Biochemical Pharmacology, Vol. 27, pp. 1767–1769.

0006-2952/78/0701-1767$02.00/0

N-SUBSTITUTED CYCLOPROPYLAMINES AS INHIBITORS OF MAO-A AND -B FORMS

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(*Received* 20 *May* 1977; *accepted* 31 *October* 1977)

Abstract—Monoamine oxidase (MAO) activity in mouse neuroblastoma homogenates was preferentially inhibited by *N*[2-(*o*-chlorophenoxy)-ethyl]cyclopropylamine (L-51641) and a series of structurally related compounds. Two other congeners (L-54761 and L-54748) from this series, however, demonstrated preferential inhibition of MAO in human platelet homogenates. As neuroblastoma and human platelet cells have been identified as possessing apparently exclusive MAO-A and MAO-B characteristics, respectively, L-54761 and L-54748 appear to be preferential MAO-B inhibitors. In keeping with this conclusion, a greater proportional inhibition of phenylethylamine oxidation than serotonin oxidation by these two compounds was found in rat brain homogenates, while contrasting results were obtained with L-51641. Preferential effects of L-54761 and L-51641 on phenylethylamine oxidation and, to a lesser extent, on serotonin oxidation were also observed *in vivo*.

The observation that tyramine deamination was inhibited in a biphasic fashion by clorgyline [1] led to the recent delineation of two forms of monoamine oxidase (MAO) activity on the basis of differences in substrate specificity and sensitivity to inhibitors [2, 3]. Most MAO inhibitors, however, including all but one of those currently approved in the U.S. for clinical use, are non-selective and inhibit the MAO-A and MAO-B forms approximately equally.

A series of *N*-substituted cyclopropylamines, especially *N*[2-(*o*-chlorophenoxy)-ethyllcyclopropylamine (L-51641) have also been reported to be irreversible MAO inhibitors with clorgyline-like MAO-A specificity [4, 5]. L-51641 inhibited the deamination of serotonin (an MAO-A substrate) in rat liver mitochondria at concentrations 400 times lower than those required to inhibit the deamination of phenylethylamine, an MAO-B substrate. We have now examined a series of *N*-substituted cyclopropylamines with side chain modifications and report comparative studies of L-51641 and L-54761 (*N*-phenacyl-cyclopropylamine), the latter compound exhibiting some measure of MAO-B-selective inhibitory properties. For these studies, mouse neuroblastoma and human platelets were used as sources of MAO-A and MAO-B enzyme, respectively, following previous data from multiple substrate and inhibitor comparisons indicating that these two tissues manifest apparently exclusive MAO-A or MAO-B characteristics [6, 7].

METHODS

Mouse neuroblastoma N1E-115 cells, human platelet concentrates, and rat brain homogenates were prepared and sonicated as described previously [6, 7]. Monoamine oxidase activity was assayed in these tissues in duplicate or triplicate using [^{14}C]tyramine HCl, [^{14}C]5-hydroxytryptamine creatinine sulfate or [^{14}C]β-phenylethylamine HCl as substrates and incubation conditions and column separation procedures identical to those reported in our earlier studies [6, 7].

In the studies *in vitro*, all inhibitors were preincubated for 30 min at 25°C prior to the addition of substrate. The pI_{50} values (the negative logarithm of the molar concentrations producing 50 per cent enzyme inhibition) were obtained using seven concentrations of each inhibitor. Most of the inhibitors studied were synthesized in the Lilly Research Laboratories, Indianapolis, IN. Other inhibitors were kindly supplied by Prof. J. Knoll, Semmelweis University of Medicine, Budapest, Hungary (deprenyl) and May & Baker, Ltd., Essex, England (clorgyline).

RESULTS

Among the various *N*-substituted cyclopropylamines with side chain modifications examined, most resembled L-51641 in more effectively inhibiting mouse neuroblastoma (MAO-A) activity than human platelet (MAO-B) activity, when tyramine was used as substrate (Table 1). None showed greater specificity for MAO-A than L-51641. Two compounds, L-54761 and L-54748, showed some evidence of reverse specificity in inhibiting the platelet enzyme at lower concentrations than the neuroblastoma MAO.

