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FORMATION *IN VITRO* OF *N*-ACETYL-3,4-DIMETHOXYPHENETHYLAMINE BY PINEAL HYDROXY-INDOLE-*O*-METHYL TRANSFERASE

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Abstract—The pineal hydroxyindole-*O*-methyl transferase (HIOMT) catalysed formation of *N*-acetyl-3,4-dimethoxyphenethylamine (NADMPEA) from both *N*-acetyl-4-hydroxy-3-methoxyphenethylamine and *N*-acetyl-3-hydroxy-4-methoxyphenethylamine have been demonstrated *in vitro*. Dimethylation was not achieved. The formation of NADMPEA is shown also to be inhibited by haloperidol. It is suggested that, in schizophrenia, pineal HIOMT might get out of phase with its normal substrate and so act on other substrates, thus producing abnormally methylated catecholamine derivatives.

THE THEORY that transmethylation is implicated in the etiology of schizophrenia has wide support.[1,2] Abnormal methylated metabolites reported to be present in schizophrenic urine but not in normal controls include *N*-methylated indoles exemplified by dimethyl tryptamine (DMT)[3] and *O*-methylated catecholamines, such as 3,4-dimethoxyphenethylamine (DMPEA).[4] In the latter case, however, other workers have put forward conflicting evidence.[5] Furthermore, the drugs mescaline, an *O*-methylated catecholamine, and DMT produce psychoses in normal subjects similar to those observed in schizophrenia.[6]

In studies on the *in vitro* formation of DMPEA and its *N*-acetyl derivative NADMPEA, there appears to be no evidence of an enzyme system capable of dimethylating the catecholamine precursors. However, such dimethylated compounds can be produced in successive steps from the monomethylated compounds by incubation with liver and brain homogenate.[7]

Moreover, there exists uniquely in the pineal gland an analogous enzyme, hydroxyindole-*O*-methyltransferase (HIOMT), which is capable of *O*-methylating *N*-acetyl-5-hydroxytryptamine to produce melatonin.[8] This report investigates the *in vitro* effect of HIOMT on *N*-acetyl dopamine, (NADA) *N*-acetyl-4-hydroxy-3-methoxyphenethylamine (NA-4HMPEA) and *N*-acetyl-3-hydroxy-4-methoxyphenethylamine (NA-3HMPEA). It is shown that the enzyme will produce NADMPEA from the monomethylated catecholamines but not from NADA. It is also demonstrated that Haloperidol, a drug used in the successful treatment of schizophrenia, inhibits the *in vitro* formation of NADMPEA.

MATERIALS

N-acetyl-4-hydroxy-3-methoxyphenethylamine, *N*-acetyl 3-hydroxy-4-methoxy phenethylamine and *N*-acetyl dopamine were generously donated by A. J. Friedhoff

and E. Van Winkle, Department of Psychiatry and Neurology, New York University School of Medicine, New York, U.S.A. *S*-adenosyl-5′-L-methionine chloride was purchased from Koch-Light Laboratories, haloperidol HCl was supplied by Janssen Pharmaceutica, Beerse, Belgium. Fresh bovine pineals were supplied by the City Abbatoir, Bradford.

METHODS

Enzyme preparations. The methylation of the isomeric *N*-acetyl methyl ethers of dopamine was accomplished by HIOMT prepared according to the method of Axelrod *et al.*[8] from fresh bovine pineal glands. Enzyme protein was estimated by the method of Lowry *et al.*[9]

Formation of NADMPEA. Incubations were carried out at 37° for periods of time extending up to 6 hr. A total assay volume of 3 ml contained NA-4HMPEA or NA-3HMPEA or N–Ac dopamine ($7{\cdot}5 \times 10^{-4}$ M, 2·25 μM) *S*-adenosylmethionine (SAM $3{\cdot}3 \times 10^{-4}$ M, 0·99 μM), phosphate buffer pH 7·9 (1×10^{-1} M, 300 μM) and pineal homogenate 100 mg pineal tissue (equivalent to 2550 μg protein per assay).

