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INFLUENCES OF INTRAVENTRICULARLY ADMINISTERED 5-HYDROXYTRYPTAMINE ON NORMAL AND AMINE-DEPLETED RABBIT: SUBCELLULAR DISTRIBUTION OF 5-HYDROXYTRYPTAMINE IN THE BRAIN STEM, AND ANIMAL BEHAVIOUR

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Abstract—Normal and *p*-chlorophenylalanine (PCP) pretreated rabbits were subjected to intraventricular injection of 5-hydroxytryptamine (5-HT), and subcellular distribution of 5-HT in the brain stem, drug effects and behavioural changes were investigated. 5-HT in cytoplasmic S_3-fraction was remarkably elevated by intraventricular 5-HT while those of mitochondrial P_2-, microsomal P_3- and synaptic vesicular P_2V-fractions were not increased significantly unless animals were pretreated with PCP. In PCP-treated rabbits, intraventricular 5-HT produced characteristic sedation in animals. Intraperitoneal injection of imipramine (IM) prevented 5-HT-induced increase of 5-HT in P_2-fraction whereas desmethylimipramine (DMI) had no effect. DMI was found to elevate endogenous 5-HT in P_2-fraction and 5-hydroxyindole acetic acid (5-HIAA) in the brain stem while IM caused a significant decrease of 5-HIAA in the brain stem. Reserpine (Re) clearly blocked the increase of 5-HT in P_2V-fraction. Re-induced sedation could not be counteracted by intraventricular injection of 5-HT. The results are discussed.

IN PREVIOUS publications[1,2] we have shown that nerve-ending particles (NEPs) or synaptic vesicles (sv) isolated from rabbit brain stem homogenates, when incubated in a medium containing 5-hydroxytryptamine (5-HT), could take up 5-HT from the surroundings, and reserpine (Re), desmethylimipramine (DMI) and imipramine (IM) were found to be capable of inhibiting the uptake both at NEPs and sv while cocaine could block only at NEPs. The finding is of interest because this gives some information as to the mode of action of neuropharmacologic drugs in relation to centrally mediated serotonergic function.

An extension of the study has been carried out to see if a correlation exists between *in vitro* and *in vivo*.[3] Because 5-HT is unable to penetrate into the central nervous system through blood–brain barrier, the precursor 5-hydroxytryptophan (5-HTP) was injected intravenously and after that subcellular distribution of 5-HT in the brain stem and drug effects were investigated.[3] 5-HTP-induced increase in 5-HT was more prominent in microsomal and supernatant fractions than in synaptic fractions. Re-inhibited the increase in 5-HT at NEPs and sv while DMI inhibited at sv. Therefore, parts of the results *in vivo* were in line with *in vitro* but the possibility cannot be ruled out that drugs may affect 5-HTP transport through neuronal membrane.

The technique of intraventricular administration is of advantage in studying the effect of amine on the central nervous system because we need not take blood–brain barrier into consideration. With the help of the histochemical fluorescence method Fuxe and Ungerstedt[4,5] have shown that intraventricularly administered 5-HT was specifically taken up into central 5-HT neurons. Similarly, Aghajanian and Bloom,[6] using electron microscopic autoradiography, have reported that intraventricular tritiated 5-HT was efficiently taken up by the areas that are rich in the endogenous 5-HT. Palaić *et al.*,[7] have found that perfusion of ventricles with labelled 5-HT resulted in increase of not only labelled 5-HT but endogenous 5-HT. However, we have as yet little information as to the correlation between subcellular distribution of 5-HT in central neurons and animal behaviour after intraventricular administration of 5-HT. Therefore, in an attempt to throw further light upon the physiological significance of 5-HT in central serotonergic function, in this paper the brain stem was fractionated into crude mitochondrial P_2-, cytoplasmic supernatant S_3-, microsomal P_3- and synaptic vesicular P_2V-fractions after intraventricular administration of 5-HT, and the relationship between 5-HT amounts in the subcellular fractions and animal behaviour were studied.

