Localization of 5-hydroxytryptamine uptake in rat brain after intraventricular injection

SIR,—With the help of the histochemical fluorescence method for the demonstration of catecholamines and 5-hydroxytryptamine (5-HT) (see review by Hillarp, Fuxe & Dahlström, 1966; Corrodi & Jonsson, 1967), it has recently been possible to show that intraventricularly administered catecholamines are specifically taken up into those parts of the central catecholamine neurons lying in a zone around the ventricles and the ventral part of the subarachnoid space (Fuxe & Ungerstedt, 1966, 1967a). The facts support the view that there exists in the central catecholamine neurons a reserpine-resistant uptake-concentration mechanism for catecholamines probably localized at the level of the nerve cell membrane (Hamberger, 1967). Whether there exist similar uptake mechanisms in the central 5-HT neurons is the object of this report (Dahlström & Fuxe, 1964; Fuxe, 1965). Light microscopic autoradiography of intraventricularly administered tritiated 5-HT suggested an accumulation of amine in nerve terminals (Aghajanian, Bloom & others, 1966). To avoid the blood-brain barrier the intraventricular route was used.

Female Sprague-Dawley rats, 150 g, were given intraventricular injections (20 μl) stereotaxically into the left lateral ventricle. The rats were operated on in fluothane-nitrous oxide-oxygen anaesthesia which enabled the rats to wake up within 15 min after the injection. Body temperature was maintained within normal limits during the experiment. The intraventricular injections were made on normal rats, on reserpine pretreated rats (10 mg/kg, i.p., 12 hr before death) and also on rats pretreated with reserpine (10 mg/kg, i.p., 12 hr before death) and nialamide (500 mg/kg, i.p., 1 hr before death). 5-HT was injected in doses of 1 to 5 μg and the rats were killed 30 min afterwards. Some rats in this last group were also pretreated with desipramine (25 mg/kg, i.p., 1 hr before the 5-HT), (+)-amphetamine (10 mg/kg, i.p., 1 hr before the 5-HT), or tryptamine (50–100 mg/kg, i.p., 20 min before the 5-HT). All doses given refer to the base. The rats were killed by decapitation under light chloroform anaesthesia. The telencephalon, the diencephalon, the mesencephalon, the pons, the medulla oblongata and parts of the spinal cord were rapidly dissected, quickly frozen in liquid propane cooled by liquid nitrogen, freeze-dried, embedded in paraffin, sectioned, and mounted as previously described (Dahlström & Fuxe, 1964, Hamberger, Malmfors & Sachs, 1965) although modified to provide optimum reaction conditions for 5-HT (Fuxe & Jonsson, 1967).

5-Hydroxytryptamine. When 5-HT was given to untreated rats there was a clear accumulation of amine in very fine varicose fibres and nerve cell bodies lying close to the ventricles and the ventral part of the subarachnoid space. This was seen as an increased yellow fluorescence in nerve terminals, for example in the nucleus suprachiasmaticus, and in nerve cell bodies, for example in the nucleus raphe dorsalis. These neuronal structures were in all probability identical with the 5-HT nerve terminals and cell bodies, since they had the same appearance and distribution as the weakly fluorescent 5-HT neurons of the untreated control animals. The catecholamine neurons present in this zone did not seem to accumulate 5-HT.

Reserpine–5-HT. When 5-HT (1–5 μg) was given to reserpine pretreated rats there was no clear accumulation of amine within the 5-HT neurons, except in the area close to the injection site (5 μg), where the exogenous 5-HT concentration was probably high. Medium to strongly yellow-fluorescent nerve terminals could for example be observed in the septal area and a moderate diffuse yellow fluorescence was present in the part of the nucleus caudatus and putamen lying

close to the lateral ventricle on the side of injection. We could not say with certainty whether this diffuse yellow fluorescence represented an accumulation of amine in very fine, densely packed 5-HT or dopamine nerve terminals.

Reserpine–nialamide–5-HT. When 5-HT (1–5 μg) was given to reserpine-nialamide pretreated rats there was a clear to marked accumulation of amine within 5-HT cell bodies, non-terminal axons, for example, those in the spinal cord, and nerve terminals in the zones mentioned above. There was no certain accumulation of 5-HT within those parts of the central catecholamine neurons lying in the above mentioned zones with the possible exception of the yellow fluorescence observed in the nucleus caudatus and putamen close to the injection site. With the highest doses there was also observed a yellow fluorescence in the cells of the capillary walls.

