homogenate supernatants is found in fraction A. Even in experiments without eserine, acetylcholine is found in this fraction, indicating either synthesis of acetylcholine or, less likely, inaccessibility to acetylcholinesterase. However, fractions collected in 1.8M-sucrose always contain a small amount of acetylcholine with high specific activity. Choline acetylase is not found in these fractions. It is therefore possible that this acetylcholine occurs in structures such as vesicles or granules. Lactate dehydrogenase, a cytoplasmic marker, is not found in the fraction (Fig. 1), excluding formation of artifact vesicles during homogenization. Electron micrographs show small vesicles; a few of them may be dense structures. No difference in this respect has as yet been observed between fractions from ligated and control nerves.

An effort to trace cholinergic vesicles above a ligature was made with the aid of antiserum against such vesicles, prepared in our laboratory in 1971 and stored in the deep-freezer (Widlund et al., 1973). Immunofluorescence tests with this material showed intense fluorescence proximal to a ligature, whereas the distal side nearest to the ligation or a control with normal rabbit serum showed little light.

The experiments suggest synthesis of acetylcholine in cholinergic axons and transport of material belonging to cholinergic vesicles. They leave open the question whether or not heavy acetylcholine-granules exist.

It should be pointed out that acetylcholine determined with mass fragmentography usually gives lower values than determinations on the leech, and that the contractions of the leech do not disappear completely after incubation with acetylcholinesterase. This matter needs further clarification.


Effects of Lesions and Drugs on Tryptamine and 5-Hydroxytryptamine in Rat Brain
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It is established that there is a small amount of tryptamine in the rat brain (Boulton & Majer, 1971; Saavedra & Axelrod, 1972; Horn & Snodgrass, 1973). Its regional distribution is not dissimilar to that of 5-hydroxytryptamine with highest amounts in the hypothalamus and lowest in the cerebellum, although the striatum has a relatively high tryptamine content (Knott et al., 1973). In the present study the effects of various brain lesions and drugs on rat brain tryptamine and 5-hydroxytryptamine were compared.

Sprague-Dawley CFY strain rats (Carworth, Alconbury) were used in all experiments. Electrolytic lesions were placed in the dorsal and medial raphe nuclei of a group of rats by using co-ordinates from Pellegrino & Cushman (1967). 6-Hydroxydopamine (3,4,6-trihydroxyphenethylamine) (250 µg in 25 µl of 0.90% saline containing 0.1% ascorbic acid) was injected into the left ventricles of another group. Tryptamine (Saavedra & Axelrod, 1972) and 5-hydroxytryptamine (Curzon et al., 1972) were assayed 14 days after making the lesions and 21 days after the injection of 6-hydroxydopamine. 5-Hydroxytryptamine (cerebral cortex) and tryptamine (remainder of brain) were also determined after intraperitoneal injection of the following drugs: pargyline (N-benzyl-N-methylprop-2-ynylamine) hydrochloride (75 mg/kg), para-chlorophenylalanine (250 mg/kg) and reserpine (10 mg/kg). The uptake of 3H-labelled tryptamine and 3H-labelled 5-hydroxytryptamine into synaptosome-rich preparations from sham and raphe-lesioned rats (Kuhar et al., 1972) was measured.

A low concentration [35 ng/g ± 9(12)] of tryptamine was found in the rat brain. The monoamine oxidase inhibitor pargyline markedly increases tryptamine concentration
after 2h (+240 %) but only increases 5-hydroxytryptamine by 64%. Reserpine leads to a 70 % decrease in 5-hydroxytryptamine after 16h, but has no significant effect on tryptamine. p-Chlorophenylalanine an inhibitor of tryptophan hydroxylase (Koe & Weissman, 1966) decreases 5-hydroxytryptamine concentrations after 24h (−71 %) but significantly increases tryptamine concentration (+48 %).

The absence of complete parallelism between the distributions of tryptamine and 5-hydroxytryptamine in the rat brain suggests that tryptamine is not synthesized specifically within 5-hydroxytryptamine-containing neurones. This is also indicated by the experiments on rats with raphe lesions given pargyline (75mg/kg intraperitoneally) 2h before killing. These had a 70 % decrease in cerebral cortex 5-hydroxytryptamine but tryptamine in the remainder of the brain decreased by only 30 %. These decreases compare respectively with decreases after raphe lesions, of 70 % in tryptophan hydroxylase and 10–20 % in amino acid decarboxylase activities (Kuhar et al., 1971). The latter enzyme decarboxylates tryptophan to tryptamine (Lovenberg et al., 1962). Evidence that tryptamine may be formed in other neurones containing amino acid decarboxylase was also obtained by using 6-hydroxydopamine, which causes a specific degeneration of catecholamine terminals (Ungerstedt, 1971) and decreases dopa (3,4-dihydroxyphenylalanine) decarboxylase (Uretsky & Iversen, 1970) but not tryptophan hydroxylase activity (Smith et al., 1973). Thus after intraventricular injection of 6-hydroxydopamine and administration of pargyline (75mg/kg intraperitoneally) 2h before killing, striatal dopamine decreased by 56 % and tryptamine in the remainder of the brain by 28 % whereas striatal 5-hydroxytryptamine concentration did not change.

Results suggest that whereas formation of 5-hydroxytryptamine is specific to 5-hydroxytryptamine-containing neurones as tryptophan hydroxylase is localized therein (Kuhar et al., 1971) tryptamine may be formed in both 5-hydroxytryptamine and catecholamine-containing neurones as both contain amino-acid decarboxylase and presumably the substrate tryptophan.

Lesions in the raphe nuclei selectively depressed endogenous 5-hydroxytryptamine by about 55 % and decreased uptake of 3H-labelled 5-hydroxytryptamine (40nm) into a synaptosomal preparation by 60 %. However, 3H-labelled tryptamine (10 µm to 2 nm) appears not to be taken up actively, as a linear relationship between uptake and homogenate volume or incubation time could not be shown. Also raphe lesions had no effect on the very low 3H-labelled tryptamine count recovered from the synaptosomal pellet.

The apparent absence of a synaptosomal uptake mechanism for tryptamine together with its lack of susceptibility to reserpine is evidence against intraneuronal storage of tryptamine. This is consistent with the rapid disappearance of intracerebrally injected tryptamine from brain (Meek et al., 1970).

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