MATERIALS AND METHODS

Both sexes of rabbits weighing about 2·5 kg were used. In an aseptic operation under Pentobarbital-Na anesthesia the injection needle of about 6 mm long with an external diameter of 1 mm was implanted stereotaxically into the brain in the co-ordinates A, 1; L, 2·5; H, 5. The co-ordinates are based on the Atlas of Sawyer *et al.*[8] When cerebro-spinal fluid welled up with respiratory and pulse movements the needle was fixed with dental cement on the cranium. An interval of at least 1 week was allowed between the implantation and the injection of drugs. The drugs were dissolved in pyrogen-free saline and were injected into the lateral ventricle through the implanted needle in a volume of 0·2 ml.

After intraventricular administration of 5-HT, the animal was killed, the brain stem was removed, homogenized and fractionated by the same method as that employed previously in this laboratory.[1,2] Because previous studies[9] from this laboratory have shown that on subfractionation of crude mitochondrial P_2-fraction in the sucrose density gradient not all of 5-HT in P_2-fraction was recovered from myelin, NEPs and mitochondria, in this study P_2-fraction was not subfractionated but total 5-HT in P_2-fraction was used as a basis for discussing the changes in 5-HT in synaptic region.

Electron microscopical structures of particulate P_2-, P_3- and P_2V-fractions have already been reported.[10]

5-HT was extracted and assayed spectrophotofluorometrically by the method of Snyder *et al.*[11] 5-Hydroxyindole acetic acid (5-HIAA) was assayed by the method of Ashcroft and Sharman.[12]

RESULTS

Increase in 5-HT content in normal rabbit

Rabbits received an intraventricular injection of 5-HT (200 μg/0·2 ml/animal) and were killed 1, 1·5 and 2·5 hr later and 5-HT contents in P_2-, P_3-, S_3- and P_2V-fraction were measured. There was a remarkable increase of 5-HT in S_3-fraction mainly

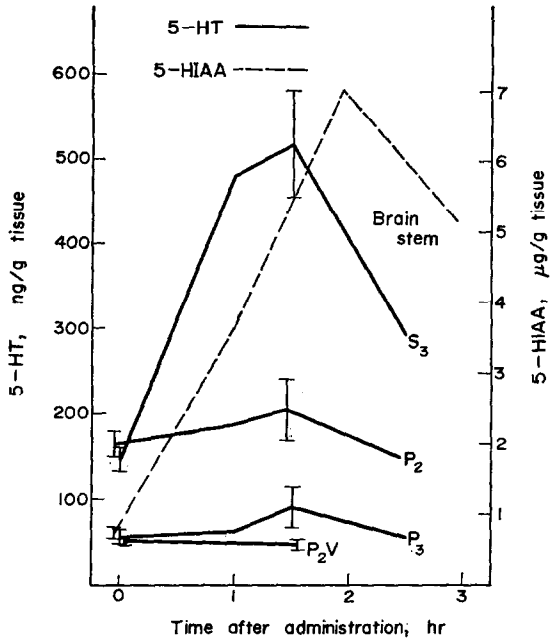

FIG. 1. Changes of 5-HT in brain stem subcellular fractions and 5-HIAA in brain stem as a function of time after intraventricular administration of 5-HT into rabbits. Vertical bars represent the standard error of the mean of four assays. Values without standard error are mean of two determinations.

derived from cell body and axon (Fig. 1). Thus, within 1 hr after administration, 5-HT content increased to a level which was about three times the endogenous amounts and 1·5 hr after injection it reached a maximum level. Thereafter it decreased rapidly and at the end of 2·5 hr less than twice the endogenous level remained in S_3-fraction. In contrast, the increase of 5-HT contents in particulate fractions was less prominent. As is shown in Fig. 1 a slight increase of 5-HT amounts in both P_2- and P_3-fractions was observed 1·5 hr after administration of 5-HT but it returned to normal value within 2·5 hr. Practically no increase of 5-HT was observed in P_2V-fraction. The concentration of 5-HIAA in total brain stem homogenates as a function of time after intraventricular 5-HT is also presented in Fig. 1. Within 2 hr 5-HIAA reached to a maximum value of 7 μg/g tissue which was about 12 times the endogenous 5-HIAA and more than 5 μg/g tissue of 5-HIAA still remained in the brain stem even after 3 hr.

