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PROPERTIES OF 8,9-DICHLORO-2,3,4,5-TETRAHYDRO-1H-2-BENZAZEPINE, AN INHIBITOR OF NOREPINEPHRINE *N*-METHYLTRANSFERASE

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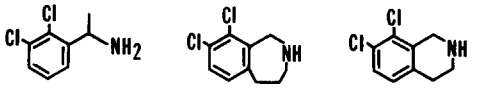
Abstract—LY134046, 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine hydrochloride, was a potent inhibitor of norepinephrine *N*-methyltransferase (NMT) from rat brain or rabbit adrenal glands *in vitro*. The inhibition was competitive with respect to the methyl-accepting substrate, (-)-norepinephrine, the K_i for LY134046 being 2.4×10^{-8} M. LY134046 inhibited the NMT activity in rat brain stem and hypothalamus *in vivo* at doses of 10–40 mg/kg, i.p., and lowered the epinephrine (but not norepinephrine or dopamine) concentration in these brain regions. The epinephrine reduction produced by a single 40 mg/kg, i.p. dose of LY134046 persisted at 24 hr and daily injections of 10–40 mg/kg doses for 5 days produced cumulative reductions in epinephrine concentration. LY134046 was similar to SK&F 64139 (7,8-dichloro-1,2,3,4-tetrahydroisoquinoline hydrochloride), a structurally related compound, as an inhibitor of NMT *in vitro* and *in vivo*, but the two compounds differed in their relative abilities to block α_2 receptors. SK&F 64139 was 20- to 50-fold more potent than LY134046 in antagonizing [^{3}H]clonidine binding to rat brain membranes and phenylephrine-induced contractions of rat aortic strips, but it was only about twice as potent as LY134046 in inhibiting NMT activity. LY134046 seems to be more selective than other currently known inhibitors of NMT and may be useful for pharmacologic intervention in the function of epinephrine-forming neurons in brain.

Among inhibitors of norepinephrine *N*-methyltransferase (NMT, EC 2.1.1.28) that we have described previously, some are conformationally rigid analogs of chlorine-substituted benzylamines [1, 2]. In particular, several chlorine-substituted 2,3,4,5-tetrahydro-1H-2-benzazepines were shown to be potent inhibitors of rabbit adrenal [1] and rat brain [3] NMT. Three of these, the 8-chloro, 7,8-dichloro and 8,9-dichloro compounds, were shown to lower hypothalamic concentrations of epinephrine in rats through inhibition of NMT *in vivo* [3–5]. The 8,9-dichloro compound is a conformationally rigid analog of 2,3-dichloro-α-methylbenzylamine (DCMB) [6] and is structurally related to another NMT inhibitor, SK&F 64139 (7,8-dichloro-1,2,3,4-tetrahydroisoquinoline hydrochloride), developed by Pendleton *et al.* [7] (see Fig. 1). In this communication we describe further studies on the *in vitro* and *in vivo* properties of LY134046, 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine hydrochloride, as an NMT inhibitor and present some comparative data on SK&F 64139.



DCMB LY 134046 SK+F 64139

Fig. 1. Structures of three NMT inhibitors.

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MATERIALS AND METHODS

LY134046 was synthesized by borane reduction of 8,9,-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepin-3-one [8]. In an earlier publication [1], we had reported a pI_{50} value of 6.37 for 8,9-dichloro-2,3,4,5-tetrahydro-1H-2-benzazepine as an inhibitor of rabbit adrenal NMT. The material used in that earlier study had been synthesized by a different procedure and contained a structural isomer, 7, 8-dichloro-4-methyl-1,2,3,4-tetrahydroisoquinoline, formed in the synthetic procedure (see Ref. 9). Since the latter compound is a weaker NMT inhibitor, the earlier pI_{50} was erroneously low. The identity and purity of the material used in the present study were verified by physicochemical methods. (±)-2,3-Dichloro-α-methylbenzylamine hydrochloride (DCMB) and various other compounds representing partial molecular structures of LY134046 and SK&F 64139 were synthesized in the Lilly Research Laboratories Indianapolis, IN). The compound referred to throughout as SK&F 64139 was obtained as a gift from Smith Kline & French Laboratories (Philadelphia, PA) or was synthesized in the Lilly Research Laboratories.

