3. M. G. Hadfield, *Archs Neurol., Chicago* **26**, 78 (1972).
4. M. G. Hadfield and M. E. Boykin, *Res. Commun. Chem. Path. Pharmac.* **7**, 209 (1974).
5. J. H. Pincus and S. H. Lee, *Archs Neurol., Chicago* **29**, 239 (1973).
6. A. J. Azzaro and J. A. Gutrecht, *Biochem. Pharmac.* **21**, 2719 (1973).
7. G. Chen, B. Bohner and A. C. Bratton, Jr., *Archs int. Pharmacodyn. Thér.* **142**, 30 (1963).
8. R. E. Tedeschi, D. H. Tedeschi, A. Mucha, L. Cook, P. Mattis and E. J. Fellows, *J. Pharmac. exp. Ther.* **125**, 28 (1959).
9. L. A. Gottschalk, L. Covi, R. Uliana and D. E. Bates, *Compreh. Psychiat.* **14**, 503 (1973).
10. J. H. Stephens and J. W. Shaffer, *Psychophamocologia* **17**, 169 (1970).
11. A. D. Jonas, *Int. J. Neuropsychiat.* **3**, (Suppl.), 21 (1967).
12. V. P. Whittaker, Methods of Separation of Subcellular Structural Components, p. 109. Univ. Press, Cambridge, Mass. (1963).
13. A. G. Gornall, C. J. Bardawill and M. M. David, *J. biol. Chem.* **177**, 75 (1949).
14. N. Kirschner, *J. biol. Chem.* **237**, 2311 (1962).
15. A. Carlsson, N. A. Hillarp and B. Waldeck, *Acta. physiol. scand.* **59**, (Suppl. 215), 1 (1962).
16. B. W. Festoff and S. H. Appel, *J. clin. Invest.* **47**, 2752 (1968).
17. J. H. Pincus and N. J. Giarman, *Biochem. Pharmac.* **16**, 600 (1967).
18. D. M. Woodbury, *J. Pharmacol. exp. Ther.* **115**, 74 (1955).
19. J. H. Pincus, I. Grove, B. B. Marino and G. E. Glaser, *Archs. Neurol., Chicago* **22**, 566 (1970).

Biochemical Pharmacology, Vol. 24, pp 1540–1542. Pergamon Press, 1975. Printed in Great Britain.

Accumulation of 4-hydroxyamphetamine by rat striatal homogenates*

(*Received* 27 *September* 1974; *accepted* 15 *January* 1975)

4-Hydroxyamphetamine (p-OHA) [1-(4-hydroxyphenyl)-isopropylamine], one of several amphetamine metabolites [1], is a sympathomimetic amine that has been implicated in some of the pharmacological actions of amphetamine [2–5]. The compound is a major metabolite of amphetamine in the rat and is found in the brain as well as the heart after systematic administration [4–6]. In addition, *p*-hydroxyamphetamine is an effective inhibitor of neuronal uptake of catecholamines in peripheral [7] and central neurons [8].

This report describes the behavior of *p*-hydroxyamphetamine-^{3}H(p-OHA-^{3}H) in rat striatal preparations that accumulate dopamine-^{3}H(DA-^{3}H). In this system, p-OHA is comparable to amphetamine as an inhibitor of dopamine uptake and is itself accumulated by the tissue. The nature of the accumulation suggests that it is the neuronal uptake process that is responsible.

The striata from six male Sprague–Dawley rats weighing 180–200 g were dissected and homogenized in 8 ml of 0·32 M sucrose. The homogenate was centrifuged for 10 min at 1000 *g* and the supernatant recentrifuged for 20 min at 11,000 *g*. The pellet obtained was resuspended in the same volume of isotonic sucrose and again centrifuged for 20 min at 11,000 *g*. The resulting pellet was resuspended by hand in 0·32 M sucrose to a final volume of 6 ml (protein concentration [9], 2·0–2·5 mg/ml).

