Distribution and Metabolism of Tryptamine in Rat Brain

P. H. WU AND ALAN A. BOULTON
Psychiatric Research Unit, University Hospital, Saskatoon, Saskatchewan

Received January 26, 1973

WU, P. H., and BOULTON, A. A. Distribution and metabolism of tryptamine in rat brain. Can. J. Biochem. **51**, 1104–1112 (1973).

Following intraventricular injection in untreated rats, the only metabolites of tryptamine isolated were indoleacetic acid and indoleacetaldehyde; the indication is that the acid is formed from the aldehyde during the isolation procedure. Additional metabolites (*N*-methyltryptamine and *N,N*-dimethyltryptamine) were formed in small quantities if the animals had been pretreated with pargyline. *N*-acetyltryptamine was not found if acetate ions were excluded during the isolation of the amine fraction.

More tryptamine remained in the hypothalamus than any other region at all times following injection, both in the presence and absence of pargyline. Incubation with slices indicated a temperature dependent active uptake process in all regions.

The loss of injected tryptamine from all regions in the presence of pargyline, and from the hypothalamus, cerebellum, and 'rest' in the absence of pargyline followed a pattern of rapid initial loss and then slower decline. This pattern was neither linear nor exponential. All the data, however, indicate a rapid turnover of the injected amine.

WU, P. H., et BOULTON, A. A. Distribution and metabolism of tryptamine in rat brain. Can. J. Biochem. **51**, 1104–1112 (1973).

Après une injection intraventriculaire de tryptamine à des rats non traités, les seuls métabolites isolés sont l'acide indoleacétique et l'indoleacétaldéhyde; l'acide se formerait à partir de l'aldéhyde durant le processus d'isolation. Chez les animaux d'abord traités à la pargyline, il se forme des métabolites additionnels (*N*-méthyltryptamine et *N,N*-diméthyltryptamine) en petites quantités. Si les ions acétate sont exclus lors de l'isolation de la fraction amine, on ne trouve pas d'*N*-acétyltryptamine.

Après une injection de tryptamine en présence ou en absence de pargyline, on retrouve en tout temps plus de tryptamine dans l'hypothalamus que dans toute autre région. L'incubation avec des tranches minces montre une incorporation active et dépendante de la température dans toutes les régions.

Dans toutes les régions du cerveau en présence de pargyline et dans l'hypothalamus, le cervelet et le "reste" en absence de pargyline, la tryptamine injectée disparaît d'abord rapidement et, par la suite, diminue plus lentement. Cette disparition n'est ni linéaire ni exponentielle. Cependant, tous les résultats montrent un turnover rapide de l'amine injectée.

[Traduit par le journal]

Introduction

Tryptamine has been identified in human urine (1–3) and certain tissues, including brain (4–7). It has also been claimed to be involved in the etiology of schizophrenia (8) and depression (9), and some methylated analogs of the indolealkylamines certainly possess psychotomimetic properties (10, 11). In animals, tryptamine, which readily crosses the blood–brain barrier (12), has been shown to be behaviorally active and to possess amphetamine-like properties (13–15).

It is presumed that tryptamine is formed exclusively by decarboxylation of tryptophan and such a decarboxylation has been demonstrated in mammalian tissues (16–18), in bacteria (19, 20), and with purified aromatic L-amino acid decarboxylase isolated from hog kidney (21).

The catabolism of tryptamine appears to be predominantly oxidation to indoleacetaldehyde and/or 3-indoleacetic acid (16, 22, 23). Recently a minor pathway involving *N*-methylation has been demonstrated (24–29) and the possibility exists that *N*-acetylation and/or conjugation might occur, as in the metabolism of serotonin (30).

The purpose of this study was to investigate the regional distribution in the rat brain and clearance of intraventricularly introduced labelled tryptamine, both in the presence and absence of the monoamine oxidase inhibitor pargyline, and to attempt to isolate and identify any other metabolites formed.

Materials and Methods

Radioactive Substrates and Their Purification

Tryptamine-^{3}H hydrochloride (specific activity, 535 mCi/mmol; Amersham/Searle, Don Mills, Ontario) was dissolved in physiological saline (final concentration, 10 μCi/40 μl) and stored at −20 °C until use. Tryptamine-2-^{14}C bisuccinate (specific activity, 47.3 mCi/mmol; New England Nuclear Canada Ltd., Dorval, Quebec) was used for a study of the metabolites and hence was purified before use. The tryptamine was dissolved in a small volume of ethanol, transferred to the origin of a Whatman No. 2 paper chromatographic strip, and developed during 14 h in the solvent system butanol – acetic acid – water, 120:30:50 (v/v). The radioactive zone corresponding to tryptamine (as assessed by a standard run alongside and visualized by the ninhydrin – acetic acid fluorometric procedure described by Jepson and Stevens (31)) was excised, eluted (2 × 5 ml) in 4 *N* NH_4OH in 65% ethanol, and dried under a stream of N_2 at 45 °C. The dried residue was stored at −20 °C until required. For use it was dissolved in Krebs–Ringer solution, pH 7.4. The recovery of tryptamine through this procedure was approximately 76%.