Increase in 5-HT content in p-chlorophenylalanine-treated rabbit

Because a maximum increase in 5-HT contents was obtained 1·5 hr after intraventricular administration of 5-HT, in the following experiments 5-HT in each fraction was measured at this time interval after 5-HT administration.

p-Chlorophenylalanine (PCP) is known to lower concentration of 5-HT in the brain. PCP was injected intraperitoneally in a dose of 150 mg/kg and the rabbits were killed 60 hr afterwards. As shown in Fig. 2 there was marked reduction of 5-HT in P_2-, P_3-, S_3- and P_2V-fractions. Practically, the values in S_3 and P_2V were nil. In order to see if intraventricular administration of 5-HT is able to restore the 5-HT content in

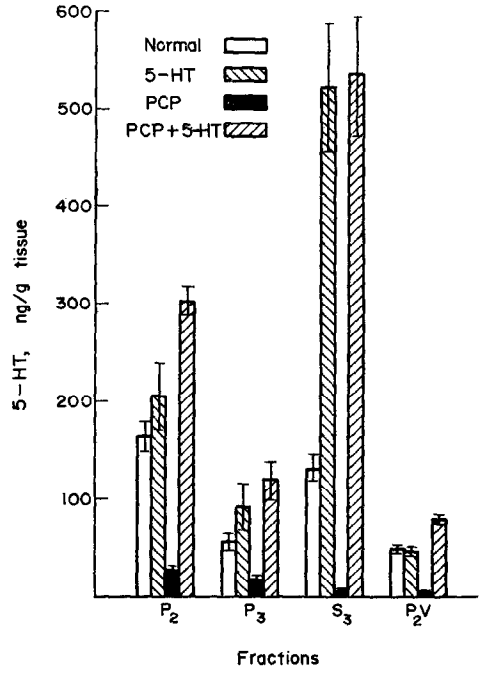

FIG. 2. Increase of 5-HT in brain stem subcellular fractions in normal and *p*-chlorophenylalaniner treated rabbits after intraventricular administration of 5-HT. Values are mean ± standard error of four determinations.

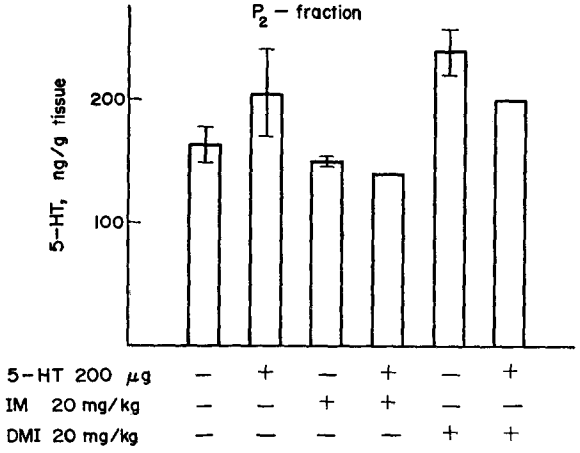

FIG. 3. Effects of imipramine and desmethylimipramine on the endogenous 5-HT and intraventricula 5-HT-induced increase of 5-HT in P_2-fraction from rabbit brain stem. Vertical bars represent th standard error of the mean of four assays. Values without standard error are mean of two deter minations.

PCP-treated animals 5-HT was given intraventricularly to rabbits treated with PCP 58·5 hr before and the animals were killed after another 1·5 hr. Unexpectedly, the increase of 5-HT was remarkable in all fractions examined. Actually, it exceeded the value obtained by intraventricular 5-HT in corresponding untreated normal fractions. The increase was especially noticeable in particulate fractions. For example, the net increase of 5-HT in PCP-treated P_2-fraction calculated by subtracting 5-HT value in PCP-treated P_2-fraction from 5-HT value after intraventricular injection of 5-HT into PCP-treated animals was about six times as high as the net increase in normal P_2-fraction. There was also a considerable increase of 5-HT in P_2V-fraction. As described above no increase was obtained in normal P_2V-fraction.