A 0·1-ml aliquot of the suspension was added to 1·4 ml Krebs–Ringer phosphate containing 12·5 μM nialamide, 8·4 mM glucose, 1·1 mM l-ascorbate and the appropriate inhibitor. This mixture was allowed to preincubate for 3 min at 25° in a Dubnoff metabolic shaker. The tritiated amine was then added to a final concentration of 1 μM and the mixture incubated for 3 min at 25°. Uptake was quenched by the addition of 5 ml cold (0°) 0·9% NaCl that was 100 μM in unlabeled amine; this was followed immediately by filtration through a Millipore filter assembly. The incubation vessels and filters were washed with two 5-ml portions of quenching solution. The 0·8 μm Millipore filters were removed, placed in scintillation vials containing Bray's solution [10], and counted. Net uptake was calculated as the radioactivity retained by the filter less the radioactivity retained when the incubation was carried out at 0°. Each value was the mean of triplicates which usually had a standard deviation of 10 per cent.

Dopamine-^{3}H(10 Ci/m-mole) and amphetamine-^{3}H(6·2 Ci/m-mole) were obtained from the New England Nuclear Co., and *p*-hydroxyamphetamine-^{3}H (20 mCi/mg) was a generous gift from Dr. J. V. Dingell of the Department of Pharmacology, Vanderbilt University. The radiochemical purity of the *p*-hydroxyamphetamine was established to be greater than 95 per cent by thin-layer chromatographic (TLC) procedures.

The uptake described in these studies is the net accumulation occurring at 25° after subtraction of the accumulation at 0°. The rationale for this subtraction is the high temperature coefficient for membrane transport processes relative to diffusion [11]. In these experiments, the accumulation of DA and p-OHA at 0° was 10–20 per cent of that at 25°, while the accumulation of amphetamine at 0° was 90 per cent of that at 25°. The relative accumulations of DA, p-OHA and amphetamine are shown in Fig. 1. The uptake of DA is about 3 times that for p-OHA and 150 times that for amphetamine. The uptake of DA and p-OHA was not linear with time for more than 1 min, although uptake was still increasing at 3 min.

The effects of different inhibitory agents and experimental conditions on DA and p-OHA accumulation are summarized in Table 1. The accumulation was sensitive to osmotic shock, although the fraction liberated upon lysis was different for the two compounds in that p-OHA was much more completely released (77 per cent) than DA (46 per cent).

Table 1 also shows the results of experiments examining the interaction of the three compounds, p-OHA, ampheta-

* This research was supported by USPHS Grant MH-23839.

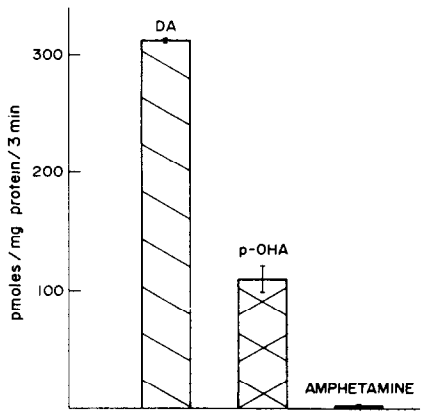

Fig. 1. Temperature-sensitive accumulation of dopamine, 4-hydroxyamphetamine and amphetamine by striatal homogenates. The accumulation of dopamine (DA), 4-hydroxyamphetamine (p-OHA) and amphetamine measured after a 3-min incubation at 25° was as described in the text. All the amine concentrations were 1 μM.

mine and DA, as substrates and inhibitors in this system. With DA as the substrate, p-OHA and amphetamine were equipotent as inhibitors. DA is a potent inhibitor of p-OHA uptake. Amphetamine appears to be more effective as an inhibitor of DA uptake than of p-OHA uptake, with calculated I_{50} values of 3·4 μM and 9·9 μM respectively.