It was not possible to obtain any observable blockade of this accumulation with desipramine or amphetamine which, however, in the doses used, clearly blocked the accumulation of intraventricularly administered noradrenaline in the central catecholamine neurons (Fuxe & Ungerstedt, 1967b). Tryptamine, on the other hand, partly prevented the accumulation of 5-HT within the 5-HT neurons. Thus the zone in which yellow fluorescent 5-HT nerve terminals were observed was clearly decreased in thickness, and the intensity of the yellow fluorescence that appeared was reduced compared to reserpine-nialamide-5-HT treated rats.

These experiments support the view that there exists a reserpine-sensitive uptake-storage mechanism in the 5-HT neurons (see Dahlström, Fuxe & Hillarp, 1965; Carlsson, 1966), since 5-HT did not accumulate in the 5-HT neurons of reserpine-pretreated rats unless the exogenous 5-HT concentrations were very high. Furthermore, there seems to exist a reserpine-resistant uptake-concentration mechanism for 5-HT in all parts of the 5-HT neurons since 5-HT accumulated in such neurons in rats pretreated with reserpine and a monoamine oxidase inhibitor. This mechanism does not seem to be related to the reserpine-sensitive uptake-storage mechanism because it appears as efficient in the non-terminal axons as in the terminals. Thus, it is probably localized to the level of the nerve cell membrane as was suggested earlier for the catecholamine neurons (for references, see Hamberger, 1967). The 5-HT accumulation occurred selectively in the 5-HT neurons and not in the catecholamine neurons. Finally the reserpine resistant uptake-concentration mechanism for 5-HT is not blocked to any observable degree by desipramine or amphetamine in the doses used. The experiments also indicate that the hallucinogenic agent, tryptamine (Sai-Halasz, Brunnecker & Szara, 1958) may have indirect actions via the 5-HT neurons either by blocking the reserpine-resistant uptake-concentrating mechanism or by releasing accumulated amines.

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Fenfluramine and critical flicker frequency

SIR,—Fenfluramine hydrochloride (Ponderax) is a recently marketed appetite-suppressant agent which, although having chemical resemblances to amphetamine, does not appear clinically to produce central stimulation (Traherne, 1965; Munro, Seaton & Duncan, 1966). It has been claimed to possess sedative acitivity and has been used for this reason in patients with anxiety states (Raich, Richels & Raab, 1966).

Amphetamine, phenmetrazine and diethylpropion are appetite suppressant agents possessing central stimulant properties, and they have been shown to increase the critical flicker frequency in normal subjects (Smart & Turner, 1966; Turner, 1967). This method is a valuable test of central function which has been shown to be sensitive in assessing the action of several centrally-acting drugs when administered in modest therapeutic doses (Turner, 1967). In a double-blind experiment, identical tablets of fenfluramine 20 and 40 mg and a placebo were administered in random order in a latin square design and at intervals of not less than 3 days to 6 young adult subjects of either sex. The critical flicker frequency was measured before dosing and at 1½ and 3 hr thereafter by a technique (Turner, 1965a; Smart & Turner, 1966) which involved exposing the subjects in random order to intermittent light at 25 and 50 c/sec for 1 min before measuring the ascending and descending thresholds of critical flicker frequency.

The results were submitted to an analysis of dispersion (Rao, 1952) which is the multivariate analogue of the analysis of variance. This permits a more accurate evaluation than does an analysis of variance of the responses to drug and placebo over time.

No significant difference was demonstrated between the effects of fenfluramine 20 and 40 mg and placebo on mean critical flicker frequency at either 1½ or 3 hr. There was a significant difference between ascending and descending thresholds ($P < 0.001$), and between thresholds after adaptation to light at 25 and 50 c/sec ($P < 0.05$), but these were not influenced by either dose of drug or placebo. This is consistent with the stability of these factors which has been previously demonstrated (Turner, 1965b; Turner, Patterson & Smart, 1966).

These findings indicate that fenfluramine in therapeutic doses does not influence the critical flicker frequency, and this is in keeping with the clinical absence of central stimulation associated with its use.

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