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Lowering of dopamine metabolites in rat brain by harmaline

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Harmaline is a competitive, reversible inhibitor of monoamine oxidase (MAO; EC 1.4.3.4) (Udenfriend et al 1958). Early studies with harmaline revealed greater inhibition when 5-hydroxytryptamine (5-HT) was substrate than when other substrates like phenethylamine or tyramine were used to assay MAO (Long 1962; Fuller 1968a). Subsequent studies of MAO led to the definition of two forms, type A which is particularly susceptible to inhibition by clorgyline and prefers 5-HT as its substrate, and type B which is relatively resistant to inhibition by clorgyline and prefers phenethylamine or benzylamine as substrate (Johnston 1968; Neff & Yang 1974). Harmaline was recognized to be a selective inhibitor of type A MAO.

In recent years attempts have been made to elucidate which MAO form is responsible for the in vivo oxidation of dopamine in rat brain, since dopamine can be oxidized by both forms of MAO in vitro (Neff & Yang 1974). Results from these studies, which have generally involved the use of irreversible inhibitors of MAO, have mostly agreed that MAO type A is primarily involved.

We now describe a study showing that no inhibition of MAO type B occurs in vivo after harmaline injection into rats at a 30 mg kg^{-1} dose, whereas virtually complete inhibition of MAO type A occurs for several h, though enzyme activity has returned essentially to the untreated level within 24 h. The concentration of two dopamine metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), is decreased markedly over a time course that parallels the inhibition of MAO type A. These results strengthen the belief that DOPAC and HVA formation in rat brain occur predominantly through the action of type A MAO.

Male Wistar rats (130–150 g) from Harlan Industries, Cumberland, Indiana were housed in groups of 5 with food and water freely available. Harmaline hydrochloride (Sigma) was injected at 30 mg kg^{-1} i.p., and rats were decapitated at 2, 4, 8 or 24 h thereafter. Whole brains were quickly removed, frozen on dry ice, and stored at −15 °C before analysis. The tissue was homogenized in 0·1 M sucrose containing 0·5 mg cysteine ml^{-1}. An aliquot of the homogenate was used for radiometric MAO assay (Wurtman & Axelrod 1965; Fuller 1968b) with 100 μM [^{14}C]-5-hydroxytryptamine or 12·5 μM [^{14}C]phenethylamine (isotopes from New England Nuclear) as substrates for type A and type B MAO, respectively. This concentration of 5-HT has been found to be optimum for deamination by rat brain MAO (R. W. Fuller & B. W. Roush, unpublished data), and 5-HT is accepted as a selective substrate for type A MAO in rat brain (Fowler et al 1978). The concentration of phenethylamine was chosen to be selective for type B MAO, since at higher concentrations phenethylamine can be deaminated partially by type A MAO (Dial & Clarke 1979; Suzuki et al 1979). Dopamine metabolites in the homogenates were assayed by high performance liquid chromatography with electrochemical detection (Perry & Fuller 1979).

Fig. 1a shows that type B MAO measured with 12·5 μM [^{14}C]phenethylamine as substrate remained unaffected after harmaline injection. In contrast, the inhibition of type A MAO was virtually complete at the early times after harmaline (2 and 4 h) and remained substantial at 8 h, but was essentially gone by 24 h. This degree of in vivo selectivity with harmaline is greater than has been obtained with irreversible type A MAO inhibitors like clorgyline (Waldmeier et al 1976) and LY51641 {*N*-[2-(*o*-chlorophenoxy)-ethyl]-cyclopropylamine} (Fuller & Hemrick 1978b).

The activity of MAO measured in vitro in tissue homogenates after administration of a reversible inhibitor may not reflect the exact percentage inhibition that occurred in vivo, since dilution of the inhibitor has occurred and cellular compartmentation has been destroyed. The time course and relative selectivity of inhibition measured in this way would be expected to parallel closely the in vivo situation, however, and the selectivity of harmaline has been established in other ways. For instance, the high degree of selectivity of harmaline as an inhibitor of type A MAO in vitro has been amply documented (Long 1962; Fuller 1968a). The selectivity has also been shown by in vivo experiments in which harmaline protected against the inactivation of type A but not type B MAO by an irreversible inhibitor of MAO (Fuller & Hemrick 1978a). Experiments of the latter type do not permit studies of the time course of MAO inhibition by harmaline, since the measurements must be made after the inhibitory effects of the reversible inhibitor has disappeared and only the inactivation by the irreversible inhibitor remains.

Fig. 1b shows that both dopamine metabolites measured, DOPAC and HVA, were lowered by harmaline over a time course remarkably similar to the inhibition of type A MAO. That is, approximately 90% lowering occurred at 2 and 4 h with partial recovery by 8 h and nearly complete recovery at 24 h.

