The relevance of this blockade of adenosine to the normal pharmacology of clonidine will require further work. However, although the drug is normally considered to be a central α-adrenoceptor agonist (Anderson & Stone, 1974; Schmitt, 1976) there are results not entirely compatible with such an action. Thus, clonidine is no longer effective after destroying central noradrenergic neurons (Dollery & Reid, 1973), its effects are not reduced by several α-blocking drugs (Schmitt, 1976) and it does not share with α-agonists the ability to activate cerebral adenylate cyclase (Skolnick & Daly, 1976).

This work was supported by the Medical Research Council. We thank Miss C. Hollins for technical assistance.

May 8, 1978

REFERENCES

ANDERSON, C. & STONE, T. W. (1974). *Br. J. Pharmac.*, **51**, 359–365.
DOLLERY, C. T. & REID, J. L. (1973). *Ibid.*, **47**, 206–216.
MCILWAIN, H. (1972). *Biochem. Soc. Symp.*, **36**, 69–85.
PHILLIS, J. W., KOSTOPOULOS, G. K. & LIMACHER, J. C. (1974). *Can. J. Physiol. Pharmac.*, **52**, 1226–1229.
SATCHELL, D., BURNSTOCK, G. & DANN, P. (1973). *Eur. J. Pharmac.*, **23**, 264–269.
SCHMITT, H. (1976). In: *Drugs and Central Synaptic Transmission*, pp. 63–88. Editors: Bradley P. B. & Dhawan, B. N. London: MacMillan.
SKOLNICK, P. & DALY, J. W. (1976). *Eur. J. Pharmac.*, **39**, 11–21.
STONE, T. W. (1973). *J. Physiol., London*, **233**, 211–225.
STONE, T. W. (1978). *Biochem. Soc. Trans.*, in the press.
STONE, T. W. & TAYLOR, D. A. (1977). *J. Physiol., Lond.*, **266**, 523–543.
TOMITA, T. & WATANABE, H. (1973). *Ibid.*, **231**, 167–177.

On the formation of 6-hydroxyindoles in rat brain

N. AWAZI, H. C. GULDBERG*, *Department of Pharmacology and Clinical Pharmacology with Toxicology, University of Trondheim, Regional Hospital, N-7000 Trondheim, Norway*

As a major route of metabolism, tryptamine is oxidatively deaminated to 3-indoleacetic acid and, in addition, it has been shown that *in vitro* rat and rabbit liver microsomes can hydroxylate tryptamine to form 6-hydroxytryptamine (Szara & Axelrod, 1959; Jepson, Zaltzman & Udenfriend, 1962). More recently, Lemberger, Axelrod & Kopin (1971) have demonstrated the *in vivo* formation of 6-hydroxytryptamine in rabbit kidney and ileum as well as its occurence in urine. *N*-Methylated tryptamine has been demonstrated in rat brain *in vivo* and *in vitro* (Saavedra & Axelrod, 1972; Boulton & Baker, 1974).

The formation of 6-hydroxytryptamine in brain has not been demonstrated and, therefore, we considered it of interest to investigate the metabolic fate of tryptamine in rat brain with particular reference to oxidative hydroxylation as a metabolic pathway for cerebral tryptamine.

We now report that 6-hydroxytryptamine as well as 6-hydroxy-*N*-methyltryptamine can be formed from tryptamine in rat brain homogenates as well as 6-hydroxyindoleacetic acid and 3-indole-acetic acid which can be formed both *in vivo* and *in vitro*.

For the *in vitro* studies Albino rats (Wistar), 200–250 g were decapitated and the brains rapidly removed. After separation of the cerebral hemispheres and the cerebellum the remainder of the brain was homogenized in ten vol 0·32 M sucrose at 4°. The subcellular fractionation was done according to Michaelson & Whittaker (1963). The homogenates were centrifuged at 1000*g* for 10 min and the supernatants were centrifuged again at 17 000 *g* for 20 min to yield crude mitochondrial pellets. The crude microsomal pellets were obtained by centrifuging the remaining supernatant at 100 000 *g* for 60 min. All centrifugations were in a Sorvall refrigerated centrifuge or Spinco ultra-centrifuge at 4°. Mitochondrial or microsomal preparations were suspended in 3 ml incubation medium containing: 0·5 M phosphate buffer (pH 6·8), 1 μmol ATP (Sigma), 100 μg of tryptamine (Sigma), 0·5 μmol nicotinamide (Sigma). The medium was gassed with 5% CO_2 in oxygen. The mixture was incubated in a metabolic shaking incubator for 1·3 h at 37°. After adjusting the pH to 5·8, the mixture was allowed to react with sulphatase (arylsulphatase in combination with β-glucuronidase, Sigma). The incubation was for 6 h at 37°. To the medium containing the microsomal fraction was added 1 vol 0·5 M borate buffer (pH 10) and the mixture was extracted with 2 vol of benzene to remove unreacted tryptamine. The aqueous layer was saturated with sodium chloride and extracted with a mixture of n-butanol and isoamylalcohol (9 : 1).