Injection and Dissection Procedures

Male Wistar rats (body weight approximately 150–200 g; some pretreated intraperitoneally with pargyline, 75 mg/kg, 45 min before injection) were lightly anesthetized with ether, and labelled tryptamine (in 40 μl) was injected into the right lateral ventricles. After 30 min the rats were decapitated and the brains removed and used, either whole or after dissection, as previously described (32).

Homogenization Procedure and the Isolation of Some Indolealkylamines

Whole brains, or the various regions, were homogenized at 4 °C in 15 ml of 0.4 *N* $HClO_4$. After adding tryptamine, 3-indoleacetic acid, 3-indoleacetaldehyde, *N*-methyltryptamine, *N*,*N*-dimethyltryptamine, and *N*-acetyltryptamine (10 μg of each) as carriers, the suspension was centrifuged at 10 000 × *g* for 20 min. After transferring the supernatant (50 μl removed for measurement of total radioactivity) to a beaker containing sodium thiosulfate (100 mg), Triton X-100 (J. T. Baker Chemical Co., Phillipsburg, New Jersey) was carefully added with stirring to a final concentration of 0.5% (w/v) in order to prevent loss of amines by association with particulate materials (33), and the mixture was then adjusted to pH 7.0 with 1 *N* NaOH. After percolation through Biorad AG50-X2 (H^+) resin, the resin column was washed with 10 ml of 0.1 *M* Tris buffer solution, pH 8.0, and 10 ml of glass distilled water. The amine fraction was then eluted with 10 ml of 4 *N* NH_4OH in 65% ethanol. This eluate, after rotatory evaporation to dryness at 45 °C, was dissolved in 200 μl methanol. Aliquots (100 μl) were transferred to the origin of Whatman No. 2 paper strips and developed during 20 h in the solvent system butanol – acetic acid – water 120:30:50 (v/v). After air drying, the chromatograms were dipped either into the ninhydrin – acetic acid solution described by Jepson and Stevens (31), or Ehrlich's reagent (34), and the radioactivity in zones corresponding to tryptamine and *N*-acetyltryptamine (R_f values 0.70 and 0.83, respectively) were recorded using a radiochromatogram scanner (Packard model 7200). In the case of the distribution of tryptamine following intraventricular injection (see Results section), the tryptamine fluorophore was eluted (2 ml 0.4 *N* $HClO_4$) from the appropriate zone, mixed with 15 ml Aquasol, and counted in the liquid scintillation counter (Nuclear Chicago Isocap 300). The overall recovery of tryptamine through this procedure was approximately 40%.

A further 25 μl aliquot of the methanol solution was transferred to the origin of a two dimensional silica gel plate (20 × 20 cm) (Mondray Ltd., Montreal) activated by heating at 90 °C for 30 min just prior to use, and separated first in butanol – acetic acid – water, 120:30:50 (v/v), and then isopropanol–ammonia–water, 200:10:20 (v/v). After air drying, the plate was sprayed with Ehrlich's reagent and the zones corresponding to tryptamine, *N*-acetyltryptamine, *N*-methyltryptamine, and *N*,*N*-dimethyltryptamine (zones located by comparison with standards run in parallel) were scraped off, suspended in 2 ml 0.4 *N* $HClO_4$, mixed with 15 ml Aquasol (New England Nuclear), and counted in a liquid scintillation counter.

Isolation and Separation of Acidic Metabolites

The initial effluent and washes from the Biorad column were combined in the presence of sodium thiosulfate (100 mg) and rotatory evaporated to dryness at 60 °C. The residue was dissolved in 5 ml 2 *N* HCl saturated with NaCl and then extracted with butyl acetate (2 × 5 ml). This mixture was centrifuged (3000 × *g* for 5 min) and the organic phase, exhibiting at this stage a slight pink color, was removed and rotatory evaporated to dryness. After dissolving the residue in 500 μl acetone, 50 μl was removed, mixed with Aquasol, and counted in a liquid scintillation counter to indicate total acidic metabolites; a further 50 μl aliquot was transferred to the origin of a Whatman No. 3 paper chromatographic strip, developed during 14 h in isopropanol–ammonia–water, 200:10:20 (v/v), air dried, and then dipped through Ehrlich's reagent. Indoleacetic acid and 3-indoleacetaldehyde exhibit R_f values of 0.23 and 0.73, respectively.