Comparisons of the inhibition of mouse neuroblastoma and human platelet MAO activity by L-51641 and L-54761 are presented graphically in Fig. 1. As indicated, L-51641 inhibited neuroblastoma MAO activity at concentrations over 1000-fold lower than those required for inhibition of the platelet enzyme; in contrast, the platelet enzyme was inhibited at approximately 15-fold lower concentrations by L-54761 than those required to inhibit neuroblastoma MAO. Generally similar differential effects were also observed for rat brain MAO when

Table 1. Inhibition by *N*-substituted cyclopropylamines of MAO activity in mouse neuroblastoma and human platelet homogenates

Drug number	Drug structure X =	Inhibition of tyramine deamination (pI_{50})* Mouse neuroblastoma	Human platelet	Difference
L-51641	—OCH_2CH_2—†	8.3	5.7	2.5
L-49393	—SCH_2CH_2—	8.0	6.2	1.8
L-54746	—OCH_2CH_2—	6.6	5.0	1.6
L-54810	—CH_2CH_2—	5.9	4.5	1.4
L-54922	—$CH_2CH_2CH_2$—	6.3	5.0	1.3
L-51988	—$C(=O)CH_2CH_2$—	6.5	5.3	1.2
L-54748	—$CH_2CH(CH_3)$—	4.5	5.6	−1.1
L-54761	—$C(=O)CH_2$—	4.5	5.7	−1.2

* pI_{50} is the negative log of the molar concentration of the drug producing 50 per cent inhibition. Specific activities for tyramine deamination were 32 and 36 nmoles/mg of protein/hr for neuroblastoma and platelet respectively. The means from three experiments are presented.

† *o*-Chloro substituent on the phenyl ring.

the MAO-A selective substrate, 5-hydroxytryptamine, was compared to the MAO-B selective substrate, phenylethylamine (Table 2), although the differences between the effects of the two drugs were somewhat less marked than in the comparisons of neuroblastoma and platelet MAO with tyramine as substrate.

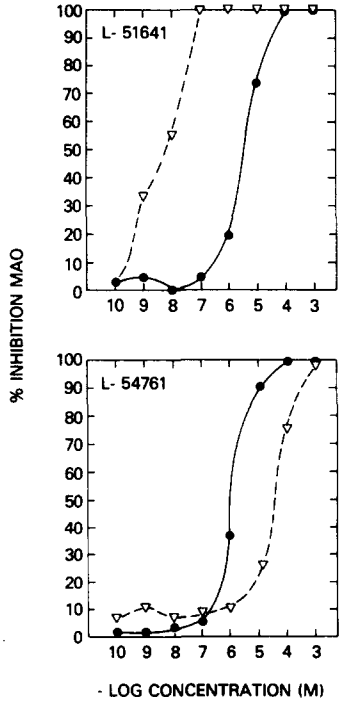

Fig. 1. L-51641 and L-54761 inhibition of MAO activity using 10^{-3} M tyramine as substrate in neuroblastoma N1E-115 cells (▽– –▽) and human blood platelets (●—●). Specific activities for tyramine deamination were 32 and 36 nmoles/mg of protein/hr for neuroblastoma and platelet respectively.

Table 3 shows that MAO activity in brain homogenates was selectively inhibited when L-51641 and L-54761 were given to rats *in vivo*. L-54761 caused markedly greater inhibition of phenylethylamine oxidation than did L-51641, whereas L-51641 caused somewhat greater inhibition of serotonin oxidation than did L-54761. Thus, L-51641 showed marked preferential inhibition of serotonin oxidation over phenylethylamine oxidation, whereas L-54761 showed only slight preferential inhibition of phenylethylamine oxidation, in keeping with the differences observed *in vitro* with these two inhibitors.

