The occurrence of tryptamine in rat brain and its pharmacological manipulation

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The occurrence of tryptamine in the mammalian CNS is a subject of some controversy (Green & Sawyer, 1960; Hess & Doepfner, 1961; Eccleston, Ashcroft, Crawford & Loose, 1966; Saavedra & Axelrod, 1972). We have measured tryptamine as its dansyl derivative employing [^{3}H]-5-dimethylamino-1-naphthalenesulphonyl chloride (Amersham). Male rats were decapitated and their brains homogenized in cold 10% trichloroacetic acid solution. After centrifugation the supernatant was extracted with chloroform and then adjusted to pH 11·0 and extracted twice with tolueneisoamyl alcohol (3:2). The organic phase was back extracted with 0·1 N hydrochloric acid. Tryptamine in the aqueous phase was adsorbed onto non-ionic polystyrene resin, Porapak Q (Waters Associates, Framingham, Mass. U.S.A.) from which it was eluted with acetone methanol (3:1). The eluate was dried under a stream of nitrogen and suspended in 0·03 M sodium carbonate pH 9·7 to which was added tetrahydrofuran containing 8 mM [^{3}H]-dansyl chloride. The mixture was spotted onto silica gel plates and chromatographed in cyclohexanone:n-heptane:di-isopropyl ether (3:10:4, v:v) and then in the second direction with acetone:methanol:aqueous 0·05 N sodium carbonate (3:5:20, v:v). The fluorescent dansyl derivative was located with a U.V. light and the radioactivity in the dansyl tryptamine spot counted by liquid scintillation spectrometry. The spot presumed to be dansylated tryptamine was isolated from the plate and shown to have an identical Rf in 4 different solvent systems to authentic dansyl tryptamine and identical fluorescence spectra. The average overall recovery of added internal standards of tryptamine was $47{\cdot}5 \pm 3{\cdot}6\%$ (S.E. of mean) and results were corrected accordingly. Tryptamine levels were not increased by the addition to brain homogenates of up to 20 times the normal level of L-tryptophan, indicating that no non-specific decarboxylation occurred during the extraction and assay procedures.

Using this method, tryptamine was found to be present in small amounts in rat brain (Table 1) and in higher concentrations in spinal cord ($2{\cdot}23 \pm 0{\cdot}31$ nmol/g, $n=7$). The effects of various drugs known to alter the levels of biogenic amines in rat brain are summarized in Table 1.

TABLE 1.

	Brain tryptamine (n mol/g)	No. of animals
Controls	$0{\cdot}51 \pm 0{\cdot}08$	8
Reserpine (4 mg/kg, I.P., 16 h)	$0{\cdot}38 \pm 0{\cdot}05$	4*
Reserpine (7·5 mg/kg, I.P., 16 h)	$0{\cdot}33 \pm 0{\cdot}07$	5*
Pheniprazine (10 mg/kg, I.P., 16 h)	$1{\cdot}30 \pm 0{\cdot}31$	4***
Pheniprazine (10 mg/kg, I.P., 16 h + L-tryptophan 100 mg/kg, I.P., 1½ h)	$1{\cdot}31 \pm 0{\cdot}19$	7***
d-Amphetamine (10 mg/kg, I.P., 1½ h)	$0{\cdot}53 \pm 0{\cdot}13$	3
p-Chlorophenylalanine (100 mg/kg, I.P., 16 h)	$0{\cdot}60 \pm 0{\cdot}16$	4

Values are means ±S.E.M. For each drug, the dose and time interval from injection to decapitation are given. *=Difference from controls significant at $0{\cdot}10 \geq P \geq 0{\cdot}05$, ***=difference significant at $P \leq 0{\cdot}001$ (analysis of variance).

REFERENCES

ECCLESTON, D., ASHCROFT, G. W., CRAWFORD, T. B. B. & LOOSE, R. (1966). Some observations on the estimation of tryptamine in tissues. *J. Neurochem.*, **13**, 93–101.

GREEN, H. & SAWYER, J. L. (1960). Correlation of tryptamine induced convulsions in rats with brain tryptamine concentration. *Proc. soc. exp. Biol. (N.Y.)*, **104**, 153–155.

HESS, S. M. & DOEPFNER, W. (1961). Behavioural effects and brain amine content in rats. *Arch. Int. Pharmacodyn.*, **134**, 89–99.

SAAVEDRA, J. M. & AXELROD, J. (1972). Psychotomimetic N-methylated tryptamines: formation in brain *in vivo* and *in vitro*. *Science*, **172**, 1365–1366.