Incubation of Tryptamine-^{3}H with Slices Obtained from Various Regions

Groups of rats were sacrificed 45 min after an intraperitoneal injection of pargyline (75 mg/kg), the brains removed, chilled, and washed in 0.25 *M* sucrose solution at 0 °C, then dissected into the hypothalamus, caudate nucleus, cerebellum, stem, and 'rest,' as previously described (32). These regions were sliced, incubated, and washed essentially according to the procedures described by Steinberg and Smith (35) and Baldessarini and Vogt (36). Briefly, each region was manually sliced to yield pieces 1–2 mm thick; two of these were selected, weighed, and placed in 0.25 *M* sucrose solution at 0 °C for a period not exceeding 10 min. The slices (hypothalamus, 24.7 ± 4.8; caudate nucleus, 31.1 ± 5.2; stem, 49.5 ± 5.0; cerebellum,

50.6 ± 5.1; 'rest,' 56.7 ± 11.8; mean ± standard deviation, respectively, in milligrams) were then transferred to 2 ml Krebs–Ringer phosphate buffer solution (pH 7.4) (35) containing 1 μCi tryptamine-^{3}H. During the next 20 min incubation proceeded with shaking at 37.5 °C. On termination of this period the supernatant was decanted and the slices were incubated twice more at 37.5 °C with 2 ml Kreb–Ringer solution for 6 and 4 min, respectively. After the final incubation, 2 ml of chilled 0.4 *N* $HClO_4$ was added, and after homogenization, the homogenate was mixed with 15 ml Aquasol and counted in the liquid scintillation counter. Control samples were prepared by incubating and washing slices in a similar fashion with 1 μCi tryptamine-^{3}H at 0 °C.

Results

Metabolism of Tryptamine-^{14}C in Whole Brain

Groups of pargyline treated rats were sacrificed 30 min after an intraventricular injection of purified tryptamine-^{14}C (7.6 μCi). The amines tryptamine (TRYPT), *N*-methyltryptamine (NMT) and *N,N*-dimethyltryptamine (DMT) were isolated from a two dimensional silica gel plate as described in the Materials and Methods section, and the amount of associated radioactivity was assessed by liquid scintillation counting. The values obtained (TRYPT, 140 000 c.p.m.; NMT, 6000 c.p.m.; DMT, 240 c.p.m.), although low, are in quite close agreement with those listed by Saavedra and Axelrod (25). No label was found to be associated with the NMT and DMT zones when purified TRYPT was separated in a similar manner.

During the isolation of the amine fraction from whole brain homogenates, the resin column was washed with 0.1 *N* sodium acetate, as originally described by Kakimoto and Armstrong (37), a radioactive zone corresponding to *N*-acetyltryptamine was located when scanning a paper chromatogram on which the amine fraction was separated in butanol – acetic acid – water, 120:30:50 (v/v). On substituting 0.1 *M* Tris buffer (pH 8.0) for the 0.1 *N* sodium acetate, however, this zone disappeared. The artifactual formation of *N*-acetyltryptamine arising during extraction procedures has been described by Hutzinger (38).

In another experiment, rats, not treated with pargyline, were sacrificed 30 min after an intraventricular injection of 5 μCi of purified tryptamine. Both 3-indoleacetic acid and 3-indoleacetaldehyde were deemed to be present, as revealed by the association of radioactivity with the appropriate zones identified both on the basis of the chromogenic changes occurring after dipping and from the location of standards run in parallel. It would appear, based on a visual assessment of the chromatograms, that the conversion of the aldehyde to acid occurs predominantly, if not exclusively, during the extraction procedure. If the antioxidant sodium thiosulfate is excluded, virtually all the radioactivity is found to be associated with the acid zone. No other minor metabolites from the acidic fraction could be located on the chromatogram.

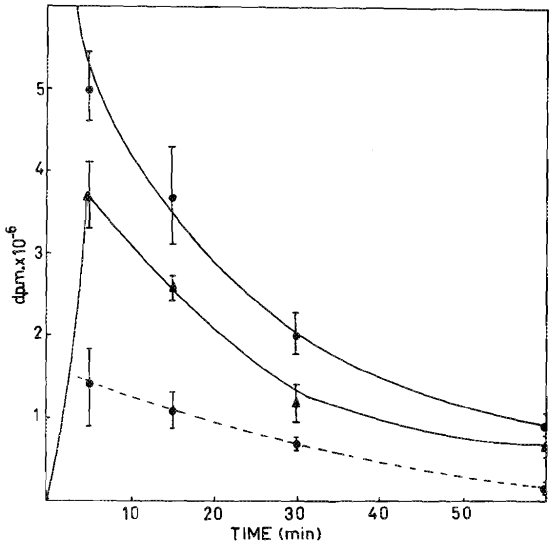

FIG. 1. Disappearance of total radioactivity (●——●), various acid metabolites (▲——▲), and tryptamine (●- - -●) from untreated rat brain. Total radioactivity and acid metabolites, mean ± standard deviation (n = 8); tryptamine, mean ± mean deviation (n = 4).