NMT activity was assayed radiometrically with (-)-norepinephrine as the methyl-accepting substrate and *S*-adenosyl-L-methionine[methyl-^{14}C] (New England Nuclear Corp., Boston, MA) as the methyl donor. The enzyme from rabbit adrenal glands was prepared and assayed by the reineckate precipitation method as we have described previously [10]. The concentration of (-)-norepinephrine was 80 μM, and the concentration of *S*-adenosyl-L-methionine-[methyl-^{14}C] was 20 μM. NMT activity from rat brain

was assayed using the methodology of Henry *et al.* [11] as we have described recently [2]. In this case, the concentration of (-)-norepinephrine was 100 μM, and the concentration of *S*-adenosyl-L-methionine[methyl-^{14}C] was 50 μM.

The *in vivo* effects on NMT activity and catecholamine concentration in brain were evaluated in male Wistar rats (approximately 150 g) from Harlan Industries, Cumberland, IN. Aqueous solutions of the compounds were injected i.p., rats were decapitated, and brains were quickly excised and dissected. NMT activity was assayed radiometrically as described above. Epinephrine (and dopamine and norepinephrine) concentrations were measured by high performance liquid chromatography with electrochemical detection as we have described earlier [12].

For studies of radioligand binding, the frontal cerebral cortices from male Wistar rats were homogenized in 20 vol. of 50 mM Tris–HCl (pH 7.4), using a Brinkman polytron. The homogenate was centrifuged for 20 min at 49,000 *g*, and the pellet was resuspended in the same buffer and centrifuged for 20 min at 49,000 *g*. Aliquots of the membranes in the washed pellet (0.5 mg protein) were incubated with 0.8 nM [^{3}H]clonidine or 0.5 nM[^{3}H]WB4101 at 20° (30 min for clonidine and 15 min for WB4101) in a 2.0 ml incubation mixture containing 0.1 mg/ml ascorbic acid and 10 μM pargyline. The binding was terminated by filtration with Whatman GFC glass fiber filters. The fibers were washed eight times with 2.5 ml of ice-cold 50 mM Tris–HCl (pH 7.4). Membrane bound ^{3}H-radioligand was measured by transferring the filters to scintillation vials containing 10 ml of PCS counting fluid (Amersham/Searle, Arlington Heights, IL) and counting in a liquid scintillation counter. "Specific" binding was defined as total radioactivity bound minus "nonspecific" binding and was generally 85 per cent of the total radioactivity for clonidine and 70 per cent for WB4101. "Non-specific" binding was defined as radioactivity not displayed by 10^{-4} to 10^{-5} M (-)-norepinephrine.

For physiological studies of α-receptor antagonism, the thoracic aorta was removed from male Wistar rats (220–350 g) and dissected free of fat and connective tissue in physiological salt solution (PSS, pH 7.4) at room temperature. Helically cut strips, approximately 2 mm wide and 30 mm long, were prepared as described by Furchgott and Bhadrakom [13]. Aortic strips were suspended in 10 ml organ baths containing PSS maintained at 37.5° and aerated with a 5% CO_2–95% O_2 mixture. The composition of PSS was (mM): NaCl, 118; KCl, 4.7; $MgCl_2 \cdot 6H_2O$, 0.54; $CaCl_2 \cdot 2H_2O$, 2.5; $NaHPO_4$, 1.0; $NaHCO_3$, 25; and glucose, 11; dissolved in demineralized water. The aortic strips were attached to Statham Universal UC-3 isometric transducers connected to a Beckman R411 Dynograph recorder and were allowed to equilibrate under a resting tension of 2 g for at least 2 hr before drug addition. Dose–response curves were constructed by increasing bath concentrations of agonist approximately 3-fold [14]. The concentration of agonist was increased only after the previous concentration had produced a maximum response that remained constant. After completion of a dose–response curve, drugs were washed from the preparation at regular intervals by the overflow method. Consecutive dose–response curves on a given tissue were always spaced at least 1 hr apart to ensure maximum washout of agonists and to minimize receptor desensitization. In all experiments, at least one aortic strip was run in parallel with the experimental strips, but received no antagonists, and was used to correct for time-dependent changes in agonist sensitivity [15].

