Lobeline: Structure−Affinity Investigation of Nicotinic Acetylcholinergic Receptor Binding

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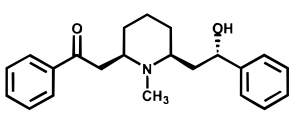
(−)Lobeline (**1**) and (−)nicotine (**2**) bind at neuronal nicotinic cholinergic (nACh) receptors with high affinity (K_i = 4 and 2 nM, respectively). Previous attempts to determine whether lobeline fits the currently accepted nicotinic pharmacophore model have led to suggestions that the carbonyl function, rather than the hydroxyl group, is a major contributor to binding. Interestingly, however, it has never been empirically demonstrated that *either* oxygen function is actually required for interaction with the receptor. In the present investigation we systematically examined a number of abbreviated analogues of lobeline and found that removal of either one or both oxygen functions reduces the affinity of lobeline by at least 25-fold; furthermore, oxidation of the (−)lobeline hydroxyl group (to afford lobelanine) or reduction of the carbonyl group (to afford lobelanidine) also resulted in decreased affinity. Although it is likely that both oxygen functions contribute to the high affinity of (−)lobeline at nACh receptors, it is concluded that the presence of both oxygen functions is not a requirement for binding; that is, replacement of the (−)lobeline hydroxyl group with a chloro group had no effect on affinity. Another finding of the present investigation is that removal of either one or both oxygen functions of lobeline results in compounds that retain the analgesic activity and potency of (−)lobeline, indicating that there is no direct relationship between neuronal nicotinic cholinergic (primarily $\alpha_4\beta_2$ type) receptor affinity and spinal analgesia as measured in the tail-flick assay.

Introduction

(−)Lobeline (α-lobeline, **1**), originally isolated from *Lobelia inflata*, has long been regarded as a nicotinic agent. The agent is of particular interest for several reasons: (i) (−)lobeline binds with high affinity at nicotinic acetylcholinergic receptors, (ii) lobeline produces some, but not all, effects elicited by (−)nicotine (**2**), and (iii) lobeline is structurally distinct as compared to nicotine. Our interests are primarily associated with the effect of lobeline and other nicotinic agents in pain management.[1] There also are indications that lobeline may be useful in memory and learning disorders[2] and for the treatment of anxiety.[3]



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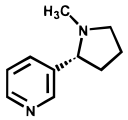

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Nicotinic acetylcholinergic (nACh) receptors are composed of different subunits, and multiple populations of nACh receptors exist; brain nACh receptors are primarily of the $\alpha_4\beta_2$ and α_7 type.[4] (−)Lobeline (**1**), like (−)nicotine (**2**), displays very low affinity (K_i > 10 000 nM) for α_7 receptors, but high affinity for rodent and human $\alpha_4\beta_2$ receptors (K_i = 1.4−2 nM).[4] Although (−)-lobeline (**1**) has been reported to bind with as much as 7 times the affinity of (−)nicotine (IC_{50} values = 0.7 and 5 nM, respectively),[5] the two agents are most often found to bind with roughly comparable affinity ($K_i \approx$ 1−5 nM) at nACh receptors in rodent brain homogenates.

(−)Lobeline can mimic some of the pharmacological effects produced by (−)nicotine. For example, both are self-administered by animals,[6] both produce antinociceptive effects,[1,7,8] and both produce hypolocomotion in rats and mice.[9] However, there are also some significant differences between the two agents. For example, in drug discrimination studies with rats trained to discriminate (−)nicotine from saline vehicle, the nicotine stimulus failed to generalize to (nor was it antagonized by) (−)lobeline despite the fact that lobeline achieved brain-to-plasma levels greater than that attained by nicotine.[10] While nicotine readily produces antinociception following peripheral administration, (−)lobeline does not. Actually, (−)lobeline has been shown to at least partially antagonize the antinociceptive[11] and hyperlocomotor[12] effects of nicotine in rats, but in other cases (−)lobeline has potentiated the analgesic effects of nicotine.[8] Some effects of nicotinic agents may be indirectly mediated by other neurotransmitter systems. For example, both nicotine and lobeline cause release of norepinephrine[13,14] and dopamine[15] and can antagonize *N*-methyl-D-aspartate-induced responses in rat cortical neurons.[16] Lobeline also causes release of 5-HT from hippocampal slices.[17] Even in the above examples where nicotine and lobeline were shown to produce similar effects, there is some evidence that there might exist subtle mechanistic differences; that is, some effects produced by nicotine and lobeline are antagonized to a