The data presented show that p-OHA at a concentration of 1·0 μM is accumulated by washed P_2 preparations by a process that is temperature as well as sodium dependent and is similar to the highly active DA uptake [12] in its sensitivity to a variety of different inhibitors. Thus, it appears that p-OHA can be taken up by the DA neuronal uptake system. In contrast, amphetamine is not appreciably accumulated by this system, as evidenced by a very small difference in 25° and 0° uptake. These data are analogous to those of Baldessarini and Vogt [13] who showed *p*- and *m*-tyramine to be taken up by this type of preparation but not phenylethylamine. Similar data on uptake and retention have been shown for α-methyl-*m*-tyramine and metaraminol [14], as well as tyramine [15]. Ross and Renyi [16] have shown accumulation of phenylethylamine and amphetamine as well as tyramine by mouse cortical slices. In this case, however, the accumulation of the non-phenolic amines was not sensitive to cocaine or desmethylimipramine (DMI) and may represent a non-specific process. Thus, the presence of a phenolic hydroxyl function appears to be necessary for retention of these types of sympathomimetics by neuronal tissue, but it is not necessary for the inhibition of the DA uptake system.

Table 1. Interaction of DA, p-OHA and amphetamine and the effects of various inhibitors and conditions*

Substrate	Inhibitor	% Inhibition
DA (1 μM)	p-OHA (10 μM)	82·3 ± 0·9
	p-OHA (5 μM)	66·7 ± 0·3
	p-OHA (1 μM)	24·7 ± 2·2
	Amphetamine (10 μM)	79·0 ± 1·0
	Amphetamine (5 μM)	63·0 ± 1·0
	Amphetamine (1 μM)	18·2 ± 2·2
	Na (23 mM)	57·3 ± 2·4
	Ouabain (0·1 mM)	66·3 ± 1·5
	Dinitrophenol (0·1 mM)	49·3 ± 1·5
	Cocaine (0·01 mM)	81·7 ± 1·2
	DMI (0·01 mM)	41·0 ± 2·6
p-OHA	DA (2 μM)	54·8 ± 0·7
(1 μM)	DA (1 μM)	46·3 ± 2·6
	DA (0·5 μM)	34·0 ± 1·7
	Amphetamine (10 μM)	50·7 ± 8·8
	Amphetamine (5 μM)	37·0 ± 6·1
	Amphetamine (2·5 μM)	14·8 ± 3·0
	Amphetamine (1 μM)	5·8 ± 2·0
	Na (23 mM)	60·0 ± 3·6
	Ouabain (0·1 mM)	60·5 ± 1·4
	2,4-Dinitrophenol (0·1 mM)	53·3 ± 6·3
	Cocaine (0·01 mM)	60·0 ± 1·0
	DMI (0·01 mM)	57·7 ± 4·9

* DA or p-OHA at 1 μM was incubated with the striatal preparation in the presence of the various inhibitor concentrations under conditions described in the text. The accumulation was measured and is expressed as per cent decrease of the control uptake. The data represent the mean ± S.E. of at least three experiments.

Consistent with the work of Harris and Baldessarini [12], the effect of p-OHA and amphetamine of DA uptake (Table 1) suggests that a phenolic hydroxyl has little effect on inhibitor potency. However, with the poorer substrate, p-OHA, DA is a substantially better inhibitor than amphetamine. The I_{50} of amphetamine for p-OHA uptake, about 10 μM, is three times higher than that for DA uptake.

The uptake of amphetamine reported by Azzara *et al.* [17] may seem to conflict with the present observations but the washes performed in our work-up procedure may have removed the readily diffusable amphetamine. Amphetamine appears to diffuse freely through tissue, since it readily penetrates the brain after peripheral administration and leaves the brain quite rapidly [4, 5]. Furthermore, in studies on the distribution of amphetamine-^{3}H in brain fractions after peripheral administration, very little of the drug is found with the synaptosomal fraction [18, 19]. Free diffusion might also account for the non-specific accumulation of amphetamine described by Ross and Renyi [16]. The accumulation observed by these workers could be the result of a partition of a weak electrolyte between the intracellular and extracellular space with the lower intracellular pH [20] causing accumulation of the basic compound.