Dissociation constants (K_B) and pA_2 values (i.e. $-\log K_B$) were determined by the method of Arunlakshana and Schild [16] using phenylephrine as the agonist. The incubation period for antagonists was 30 min. In this method, the dose-ratio produced by the blocker (i.e. the ratio of concentrations of phenylephrine giving equal responses in the presence and in the absence of the competitive antagonist, measured at the ED_{50}) is determined at various concentrations of antagonist. According to Arunlakshana and Schild [16], if blockade is competitive, a plot of the logarithm of (dose-ratio -1) against the negative logarithm of the molar concentration of antagonist should yield a straight line whose slope is 1 and the intercept of which along the abscissa is the pA_2, which is equal to the $-\log K_B$ (under equilibrium conditions). The K_B was also determined for statistical purposes at each concentration of antagonist by the dose-ratio method described by Furchgott [15]. The equation relating the dissociation constant to the dose-ratio and the antagonist concentration (molar) is:

$$K_B = [\text{Antagonist}]/(\text{Dose ratio} - 1).$$

RESULTS

A comparison of LY134046, SK&F 64139, and DCMB as inhibitors of rat brain and rabbit adrenal NMT is shown in Fig. 2. The IC_{50} values (concentration producing 50 per cent inhibition of NMT activity) of these three compounds were 7×10^{-8} M, 3×10^{-8} M, and 3×10^{-6} M, respectively, for the rat brain enzyme, and 2×10^{-7} M, 6×10^{-8} M, and 4×10^{-7} M, respectively, for the rabbit adrenal enzyme.

A kinetic analysis of the inhibition by the new compound of this group, LY134046, is shown in Fig. 3. This compound was a competitive inhibitor with (-)-norepinephrine as the variable substrate, and the K_i value was established by a Dixon plot to be 2.4×10^{-8} M.

The *in vivo* inhibition of NMT in brain stem and in hypothalamus also showed competitive kinetics with (-)-norepinephrine as the variable substrate (Fig. 4). Doses of 10 and 40 mg/kg inhibited NMT activity approximately to the same degree in both brain regions, and Lineweaver–Burk plots revealed the kinetics of the inhibition to be the same as found *in vitro*.

The inhibition of NMT activity in brain stem (a region rich in NMT-containing cell bodies) [17] and the lowering of epinephrine concentration in hypothalamus (a region rich in NMT-containing nerve terminals) [17] by LY134046 and SK&F 64139 are shown in Table 1. Both variables showed statistically significant reductions 6 hr after the injection of 10, 20 and 40 mg/kg, i.p., doses of the two compounds.

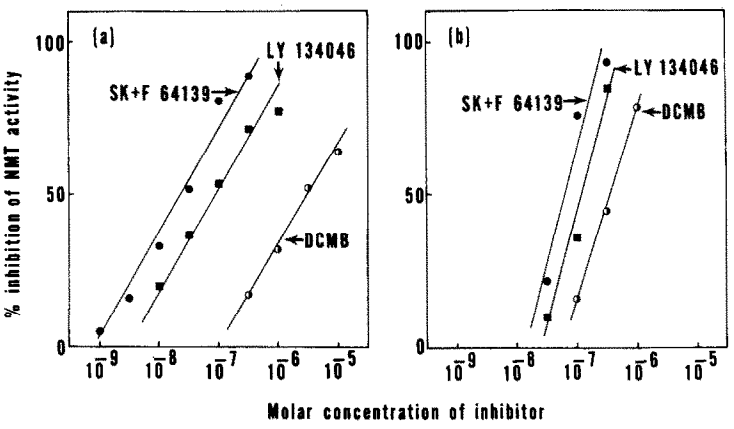


Fig. 2. *In vitro* inhibition of (a) rat brain and (b) rabbit adrenal NMT.