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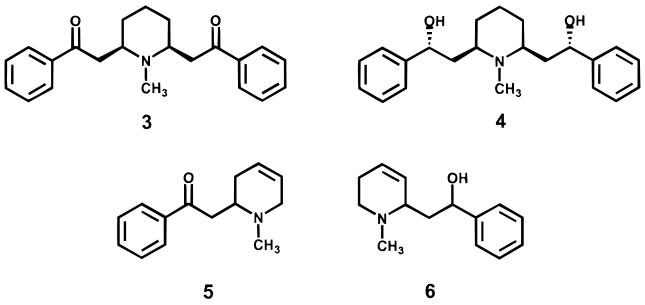
different extent by nicotinic antagonists such as mecamylamine and dihydro-β-erythroidine suggesting that different subpopulations of nicotinic receptors might be involved. Certain other common effects produced by these agents are not antagonized by nicotinic antagonists suggesting the involvement of antagonist-insensitive nACh receptors or even of nonnicotinic receptors. From this perspective, lobeline might serve as a useful template for the development of novel nACh receptor ligands that could perhaps produce some, but not necessarily all, of the effects produced by nicotine. Alternatively, some of the beneficial but non-nACh receptor-mediated effects of lobeline might be exploited for their therapeutic potential.

A feature shared by (−)nicotine and (−)lobeline is their high affinity for nACh receptors, and in particular $\alpha_4\beta_2$ nACh receptors. A nicotinic pharmacophore model was proposed nearly 30 years ago by Beers and Reich[18] and was later elaborated upon by Sheridan and coworkers.[19] The pharmacophore identifies structural features common to various nicotinic agents: an onium site (***A***) (e.g., the pyrrolidine nitrogen atom of nicotine), a hydrogen-bond acceptor site (***B***) (e.g., the pyridine nitrogen atom of nicotine), and a point ***C*** that forms a dipole with the hydrogen bond site (e.g., a near-centroid pyridine-ring dummy atom for nicotine). These three features are arranged in a triangular fashion with idealized distances for ***A***−***B***, ***B***−***C***, and ***C***−***A*** of 4.8, 1.2, and 4.0 Å, respectively (see Glennon and Dukat for a recent review[20]). Does lobeline comply with the proposed pharmacophore model? Beers and Reich[18] actually considered lobeline in the construction of their pharmacophore model and commented, without elaboration, that it possesses the necessary pharmacophoric features. In the absence of a detailed analysis it might be assumed that the piperidine-ring nitrogen atom represents the onium feature ***A***; however, lobeline possesses two potential hydrogen bond acceptors: a carbonyl group and a hydroxyl group. This problem was recognized by Barlow and Johnson;[21] although they favored a somewhat modified pharmacophore, they suggested on the basis of X-ray crystallographic studies on (−)-lobeline hydrochloride that the carbonyl oxygen rather than the hydroxyl group likely represents the hydrogen bond acceptor feature. Glaser et al.[22] determined the X-ray crystal structure of (−)lobeline hydrobromide and found that it differed somewhat from that of its hydrochloride salt. The latter investigators also examined the solution conformation of (−)lobeline, (−)lobeline hydrochloride, and (−)lobeline hydrobromide in various solvents using proton and ^{13}C NMR and concluded that (i) lobeline is a highly flexible molecule with a number of accessible conformations, (ii) the conformations are state- and solvent-dependent, and (iii) the most likely bioactive conformation of (−)lobeline is not accurately represented by any of the crystal structures. However, molecular mechanics manipulation of prevalent conformers identified one that seemed consistent with the Sheridan pharmacophore. That is, one identified conformer was demonstrated to possess ***A***−***B***, ***B***−***C***, and ***C***−***A*** distances of 4.54, 1.22, and 3.89 Å, respectively.[22] The feature used for ***B*** was the carbonyl oxygen atom. Thus, scant though it might be, evidence suggests that the carbonyl function of lobeline serves as the hydrogen bond acceptor.