Disappearance of Tryptamine-^{3}H and Certain Metabolites in Whole Brain Following an Intraventricular Injection

Groups of rats, either untreated or pretreated with pargyline, were sacrificed at various time intervals (5, 10, 15, 30, and 60 min) following an intraventricular injection of tryptamine-^{3}H (10 μCi). After removal of the brain and homogenization, total radioactivity, tryptamine-^{3}H, and labelled acid metabolites were isolated and assessed as previously described. From Fig. 1 it is apparent that the amount of radioactivity remaining in the untreated brain decreased nonlinearly to approximately 4.1%

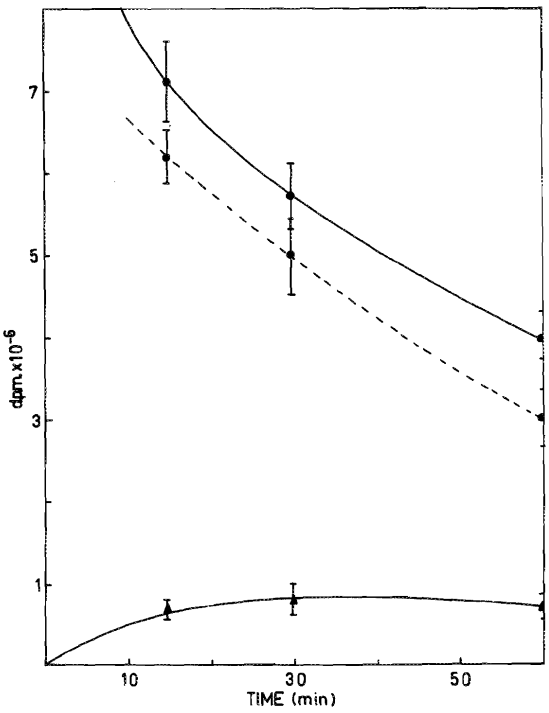

FIG. 2. Disappearance of total radioactivity (●——●), various acid metabolites (▲——▲), and tryptamine (●- - -●) from pargyline treated rat brain. Each point with bar indicates mean ± mean deviation (n = 4).

TABLE 1. Distribution of tryptamine-^{3}H in various regions of the untreated and pargyline treated rat brain 15 min after intraventricular injection

	Tryptamine-^{3}H	
	Untreated	Treated
Region	(d.p.m. × 10^{-5}/g)	(d.p.m. × 10^{-6}/g)
Hypothalamus	3.65 ± 0.56[a]	4.05 ± 0.45
Caudate nucleus	1.41 ± 0.14	2.17 ± 0.17
Stem	1.95 ± 0.84	1.72 ± 0.27
Cerebellum	1.70 ± 0.18	2.64 ± 0.23
'Rest'	2.63 ± 0.75	2.56 ± 0.46

[a]Each value mean ± mean deviation (n = 4).

of the amount injected after 60 min. The acid metabolites, after increasing rapidly in the first 15 min, then decreased in step with the total radioactivity, being approximately 70% of the total radioactivity at all times after 15 min. In the case of pargyline-treated rats, after an initial fast decrease during the first 15 min, the total radioactivity was lost in a linear fashion (Fig. 2). In this case the tryptamine equalled approximately 80% of the total radioactivity. As was expected, the total acid metabolite level was very low. In both pargyline treated and untreated rats, the loss of labelled tryptamine (see Figs. 1, 2) after the first 10 min was approximately linear, with an approximate half-life of 52.5 min and 33.5 min, respectively (Table 2). As the amount of tryptamine in the brain at any time after injection in the pargyline-treated rat was about five times the value in controls, it seems reasonable to conclude that monoamine oxidase is the primary route of cerebral tryptamine catabolism.

Distribution and Disappearance of Tryptamine-^{3}H from Certain Rat Brain Regions after an Intraventricular Injection

The distribution of tryptamine in regions of the rat brain after 15 min is listed in Table 1. At this time it is apparent that the tryptamine (expressed as disintegrations per minute per gram of tissue) in the absence of pargyline is concentrated primarily in the hypothalamus, followed respectively by the 'rest,' stem, cerebellum, and caudate nucleus. In the presence of pargyline, although the pattern of distribution does not appear to change very much, the amount of tryptamine retained is increased approximately by a factor of 10.

If the amount of tryptamine-^{3}H remaining in each region is plotted against time (Figs. 3, 4), there appears to be a linear loss of label in the caudate nucleus, stem, and possibly cerebellum of untreated rats. A nonlinear and nonexponential loss occurred in the hypothalamus and in the rest of the brain of such rats and in all areas of pargyline-treated rats. In order to calculate the approximate half-life of the label, the nonlinear curves were considered in two sections, the initial rapid loss and the later slower decline, and straight lines were fitted as shown in Figs. 3 and 4. Half-life estimates obtained in this way are listed in Table 2.