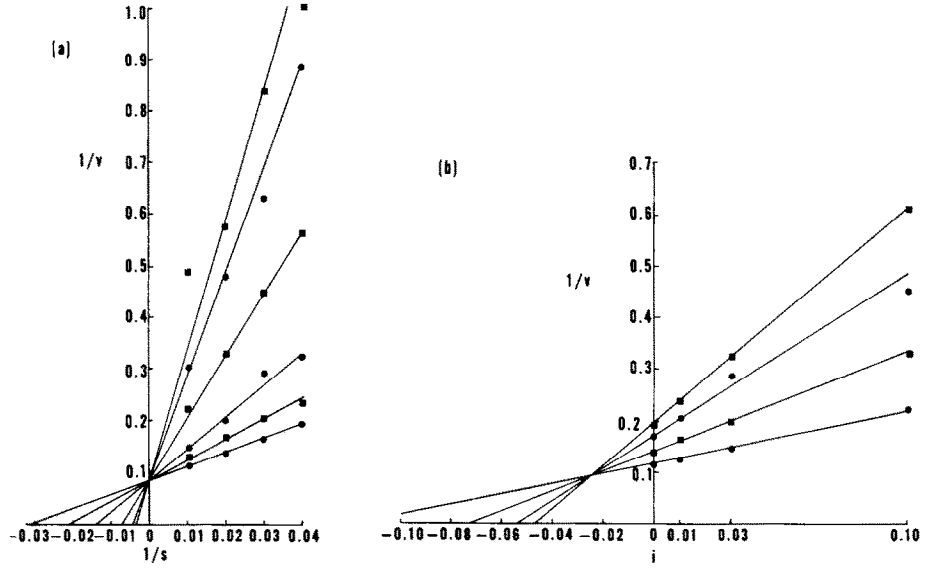


Fig. 3. Kinetic analysis of the inhibition of rat brain NMT by LY134046 *in vitro*. (a) Lineweaver–Burk plot showing competitive inhibition with (-)-norepinephrine as the variable substrate. Key: v velocity of enzyme reaction (pmoles/60 min); and s substrate concentration (micromolar). From bottom to top, lines represent 0, 0.01, 0.03, 0.1, 0.3 and 1 μM concentrations of LY134046. Panel (b) Dixon plot showing a K_i value of 2.4×10^{-8} M for LY134046. Key: v velocity of enzyme reaction (pmoles/60 min); and i inhibitor concentration (micromolar). From top to bottom, lines represent 25, 33.3, 50 and 100 μM concentrations of the competitive substrate [(-)-norepinephrine bitartrate].

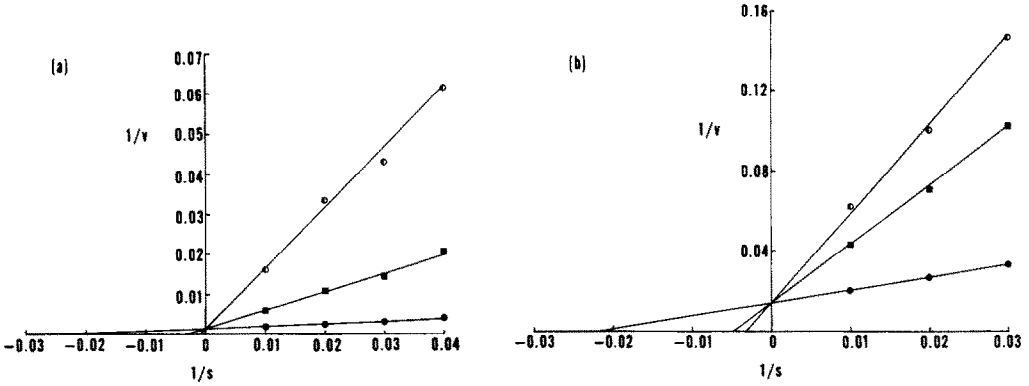


Fig. 4. *In vivo* inhibition of (a) brain stem and (b) hypothalamic NMT activity after the injection of LY134046 (10 or 40 mg/kg, i.p., 2 hr before the rats were killed). Lineweaver–Burk plots show competitive inhibition with (-)-norepinephrine as the variable substrate. Key: v velocity of enzyme reaction (nmoles/hr) per g (wet weight) of brain tissue; s micromolar substrate [(-)-norepinephrine bitartrate] concentration; (●), controls; (■), 10 mg/kg; and (◐) 40 mg/kg.