Relatively little empirical data are available to either support or refute the concept that the carbonyl group of (−)lobeline is an important contributor to binding. Lobelanine (**3**) and lobelanidine (**4**) are naturally occurring analogues of (−)lobeline where the hydroxyl group is oxidized or where the carbonyl group is reduced, respectively. Both lobelanine (**3**) and lobelanidine (**4**) (IC_{50} values = 5−20 nM depending upon the radioligand employed) bind with comparable affinity and with an affinity similar to that reported for (−)lobeline (IC_{50} = 5 nM against [^{3}H]nicotine as radioligand).[5] Assuming that the presence of an oxygen atom is required for binding, these results suggest that both the carbonyl oxygen *and* the hydroxyl group can serve as the hydrogen bond acceptor. Two related analogues have also been examined (i.e., **5** and **6**; K_i > 5 000 nM[23]), but their low affinity, the presence of ring unsaturation, and the undefined stereochemistry of the agents obscures any conclusions that might be drawn.



A question that has never been specifically addressed is whether the presence of either oxygen atom is actually required for the binding of (−)lobeline at nACh receptors. Indeed, almost nothing is known regarding the structure−affinity relationships for the binding of lobeline at nACh receptors. To address this issue, we undertook an investigation of (−)lobeline where the structure was severely abbreviated and then various substituents were subsequently reintroduced in a systematic manner in order to determine the contribution to nACh receptor binding of the different structural components.

Chemistry

Several of the required compounds were prepared by previously reported methods (see Experimental Section for details). Compound **18** was prepared by dehydration of (−)lobeline under acidic conditions using the literature method described for the dehydration of (+)-lobeline.[24] Catalytic reduction of **18** afforded **16**; the hydroxyl analogue **16** was oxidized in the presence of pyridinium dichromate to ketone **15**. The styryl analogue **19** was obtained by Clemmensen reduction of (−)-lobeline, by taking advantage of a previous report that Clemmensen reduction of benzylic ketones gives alkenes as the product.[25] Reduction of the double bond of **19** by catalytic hydrogenation gave **17**. Synthesis of chloro analogue **20** has been reported in the literature by reacting (−)lobeline with PCl_3;[26] in the present study **20** was synthesized in 80% yield by reacting (−)lobeline with thionyl chloride. The quaternary analogue **21** was

Table 1. Summary of nACh Receptor Affinities of Lobeline Analogues

compd	nACh receptor affinity K_i, nM (±SEM)[a]
1	4 (±2)
3	7 800 (±370)
4	300 (±35)
7	5 900 (±1040)
8	>10 000
9	2 200 (±800)
10	>10 000
11	2 490 (±425)
12	>10 000
13	>10 000
14	>10 000
15	110 (±26)
16	340 (±30)
17	235 (±40)
18	1 085 (±160)
19	1 315 (±275)
20	5 (±1)
21	2 035 (±500)

[a] K_i values represent triplicate determinations. SEM not calculated where K_i > 10 000 nM.

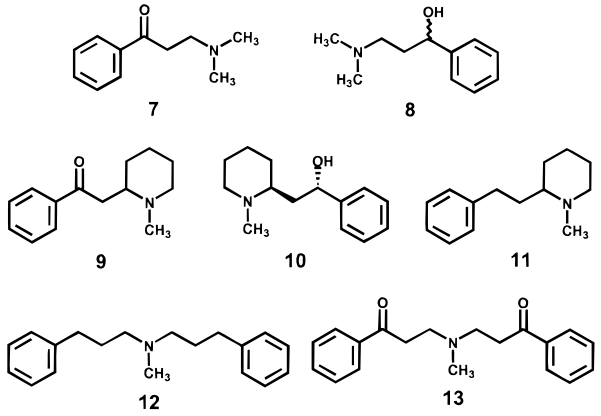


Figure 1. Simple analogues of lobeline.

prepared in 5% yield by methylation of (−)lobeline with excess methyl iodide. The unexpectedly low yield might be attributed to steric hindrance around the nitrogen atom.

