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THE EFFECT OF α-METHYL DOPA AND SOME OTHER DECARBOXYLASE INHIBITORS ON BRAIN 5-HYDROXYTRYPTAMINE

D.J. Drain, M. Horlington, R. Lazare and G.A. Poulter

Smith & Nephew Research Limited, Hunsdon Laboratories

Ware, Hertfordshire

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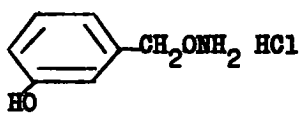
STUDIES in recent years have indicated that the clinical and pharmacological effects of certain drugs active on the central nervous system may be connected with the effects of these substances on levels of catecholamines and/or 5-hydroxytryptamine (5HT) in brain; thus iproniazid and related hydrazine derivatives are powerful inhibitors of monoamine oxidase, the enzyme presumed to be responsible for metabolic inactivation of catecholamines and 5HT, and they increase the levels of these amines in brain and other organs. Reserpine lowers tissue amine levels, probably by inhibiting an active storage process.

Another approach in the search for drugs which may modify tissue amines is the development of inhibitors of their synthesis, and in this connection inhibitors of DOPA/5HTP decarboxylase have been studied.

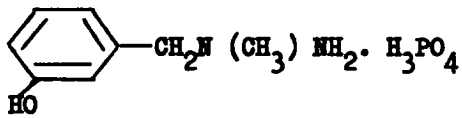
α-Methyl DOPA was first reported by Sourkes[1] to be an inhibitor of DOPA decarboxylase in vitro and subsequent work showed that this compound caused a decreased concentration of 5HT[2,3,4] and noradrenaline[3,4] in brain of rats, mice and guinea pigs. These observations were originally interpreted as a manifestation of the anti-decarboxylase action of α-methyl DOPA, but more recent work[5,6] based on the different time-course of noradrenaline depletion and decarboxylase inhibition has made it clear that the effects of α-methyl DOPA on brain and heart noradrenaline cannot be explained on the basis of decarboxylase inhibition alone. Recent studies by Carlsson[7] with α-methyl DOPA and Costa and Brodie[8] with the related α-methyl-m-tyrosine have shown that these

α-methyl-α-amino acids are decarboxylated *in vivo* to yield the corresponding α-methylamines and that these amines (or their β-hydroxylated analogues) produce a long-lasting depletion of tissue noradrenaline, presumably by impairment of, or displacement at, storage sites. We wish to report some experiments which suggest that the action of α-methyl DOPA in reducing 5HT levels is also not explicable on grounds of decarboxylase inhibition alone.

Recent work in these laboratories aimed at the design of inhibitors of DOPA/5HTP decarboxylase has resulted in the synthesis of a large number of hydrazine and hydroxylamine derivatives, many of which are extremely powerful inhibitors of this enzyme. Two such compounds are 3-hydroxybenzyloxyamine hydrochloride (NSD 1024) and N-(3-hydroxybenzyl)-N-methyl hydrazine dihydrogen phosphate (NSD 1034). In vitro activity against DOPA decarboxylase was measured



NSD 1024



NSD 1034

using the method of Hartman *et al.*[9] and 50% inhibition concentrations are given in Table 1, the units being in terms of molarity relative to substrate (RM) as used by these authors. N.S.D. 1024 and 1034 are also powerful *in vivo* inhibitors

TABLE 1

Effect on DOPA Decarboxylase *in vitro*

Compound	DOPA decarboxylase 50% i.c. (R.M.)
N.S.D. 1024	6.7×10^{-4}
N.S.D. 1034	2×10^{-3}
α-Methyl DOPA	2.5×10^{-1}

of 5HTP decarboxylase, an effect which was measured by two methods. The first method utilizes the central excitation produced in mice by injection of 5HTP following pretreatment with a monoamine oxidase inhibitor, a phenomenon first

reported by Bogdanski et al.[10] In our experiments groups of mice were injected with a monoamine oxidase inhibitor [(N-(1-methyl-2-phenoxyethyl) hydrazine hydrogen maleate (5 mg/kg i.p.)] followed 2 hr later by the decarboxylase inhibitor (controls received saline) followed 30 min later by 5HTP (75 mg/kg s.c.). Animals were examined 30 min after the 5HTP injection, at which time the controls displayed characteristic tremors, whereas those animals receiving effective doses of NSD 1024, NSD 1034 or α-methyl DOPA were protected. Minimum effective doses (M.E.D.) are shown in Table 2. The second method involved comparison of 5HTP

TABLE 2

Effect on 5HTP Induced Tremors in Mice

Compound	Route	M.E.D. (mg/kg)
NSD 1024	s.c.	2
NSD 1024	oral	10
NSD 1034	s.c.	0.5
NSD 1034	oral	1
α-Methyl DOPA	s.c.	100-200
α-Methyl DOPA	oral	>500

decarboxylase activity of mouse brain homogenates from treated animals with that of untreated control animals. Enzyme activity was measured by the method of Bogdanski et al.[11] Table 3 gives the doses required to produce 50% (1D 50) and 80% (1D 80) inhibition. Following the demonstration of in vivo decarboxylase inhibition, the effects of these compounds on concentrations of brain 5HT was investigated. We have confirmed the 5HT lowering effect of α-methyl DOPA reported by Smith.[2] In our experiments ca. 30% reduction in mouse brain 5HT was obtained 2 hr following a dose of 400 mg/kg s.c.

Many experiments with NSD 1024, using doses up to 64 mg/kg by oral, intraperitoneal, subcutaneous and intravenous administration in both rats and mice have failed to demonstrate any significant effect on brain 5HT. Similar experiments with NSD 1034 have shown that this compound at fairly high doses (ca. 50 mg/kg) produced a transient fall of ca. 30% in mouse brain 5HT, the level

TABLE 3

Effect on 5HTP Decarboxylase in Mouse Brain

Compound	Route	ID 50 (mg/kg)	ID 80 (mg/kg)
NSD 1024	i.p.	3	7
NSD 1024	oral	30	50
NSD 1034	oral	1	4
α-Methyl DOPA	i.p.	120	400

returning to normal 4 hr after administration of the drug. This is in contrast to the decarboxylase inhibition produced by NSD 1034 which has been shown to persist at high level for at least 8 hr.

These results, in particular those with 3-hydroxybenzyloxyamine (NSD 1024) indicate that a drug may produce a high percentage inhibition of 5HTP decarboxylase and yet have no effect on 5HT levels in the brain. It may be that the enzyme is normally present in a quantity considerably in excess of the functional requirements and that virtually 100% inhibition is necessary before the normal rate of 5HT synthesis is affected, and that the compounds here reported are not producing a sufficiently high percentage inhibition to prevent 5 HT synthesis. Recent work by Hirsch *et al.*[12] would seem to confirm this suggestion.

Thus it seems unlikely that the 5HT lowering effect of α-methyl DOPA, a considerably weaker decarboxylase inhibitor than NSD 1024 and NSD 1034, is mediated wholly *via* decarboxylase inhibition, although this may be a contributory factor.

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