Table 1. Dose-dependent inhibition of brain stem NMT activity and reduction of hypothalamic epinephrine concentration by LY134046 and SK&F 64139 in rats*

Dose (mg/kg)	Brain stem NMT activity (nmoles·hr^{-1}·g^{-1})		Hypothalamic epinephrine (pmoles/g)	
	LY134046	SK&F 64139	LY134046	SK&F 64139
0	41 ± 1	59 ± 3	222 ± 22	175 ± 5
10	15 ± 0.4† (−64%)	19 ± 1† (−68%)	132 ± 6† (−40%)	95 ± 14† (−46%)
20	12 ± 0.8† (−71%)	13 ± 1† (−78%)	118 ± 8† (−47%)	115 ± 9† (−49%)
40	8 ± 0.2† (−80%)	9 ± 0.6† (−85%)	96 ± 10† (−57%)	89 ± 7† (−49%)

* The compounds were injected, i.p., 6 hr before rats were killed. Mean values ± S.E. for five rats per group are shown.

† Significant change from control ($P < 0.05$).

Inhibition of NMT activity and a lowering of epinephrine concentration were in general dose-related. The magnitude of the effects by the two compounds was similar to all doses.

Table 2 shows the time course of these effects of LY134046. Within 1 hr after injection of the 40 mg/kg, i.p., dose, NMT activity in brain stem was maximally inhibited, and epinephrine concentration in hypothalamus was significantly less. The reduction of epinephrine was maximum at about 8 hr but was similar at all time points, even at 24 hr at which time NMT inhibition appeared to be subsiding slightly. Norepinephrine concentration was not changed significantly at any time point. Dopamine concentration was elevated transiently (significant statistically only at 1 hr).

Table 3 shows the effects of SK&F 64139 at various times. Again, maximum inhibition of NMT activity occurred within 1 hr. Epinephrine concentration was reduced at all times, the greatest reduction being at 8 hr. Longer times were not studied due to limited availability of this compound. Although norepinephrine concentration was not affected significantly at any time, dopamine concentration was elevated to a greater degree and for a longer duration than had been observed with LY134046.

The effects of repeated daily injections of LY134046 are shown in Table 4. After five daily doses, the epinephrine concentration in hypothalamus was reduced to a greater extent than had been observed after single doses (compare to Table 1). Epinephrine concentration was also decreased in the brain stem, though the per cent decrease was less at each dose than in hypothalamus. NMT activity was inhibited in a dose-dependent manner in both brain regions. The magnitude of this inhibition in brain stem was greater than had been measured after single doses (compare to Table 1).

Figure 5 shows the abilities of LY134046 and SK&F 64139 to inhibit the *in vitro* binding of radioligands for α-adrenergic receptors to membranes from rat brain. The binding of [^{3}H]clonidine, an α_2 radioligand [18, 19], was inhibited by SK&F 64139, the IC_{50} for this compound being 4×10^{-8} M. LY134046 also inhibited [^{3}H]clonidine binding but was less potent, the IC_{50} being 2×10^{-6} M. The bind-

Table 2. NMT activity and catecholamine concentrations in rat brain at various times after the injection of LY134046*

Hours	Hypothalamic catecholamine concentration (pmoles/g)			Brain stem NMT activity (nmoles·hr^{-1}·g^{-1})
	Epinephrine	Norepinephrine	Dopamine	
0	175 ± 16	7300 ± 530	1030 ± 70	57 ± 3
1	110 ± 7† (−37%)	6760 ± 420	1360 ± 46† (+32%)	9 ± 0.4† (−84%)
2	107 ± 14† (−39%)	6930 ± 230	1120 ± 31	10 ± 0.5† (−82%)
4	104 ± 11† (−41%)	8240 ± 430	1140 ± 79	9 ± 0.4† (−84%)
8	102 ± 6† (−42%)	8190 ± 300	1190 ± 97	12 ± 1† (−80%)
24	114 ± 9† (−35%)	8350 ± 620	980 ± 36	24 ± 2† (−58%)

* LY134046 was injected, i.p., at a dose of 40 mg/kg. Mean values ± S.E. for five rats per group are shown.

