REFERENCES

ANDÉN, N-E. & STOCK, G. (1973). *J. Pharm. Pharmac.*, **25**, 346–348.

ANDÉN, N-E., BUTCHER, S. G., CORRODI, H., FUXE, K. & UNGERSTEDT, U. (1970). *Eur. J. Pharmac.*, **11**, 303–314.

ARNFRED, T. & RANDRUP, A. (1968). *Acta pharmac. tox.*, **26**, 384–394.

BARTHOLINI, G., HAEFELY, W., JALFRE, M., KELLER, H. H. & PLETSCHER, A. (1972). *Br. J. Pharmac.*, **46**, 736–740.

BERZEWSKI, H., HELMCHEN, H., HIPPIUS, H., HOFFMANN, H. & KANOWSKI, S. (1969). *Arzneimittel-Forsch.*, **19**, 495–496.

BUNNEY, B. S., WALTERS, J. R., ROTH, R. H. & AGHAJANIAN, G. K. (1973). *J. Pharmac. exp. Ther.*, **185**, 560–571.

CARLSSON, A. & LINDQVIST, M. (1963). *Acta pharmac. tox.*, **20**, 140–144.

CLEMENT-CORMIER, Y. C., KEBABIAN, J. W., PETZOLD, G. L. & GREENGARD, P. (1974). *Proc. Nat. Acad. Sci., U.S.A.*, **71**, 1113–1117.

COSTALL, B. & OLLEY, J. E. (1971). *Neuropharmac.*, **10**, 297–306.

COSTALL, B. & NAYLOR, R. J. (1974). *Eur. J. Pharmac.*, **27**, 46–58.

DE MAIO, D. (1972). *Arzneimittel-Forsch.*, **22**, 919–923.

DORRIS, R. L. & SHORE, P. A. (1974). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **33**, 511.

GROSS, H. & LANGNER, E. (1969). *Arzneimittel-Forsch.*, **19**, 496–498.

KELLY, P., MILLER, R. J. & SAHAKIAN, B. (1974). *Br. J. Pharmac.*, **52**, 430–431.

MILLER, R. J. & HILEY, C. R. (1974). *Nature, Lond.*, **248**, 596–597.

NYBÄCK, H. & SEDVALL, G. (1968). *J. Pharmac. exp. Ther.*, **162**, 294–301.

STILLE, G. & HIPPIUS, H. (1971). *Pharmakopsychiatr. Neuro-Psychopharmak.*, **4**, 182–191.

STILLE, G., LAUENER, H. & EICHENBERGER, E. (1971). *Il Farmaco, Ed. Pr.*, **26**, 603–625.

UNGERSTEDT, U. (1971). *Acta physiol. scand., Suppl.*, **367**, 49–93.

VON VOIGTLANDER, P. F. & MOORE, K. E. (1973). *Neuropharmac.*, **12**, 451–462.

Urinary 3-methoxy-4-hydroxyphenylglycol production in mice and rats following intraventricular 6-hydroxydopamine

The longitudinal study of noradrenaline metabolism in the mammalian brain is hampered by the inaccessibility of the most relevant tissue. A major metabolite in the dog (Maas & Landis, 1968), the cat (Mannarino, Kirshner & Nashold, 1963), the rat (Schanberg, Schildkraut & others, 1968) and the rabbit (Rutledge & Jonason, 1967) is 3-methoxy-4-hydroxyphenylglycol (MOPEG). In particular, the sulphate conjugate of MOPEG has been shown to increase in the brains of rats when noradrenaline synthesis is stimulated by neuroleptic drugs (Keller, Bartholini & Pletscher, 1973) and to increase or decrease after stimulation or destruction, respectively, of the locus coeruleus (Korf, Aghajanian & Roth, 1973). The measurement of urinary MOPEG has therefore been proposed as a possible indicator of brain metabolism (Maas & Landis, 1968; Gitlow, Mendlowitz & others, 1971).

Intraventricular administration of 6-hydroxydopamine (6-OH-DA) results in a prolonged depletion of brain noradrenaline (Breese & Traylor, 1970; Uretsky & Iversen, 1970), a chronic reduction of tyrosine hydroxylase (Fibiger, Fibiger & Zis, 1973) and ultrastructural damage in brain regions rich in adrenergic terminals (Bloom, Algeri & others, 1969). There are, however, conflicting reports on how this specific destruction of catecholamine neuronal processes in rat brain (Breese, Prange & others, 1972; Karoum & Costa, 1974; Hoeldtke, Rogawski & Wurtman, 1974; Bareggi, Marc & Morselli, 1974) and primate brain (Breese & others, 1972; Maas, Dekirmenjian & others, 1972) is reflected by urinary MOPEG.