Uptake and Retention of Tryptamine-^{3}H in Slices Obtained from Various Brain Regions of Pargyline-Treated Rats

Incubation of various brain slices with 1 μCi tryptamine-^{3}H at 0 °C and 37.5 °C indicated that the uptake of labelled tryptamine was temperature dependent, only about 26% being retained at 0 °C as compared with 37.5 °C. No

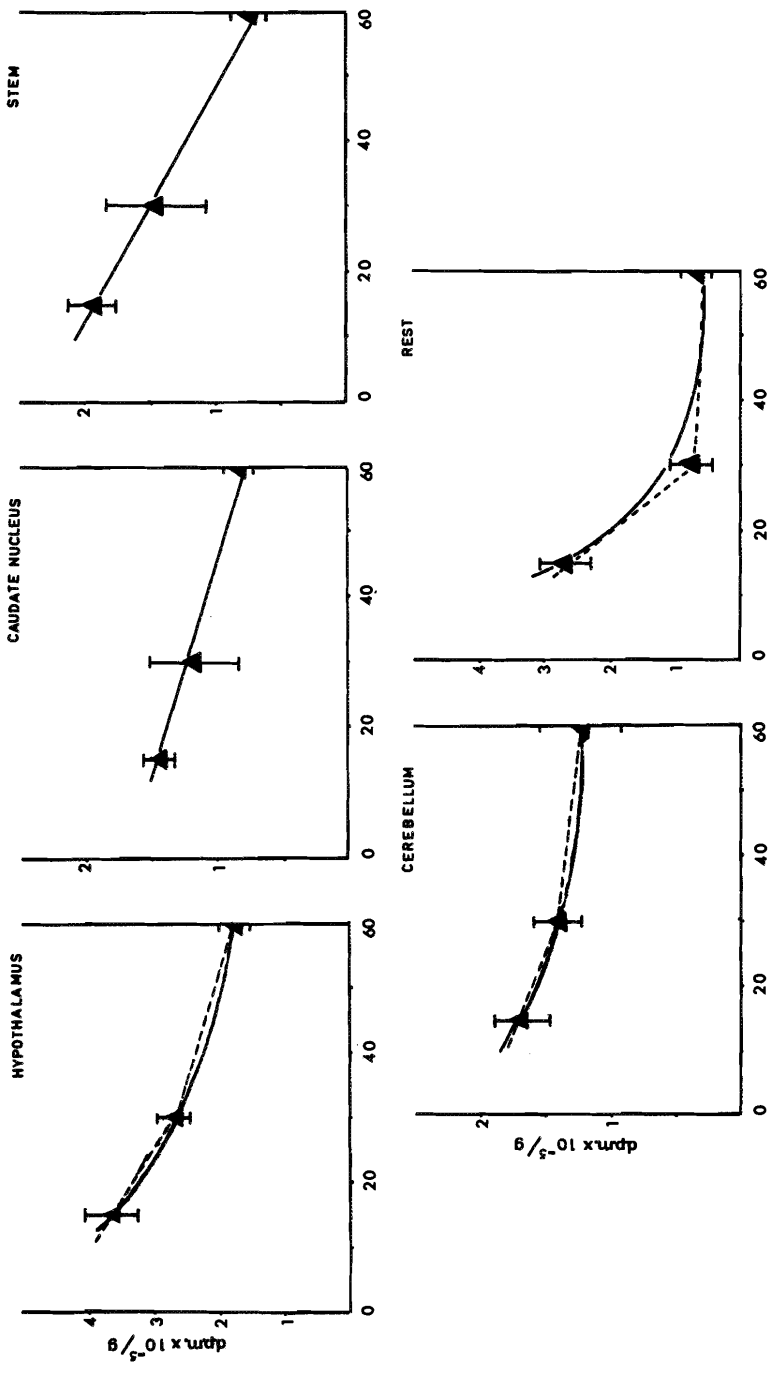

FIG. 3. Disappearance of tryptamine-^{3}H from various regions of untreated rat brain. Each point with bar indicates mean ± mean deviation (n = 4).