† Significant change from control ($P < 0.05$).

Table 3. NMT activity and catecholamine concentrations in rat brain at various times after the injection of SK&F 64139*

Hours	Hypothalamic catecholamine concentration (pmoles/g)			Brain stem NMT activity ($nmoles \cdot hr^{-1} \cdot g^{-1}$)
	Epinephrine	Norepinephrine	Dopamine	
0	150 ± 6	6790 ± 213	747 ± 32	46 ± 3
1	80 ± 9† (−47%)	6350 ± 338	1464 ± 54† (+96%)	7 ± 0.3† (−84%)
2	79 ± 10† (−47%)	7390 ± 532	1390 ± 74† (+86%)	8 ± 0.4† (−83%)
4	80 ± 14† (−47%)	7980 ± 310	1400 ± 78† (+88%)	8 ± 0.3† (−82%)
8	66 ± 8† (−56%)	7820 ± 162	1400 ± 76† (+88%)	11 ± 0.6† (−76%)

* SK&F 64139 was injected, i.p., at a dose of 40 mg/kg. Mean values ± S.E. for five rats per group are shown.
† Significant change from control ($P < 0.05$).

Table 4. Epinephrine concentration and NMT activity in brain regions after five daily doses of LY134046*

Dose (mg/kg)	Hypothalamus		Brain stem	
	Epinephrine (pmoles/g)	NMT ($nmoles \cdot hr^{-1} \cdot g^{-1}$)	Epinephrine (pmoles/g)	NMT ($nmoles \cdot hr^{-1} \cdot g^{-1}$)
0	73 ± 6	942 ± 34	72 ± 9	1023 ± 65
10	27 ± 11† (−63%)	360 ± 24† (−62%)	45 ± 5† (−37%)	311 ± 20† (−70%)
20	11 ± 11† (−85%)	295 ± 22† (−69%)	34 ± 7† (−53%)	214 ± 21† (−79%)
40	0† (−100%)	222 ± 9† (−76%)	15 ± 2† (−79%)	137 ± 13† (−87%)

* LY134046 was injected daily at 8.00 a.m. for 5 days, and rats were killed at 2.00 p.m. on day 5. Mean values ± S.E. for five rats per group are shown.
† $P < 0.01$.

ing of [^{3}H]WB4101, a radioligand for α_1 receptors [18, 19], was inhibited much less by SK&F 64139 ($IC_{50} = 4 \times 10^{-6}$ M) and not at all by LY134046 at the concentrations tested.

Interactions of these two compounds with α-receptors on the rat aorta are shown in Fig. 6. These receptors have been characterized by Ruffolo *et al.* [20] to be of the α_2 type. As with [^{3}H]clonidine binding, the potency of SK&F 64139 was more than 20-fold that of LY134046 in antagonizing these α_2 receptors.

The effects of these two compounds, and others

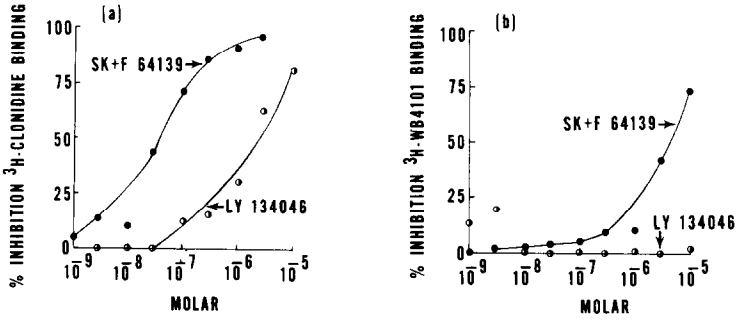


Fig. 5. *In vitro* inhibition of (a) binding of [^{3}H]clonidine, an α_2 radioligand, and (b) binding of [^{3}H]WB-4101, an α_1 radioligand, to rat brain membranes.