Department of Pharmacology, UCLA School of Medicine, Center for the Health Sciences, Los Angeles, Calif. 90024, *U.S.A.*

ARTHUR K. CHO
JAMES C. SCHAEFFER
JOSEPH F. FISCHER

REFERENCES

1. L. G. Dring, R. L. Smith and R. T. Williams, *Biochem. J.* **116**, 425 (1970).
2. B. B. Brodie, A. K. Cho and G. C. Gessa, in *Int. Symposium on Amphetamine and Related Compounds* (Eds. E. Costa and S. Garrattini), p. 217. Raven Press, New York (1970).
3. A. Groppetti and E. Costa, *Life Sci.* **8**, 653 (1969).
4. G. A. Clay, A. K. Cho and M. Roberfroid, *Biochem. Pharmac.* **20**, 1821 (1971).
5. T. Lewander, *Psychopharmacologia* **21**, 17 (1971).
6. J. J. Freeman and F. Sulser, *J. Pharmac. exp. Ther.* **183**, 307 (1972).
7. A. S. V. Burgen and L. L. Iversen, *Br. J. Pharmac. Chemother.* **25**, 34 (1965).
8. A. S. Horn, *Br. J. Pharmac. Chemother.* **47**, 332 (1973).
9. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
10. G. A. Bray, *Analyt. Biochem.* **1**, 279 (1960).
11. W. D. Stein, *The Movement of Molecules Across Cell Membranes*, p. 103. Academic Press, New York (1967).
12. J. E. Harris and R. J. Baldessarini, *Neuropharmacology* **12**, 669 (1973).

13. R. J. Baldessarini and M. Vogt, *J. Neurochem.* **18**, 2159 (1971).
14. R. L. Dorris and P. A. Shore, *J. Pharmac. exp. Ther.* **179**, 15 (1971).
15. M. J. Steinberg and C. B. Smith, *J. Pharmac. exp. Ther.* **173**, 176 (1970).
16. S. B. Ross and A. L. Renyi, *J. Pharm. Pharmac.* **18**, 756 (1966).
17. A. J. Azzara, R. J. Ziance and C. O. Rutledge, *J. Pharmac. exp. Ther.* **189**, 110 (1974).
18. H. O. Obiwana, R. Stitzel and P. Lundborg, *J. Pharm. Pharmac.* **20**, 585 (1974).
19. D. T. Wong, R. M. Van Frank, J. Horng and R. W. Fuller, *J. Pharm. Pharmac.* **24**, 171 (1972).
20. W. J. Waddell and R. Bates, *Physiol. Rev.* **49**, 285 (1969).

Biochemical Pharmacology, Vol. 24, pp. 1542–1544. Pergamon Press. 1975 Printed in Great Britain.

Inhibition of indolethylamine-*N*-methyltransferase by analogs of *S*-adenosylhomocysteine

(*Received* 8 *October* 1974; *accepted* 20 *December* 1974)

Indolethylamine-*N*-methyltransferase (INMT), which was first isolated from rabbit lung [1, 2], catalyzes the transfer of the methyl group from *S*-adenosylmethionine (SAM) to the amino group of a variety of indoleamines. Recently, a similar enzyme has been isolated from human brain [3] and serum [4] and has been implicated in certain mental disorders [3–5]. Because of the recent observations by Lin *et al.* [6] that *S*-adenosylhomocysteine (SAH), a general product of all SAM-dependent methyltransferases, inhibited INMT *in vitro*, we decided to explore the specificity of this inhibition using various analogs of SAH, which had been previously prepared in our laboratory [7–9.*].

