugs act at the same sites on 5-HT uptake in 5-HT neurons as with catecholamine uptake in adrenergic neurons is still unknown. We now describe an attempt to see if these drugs have also an inhibitory effect on the uptake of 5-HT by isolated synaptic vesicles.

Male rabbits, of 2·5 kg provided three brain stems (about 7·5 g), which were homogenized in ice-cold 0·32 M sucrose with a Teflon pestle and made up to about 75 ml. The crude mitochondrial P_2-fraction, was prepared by differential centrifugation (Segawa & Kuruma, 1968). The isolation of synaptic vesicles from the disrupted nerve ending particles was made as described by DeRobertis, Rodriguez de Lores Arnaiz & Pellegrino de Iraldi (1962), DeRobertis, Rodriguez de Lores Arnaiz, & others (1963) and Maynert, Levi & De Lorenzo (1964), with some modification. The P_2-fraction was resuspended in 0·32 M sucrose (3 ml/g of original tissue) and diluted to approximately 0·08 M sucrose by the addition of three volumes of ice-cold distilled water. This fraction was homogenized again with a Teflon pestle for about 90 sec at 4° and again centrifuged at 11,500 *g* for 20 min to separate the synaptic ghosts, swollen mitochondria and myelin. The supernatant was then centrifuged at 100,000 *g* for 30 min to sediment the synaptic vesicles fraction. This fraction was separated from the supernatant simply by decantation. Under the electron microscope this fraction appeared to consist mainly of isolated synaptic vesicles contaminated by few membrane fragments.

For 5-HT uptake experiments, the fraction containing synaptic vesicles was suspended in about 40 ml of Krebs solution of pH 7·6. To 4·6 ml of the suspension, 0·2 ml of 5-HT dissolved in phosphate buffer of pH 7·0 and 0·2 ml of test drugs (final concentration of 20 μg/ml) were added. The mixture was then incubated at 37° in air for 60 min. After incubation the mixture was centrifuged at 100,000 *g* for 30 min and the supernantant fluid was decanted. The pellet of synaptic vesicles was directly (or after washing twice with Krebs solution) subjected to 5-HT estimation. 5-HT was extracted and assayed fluorimetrically (Snyder, Axelrod & Zweig, 1965). By electron microscope it was found that most of the synaptic vesicles were unchanged in structure even after 1 hr incubation.

Table 1 gives the results obtained in this experiment. Reserpine and desipr-

TABLE 1. THE EFFECTS OF DRUGS (20 μG/ML) ON THE UPTAKE OF 5-HT AT 37° BY THE FRACTION CONTAINING SYNAPTIC VESICLES FROM THE BRAIN STEM OF THE RABBIT

Drugs	5-HT in medium (μg base/ml)	wash	% change from *control*
Reserpine	2	0	−26·45*** (4)
Desipramine HCl	2	2	−84·15* (11)
Imipramine HCl	2	2	−78·70** (5)
Cocaine HCl	2	2	−27·80† (5)
	1	2	−34·50† (6)

* $P < 0.001$. ** $P < 0.01$. *** $P < 0.02$. † $P > 0.1$.

amine which inhibited the uptake of 5-HT by nerve ending particles also showed inhibitory effect on isolated synaptic vesicles. These results imply that if reserpine and desipramine are able to be transported through neuronal membrane they are capable of blocking the intracellular 5-HT concentrating mechanism located at synaptic vesicles level. On the other hand cocaine showed no significant effect on 5-HT uptake by synaptic vesicles even when the concentration of 5-HT in the medium was decreased to 1 μg/ml. This result together with the previous observation that cocaine caused an inhibition of the uptake of 5-HT by nerve ending particles (Segawa & Kuruma, 1968) indicates that cocaine selectively blocks the neuronal membrane pump in the same way as it does with catecholamine uptake. Imipramine was reported to be capable of blocking the 5-HT concentrating mechanism located at the level of cell membrane (Fuxe & Ungerstedt, 1968). Our result showed that imipramine significantly blocked 5-HT uptake by synaptic vesicles under the experimental condition described above.

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July 23, 1968

References

Carlsson, A. & Waldeck, B. (1965). *Acta pharmac. tox.*, **22**, 293–300.
DeRobertis, E., Rodriguez de Lores Arnaiz, G. & Pellegrino de Iraldi, A. (1962). *Nature, Lond.*, **194**, 794–795.
DeRobertis, E., Rodriguez de Lores Arnaiz, G., Salganicoff, L., Pellegrino de Iraldi, A. & Zieher, L. M. (1963). *J. Neurochem.*, **10**, 225–235.
Fuxe, K. & Ungerstedt, U. (1968). *J. Pharm. Pharmac.*, **20**, 150–151.
Hillarp, N.-A. & Malmfors, T. (1964). *Life Sci.*, **3**, 703–708.
Malmfors, T. (1965). *Acta physiol. scand.*, **64**, *Suppl.* 248.
Maynert, E. W., Levi, R. & DeLorenzo, A. J. D. (1964). *J. Pharmac. exp. Ther.*, **144**, 385–392.
Segawa, T. & Kuruma, I. (1968). *J. Pharm. Pharmac.*, **20**, 320–322.
Snyder, S. H., Axelrod, J. & Zweig, M. (1965). *Biochem. Pharmac.*, **14**, 831–835.

Development of antihistamine and anti-allergic activity after prolonged administration of a plant saponin from *Clerodendron serratum*

SIR,—It was of interest that both the alcoholic extract and the saponin isolated from the root bark of an indigenous plant, *Clerodendron serratum*, which has been used for the treatment of bronchial asthma, caused release of histamine from lung tissue (Gupta & Gupta, 1967). Prolonged administration of the saponin in 20 mg/kg doses caused significant depletion of the amine from the lungs of rats treated with the drug (Gupta, Mahesh Rai & Gupta, 1967). The saponin fraction like other histamine releasers was not found to manifest any antihistamine activity or to give protection against anaphylactic shock in sensitized guinea-pigs exposed to egg albumin (antigen) micro-aerosols (Mongar & Schild, 1952). However, the continued daily administration of the drug, 20 mg/kg (1/15 of the LD50 dose 307·7 mg/kg), intramuscularly for 20 days to sensitized guinea-pigs was found to gradually develop protection against anaphylaxis. This became evident from the significant ($P < 0{\cdot}05$) delay in onset of dyspnoea in treated animals exposed to 1·0% egg albumin micro-aerosol as compared to the controls. At the end of 20 days, the treated animals on continued exposure to the micro-aerosol for 10 min, did not manifest air hunger or asphyxial convulsion as was observed in untreated controls before collapse. The results are summarized in Table 1.