In the rat 80% of the urinary MOPEG is found as the sulphate conjugate (Karoum, Lefevre & others, 1973), whereas the mouse (Howlett, unpublished) like man (Karoum & others, 1973; Bond & Howlett, 1974) excretes approximately equal amounts of the sulphate and glucuronide conjugates. This report describes the effect of the intraventricular administration of 6-OH-DA on mouse brain noradrenaline metabolism and also re-examines the effect on the rat.

Male albino mice (20–25 g) were treated with a single intraventricular injection of 6-OH-DA equivalent to 60 μg free base in 0·9% w/v saline. Male CFY rats (150–200 g) received 300 μg in a single dose. Both types of control animal were given intraventricular injections of the saline vehicle. 24 h urine collections were made 6 to 8 weeks after the injections and analysed for MOPEG conjugates (Bond & Howlett, 1974). After the last urine collection the animals were killed and brain MOPEG was measured by a modification of the urine method and brain and heart noradrenaline extracted and separated by the method of Schellenberger & Gordon (1971) and measured by the method of Anton & Sayre (1962).

The intraventricular administration of 6-OH-DA caused a 65 and 80% decrease in mouse and rat brain noradrenaline respectively (Table 1). Rat heart noradrenaline concentrations show that there was no significant effect on peripheral stores of the amine. In the mouse, no conjugated MOPEG was detected in the brain, but a 50% decrease in free MOPEG was observed in the 6-OH-DA treated animals. These mice also showed decreases in urinary free MOPEG while the excretion of both MOPEG conjugates remained unchanged. In rat brain we find MOPEG almost entirely as the sulphate conjugate and this was depleted by 75% after 6-OH-DA. We also noted apparent post mortem increases in free MOPEG in rat brain. This possibly explains the greater proportion of free MOPEG observed in rat brain by other workers (Braestrup, 1973). There were no statistically significant changes in urinary MOPEG although the *P* value for the difference in MOPEG excretion between the two groups was only slightly greater than 0·05.

Breese & others (1972) found that an 82% depletion of rat brain noradrenaline stores, following 6-OH-DA administration, resulted in a 29% decrease in total urinary MOPEG, whereas the experiments of Karoum & Costa (1974); Hoeldtke & others (1974) and Bareggi & others (1974), which depleted rat brain noradrenaline by 66%, produced no change in urinary MOPEG excretion. In primates, Maas & others (1972) found that a 72% decrease in brain noradrenaline stores following 6-OH-DA administration resulted in a 32% decrement in total urinary MOPEG. The experiments of

Table 1. *The effect of intraventricular 6-OH-DA on tissue noradrenaline (NA) and MOPEG and on urinary MOPEG in mice and rats.* Two mouse brains were pooled for each chemical estimation. The mean for each group is expressed with standard deviation. The data was analysed by the 2-tailed *t*-test. $P>0{\cdot}05$ is written as not significant (N.S.) for the comparison of the treated animals and the saline controls.

	Tissue Brain ng g^{-1}		Heart ng g^{-1}		Urine Creatinine μg mg^{-1}		
	NA	MOPEG sulphate	NA		MOPEG sulphate	MOPEG glucuronide	Free MOPEG
Rat							
Saline (n = 6)	356 s.d. 67	112·2 s.d. 25·8	302 s.d. 89	Saline (n = 6)	4·22 s.d. 1·67	0·35 s.d. 0·22	0·13 s.d. 0·03
6-OH-DA (n = 5)	69 19	28·3 10·9	272 90	6-OH-DA (n = 5)	2·77 0·64	0·37 0·11	0·11 0·02
P	<0·001	<0·001	N.S.		N.S.	N.S.	N.S.
	NA	Free MOPEG					
Mouse							
Saline (n = 4)	420 s.d. 43	28·2 s.d. 3·0		Saline (n = 8)	1·06 s.d. 0·17	1·44 s.d. 0·25	0·27 s.d. 0·08
6-OH-DA (n = 4)	149 31	14·2 6·6		6-OH-DA (n=7)	1·22 0·23	1·57 0·29	0·16 0·04
P	<0·001	<0·02			N.S.	N.S.	<0·01

Breese & others (1972), however, which depleted primate brain noradrenaline by 59%, did not produce significant changes in urinary MOPEG. The effects on MOPEG excretion may therefore be dependent on the extent of the catecholaminergic neuronal destruction. Bareggi & others (1974) showed that a 67% depletion of rat brain noradrenaline resulted in a 63% decrease in brain MOPEG SO_4 concentrations. The present experiments have produced 80 and 75% decreases respectively of noradrenaline and MOPEG-SO_4 in rat brain. It would therefore appear that although MOPEG-SO_4 is the major noradrenaline metabolite in rat brain its contribution to the body pool of MOPEG-SO_4 is so small that even drastic changes in brain MOPEG-SO_4 are difficult to observe in the urine.