Results and Discussion

Radioligand Binding Studies. Binding data obtained in this study are summarized in Table 1. The investigation began by abbreviation of (−)lobeline to two simple structures that retain the putative pharmacophoric features delineated by the Sheridan pharmacophore: **7** and **8** (Figure 1). Compound **7** (K_i = 5 900 ± 1 040 nM) binds with nearly 1 500-fold lower affinity than (−)-lobeline (K_i = 4 ± 2 nM); compound **8** (K_i > 10 000 nM) lacked affinity for nACh receptors. The absence of the piperidine ring might account for conformational differences between lobeline and the structurally simpler analogues; hence, the piperidine ring was reintroduced to afford **9** and **10**. The affinity of **9** (K_i = 2 200 ± 800 nM) was slightly enhanced relative to that of **7**, but compound **10** (K_i > 10 000 nM) was still without affinity. Compound **11** (K_i = 2 490 ± 425 nM), the des-keto analogue of **9**, was found to bind with comparable affinity suggesting that the ketone function might not be making a significant contribution to binding. On the other hand, the affinities of **9** and **11** are still well below that of (−)lobeline. Accordingly, the second "arm" was introduced to afford compounds **12** and **13**; both compounds lacked affinity for nACh receptors (i.e., K_i > 10 000 nM).

At this point, evidence suggested that the piperidine ring and both arms should be retained in subsequent compounds. This led to an examination of the series of compounds shown in Figure 2. Compound **14** (lobelan; K_i > 10 000 nM), the piperidine counterpart of **12**, was without significant affinity indicating that at least one of the two oxygen functions might be an important determinant for binding. Introduction of two carbonyl groups (i.e., **3**; K_i = 7 800 ± 370 nM) afforded the piperidine counterpart of **13**. Surprisingly, **3** (i.e., lobelanine) was found to bind with much lower affinity than previously reported by Abood and co-workers.[5] Compound **4** (i.e., lobelanidine; K_i = 300 ± 35 nM) was found to bind with enhanced affinity, but again with significantly lower affinity than previously reported.[5] Although earlier binding data on lobelanine (**3**) and lobelanidine (**4**) suggested that both the carbonyl and hydroxyl groups might have served as hydrogen bond acceptors to satisfy the Sheridan pharmacophore model, the present results suggest that this might not be the case.

Compounds **15** and **17** represent (−)lobeline minus the hydroxyl group or the carbonyl group, respectively. Compound **15** (K_i = 110 ± 26 nM) binds with about 25-fold lower affinity than (−)lobeline (**1**), and compound **17** (K_i = 235 ± 40 nM) binds with about 60-fold lower affinity than (−)lobeline (**1**); both compounds bind with higher affinity than the deoxygenated analogue **14** (K_i > 10 000 nM). These results suggest that both oxygen functions are somehow contributing to binding and that the presence of either oxygen function alone cannot account for the high affinity associated with (−)lobeline itself. Interestingly, reduction of the carbonyl group of **15** to **16** (K_i = 340 ± 30 nM) had relatively little influence on affinity and, together with **17**, likely explains the affinity observed for lobelanidine (**4**).

Several additional analogues of lobeline were prepared and examined. The two unsaturated derivatives **18** and **19** (Figure 2) both displayed modest affinity (K_i = 1 085 ± 160 and 1 315 ± 275 nM, respectively). Up to this point, it might be concluded that both oxygen functions of (−)lobeline (**1**) are required for binding. Interestingly, however, compound **20** (K_i = 5 ± 1 nM) binds with an affinity comparable to that of (−)lobeline itself. The possibility exists that **20** might undergo cyclization to a quaternary amine under conditions of the binding assay, and the simple *N*-methyl quaternary amine derivative of (−)nicotine is known to bind at nACh receptors with an affinity at least comparable to that of (−)nicotine.[27] For this reason, we prepared and examined a simple *N*-methyl quaternary amine derivative of (−)lobeline (**1**) (i.e., **21**); compound **21** (K_i = 2 035 ± 500 nM) displayed 500-fold reduced affinity relative to its parent compound. It would seem unlikely that the enhanced affinity of **5** is due to formation of the quaternary amine.