Effects of imipramine and desmethylimipramine

Our previous results[1–3] have shown that IM and DMI inhibited the 5-HT uptake both at NEPs and sv *in vitro* and DMI inhibited 5-HTP-induced increase in 5-HT at sv, therefore our next experiment was performed to see if there is similarity between the above-mentioned results and the effects on intraventricularly injected 5-HT-induced increase in 5-HT.

Rabbits were treated intraperitoneally with 20 mg/kg of IM or DMI and 2 hr later 5-HT amount in P_2-fraction was assayed. As is shown in Fig. 3 treatment with IM had no influence on the amount of 5-HT in P_2-fraction. In contrast, there was a marked increase in the endogenous amount of 5-HT when rabbits were treated with DMI.

The effects of IM and DMI on the intraventricular 5-HT-induced increase of 5-HT in P_2-fraction are shown in Fig. 3. In this experiment IM or DMI was injected into rabbits intraperitoneally and 30 min later rabbits were treated with intraventricular 5-HT. IM pretreatment resulted in clear prevention of the increase in 5-HT. On the other hand, pretreatment with DMI did not seem to affect the 5-HT increase induced by intraventricularly injected 5-HT because there was no difference between 5-HT value in P_2-fraction from intraventricular 5-HT-treated rabbits and from rabbits treated with intraperitoneal DMI and intraventricular 5-HT (Fig. 3).

Effects of IM and DMI on the amount of 5-HT in P_3- and S_3-fractions were also studied. The results are shown in Fig. 4. The schedule of IM or DMI treatment was the same as described above. In contrast to the results obtained in P_2-fraction neither IM nor DMI did affect the endogenous amount of 5-HT both in P_3- and S_3-fractions. Similarly, both drugs were found not to influence the 5-HT increase induced by intraventricularly injected 5-HT.

It seems clear from the above experiment that in P_2-fraction IM inhibits 5-HT increase caused by intraventricular 5-HT while DMI does not. Because intraventricular 5-HT-induced increase of 5-HT in P_2-fraction was more significant in PCP-treated than in non-treated animals, in the following experiment the effects of IM and DMI were studied in PCP-treated rabbits. The intraventricular injection of 5-HT was made on rabbits pretreated with PCP (150 mg/kg, i.p., 60 hr before death) followed by treatment with IM or DMI (20 mg/kg, i.p., 2 hr before death). As seen in Fig. 5 IM inhibited clearly the 5-HT increase but DMI treatment resulted in further elevation of the increase in the amount of 5-HT.

The elevation of 5-HT in P_2-fraction following administration of DMI suggests that DMI increases the metabolism of 5-HT in the brain. Accordingly, as a measure of metabolism, the amount of 5-HIAA in the brain stem following administration of

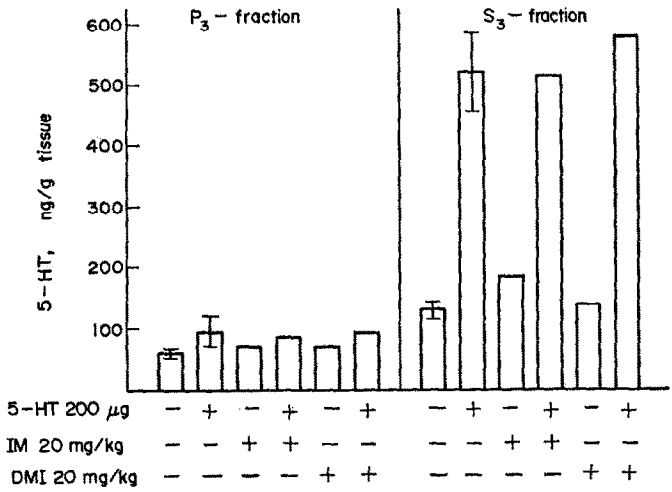

FIG. 4. Effects of imipramine and desmethylimipramine on the endogenous 5-HT and intraventricular 5-HT-induced increase of 5-HT in P_3- and S_3-fractions from rabbit brain stem. Vertical bars represent the standard error of the mean of four assays. Values without standard error are mean of two determinations.