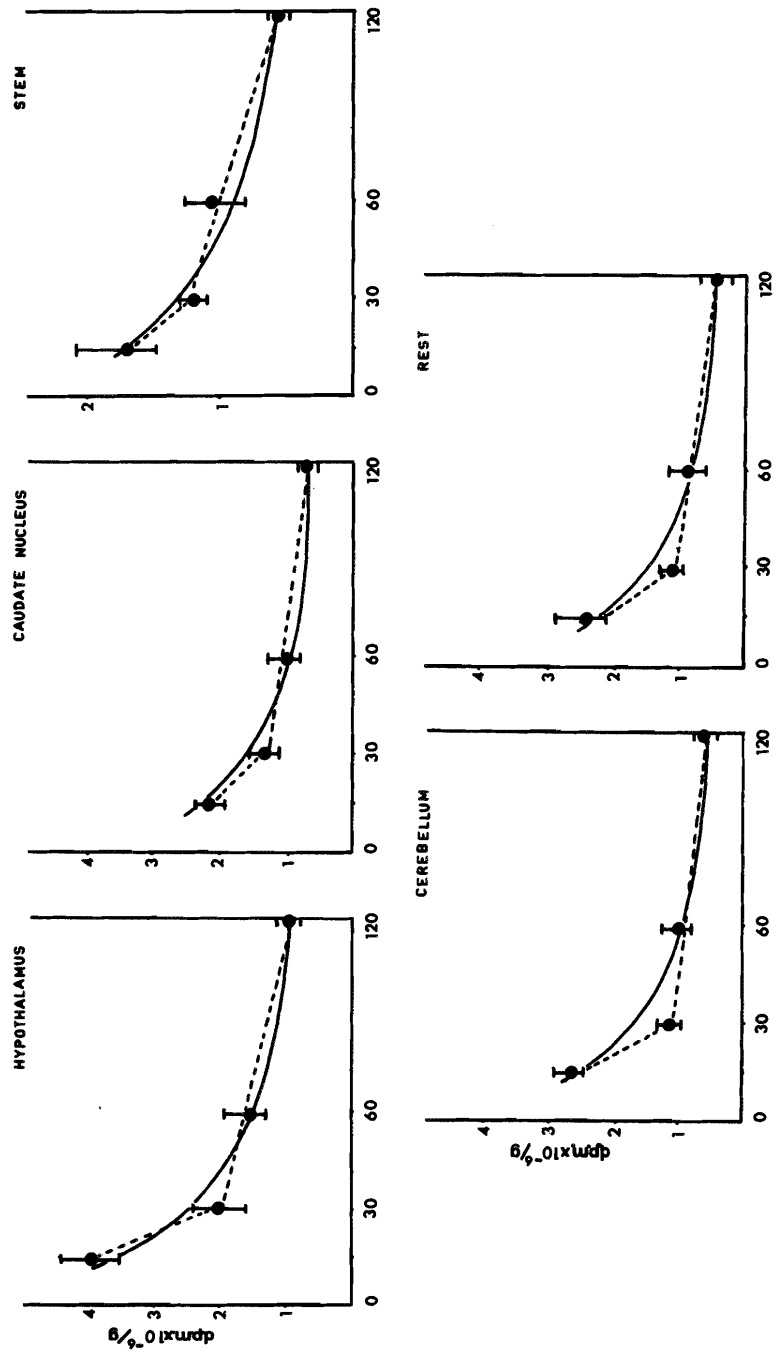

FIG. 4. Disappearance of tryptamine-^{3}H from various regions of pargyline treated rat brain. Each point with bar indicates mean ± mean deviation ($n = 4$).

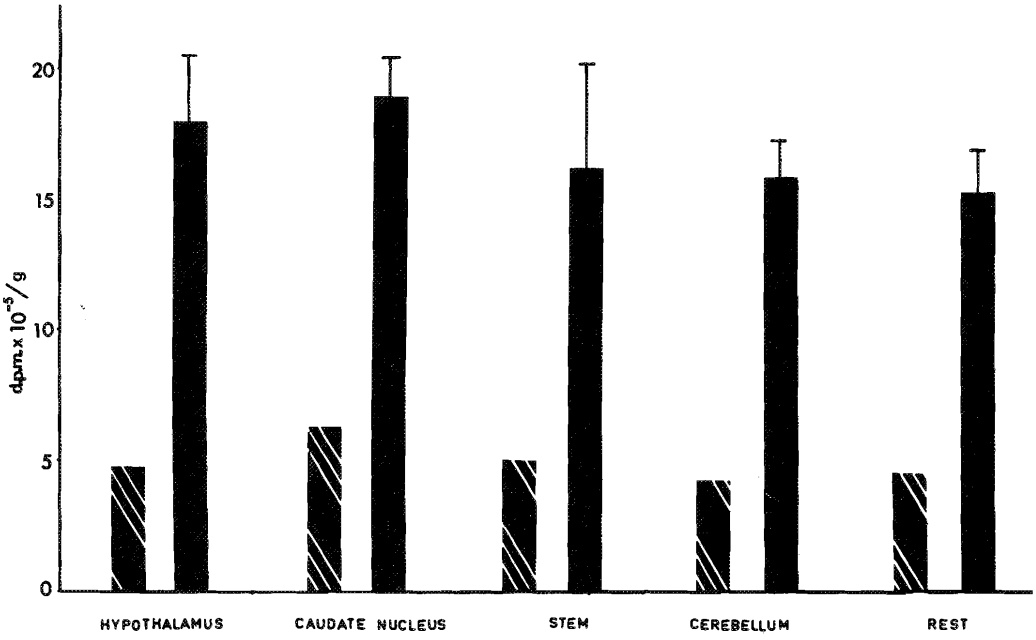

FIG. 5. Retention of tryptamine-^{3}H in tissue slices obtained from various regions of pargyline treated rat brain at 37.5 °C and 0 °C (hatched areas). Each value indicates mean ± mean deviation (n = 3).