Functional Studies. Both (−)lobeline and (−)-nicotine produce an antinociceptive effect, following intrathecal administration, as measured using the tail-flick assay with mice. Unlike (−)nicotine's effect, the

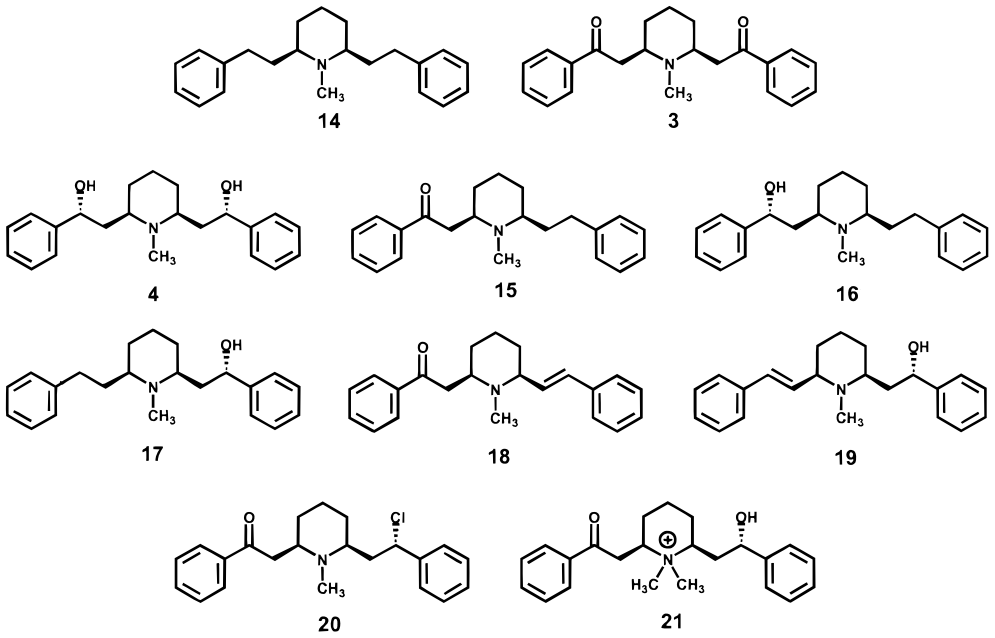


Figure 2. Two-armed piperidine analogues of lobeline.

effect produced by (−)lobeline is not attenuated by the administration of nACh receptor antagonists such as the noncompetitive antagonist mecamylamine and the competitive antagonist dihydro-β-erythroidine.[1,8] Given the comparable affinity of (−)nicotine (K_i = 2 nM) and (−)-lobeline (K_i = 4 nM) for nACh receptors of the $\alpha_4\beta_2$ type, it is thought that the two agents produce their effects via different types of nicotinic (or nonnicotinic) receptors.[1,8] The present synthetic investigation provided several novel lobeline analogues with which to further test this hypothesis. That is, compounds **1**, **14**, **15**, and **17** display a broad range of affinities for nACh receptors (i.e., K_i values = 4, > 10 000, 110, and 235 nM, respectively). All four compounds were found to produce an analgesic effect, and potencies were comparable across the series: **1**, ED_{50} = 23 (95% CL = 18−31) μmol/animal; **14**, 27 (19−38) μmol/animal; **15**, 38 (32−45) μmol/animal, and **17**, 32 (28−46) μmol/animal. Not only is there no relationship between binding affinity and analgesic potency, but it can also be concluded on the basis of the activity and potency of compound **14** that the oxygen atoms found in lobeline, **15**, and **17** are not required for this activity. Interestingly however, the high-affinity lobeline analogue **5** failed to produce the maximal possible effect up to a concentration of 50 μmol/animal. Further investigation of analgesic activity with these and other lobeline analogues should lead to an entirely different structure−activity relationship than that formulated here for nACh receptor binding.