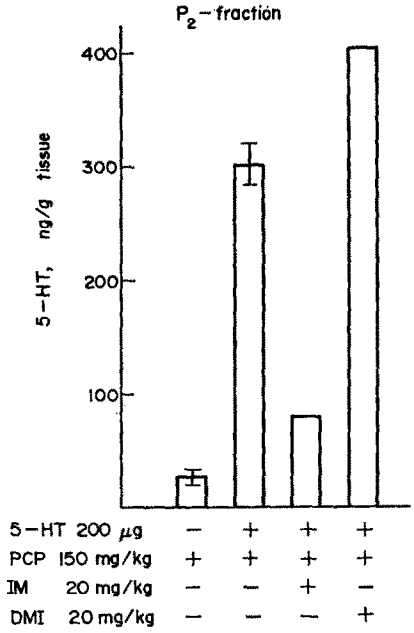

FIG. 5. Effects of imipramine and desmethylimipramine on the intraventricular 5-HT-induced increase of 5-HT in P_2-fraction from *p*-chlorophenylalanine-treated rabbit brain stem. Vertical bars represent the standard error of the mean of four assays. Value without standard error are mean of two determinations.

either IM or DMI was assayed. The results are presented in Fig. 6 in which 5-HIAA amount in the brain stem was assayed 1·5 hr after intraperitoneal administration of 20 mg/kg of IM or DMI. Compared to normal value, pretreatment with IM decreased 5-HIAA by more than 35 per cent. On the other hand, upon treating with DMI the value rose to a level which was almost three times the normal. This was comparable to the value obtained 16 hr after intraperitoneal administration of Re (Fig. 6).

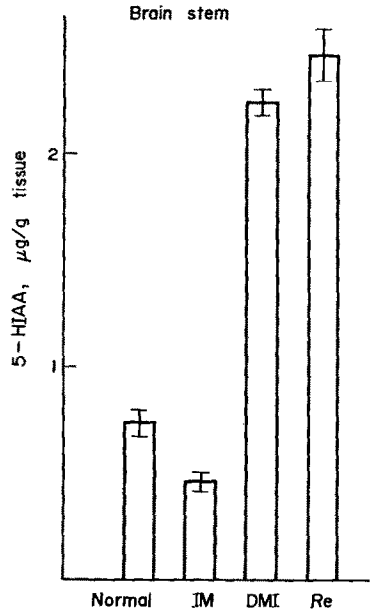

FIG. 6. Changes of 5-HIAA in rabbit brain stem after intraperitoneal administration of imipramine, desmethylimipramine and reserpine. Values are mean ± standard error of four determinations

Effect of reserpine

Re was injected intraperitoneally in a dose of 3 mg/kg and rabbits were killed 15 hr afterwards. A severe depletion of endogenous 5-HT was observed in all fractions examined (Fig. 7). The reduction was most prominent in P_2V-fraction in which practically 5-HT amount was nondetectable. When 5-HT was injected intraventricularly into Re-pretreated rabbits 14·5 hr after Re there was a marked elevation of 5-HT in P_2-, P_3- and S_3-fractions. The increase was almost the same in order of magnitude as that observed in nontreated normal animals, suggesting that amine-storing ability was restored. On the contrary, no increase in 5-HT was observed in P_2V-fraction after intraventricular administration of 5-HT (Fig. 7). This is a striking contrast to the results obtained in PCP-treated P_2V-fraction which showed a considerable increase in 5-HT by intraventricular 5-HT (Fig. 2).