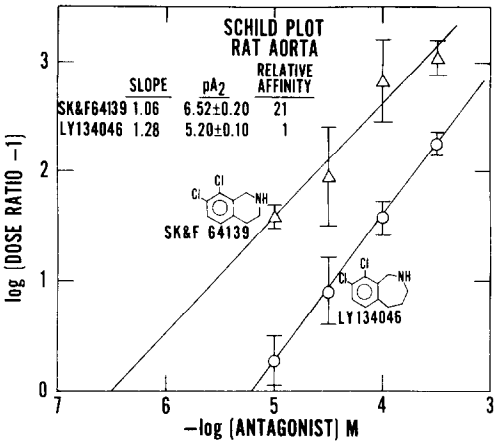


Fig. 6. Schild plot showing α-adrenergic receptor blocking activity in the rat aorta.

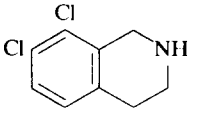
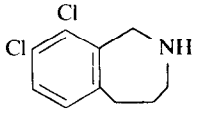
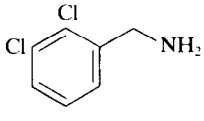
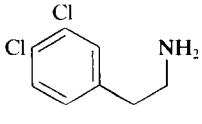
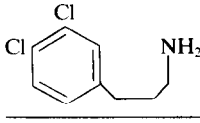
that are structurally related, on α_2 receptors in the rat aorta and rat brain are compared to their effects on NMT in Table 5. SK&F 64139 was 20- to 50-fold more potent than LY134046 in antagonizing α_2 receptors but only about twice as potent in inhibiting NMT. 2,3-Dichlorobenzylamine, a molecular fragment of both of the above compounds, was a reasonably potent NMT inhibitor but relatively weak as an α_2 blocker. 3,4-Dichloro-phenylethylamine, a fragment of the SK&F 64139 molecule, was a relatively weak NMT inhibitor but second only to SK&F 64139 itself as an α_2 blocker. 3,4-Dichlorophenylpropylamine was least potent among all the compounds in respect to all three variables.

DISCUSSION

LY134046 was a potent inhibitor of rabbit adrenal and rat brain NMT *in vitro*. With these two enzyme sources, this compound was a slightly less potent inhibitor than was SK&F 64139, but both were more potent than was DCMB, to which they are structurally related. LY134046 was a competitive inhibitor of NMT *in vitro* with (-)-norepinephrine as the variable substrate, as has been reported for SK&F 64139 [7]. With LY134046, this pattern of inhibition was also observed after *in vivo* administration of the compound.

LY134046 was effective, as was SK&F 64139, in inhibiting NMT and in reducing epinephrine concentration in rat brain *in vivo*. The dose–response curves for the two compounds were essentially the same, doses of 10–40 mg/kg, i.p., being effective.

Table 5. Comparison of SK&F 64139, LY134046, and some compounds representing molecular fragments of those NMT inhibitors*

Compound	Rabbit adrenal NMT (pI_{50})	Rat aorta (pA_2)	[^{3}H]Clonidine binding to rat brain (pI_{50})
	7.24	6.52	7.40
SK&F 64139 	6.93	5.20	5.70
LY134046 	6.23	5.02	5.70
	4.75	5.54	6.00
	3.79	4.72	4.82

* The pI_{50} values are the negative logarithms of the molar-concentrations producing 50 per cent inhibition of NMT activity or of [^{3}H]clonidine binding. The pA_2 value is defined in the Materials and Methods and was determined graphically as in Fig. 6.