DISCUSSION

Among drugs with some specificity for MAO-A vs MAO-B activity, clorgyline, L-51641, harmine, harmaline, α-ethyltryptamine and PCO [5-phenyl-3-(*N*-cyclopropyl)-ethylamine, 1,2,4-oxadiazole] inhibit MAO-A selectively, while deprenyl and pargyline are preferential inhibitors of MAO-B [2, 3, 8, 9]. Limited data have suggested that the differential effects of some of these drugs are maintained with administration *in vivo* [4, 5, 10–12]. Deprenyl [12], L-51741 (J. C. Gillin, personal communication) and clorgyline [13] are all currently being used in clinical trials to evaluate whether selective inhibition of MAO-A or MAO-B may be of greater benefit or may decrease side effects in the treatment of patients with neurologic, psychiatric and other disorders such as hypertension.

In the present study, two *N*-substituted cyclopropylamines (L-54761 and L-54748), which are

Table 2. Comparison *in vitro* of L-51641 and L-54761 as inhibitors of rat brain MAO activity using different substrates*

	pI_{50}		
	Serotonin	Phenylethylamine	Tyramine
L-51641	8.7	5.4	7.1
L-54761	4.8	5.8	5.6

* Specific activities for the substrates in nmoles/mg of protein/hr were: serotonin, 158; phenylethylamine, 36; and tyramine, 172. The means from three experiments are presented.

Table 3. Comparison *in vivo* of L-51641 and L-54761 as inhibitors of rat brain MAO activity using different substrates*

Treatment group	MAO activity (nmoles/min/g brain)	
	Phenylethylamine	Serotonin
Control	59 ± 1	43 ± 1
L-51641	35 ± 1 (− 41%)	1 ± 0.1 (− 98%)
L-54761	6 ± 0.2 (− 90%)	6 ± 0.2 (− 86%)

* Inhibitors were injected i.p. at 30 mg/kg 8 hr before groups of five male Wistar rats (130–150 g) were killed. MAO activity in whole homogenates was measured with [^{14}C]phenylethylamine (80 μM) or [^{14}C]serotonin (100 μM) as substrate. Mean values ± standard errors are shown. Differences from control were significant at the $P < 0.001$ level.

structurally similar to L-51641, were identified as relatively selective MAO-B inhibitors. It is of note that these two agents are relatively less selective for MAO-B than L-51641 is for MAO-A. This also appears to be the case among the propargylamine MAO inhibitors, where clorgyline inhibits MAO-A in 400-fold lower concentrations than required for MAO-B, while deprenyl inhibits MAO-B compared to MAO-A with only a 60-fold concentration difference [6, 7].

Although close relationships between inhibitor sensitivity and substrate specificity for MAO-A and MAO-B have been observed in many rodent tissues and also across several other species, a number of discrepancies indicative of greater complexity have been reported recently [3]. For example, in bovine heart and in liver from various species, 5-hydroxytryptamine deamination has been reported to be partially sensitive to low concentrations of deprenyl, and hence it has been suggested that 5-hydroxytryptamine deamination is contributed to by MAO-B activity, rather than representing a property of MAO-A exclusively [14]. In our study, the results from the comparison of 5-hydroxytryptamine and phenylethylamine as substrates in rat brain were in general agreement with the studies with tyramine in the other tissues.

In one previous comparison of L-51641 with L-54761, these drugs did not differ in their inhibitory effects on rat liver MAO measured with kynuramine as the substrate [15]. It is of note that clorgyline and pargyline did not have differential effects on kynuramine deamination either, although the central nervous system (CNS) stimulant effects of L-dopa were markedly more potentiated by L-51641 than by L-54761 and pargyline [15].

It has been recommended by Tipton *et al.* [16] that the terms MAO-A and MAO-B be reserved for the description of clorgyline-sensitive vs clorgyline-insensitive MAO forms, as originally suggested by Johnston [1]. The two pure cell lines compared here, the mouse neuroblastoma and the human platelet, have previously been identified as exhibiting a clear differentiation between MAO-A and MAO-B characteristics with both substrates and inhibitors [6, 7]. In the present study, they provided the greatest distinction between the different *N*-substituted cyclopropylamines.

Acknowledgement—We thank Nancy Garrick for valuable assistance in these studies.

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