Behavioural changes

When 5-HT was injected intraventricularly into the nontreated rabbits no behavioural signs were observed at times of considerable increase of 5-HT amount in S_3-

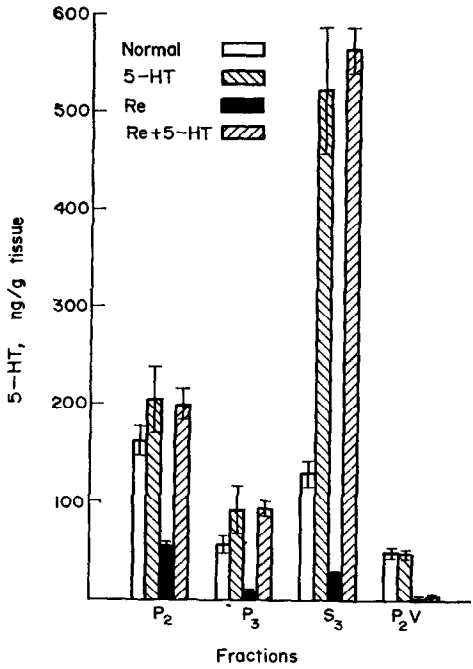

FIG. 7. Increase of 5-HT in brain stem subcellular fractions in normal and reserpine-treated rabbits after intraventricular administration of 5-HT. Values are mean ± standard error of four determinations.

fraction. Also, treatment with PCP which led to the complete depletion of 5-HT in all fractions did not produce noticeable changes in behaviour. On the other hand, however, when PCP-treated animals were subjected to further intraventricular injection of 5-HT there was evidence of sedation which was characterized by decrease in spontaneous motor activity, hunchbacked posture, slight ptosis and decreased responsiveness to tactile stimuli.

In nontreated and PCP-treated animals, certain behavioural changes were noted after 20 mg/kg of either IM or DMI were administered. The animals extended their forelegs until the abdomen almost touched the ground.

It is noteworthy that intraventricular 5-HT-induced sedation in PCP-treated rabbits was counteracted completely by 20 mg/kg of DMI while the same dose of IM was less effective in reducing 5-HT sedation. For example, PCP-treated rabbits receiving DMI followed by intraventricular 5-HT showed only DMI-induced peculiar posture described above, whereas PCP-rabbits treated with IM showed some degree of sedation after intraventricular injection of 5-HT.

A dose of 3 mg/kg of intraperitoneal Re produced typical Re syndrome characterized by ptosis and hunchbacked posture. These were unaffected by intraventricularly injected 5-HT.

DISCUSSION

In an electron microscopic study, Aghajanian and Bloom[6] have reported that administration of [^{3}H]5-HT, the most intense autoradiographic activity was found in the central grey where endogenous 5-HT is very high, whereas the paraventricular

nucleus of the hypothalamus had little activity. The reverse was found to be the case for [^{3}H]noradrenaline (NA).[6] Independently, with the help of a histochemical fluorescence method Fuxe and Ungerstedt[4,5] have shown that intraventricularly injected 5-HT was selectively accumulated in the 5-HT neurons and not in the catecholamine neurons. These findings suggest that most of the uptake occurred intraneuronally and that there is a high degree of chemical specificity of different monoamines with respect to uptake. Therefore, it may be said that the subcellular changes in 5-HT after intraventricular administration of 5-HT represents the phenomena that occurred in 5-HT neurons. However, it should be taken into consideration that exogenously administered 5-HT may itself modify the metabolism and turnover rate of endogenous 5-HT. Therefore, an increase in 5-HT content following intraventricular administration of 5-HT does not always correspond to the 5-HT amount taken up into 5-HT neurons.

5-HT content in S_3-fraction was remarkably elevated by intraventricular administration of 5-HT irrespective of whether rabbits were nontreated or treated previously with PCP. This may indicate that the uptake of 5-HT into cell body or axon through neuronal membrane is due to diffusion. On the contrary, there was no significant increase of 5-HT in P_2-, P_3- and P_2V-fractions unless animals were pretreated with PCP. One possible explanation for this is that at the synapse, intravesicular 5-HT levels control the amine uptake through neuronal membrane by the so-called membrane pump mechanism located at the synaptic membrane. If a certain amount of 5-HT exists in synaptic vesicles, the membrane pump works so that only a small amount of 5-HT can penetrate into nerve terminals. On the other hand, when endogenous stores are lowered by pretreatment with PCP the mechanism becomes ineffective, thereby enabling a considerable amount of 5-HT to be taken up through the neuronal membrane. Another possibility is that an endogenous amount of 5-HT in terminal is decreased owing to a decreased nervous activity which is the result of a nervous negative feed-back mechanism initiated by the increased 5-HT receptor activity caused by intraventricularly injected 5-HT. However, the possibility of such an increase of 5-HT receptor activity is unlikely since there were no behavioural signs after intraventricular administration of 5-HT.