The medium containing the mitochondrial fraction was adjusted to pH 1 (adding a few drops of con-

* Correspondence.

centrated HCl) and extracted with ethylacetate. The organic extracts were dried under a stream of nitrogen. The dried residues were dissolved in 50% methanol and subjected to thin-layer chromatography (Silica gel G, type 60 Merck) or paper chromatography (Whatman No 1) using n-butanol–acetic acid–water (4:1:5) or isopropanol–ammonia–water (1:20:2) as solvent systems.

To further identify unknown substances they were examined as their pentafluoropropionyl derivatives by combined gas chromatography-mass spectrometry using a Finnigan Model 3000 gas chromatograph-mass spectrometer system.

The compounds were eluted from Silica gel with a mixture of methanol and 0·1 M HCl (1:1) and, after evaporation of the solvent, the residues were dissolved in 20% (v/v) solution of the appropriate fluoroacyl-anhydride (Fluka, A.G.) in acetonitrile and the solution was allowed to stand at room temperature (20°) for 2 h.

The acetylating agent was removed under a stream of nitrogen, the residues were dissolved in a small volume of benzene and submitted to mass spectrometry (3% QV–7 column).

In the second series of experiments the presence of tryptamine in excess in the brain was studied to investigate the possible conversion of this amine to 6-hydroxy-derivatives *in vivo*. The rats were injected intracisternally with 5 μCi of [^{14}C]tryptamine (Amersham, tryptamine-bisuccinate-2-^{14}C, spec. act. 47 m Ci mmol^{-1}) in combination with 70 μg of unlabelled tryptamine diluted in an appropriate volume of isotonic saline. The animals were killed 45 min after drug administration. The brains were removed immediately, dissected and homogenized in 3 volumes of ice-cold 0·4 M perchloric acid containing ascorbic acid 10 mg g^{-1} tissue. The homogenates were centrifuged at 15 000*g* for 20 min at 0°. After centrifugation, the supernatant was adjusted to pH 10·0 and extracted into 2 vol of benzene. The aqueous layer was saturated with sodium chloride and extracted with 3 vol of n-butanol containing 10% isoamylalcohol.

The organic extracts were dried under a stream of nitrogen and subjected to paper chromatography.

The results show that when the extract containing the microsomal fraction was subjected to paper chromatography using n-butanol–acetic acid–water as the solvent system, five spots were detected under ultraviolet light. The chromatogram was sprayed with Ehrlich reagent, which is most useful for the general tests of indoles or related compounds. Three indolic spots appeared on the chromatogram, dark green (R_F: 0·83), pale blue (R_F: 0·63) and pale purple (R_F; 0·53), one of which had the same R_F value (0·63) and colour reaction as the authentic 6-hydroxytryptamine. One of the remaining spots gave a red colour reaction with van den Bergh reagent, which is characteristic for 6-hydroxyindoles. These observations suggest that two of the metabolites of tryptamine might be 6-hydroxylated derivatives.

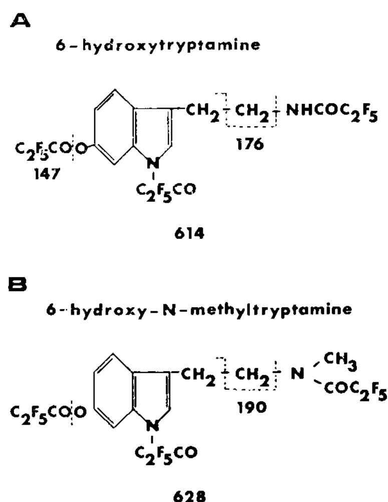

FIG. 1. Illustration of the fragmentation patterns proposed for 6-hydroxytryptamine (part A) and 6-hydroxy-*N*-methyltryptamine (part B).

The mass spectrum of 6-hydroxytryptamine showed the presence of a molecular ion peak at *m/e* 614 and a fragment ion peak at *m/e* 438, 452, 176, 147 corresponding to the fragmentation pattern shown in Fig. 1, part A. The mass spectrum of unknown metabolites showed the presence of a molecular ion peak at *m/e* 628 and fragment ion peaks at *m/e* 613, 466, 452, 190, 176, 147. The finding of an increase of 15 a.m.u. in the value of the molecular ion in relation to 6-hydroxytryptamine, as well as the occurrence of fragment ions at *m/e* 190 and *m/e* 613 were consistent with the hypothesis that the unknown metabolite was the *N*-methylated derivative of 6-hydroxytryptamine (Fig. 1, part B). Mass spectrometric data of the unknown metabolites which had the same R_F value as the authentic 6-hydroxytryptamine and of authentic 6-hydroxy-*N*-methyl tryptamine confirmed the identity of the two compounds.

When the acidic metabolites (mitochondrial fraction) were subjected to paper chromatography as described above, a major compound was found corresponding to authentic indoleacetic acid and traces of a compound having the same chromatographic mobilities (R_F values) as authentic 6-hydroxyindoleacetic acid. Chromatographic identification of 6-hydroxyindoleacetic acid was made using the solvent systems isopropanol–ammonia–water (4:1:1) and n-butanol-pyridine–water (1:1:1).