TABLE 2. Half-life of tryptamine-^{3}H in whole brain and various regions as obtained from untreated and pargyline treated rats

	Half-life (min)	
	Untreated	Pargyline treated
Hypothalamus	40 (15–30)[a]	30 (15– 30)
	60 (30–60)	85 (30–120)
Caudate nucleus	60 (15–60)	35 (15– 30)
		100 (30–120)
Stem	28.5 (15–60)	42 (15– 30)
		90 (30–120)
Cerebellum	63 (15–60)	22 (15– 30)
		105 (30–120)
Rest	17 (15–30)	18 (15– 30)
	∼∞ (30–60)	100 (30–120)
Whole brain	33.5 (10–60)	52.5 (10– 60)

[a]Figures in parentheses indicate the time period over which half-lives were calculated (see also Figs. 3, 4).

statistically significant difference between the regions could be found (Fig. 5).

Discussion

The primary route of tryptamine catabolism appears to be oxidation by monoamine oxidase. Previous reports in the literature (16, 23, 39) have indicated conversion to indoleacetic acid, presumably via indoleacetaldehyde. We have shown that the inclusion of sodium thiosulfate during isolation resulted in the identification of significant quantities of indoleacetaldehyde. It is quite possible that the autoxidation of the aldehyde occurs primarily during the isolation procedure. If this were the case, then the behavioral properties of tryptamine may result from the amine itself, one of its metabolites, or from an alkaloid type of molecule produced by condensation or complexing (40–42) between the aldehyde and some other molecule.

Although, during monoamine oxidase blockade, some methylated derivatives (*N*-methyltryptamine and *N*,*N*-dimethyltryptamine) of tryptamine were located on thin layer chromatograms, they were formed only in relatively tiny quantities. The absence of *N*-acetyltryptamine in the ion exchange amine eluate when acetate ions were excluded from the washing procedure suggests that this metabolite is only formed in certain circumstances during the isolation procedure.

Because of the very low levels of tryptamine in rat brain, 24 ng/g according to Saavedra and Axelrod (7), we chose to calculate the approximate half-life by simple means, and according to

the expression $d(T\text{-}^3H)/dt = -K$. The rate of disappearance of the labelled tryptamine indicated a half-life for the label in whole brain of 52.5 min in the presence of pargyline and of 33.5 min in its absence. The data on the disappearance of labelled tryptamine from various regions of the brain indicate a more complex pattern than those data for whole brain. The asymptotic nature of the curve, particularly in the 'rest' of the brain (see Figs. 3 and 4 and compare with Figs. 3 and 2 in Refs. 44 and 45), suggests that phenomena such as the complex formation between tryptamine and membranous material, which we have recently shown both *in vivo* and *in vitro* (33), may be playing a significant role. In any case, the amount of labelled tryptamine injected is considerably above endogenous levels, and these data cannot, therefore, necessarily be interpreted as reflecting the half-life of the endogenous material. The half-life values obtained here are, however, considerably less than those reported for amines such as 5-hydroxytryptamine and the catecholamines (43) or *p*-tyramine and octopamine (44; Wu, P.H., and Boulton, A.A.: unpublished observations).

The distribution of labelled tryptamine following an intraventricular injection is somewhat similar to that of other amines (46). This does not necessarily reflect the actual endogenous distribution, however, since the ability of amines to concentrate in areas in close proximity to the site of injection is known (47), and the extent of exogenous/endogenous mixing is not known.

The uptake and retention pattern as obtained from slices (Fig. 5) did not exhibit the regional differences obtained following intraventricular injection. In this respect tryptamine appears to differ from other amines; a temperature dependent active uptake process, however, would appear to be operative.

We thank the Psychiatric Services Branch, Province of Saskatchewan, and the Medical Research Council for continuing financial support.