The absence of MOPEG-SO_4 in mouse brain has also been noted by Ceasar, Hague & others (1974) using a fluorimetric method of estimation. In the mouse, therefore, free MOPEG would appear to be the major *O*-methylated glycol metabolite of brain noradrenaline. The presence of free MOPEG in mouse brain and urine and the simultaneous drop of free MOPEG observed in each suggests that peripheral conjugation may be more limited than might have been predicted. Though the brains themselves can be studied in lower animals, in the mouse at least some indication of cerebral changes in ongoing behavioural studies might be correlated with free MOPEG excretion.

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REFERENCES

ANTON, A. H. & SAYRE, D. F. (1962). *J. Pharmac. exp. Ther.*, **138**, 360–375.

BAREGGI, S. R., MARC, V. & MORSELLI, P. L. (1974). *Brain Res.*, **75**, 177–180.

BLOOM, F. E., ALGERI, S., GROPPETTI, A., REVUELTA, A. & COSTA, E. (1969). *Science*, **166**, 1284–1286.

BOND, P. A. & HOWLETT, D. R. (1974). *Biochem. Med.*, **10**, 219–228.

BRAESTRUP, C. (1973). *Analyt. Biochem.*, **55**, 420–431.

BREESE, G. R., PRANGE, A. J., Jr., HOWARD, J. L., LIPTON, M. A., MCKINNEY, W. T., BOWMAN, R. E. & BUSHNELL, P. (1972). *Nature New Biology*, **240**, 286–287.

BREESE, G. R. & TRAYLOR, T. D. (1970). *J. Pharmac. exp. Ther.*, **174**, 413–420.

CEASAR, P. M., HAGUE, P., SHARMAN, D. F. & WERDINIUS, B. (1974). *Br. J. Pharmac.*, **51**, 187–195.

FIBIGER, H. C., FIBIGER, H. P. & ZIS, A. P. (1973). *Ibid.*, **47**, 683–692.

GITLOW, S. E., MENDLOWITZ, M., BERTANI, L. M., WILK, S. & WILK, E. K. (1971). *J. clin. Invest.*, **50**, 859–865.

HOELDTKE, R., ROGAWSKI, M. & WURTMAN, R. J. (1974). *Br. J. Pharmac.*, **50**, 265–270.

KAROUM, F. & COSTA, E. (1974). *Biochem. Pharmac.*, **23**, 533–538.

KAROUM, F., LEFEVRE, H., BIGELOW, L. B. & COSTA, E. (1973). *Clin. chim. Acta*, **43**, 127–137.

KELLER, H. H., BARTHOLINI, G. & PLETSCHER, A. (1973). *Eur. J. Pharmac.*, **23**, 183–186.

KORF, J., AGHAJANIAN, G. K. & ROTH, R. H. (1973). *Ibid.*, **21**, 305–310.

MAAS, J. W., DEKIRMENJIAN, H., GARVER, D., REDMOND, D. E., JR. & LANDIS, D. H. (1972). *Brain Res.*, **41**, 507–511.

MAAS, J. W. & LANDIS, D. H. (1968). *J. Pharmac. exp. Ther.*, **163**, 147–162.

MANNARINO, E., KIRSHNER, H. & NASHOLD, B. S., JR. (1963). *J. Neurochem.*, **10**, 373–379.

RUTLEDGE, C. O. & JONASON, J. (1967). *J. Pharmac. exp. Ther.*, **157**, 493–502.

SCHANBERG, S. M., SCHILDKRAUT, J. J., BREESE, G. R. & KOPIN, I. J. (1968). *Biochem. Pharmac.*, **17**, 247–254.

SCHELLENBERGER, M. K. & GORDON, J. H. (1971). *Analyt. Biochem.*, **39**, 356–372.

URETSKY, N. J. & IVERSEN, L. L. (1970). *J. Neurochem.*, **17**, 269–278.