INMT was isolated from rabbit lung (Pel-Freez Biologicals) according to the procedure of Mandel *et al.* [10] and purified through the Sephadex G-150 step, which resulted in a 28-fold purification of the enzyme with a sp. act. of 16·8 nmoles product/mg of protein/hr. For the INMT assay, $^{14}CH_3$-SAM (New England Nuclear, 55·0 mCi/m-moles) was diluted to a concentration of 10 μCi/ml and stored at −20° F. SAM iodide (Sigma) and *N*-methyltryptamine (Aldrich) were each stored as 0·01 M aqueous stock solutions. Enzyme activity was measured by a previously described radiochemical assay [1] and a normal incubation mixture consisted of the following components (in μmoles): water, so that the final vol was 0·25 ml; *N*-methyltryptamine (0·25); SAM (variable); inhibitor (variable); 0·05 μCi $^{14}CH_3$-SAM; phosphate buffer, pH 7·9 (25); and the enzyme preparation. Assay mixtures were incubated for 60 min at 37° and the reaction was terminated by addition of 0·25 ml of 0·5 M borate buffer, pH 10·0. The aqueous layer was extracted with 10 ml isoamyl alcohol (water saturated) and an aliquot (5 ml) of the organic phase was checked for radioactivity. The enzyme activity was corrected using a *N*-methyltryptamine blank. In the kinetic experiments, inhibition constants were calculated according to the method of Cleland [11] using a Hewlett–Packard 2100A digital computer and a Fortran IV program [12–15]. The synthesis of the SAH analogs used in this study has been previously reported [7–9.*]. SAH analogs were each stored as 0·01 M aqueous stock solutions.

Table 1 shows the degree of inhibition of INMT activity produced by the various structural analogs of SAH. In discussing their inhibitory activities, the analogs have been divided into three general classes: (a) amino acid-modified derivatives; (b) base-modified derivatives; and (c) sugar-modified derivatives. From the low inhibitory activities exhibited by the various amino acid derivatives, it is apparent that INMT shows a fairly high specificity for the structural features of the homocysteine portion of L-SAH. The structural features of primary importance in binding appear to be: (1) the chirality of the amino acid asymmetric carbon; (2) the terminal amino group; (3) the terminal carboxyl group; (4) the sulfur atom; and (5) the 3-carbon distance between the sulfur atom and the terminal amino and carboxyl groups. This high specificity for the homocysteine portion of SAH appears to be a general characteristic of most methyltransferases which have been studied [7].

From the inhibitory activities of the various base-modified derivatives shown in Table 1, it can be concluded that there exists a very strict requirement for the adenine moiety of SAH, particularly the 6-amino group in binding to this enzyme. This conclusion was derived from the fact that substitution of guanine (SGH), hypoxanthine (SIH), uracil (SUH) or cytosine (SCH) in place of adenine in SAH resulted in almost complete loss of inhibitory activity. Interestingly, replacement of the adenine moiety with 3-deaza-adenine (3-deaza-SAH, compound 7, Table 1) produced a potent INMT inhibitor. Therefore, it appears that the nitrogen in the 3-position of adenine is not an absolute requirement for the binding of SAH to INMT. In fact, there may exist a general lack of importance of the adenine ring nitrogens in binding, since Coward† has observed recently that the tubercidin analog of SAH is a potent inhibitor of INMT. Mono-methylation of the 6-amino group of 3-deaza-SAH had little effect on binding, since N^6-methyl-3-deaza-SAH (compound 8) produced strong inhibition of INMT. However, the dimethylamino analog (compound 9) was completely inactive as an inhibitor of INMT. This reduction in activity is probably the result of increased steric bulk at the 6-position rather than an electronic effect [8, 9].

The sugar-modified derivatives of SAH have exhibited a very interesting inhibitory profile. The 2′-hydroxy group of SAH appears to be important in binding to INMT, since 2′-deoxy-SAH (compound 10) was completely inactive as an inhibitor, whereas the arabinose derivative (compound 12) produced strong inhibition of INMT, but less than that for SAH itself. Whether the 2′-hydroxyl group is involved directly in binding or is important in maintaining the proper relative conformation between the base and amino acid portion of SAH has yet to be resolved. 3′-Deoxy-SAH (compound 11) was also found to be a potent inhibitor of INMT. Of the methyltransferases studied in our laboratory, strong inhibition by 3′-deoxy-SAH has

* R. T. Borchardt and Y. S. Wu, *J. med. chem.* **18**, 300 (1975).

† James Coward, Yale University School of Medicine, personal communication.