After the intracisternal injection of [^{14}C]tryptamine, the radioactivities of unchanged tryptamine and its metabolites in striatum and thalamus were determined. The results, illustrated in Fig. 2, show that the thalamus had a slightly higher concentration of labelled tryptamine incorporated than that of the striatum 45 min

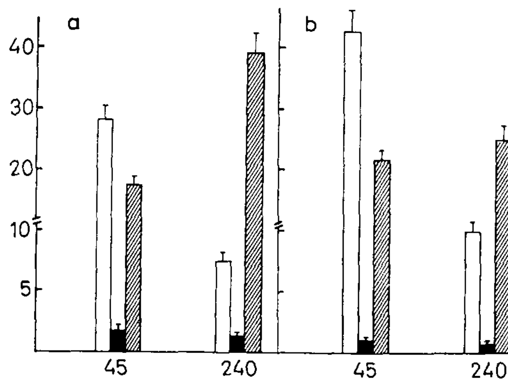

FIG. 2. The distribution of [^{14}C]tryptamine and its acidic and basic metabolites in the rat striatum (a) and thalamus (b) following the intracisternal injection of [^{14}C]tryptamine (5 μCi per rat). The results are expressed as mean values $\pm$ s.e.m. (3 determinations). Open columns: [^{14}C]tryptamine; hatched columns: acid metabolites; stippled columns: basic metabolites. Ordinate: d min^{-1} $\times$ 10^3 mg^{-1} tissue. Abscissa: Time (min).

after drug administration while the disappearance of unchanged tryptamine was more rapid in the thalamus. The results suggest that tryptamine might be taken up by serotoninergic neurons predominantly. The amounts of radioactive polar acidic metabolites were much greater than those of the polar basic metabolites. This might be explained, in part, by the fact that tryptamine is a good substrate for monoamine oxidase (Blaschko, Friedman & others, 1959).

Initial attempts at isolating the labelled 6-hydroxytryptamine *in vivo* were unsuccessful although there were amounts of radioactivity on the chromatogram corresponding to 6-hydroxy *N*-methyltryptamine. The acidic metabolites were, however, identified as 6-hydroxyindoleacetic acid and indoleacetic acid. It seems reasonable to conclude that oxidative deamination might be a main pathway for the metabolism of tryptamine *in vivo* and *in vitro*. The hydroxylation of tryptamine occurring in the presence of high exogenous amounts of tryptamine might be an abnormal route. The presence of 6-hydroxy-*N*-methyltryptamine seems most probably to be due to the hydroxylation of *N*-methylated tryptamine (Saavedra & Axelrod, 1972; Boulton & Baker, 1974).

The present finding that 6-hydroxytryptamine and 6-hydroxy-*N*-methyltryptamine can be isolated from homogenates of brain suggests that these amines are formed in rat brain by hydroxylation of tryptamine and *N*-methylated tryptamine. Some 6-hydroxylating activity in rabbit brain microsomes has been reported (Jepson & others, 1962), but evidence for the normal production of 6-hydroxy derivatives by endogenous tryptamine in brain has not been reported. However, Szara (1961) and Szara & Hearst (1962) have suggested that the psychomimetic properties of dimethyltryptamine (DMT) and diethyltryptamine are due to their conversion *in vivo* into 6-hydroxy derivatives. Although there is good experimental evidence that DMT itself crosses the blood-brain barrier (Cohen & Vogel, 1972) it remains to be determined if the 6-hydroxy-derivatives do. 6-Hydroxytryptamine has been demonstrated to be taken up into serotoninergic neurons (Hökfelt, 1969) and hence can alter amine function in brain. Whether 6-hydroxytryptamine or the *N*-methylated derivative as possible abnormal metabolites play a role in pathological functions of the nervous system remains to be answered.

July 3, 1978

REFERENCES

BLASCHKO, H., FRIEDMAN, P. J., HAWES, R. & NILSSON, K. (1959). *J. Physiol., Lond.*, **145**, 384–404.
BOULTON, A. A. & BAKER, G. B. (1974). *Can J. Biochem.*, **52**, 288–293.
COHEN, I. & VOGEL, W. H. (1972). *Biochem. Pharmac.*, **21**, 1214–1216.
HÖKFELT, T. (1969). Abstracts of the Fourth International Congress on Pharmacology, p. 70. Basel: Schwahe and Co.
JEPSON, J. B., ZALTZMAN, P. & UDENFRIEND, S. (1962). *Biochim. biophys. Acta*, **62**, 91–102.
LEMBERGER, L., AXELROD, J. & KOPIN, I. J. (1971). *J. Pharmac. exp. Ther.*, **177**, 169–176.
MICHAELSON, I. A. & WHITTAKER, V. P. (1963). *Biochem. Pharmac.*, **12**, 203–211.
SAAVEDRA, J. M. & AXELROD, J. (1972). *Science*, **175**, 1365–1366.
SZARA, S. (1961). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **20**, 885–888.
SZARA, S. & AXELROD, J. (1959). *Experientia*, **15**, 216–217.
SZARA, S. & HEARST, E. (1962). *Ann. N. Y. Acad. Sci.*, **96**, 134–141.