Earlier evidence with irreversible MAO inhibitors had supported the idea that dopamine is mainly deaminated

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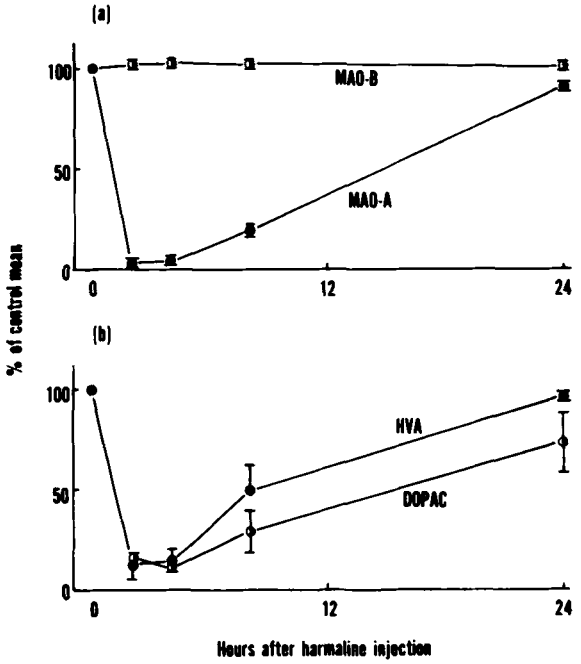

FIG. 1. (a) Time course of the inhibition of type A MAO (assayed with 100 μM [^{14}C]-5-hydroxytryptamine as substrate) but not type B MAO (assayed with 12·5 μM [^{14}C]phenethylamine as substrate) in rat brain after treatment with harmaline hydrochloride (30 mg kg^{-1} i.p.). (b) Time course of the changes in brain DOPAC and HVA in the same rats. Mean values ± standard errors for 5 rats per group are shown. All values are expressed as percentages of the control mean. Asterisks indicate significant differences from the untreated control group ($P < 0.05$), comparisons having been made with actual values not percentages.

by type A MAO in rat brain. For instance, deaminated metabolites of dopamine were lowered more by clorgyline (a selective inhibitor of type A MAO) than by deprenyl (a selective inhibitor of type B MAO) (Braestrup et al 1975; Waldmeier et al 1976). Campbell et al (1979) observed dopamine accumulation after chronic dosing with clorgyline but not pargyline (an inhibitor of type B MAO less selective than deprenyl). Demarest & Azzaro (1979) measured deamination of accumulated or newly synthesized radiolabelled dopamine in striatal synaptosomes from rat brain and found that clorgyline inhibited to a greater extent than deprenyl. Keane et al (1979) found that clorgyline, LY51641 and MD780515 (3-[4-(3-cyanophenylmethoxy)-phenyl]-5-(methoxymethyl)-2-oxazolidinone, a new reversible selective inhibitor of type A MAO) antagonized the depletion of dopamine by reserpine in rat brain, whereas deprenyl and pargyline did not. Timar et al (1979) found that chronic treatment with clorgyline restored striatal dopamine concentrations in rats with nigral lesions and antagonized the behavioural impairmentin the lesioned rats, whereas chronic treatment with deprenyl did not.

Our findings with a single dose of harmaline are among the most direct evidence available for dopamine deamination by type A MAO in rat brain since the selectivity of the inhibition of type A MAO was complete. Because harmaline is a reversible inhibitor of MAO, the relatively rapid return of type A MAO activity could be shown to parallel the return of DOPAC and HVA concentrations toward normal values.

REFERENCES

Braestrup, C., Andersen, H., Randrup, A. (1975) Eur. J. Pharmacol. 34: 181–187

Campbell, I. C., Robinson, D. S., Lovenberg, W., Murphy, D. L. (1979) J. Neurochem. 32: 49–55

Demarest, K. T., Azzaro, A. J. (1979) in: Singer, T. P., Von Korff, R. W., Murphy, D. L. (eds) Monoamine Oxidase: Structure, Function, and Altered Functions. Academic Press, New York, pp 423–430

Dial, E. J., Clarke, D. E. (1979) Pharmacol. Res. Commun. 11: 491–500

Fowler, C. J., Callingham, B. A., Mantle, T. J., Tipton, K. F. (1978) Biochem. Pharmacol. 27: 97–101

Fuller, R. W. (1968a) Arch. Int. Pharmacodyn. 174: 32–36

Fuller, R. W. (1968b) Biochem. Pharmacol. 17: 2097–2106

Fuller, R. W., Hemrick, S. K. (1978a) Life Sci. 22: 1083–1086

Fuller, R. W., Hemrick, S. K. (1978b) Proc. Soc. Exp. Biol. Med. 158: 323–325

Johnston, J. P. (1968) Biochem. Pharmacol. 17: 1285–1297

Keane, P. E., Chanoine, F., Strolin Benedetti, M. (1979) in: Singer, T. P., Von Korff, R. W., Murphy, D. L. (eds) Monoamine Oxidase: Structure, Function, and Altered Functions. Academic Press, New York, pp 341–346

Long, R. F. (1962) Acta Neurol. Scand. 38 Suppl 1: 27–28

Neff, N. H., Yang, H.-Y. T. (1974) Life Sci. 14: 2062–2074

Perry, K. W., Fuller, R. W. (1979) Soc. Neurosci. Abs. 346

Suzuki, I., Katsumata, Y., Oya, M. (1979) Biochem. Pharmacol. 28: 953–956

Timar, J., Knoll, B., Gyarmati, A., Knoll, J. (1979) Pol. J. Pharmacol. Pharm. 31: 251–260

Udenfriend, S., Witkop, B., Redfield, B. G., Weissbach, H. (1958) Biochem. Pharmacol. 1: 160–165

Waldmeier, P. C., Delini-Stula, A., Maitre, L. (1976) Naunyn-Schmiedeberg's Arch. Pharmacol. 292: 9–14

Wurtman, R. J., Axelrod, J. (1965) Biochem. Pharmacol. 12: 1439–1441