Summary

It is not known if (−)lobeline binds at nACh receptors in the same manner (i.e., avails itself of the same receptor binding features) as (−)nicotine, so it is probably premature to make attempts to fit (−)lobeline to the Sheridan pharmacophore. In any event, however, it would seem that the constructs of the pharmacophore are not sufficient to explain the affinity of (−)lobeline. Compounds such as **7**−**10** and **13** possess those features dictated by the pharmacophore model, but all bind with >500-fold lower affinity (i.e., K_i > 2 000 nM) than (−)-lobeline (K_i = 4 nM). Furthermore, it is evident that both arms are required for optimal affinity; those compounds possessing a single arm, regardless of the substituents present on the arm (i.e., carbonyl oxygen, hydroxyl oxygen, or hydrogen atom), bind with significantly lower affinity than (−)lobeline. Even compounds such as **15** and **17**, which possess both arms but simply lack one of the oxygen substituents, bind with at least 25-fold lower affinity than (−)lobeline. Although the presence of both a carbonyl group and a hydroxyl group seems optimal, the high affinity of the chloro derivative **20** indicates that the hydroxyl group is not required for binding. Glaser and co-workers have suggested on the basis of NMR studies that internal hydrogen bonding might stabilize certain conformations of lobeline; these hydrogen bonds can involve both the hydroxyl group and the carbonyl function.[22] Conceivably, such hydrogen-bonded structures might somehow contribute to binding. It is also possible that the chloro group of **20** can participate in the formation of a similar hydrogen bond with the protonated piperidine moiety. Further investigation will be required to determine how the oxygen substituents contribute to binding.

Another finding of the present investigation is that analogues of lobeline lacking either one or both oxygen functions retain analgesic activity when administered intrathecally. Because there is no direct relationship between binding and analgesic activity, these findings support the idea that the analgesic activity of lobeline in this assay system probably does not involve nACh receptors of the $\alpha_4\beta_2$ type. The present results also indicate that it should be possible to develop analogues of lobeline that will likely possess a different pharmacology than that of nicotine or, at least, analogues that will not mimic all the effects produced by nicotine.

Experimental Section

Synthesis. Melting points, determined with a Thomas-Hoover melting point apparatus, are uncorrected. Proton magnetic resonance spectra were obtained with a GE QE-300 or Varian Gemini 300 spectrometer; tetramethylsilane was

used as an internal standard, and *J* values are in Hz. Infrared spectra were recorded on a Nicolet 5ZDX FT-IR. Optical rotations were determined using a Jasco DIP-1000 polarimeter; measurements were made on 1% solutions in $CHCl_3$. Flash chromatography was performed on silica gel (Merck grade 60, 230−400 mesh, 60 Å). Elemental analysis was performed by Atlantic Microlab Inc. and determined values are within 0.4% of theory.

Some of the compounds used in the present investigation were prepared according to previously published literature procedures; these include lobelanidine HCl (**4**),[28] **7** HCl,[29] **8** HCl,[30] **9** HCl,[31] (−)sedamine HCl (**10**), [32] **11** HI,[33] **12** hydrogen oxalate,[34] and lobelan HCl (**14**).[35] Two other compounds were also prepared by literature procedures, but because their melting points differed somewhat from those reported in the literature they were submitted for elemental analysis; both analyzed correctly for C, H, and N and include lobelanine HCl (**3**; mp 203−205 °C, lit.[28] mp 197−198 °C) and **13** HCl (mp 170−171 °C, lit.[30] mp 161−162 °C). (−)Lobeline HCl was purchased from Sigma-Aldrich (St. Louis, MO).

(+)*cis*-*N*-Methyl-2-(2-phenylethyl)-6-(2-oxo-2-phenylethyl)piperidine Hydrochloride (15). Pyridinium dichromate (0.22 g, 0.58 mmol) was added to **16** (0.13 g free base, 0.39 mmol) in CH_2Cl_2 (50 mL), and the solution was allowed to stir at room temperature. After 3 h NaOH (1 N, 50 mL) was added and the organic layer was separated. The aqueous portion was extracted with Et_2O (3 × 50 mL) and the combined organic portions were dried ($MgSO_4$). The solvent was removed under reduced pressure, the remaining oil was taken up in anhydrous Et_2O, and excess HCl(g)-saturated anhydrous Et_2O was added. The precipitate was collected by filtration and recrystallized twice from absolute EtOH/anhydrous Et_2O to give **15** (0.05 g, 36%) as a white crystal: mp 166−168 °C; $[\alpha]_D^{25}$ = +12.9; ^{1}H NMR ($CDCl_3$) δ 1.4−1.8 (br m, 3H, CH), 1.8−2.2 (m, 3H, CH), 2.2−2.4 (m, 1H, CH), 2.5−3.1 (m, 6H, CH), 3.2−3.5 (m, 1H, CH), 3.7−3.8 (m, 1H, CH), 3.9−4.1 (m, 1H, CH), 4.1−4.4 (m, 1H, CH), 7.16−7.38 (m, 5H, ArH), 7.50 (t, *J* = 8.8, 2H, ArH), 7.58−7.66 (m, 1H, ArH), 8.04 (d, *J* = 7.14, 2H, ArH); IR (KBr) 1687 cm.$^{-1}$ Anal. Calcd for ($C_{22}H_{27}NO$·HCl · $0.25H_2O$) C, H, N.