The reduction of epinephrine concentration by both agents was selective, neither norepinephrine nor dopamine concentrations being reduced. In fact, dopamine concentration was markedly and persistently increased after SK&F 64139 but was only slightly and transiently affected by LY134046. The *in vivo* effects of LY134046 were characterized more fully than were those of SK&F 64139. A single dose of LY134046 was still effective at 24 hr in inhibiting NMT activity in brain stem and in lowering epinephrine concentration in hypothalamus. Multiple doses of LY134046, as would have been expected, produced cumulative effects. Both of these compounds are promising tools for depleting brain epinephrine selectivity in studies of the physiologic functions associated with epinephrine-forming neurons. Both agents appear to have advantages over DCMB in this regard in that the direct sympathomimetic effects (as judged by visible signs of CNS excitation) are less with LY134046 and SK&F 64139 than with DCMB.

A potentially important difference between LY134046 and SK&F 64139 relates to their interactions with α_2 receptors. SK&F 64139 inhibits [^{3}H]clonidine binding to rat brain membranes at essentially the same concentrations as those required for inhibition of rat brain NMT activity. The *in vitro* IC_{50} values for this compound are 4×10^{-8} M for inhibition of [^{3}H]clonidine binding and 3×10^{-8} M for inhibition of NMT activity. LY134046 is almost as potent as SK&F 64139 as NMT inhibitor but is substantially less potent as an inhibitor of [^{3}H]clonidine binding, so that some separation of these two activities exists. The *in vitro* IC_{50} values for LY134046 are 2×10^{-6} M for inhibition of [^{3}H]clonidine binding and 8×10^{-8} M for inhibition of NMT activity. This 25-fold difference in potency suggests that NMT activity can be inhibited by LY134046 without blockade of α_2 receptors necessarily occurring.

In physiological studies with the rat aorta as well as with radioligand binding, LY134046 and SK&F 64139 differed substantially. The pA_2 values for these two compounds differed by more than an order of magnitude.

The properties of these two compounds in regard to their selectivity as NMT inhibitors as opposed to α_2 blockers can apparently be accounted for as follows. Both compounds inhibit NMT primarily because they are conformationally rigid analogs of 2,3-dichlorobenzylamine. 2,3-Dichlorobenzylamine itself is a potent NMT inhibitor *in vitro* but is biologically unstable. It is substrate for monoamine oxidase and is rapidly destroyed *in vivo*, being unable to produce inhibition of NMT activity in animals [6]. The addition of an α-methyl substituent protects the molecule from oxidation by monoamine oxidase; the α-methyl analog (DCMB) is effective as an NMT inhibitor *in vivo* [4, 6], but causes some signs of CNS excitation, especially at higher doses [6], and thus may have limitations for use as a tool in manipulating epinephrine-forming neurons. LY134046 and SK&F 64139 have enhanced NMT inhibitor potency compared to 2,3-dichlorobenzylamine or to DCMB; apparently the conformation fixed by the rigidity of the second ring in these molecules is favorable for combination with NMT. LY134046 and SK&F 64139 also appear to lack some of the "side effects" of DCMB, causing less visible CNS stimulation in rats. SK&F 64139, however, apparently because it is also a conformationally rigid analog of 3,4-dichlorophenylethylamine, is a reasonably potent α_2 receptor blocker. Originally this compound had been reported to be relatively free of α-blocking activity [7], but that report was based on studies with receptors of the α_1 subtype. More recently, Svensson and Engberg [21] observed antagonism of electrophysiological effects of clonidine by SK&F 64139 and other actions (immediate increase in electrical firing of locus coeruleus neurons after intravenous administration of SK&F 64139 to rats) indicative of α_2-blocking activity. Our data on [^{3}H]clonidine binding to rat brain membranes and the antagonism of the effects of phenylephrine on the rat aorta also reveal the potent α_2-blocking activity of that compound. LY134046, because it also is a conformationally rigid analog of 2,3-dichlorobenzylamine, is an NMT inhibitor almost equal in potency to SK&F 64139, but because it is not a rigid analog of 3,4-dichlorphenylethylamine, has less α_2-blocking potency. Thus, the ratio of NMT inhibition to α_2-blocking potency is more favorable with LY134046 than with SK&F 64139.

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