The increase of brain stem 5-HIAA followed a similar time course, with a delay of 30 min, as the increase of 5-HT in S_3-fraction. This may indicate that in normal rabbits a large part of 5-HT, after being taken up into cell body or axon, is oxidized there to 5-HIAA.

In striking contrast to no behavioural signs in nontreated rabbits following intraventricular 5-HT, intraventricular 5-HT produced characteristic sedation in animals pretreated with PCP. As endogenous stores of 5-HT were lowered by PCP it is reasonable to presume that after PCP pharmacological denervation of central 5-HT neurons occurred and the areas of postsynaptic effector cells sensitive to 5-HT increased.

It is a favoured view that antidepressant drugs such as IM or DMI owe their pharmacological activity to an increase in amine concentration at the receptor site by blocking membrane pump mechanism at the synaptic membrane. However, Fuxe and Ungerstedt[4,13] and Carlsson, Fuxe and Ungerstedt,[14] using the histochemical fluorescence technique, have pointed out that IM was capable of blocking the uptake of 5-HT in 5-HT neurons, while it was not possible to obtain any observable blockade of this uptake with DMI, which, however, clearly blocked the uptake of NA in cate-

cholamine neurons. In the present study, IM prevented completely intraventricular 5-HT-induced increase of 5-HT in P_2-fraction whereas DMI had no effect. Therefore, the site and mode of action of IM must be different from those of DMI. However, it is interesting to note that *in vitro* both IM and DMI have been found to inhibit the uptake of 5-HT into NEPs.[2] Probably the difference in experimental condition has contributed to the difference in results.

Of interest is that DMI was found to elevate endogenous 5-HT in P_2-fraction and 5-HIAA in the brain stem. Previous studies from this laboratory[3] have shown that when 5-HTP was given to DMI pretreated rabbits there was more increase in 5-HT concentration in the brain stem than in normal rabbits given 5-HTP alone. In the present study, DMI, in contrast to IM, at a dose of 20 mg/kg, antagonized 5-HT-induced sedation. Therefore, it may be that after DMI turnover of brain 5-HT was accelerated through positive feed-back mechanism due to blockade of serotonergic receptor. It has to be elucidated, however, whether efflux of 5-HIAA from brain was interfered with DMI.

The present experiment also demonstrated that IM, at a dose of 20 mg/kg caused a significant decrease in 5-HIAA in the brain stem. A similar result has been reported by DaPrada and Pletscher[15] who demonstrated that IM and amitriptyline caused a slight but significant decrease of 5-HIAA in rat. There are two possible mechanisms for this: (1) IM inhibits the reuptake of 5-HT into nerve terminals, thereby decreasing 5-HT to be attacked and oxidized by mitochondria in cytoplasm. (2) IM decreases metabolism and turnover of 5-HT through negative feed-back by increasing the amount of 5-HT reaching the postsynaptic receptors. The latter has been proposed by Corrodi and Fuxe.[16]

As to the site of action of Re many investigators have favoured the view that Re selectively blocks the incorporation of 5-HT into the storage granule. The present study revealed that Re, in the dose used, clearly blocked the uptake of intraventricularly administered 5-HT into sv. Furthermore, the present experiment supports the view[4] that there exists a Re-resistant uptake mechanism of 5-HT in all parts of the 5-HT neurons because 5-HT increased in P_2-, P_3- and S_3-fractions from rabbits pretreated with Re.

It is difficult to decide from this experiment whether the effect of Re on 5-HT storage is responsible for any of its sedative effect but the fact that intraventricularly administered 5-HT could not counteract the Re-induced sedation may suggest that part of the Re-syndrome is due to a lack of catecholamine.

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