Extraction and estimation of NADMPEA. The product of the incubation was extracted into 2×6 ml aliquots of chloroform. The combined extracts were washed with 2×15 ml of N NaOH to remove the remaining phenolic substrate. Ten ml of the washed chloroform extract were evaporated to dryness; the residue taken up in 5 ml 0·1 N HCl and assayed using a Perkin–Elmer MPF-3 recording spectrofluorimeter (excitation 285 nm, emission 310 nm, uncorrected). The fluorescent intensity was linear with respect to concentration over the range 0–4 μg/ml in 0·1 N HCl. The extraction procedure gave 90·0 $\pm$ 6 per cent recovery of authentic NADMPEA and interference from the phenolic substrates were reduced to a minimum and accounted for in blanks in which SAM was omitted.

Inhibition by haloperidol. Haloperidol HCl was dissolved in 1% phosphoric acid and the pH adjusted to 7·0 with 0·5 N NaOH. The final concentration in a total assay volume of 3·0 ml was $1{\cdot}7 \times 10^{-4}$ and $3{\cdot}4 \times 10^{-5}$ M.

RESULTS AND DISCUSSION

The production of NADMPEA *in vitro*, from both mono-*O*-methylated derivatives of dopamine by pineal HIOMT, has been demonstrated although the di-*O*-methylation of *N*-acetyl dopamine was not achieved. Figure 1 indicates that a lag period of up to 1 hr is encountered although the reason for this is not yet clear. This effect is also observed to a lesser extent in the oxidation of tyrosine.[10] The rate of turnover of the two substrates was about 2:1 in favour of NA-3HMPEA after 3 hr. The final fall-off in the rates does not appear to be due to the lack of substrate but more probably to product inhibition since this enzyme is inhibited by the normal methylated product, melatonin.[11]

Table 1 indicates that 51·6 and 27·7 nmoles of NADMPEA/hr/g of gram of pineal tissue are formed from NA-3HMPEA and NA-4HMPEA, respectively. With a liver enzyme, Friedhoff *et al.*[7] obtained 5·0 and 1·2 nmoles NADMPEA from these respective substrates. By comparison, the activity of our pineal enzyme on its normal substrate *N*-acetyl-5-hydroxytryptamine is observed to be 677 nmoles melatonin/hr/g of pineal tissue although this is about twice the value reported by Axelrod.[8] These slow rates are consistent with the observation of Jackson and Lovenberg that this

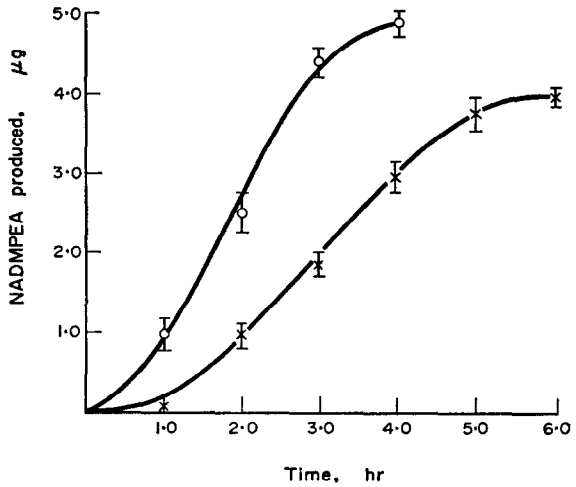

FIG. 1. The formation of NADMPEA by HIOMT from NA-3HMPEA and NA-4HMPEA. (×) NA-4HMPEA as substrate; (○) NA-3HMPEA as substrate; abscissae, time (hr); ordinate, micrograms of NADMPEA produced. Results with standard errors represent at least three determinations.

enzyme appears to have one of the lowest turnover numbers reported for any enzyme.[12] Thus although *N*-acetyl-5-hydroxytryptamine is the best substrate for pineal HIOMT, the enzyme will act on abnormal substrates.

TABLE 1.