1. RODNIGHT, R.: Biochem. J. **64**, 621 (1956).
2. PERRY, T. L., and SCHROEDER, W. A.: J. Chromatogr. **12**, 368 (1963).
3. SJOERDSMA, A., OATES, J. A., ZALTZMAN, P., and UDENFRIEND, S.: J. Pharmacol. Exp. Ther. **126**, 217 (1959).
4. ECCLESTON, D., ASHCROFT, G. W., CRAWFORD, T. B. B., and LOOSE, R.: J. Neurochem. **13**, 93 (1966).
5. MARTIN, W. R., SLOAN, J. W., CHRISTIAN, S. T., and CLEMENTS, T. H.: Psychopharmacologia, **24**, 331 (1972).
6. HESS, S. M., REDFIELD, B. G., and UDENFRIEND, S.: J. Pharmacol. Exp. Ther. **127**, 178 (1959).
7. SAAVEDRA, J. H., and AXELROD, J.: J. Pharmacol. Exp. Ther. **182**, 363 (1972).
8. BRUNE, G. G., and HIMWICH, H. E.: Arch. Gen. Psychiatr. **6**, 324 (1962).
9. DEWHURST, W. G.: Nature, **218**, 1130 (1968).
10. SZARA, S.: Fed. Proc. **20**, 885 (1961).
11. HOLMSTEDT, B.: Amines and schizophrenia. *Edited by* H. E. Himwich, S. S. Kety, and J. R. Smythies. Pergamon Press, New York. 1967.
12. OLDENDORF, W. H.: Am. J. Physiol. **221**, 1629 (1971).
13. TEDESCHI, D. H., TEDESCHI, R. E., and FELLOWS, E. J.: J. Pharmacol. Exp. Ther. **126**, 223 (1959).
14. SAAVEDRA, J. M., HELLER, B., and FISCHER, E.: Nature, **226**, 868 (1970).
15. RANDRUP, A., and MUNKVAD, C.: Acta Psychiatr. Scand. (Suppl.), **191**, 193 (1966).
16. WEISSBACH, H., LOWENBERG, W., REDFIELD, B. G., and UDENFRIEND, S.: J. Pharmacol. Exp. Ther. **131**, 26 (1961).
17. DEQUATTRO, V. L., and SJOERDSMA, A.: Clin. Chim. Acta, **16**, 227 (1967).
18. WERLE, E., and MENNICKEN, G.: Biochem. Z. **291**, 325 (1937).
19. PERRY, T. L., HESTRIN, M., MACDOUGALL, L., and HANSEN, S.: Clin. Chim. Acta, **14**, 116 (1966).
20. GALE, E. F.: Adv. Enzymol. **6**, 1 (1946).
21. CHRISTENSON, J. G., DAIRMAN, W., and UDENFRIEND, S.: Arch. Biochem. Biophys. **141**, 356 (1970).
22. BARONDES, S. H.: J. Biol. Chem. **237**, 204 (1962).
23. PUGH, C. E. M., and QUASTEL, J. H.: Biochem. J. **31**, 2306 (1937).
24. AXELROD, J.: J. Pharmacol. Exp. Ther. **138**, 28 (1962).
25. SAAVEDRA, J. M., and AXELROD, J.: Science, **175**, 1365 (1972).
26. MORGAN, M., and MANDELL, A. J.: Science, **165**, 492 (1969).
27. MANDELL, A. J., and MORGAN, M.: Nature, **230**, 85 (1971).
28. MANDEL, L. R., ROSENZWEIG, S., and KUEHL, F. A.: Biochem. Pharmacol. **20**, 712 (1971).
29. MANDEL, L. R., AHN, A. S., VANDENHEUVEL, N. J. A., and WALKER, R. W.: Biochem. Pharmacol. **21**, 1197 (1972).
30. MCISAAC, W. M., and PAGE, I. H.: J. Biol. Chem. **234**, 858 (1959).
31. JEPSON, J. B., and STEVENS, B. J.: Nature, **172**, 772 (1953).
32. BOULTON, A. A., and WU, P. H.: Can. J. Biochem. **51**, 428 (1973).
33. BOULTON, A. A., WU, P. H., and PHILIPS, S.: Can. J. Biochem. **50**, 1210 (1972).

34. SMITH, I.: Chromatographic and electrophoratic techniques. Vol. 2. Wm. Heineman, London. 1969. p. 253.
35. STEINBERG, M. J., and SMITH, C. B.: J. Pharmacol. Exp. Ther. **173**, 176 (1970).
36. BALDESSARINI, R. J., and VOGT, M.: J. Neurochem. **19**, 755 (1972).
37. KAKIMOTO, Y., and ARMSTRONG, M. D.: J. Biol. Chem. **237**, 208 (1962).
38. HUTZINGER, O.: Anal. Biochem. **30**, 153 (1969).
39. BENSON, J., WEISSBACH, H., and UDENFRIEND, S.: J. Pharmacol. Exp. Ther. **143**, 326 (1964).
40. SOURKES, T. L.: Nature, **229**, 413 (1971).
41. HEIKKILA, R., COHEN, G., and DEMBIEC, D.: J. Pharmacol. Exp. Ther. **179**, 250 (1971).
42. TABAKOFF, B., UNGER, F., and ALIVISATOS, S. G. A.: Nat. New Biol. **238**, 126 (1972).
43. COOPER, J. R., BLOOM, F. E., and ROTH, R. H.: *In* The biochemical basis of neuropharmacology. Oxford University Press, New York. 1970.
44. MOLINOFF, P. B., and AXELROD, J.: J. Neurochem. **19**, 157 (1972).
45. COSTA, E.: *In* Biochemistry of simple neuronal models. *Edited by* E. Costa and E. Giacobini. Raven Press, New York. 1970. p. 16.
46. GLOWINSKI, J., and AXELROD, J.: Pharmacol. Rev. **18**, 775–785 (1966).
47. FUXE, K., and UNGERSTEDT, U.: Life Sci. **5**, 817 (1966).