EFFECTS OF RESERPINE AND DESIPRAMINE ON THE UPTAKE AND SUBCELLULAR DISTRIBUTION OF 5-HYDROXYTRYPTAMINE IN RABBIT BRAIN STEM AFTER INTRAVENOUS ADMINISTRATION OF 5-HYDROXYTRYPTOPHAN

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Peripheral administration of 5-hydroxytryptophan (5-HTP) has been shown to increase the amount of 5-hydroxytryptamine (5-HT) in brain (1, 2). The pretreatment with reserpine inhibited this increase (2). After monoamine oxidase inhibitor a large amount of 5-HT could be found to be accumulated by the administration of 5-HTP regardless of whether the animals were treated with reserpine or not (1-3). Histochemical studies have shown that most of 5-HTP-induced 5-HT was ascribable to increased amine content in 5-HT neurons in the central nervous system (4), but part of it was found in an extraneuronal site (2). However, because the majority of these structures are small, being of submicroscopical size, it seems difficult for histochemical procedure to identify the subcellular site of uptake and storage of 5-HTP-induced 5-HT. Therefore, in this paper an attempt was made to fractionate rabbit brain stem homogenate after intravenous administration of 5-HTP and to determine 5-HT concentration in each fraction. In addition the effects of pretreatment with reserpine, pheniprazine and desipramine in the increased amount of 5-HT were also investigated.

METHODS

Male rabbits weighing about 2.5 kg were used. The intravenous injection of 5-HTP (50 mg/kg) dissolved in saline was made on normal rabbits, on reserpine pretreated rabbits (3 mg/kg, i.p., 15 hours before death), on rabbits pretreated with reserpine (3 mg/kg, i.p., 15 hours before death) and pheniprazine (3 mg/kg, i.p., 1 hour before death) and on desipramine pretreated rabbits (20 mg/kg, i.p., 50 minutes before death). The rabbits were sacrificed 30 minutes after 5-HTP injection by bleeding. The brain stem of about 2.5 g was dissected, homogenized with a Teflon pestle in an ice-cold 0.32 M sucrose which contained 5 x 10⁻⁴ M pheniprazine and was made up to about 25 ml. The method of the fractionation of P₁-fraction (debris) and P₁-fraction (crude mitochondria) was the same as that described previously (5). The microsomal P₁-fraction was separated by centrifuging supernatant from P₁-fraction at 100,000 g for 30 minutes, leaving a final super-
natant, $S_3$-fraction containing the soluble constituents of cytoplasmic material. Nerve ending particulate $P_3B$-fraction was obtained from $P_2$-fraction by means of a discontinuous density gradient centrifugation as described by Segawa and Kuruma (5). Synaptic vesicular $P,V$-fraction and soluble supernatant $P,V_S$-fraction were obtained by osmotic shock and centrifugation of $P_2$-fraction with a procedure described previously (6).

5-HT in each fraction was extracted and assayed fluorimetrically by the method of Snyder, Axelrod and Zweig (7).

RESULTS

1. Normal rabbits (Fig. 1)

The intravenous injection of 5-HTP resulted in a very marked increase in 5-HT concentration in brain stem. In total homogenate (sum of 5-HT concentration in primary fractions, i.e., $P_1$, $P_2$, $P_3$ and $S_3$) 5-HT concentration was almost six times as much as the endogenous 5-HT concentration. Among the primary fractions there was the largest increase in $S_3$-fraction followed by the increase in $P_3$, $P_2$ and $P_1$-fraction in that order, while the increase in subfractions was relatively small.

2. Reserpine treated rabbits (Fig. 2)

When the rabbits were pretreated with reserpine increase of 5-HT following administration of 5-HTP was inhibited. There was more than 40% decrease in 5-HT concentration in total homogenate in comparison with the concentration in normal rabbits brain stems given 5-HTP alone. In general the decrease was more prominent in particulate fraction than in soluble fraction except $P_1$-fraction in which the change was small. The decrease in $P,V$-fraction was most remarkable. Practically, 5-HT concentration in this
fraction was less than the endogenous 5-HT concentration, indicating that even after 5-HTP no increase occurred in P2V-fraction.