Substrate	Concn	nmoles NADMPEA/hr/g pineal tissue	Haloperidol (Concn)	Inhibition (%)
NA-4HMPEA	$7{\cdot}50 \times 10^{-4}$ M	27·7 S.E. 1·73 (4)	$3{\cdot}4 \times 10^{-5}$ M	29·14 S.E. 3·96 (2)
			$1{\cdot}7 \times 10^{-4}$ M	48·82 S.E. 0·03 (2)
NA-3HMPEA	$7{\cdot}50 \times 10^{-4}$ M	51·6 S.E. 2·22 (4)	$3{\cdot}4 \times 10^{-5}$ M	20·69 S.E. 1·94 (2)
			$1{\cdot}7 \times 10^{-4}$ M	
NAc dopamine	$7{\cdot}50 \times 10^{-4}$ M	0		

Under same conditions, 677 ± 30·3 nmoles melatonin/hr/g pineal tissue produced from *N*-acetyl-5-hydroxytryptamine.

Figures in parentheses denote the number of determinations, S.E. are shown.

Furthermore, Table 1 indicates that the neuroleptic drug haloperidol at 10^{-5} M inhibits the formation of NADMPEA from both mono-*O*-methylated substrates by about 20–30 per cent, whilst at 10^{-4} M from NA-3HMPEA the inhibition is almost 50 per cent. These results compare favourably with those reported earlier[13] for the effect of haloperidol on the enzyme HIOMT using the normal substrate, *N*-acetyl-5-hydroxytryptamine. This effect of haloperidol would offer an alternative explanation for its mechanism of action particularly since the drug is reported to be concentrated more in the pineal gland than any other part of rat brain after injection.[14]

These interesting findings lead us to propose that in schizophrenia the pineal HIOMT might act on abnormal substrates such as the isomeric methyl ethers of dopamine to produce the dimethylated metabolites already implicated in the disease. Since the enzyme normally exhibits a diurnal rhythm,[15] it is possible that in schizophrenia, the enzyme gets out of phase with its substrate and so allows such an abnormal transmethylation. Certainly this report supports other workers who have suggested that the pineal gland is implicated in the etiology of schizophrenia.[16–18]

REFERENCES

1. H. OSMOND and J. R. SMYTHIES, *J. Ment. Sci.* **98,** 309 (1952).
2. S. S. KETY, *Int. J. Psychiat.* **1,** 409 (1965).
3. H. E. HIMWICH, N. NARASIMHACHARI, B. HELLER, J. SPAIDE, M. FUJIMORI and K. TABUSHI, *Proc. Meeting of Soc. Biological Psychiatry,* San Francisco, May 3–5 (1970).
4. A. J. FRIEDHOFF and E. VAN WINKLE, *Nature, Lond.* **194,** 867 (1962).
5. J. A. G. WATT, G. W. ASHCROFT, R. T. DALY and J. R. SMYTHIES, *Nature, Lond.* **221,** 971 (1969).
6. H. E. HIMWICH, *Biochemistry, Schizophrenias and Affective Illnesses,* Chapter 5. Williams & Wilkins, Baltimore, Maryland (1971).
7. A. J. FRIEDHOFF, J. W. SCHWEITZER and J. MILLER, *Res. Commun. Chem. Path. Pharmac.* **3,** 293 (1972).
8. J. AXELROD and H. WEISSBACH, *J. biol. Chem.* **236** (I), 211 (1961).
9. O. H. LOWRY, J. N. ROSEBROUGH, A. L. FARR and R. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
10. A. B. LERNER and T. B. FITZPATRICK, *Phys. Revs.* **30,** 91 (1950).
11. B. WEISS, *Adv. Pharmac.* **64,** 152 (1968).
12. R. L. JACKSON and W. LOVENBERG, *J. biol. Chem.* **246,** 4280 (1971).
13. R. HARTLEY, D. PADWICK and J. A. SMITH, *J. Pharm. Pharmac.* **24,** Suppl., 100 P (1972).
14. R. J. NAYLOR and J. E. OLLEY, *Br. J. Pharmac.* **36** (1), 208 (1969).
15. J. AXELROD, R. J. WURTMAN and S. H. SNYDER, *J. biol. Chem.* **240,** 949 (1965).
16. A. D. ALTSCHULE, *New Eng. med. J.* **257,** 919 (1957).
17. R. L. JONES, P. L. MCGEER, A. C. GREINER, *Clin. Chem. Acta* **26,** 281 (1969).
18. A. C. GREINER, *Can. Psychiat. Ass.* **15,** 433 (1970).