Inhibition of choline acetyltransferase and hexobarbitone-metabolizing enzymes by naphthylvinyl pyridine analogues

Certain naphthylvinyl pyridine derivatives inhibit rat brain choline acetyltransferase (ChA) *in vitro* (Smith, Cavallito & Foldes, 1967; Cavallito, Yun & others, 1969). Of these, *trans*-4-(1-naphthylvinyl)pyridine HCl (4-NVP) is the most specific inhibitor of ChA. We have found that 4-NVP markedly potentiates the cholinesterase inhibitor physostigmine, instead of inhibiting its behavioral effects as might be expected (Goldberg, Sledge & others, 1970). On the basis of other drug interaction studies, we suspect that 4-NVP inhibited microsomal drug metabolizing enzymes as well as ChA. The present investigation examines whether 4-NVP, a potent inhibitor, *in vitro*, inhibits ChA *in vivo*, assesses the relative inhibitory effects on the microsomal drug metabolizing system for hexobarbitone, and also evaluates other isomers of NVP against both parameters. For these purposes, *trans*-isomers of 2-(1-naphthylvinyl)pyridine HCl (2-NVP) and 3-(1-naphthylvinyl)pyridine (3-NVP) were prepared and investigated.

ChA inhibition was measured, both *in vivo* and *in vitro*, in mouse brain (male albino, 20 g) according to McCaman & Hunt (1965). The hexobarbitone-metabolizing enzyme system (HMES) was studied *in vitro* using the 10 000 *g* supernatant fraction of rat liver homogenized in mannitol-sucrose-EDTA (225-75-0·1 mM, pH 7·4). Side-chain oxidation of hexobarbitone was estimated by substrate disappearance (Cooper & Brodie, 1955). Evidence for *in vivo* inhibition of HMES was obtained by measuring sleeping time in mice after hexobarbitone sodium (100 mg/kg, i.p.). Drugs or saline were given 30 min before the barbiturate.

Molar concentrations required to inhibit both enzymes *in vitro* by 50% (I50) were estimated graphically from the means of at least duplicate analyses using several inhibitor concentrations.

All isomers inhibited both enzymes *in vitro* (Table 1), although an I50 value for 3-NVP against ChA could not be obtained. 4-NVP was most potent against both enzymes, and was about 17 times more active against HMES than against ChA. No such correlation was observed with the other analogues. 3-NVP had one-sixth of the activity of 4-NVP on HMES, yet was about 1/100th as active against ChA. Conversely, 2-NVP had about one-half the activity of 4-NVP against ChA, yet was some 20 times weaker as an inhibitor of HMES.

4-NVP produced a dose-dependent inhibition of mouse brain ChA in the dose range of 25·0 to 100·0 mg/kg. Further inhibition was not obtained after higher doses. It also caused an inhibition which persisted for at least 8 h after 200·0 mg/kg with a peak 2 to 4 h after administration (Table 2). At this dose, signs of depression and ataxia were observed. The acute intraperitoneal LD50 for 4-NVP at 24 h was 337 (290–539) mg/kg.

The effects on the duration of hexobarbitone sleep after NVP analogues agree closely with *in vitro* inhibition of HMES (Table 3). With the lowest dose that caused

Table 1. In vitro *inhibition of choline acetyltransferase (ChA) and the hexobarbitone-metabolizing enzyme system (HMES)*. Control preparations had enzyme activities equivalent to 9·8 μmol/g h^{-1} of acetylcholine formed and 91 μ mol/g protein h^{-1} of hexobarbitone metabolized.

Compound	I50–ChA	I50–HMES
4–NVP	3×10^{-5}M	5×10^{-6}M
3–NVP	(30% at 10^{-3}M)	3×10^{-5}M
2–NVP	7×10^{-5}M	1×10^{-4}M

Table 2. In vivo *inhibition of ChA by* 4-NVP. Values given are for each group of 4–8 mice. Mean control activity of 11·1 ± 0·6 (s.e.) μmol/g h^{-1} of acetylcholine formed (n = 27).

Dose (mg/kg, i.p.)	Time of death (h)	% Inhibition of controls ± s.e.
12·5	1	2·3 ± 2·2
25·0	1	13·1 ± 3·6
50·0	1	35·7 ± 5·8
100·0	1	63·8 ± 2·3
200·0	1	65·4 ± 3·1
200·0	2	81·2 ± 4·6
200·0	4	83·0 ± 2·7
200·0	8	58·9 ± 1·1
200·0	24	0·0

Table 3. *Effects of NVP analogues on hexobarbitone sleeping time.* Mean sleeping time (min) for 6 groups of controls (10/group) was 31 ± 5 (s.e.). There were 20 mice/drug treatment and a cut-off time of 120 min was used.

Dose (mg/kg) i.p.	% Control sleeping time 4–NVP	3–NVP	2–NVP
2·5	112	—	—
5·0	158*	—	—
10·0	242*	—	—
25·0	354*	130	108
50·0	—	187*	118
100·0	—	200*	196*

* $P < 0·05$ compared with controls.

prolongation of sleep, 4-NVP was 10 times more potent than 3-NVP, which in turn was twice as potent as 2-NVP. It seems that hexobarbitone is potentiated *in vivo* by inhibition of its metabolism by NVP analogues. After parenteral administration, NVP analogues seem to be capable of entering the central nervous system where they cause a significant and prolonged inhibition of ChA. The I50 for 4-NVP in mouse brain was identical to that obtained in rat brain by Smith, Cavallito & Foldes (1967). A correlation between inhibition of ChA and HMES is not evident within this series of three compounds.

Department of Pharmacology,
Warner-Lambert Research Institute,
Morris Plains, New Jersey 07950,
U.S.A.

M. E. GOLDBERG
A. I. SALAMA
S. W. BLUM

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