3. Reserpine and pheniprazine treated rabbits (Fig. 3)
When pheniprazine was injected into the reserpine treated rabbits remarkable restoration of increase in 5-HT concentration after 5-HTP administration was obtained. 5-HT concentration in total homogenate was more than 90% of the concentration in total homogenate treated with 5-HTP alone. The restoration was more prominent in soluble fractions, i.e., in S1- and P1VS-fraction. In P2V-fraction 5-HT was restored by some 75% of the increased concentration in normal rabbit by 5-HTP alone. On the other hand almost no restoration was observed in P3-fraction.

4. Desipramine treated rabbits (Fig. 4)
When 5-HTP was given to desipramine pretreated rabbits there was about a 30% increase in 5-HT concentration in total homogenate compared to the concentration in normal rabbits given 5-HTP alone. This increase occurred in all fractions with the exception of P1V-fraction in which 5-HT concentration was 75% of that found in normal rabbits treated with 5-HTP alone.

DISCUSSION
The results of this investigation showed that intravenously administered 5-HTP could penetrate into the central nervous system and was able to be converted into 5-HT there.
The increase was more prominent in fractions derived from cell bodies and axons (P3- and S3-fraction). This observation may be explained in two ways: 1) 5-HTP decarboxylase has been shown to be highly localized in somal and axonal fractions, presumably in S3-fraction (8), therefore, intraneuronal 5-HTP is converted more readily into 5-HT in these regions than in synaptic areas. 2) Originally 5-HTP from circulation is first accumulated in the cell bodies and axons, decarboxylated to 5-HT and then transported down to the terminals. The latter speculation is based on the well-known idea that amine storage granules are formed in cell bodies and reach the terminals by soma-axonal flow.

Reserpine inhibited increase in 5-HT concentration after 5-HTP, yet more than endogenous amounts of 5-HT were observed. This indicates that at least part of 5-HTP penetrates into the neuronal cell and is converted into 5-HT even after reserpine. Of great significance is the fact that no increase was observed in P3V-fraction. This result provides the clear evidence that reserpine selectively blocks the mechanism for incorporating 5-HT into synaptic vesicles. The mechanism which underlies the relative insensitivity to reserpine of increase in 5-HT concentration in P1-fraction remains obscure since this fraction consists of the complex cellular structures. Corrodi, Fuxe and Hökfelt (2), using histochemical and biochemical techniques found that after depletion of central 5-HT store by reserpine 5-HTP was able to increase 5-HT in extraneuronal parts such as endothelial sheath and the pericytes around the capillary walls. Probably part of 5-HT in P1-fraction after reserpine and 5-HTP is due to 5-HT formed in these areas.

After treatment with monoamine oxidase inhibitor an injection of 5-HTP caused a marked increase in 5-HT in reserpine treated rabbits, in agreement with the finding of Corrodi, Fuxe and Hökfelt (2). The increase was significantly higher in soluble fraction than in particulate fraction. This could perhaps be explained by the protection of 5-HT from enzymatic destruction within the cell. Of particular interest is that the increase was also seen in P3V-fraction. This may indicate that amine uptake and concentrating mechanism located at the level of synaptic vesicles impaired by reserpine regains its ability by monoamine oxidase inhibitor, although direct evidence for this assumption is lacking. Too little is known about the microsomal amine binding mechanism but the fact that 5-HTP-induced increase of microsomal 5-HT in reserpinized rabbit brain stem was not increased significantly by monoamine oxidase inhibitor may suggest that the mechanism is different from that located at synaptic vesicles.

Another unanswered question is what kind of mechanism is responsible for the result that there was further increase in the amount of 5-HTP-induced 5-HT in rabbit brain stem after desipramine. Kivalo, Rinne and Karinkanta (9) observed a similar result that imipramine in a single dosage of 50 mg/kg body weight caused a highly significant increase in 5-HT concentration of the rat's brain. Desipramine was found to inhibit the uptake by synaptic vesicles of 5-TH formed within neurons, in agreement with our previous result that desipramine is capable of blocking the intracellular 5-HT concentrating mechanism located at synaptic level (6).
SUMMARY

Subcellular distribution of 5-HT in rabbit brain stem after intravenous administration of 5-HTP was studied. 5-HTP-induced increase was more prominent in P3- and S3-fraction. Reserpine inhibited the increase, particularly in P3V-fraction. Pretreatment with monoamine oxidase inhibitor, pheniprazine, restored 5-HT after 5-HTP even in reserpinized rabbits. Desipramine could further increase the amount of 5-HTP-induced 5-HT although it inhibited the increase in P3V-fraction.

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REFERENCES

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