(+)*cis*-*N*-Methyl-2-(2-phenylethyl)-6-(2-hydroxy-2-phenylethyl)piperidine Hydrochloride (16). Compound **18** (0.39 g, 121 mmol) was suspended in absolute MeOH (50 mL) and 5% Pd/C (0.02 g) was added. The mixture was hydrogenated on a Parr hydrogenation apparatus at 50 psi with shaking. After 1.5 h the mixture was filtered through Celite and the solvent was removed under reduced pressure. The remaining oil was taken up in H_2O (50 mL) and made basic with solid Na_2CO_3 (pH ∼ 9). The aqueous solution was extracted with Et_2O (3 × 50 mL) and the combined ethereal portion was dried ($MgSO_4$); solvent was removed under reduced pressure and the resulting oil was purified by column chromatography (silica gel, 30 g) using EtOAc/hexane (4:1). The oily product was taken up in anhydrous Et_2O and excess HCl(g)-saturated anhydrous Et_2O was added. The precipitate was collected and recrystallized from absolute EtOH/anhydrous Et_2O to give **16** (0.10 g, 26%) as colorless crystals: mp 189−190 °C; $[\alpha]_D^{25}$ = +29.6; ^{1}H NMR ($CDCl_3$) δ 1.40−2.00 (m, 8H, CH), 2.32−2.91 (m, 7H, CH), 3.51−3.58 (m, 1H, CH), 3.82−3.90 (m, 1H, CH), 5.01 (d, *J* = 11.43, 1H, C*H*OH), 7.18−7.41 (m, 10H, ArH); IR (KBr) 3285 cm^{-1}. Anal. Calcd for ($C_{22}H_{29}NO$·HCl·$0.25H_2O$) C. H. N.

(−)*cis*-*N*-Methyl-2-(2-phenylethyl)-6-(2-hydroxy-2-phenylethyl)piperidine Hydrochloride (17). Compound **19** (0.10 g, 0.31 mmol) was suspended in absolute MeOH (50 mL) and PtO_2 (0.02 g) was added. The solution was hydrogenated on a Parr hydrogenation apparatus at 1 atm with shaking. After 1 h the mixture was filtered through a Celite pad and the solvent was removed under reduced pressure. The resulting oil was taken up in NaOH (1 N, 50 mL) and extracted with Et_2O (3 × 50 mL); the combined ethereal layers were dried ($MgSO_4$) and solvent was removed under reduced pressure. The residual oil was taken up in anhydrous Et_2O and HCl(g) saturated anhydrous Et_2O was added. The precipitate was collected and recrystallized from absolute EtOH/anhydrous Et_2O to give **17** (0.09 g, 80%) as a white crystal: mp 189−190 °C; $[\alpha]_D^{25}$ = −27.9; ^{1}H NMR ($CDCl_3$) δ 1.4−2.0 (m, 8H, CH), 2.3−2.6 (m, 2H, CH), 2.66 (s, 3H, CH_3), 2.7−2.9 (m, 2H, CH), 3.5−3.6 (m, 1H, CH), 3.8−4.0 (m, 1H, CH), 4.99 (d, *J* = 10.98 1H, C*H*OH), 7.19−7.39 (m, 10H, ArH); IR (KBr) 3279 cm^{-1}. Anal. Calcd for ($C_{22}H_{29}NO$·HCl) C, H, N.

(−)*cis*-*N*-Methyl-2-(2-phenylethen-1-yl)-6-(2-oxo-2-phenylethyl)piperidine (18). (−)Lobeline HCl (**1**) (1.00 g, 2.67 mmol) in phosphoric acid (85%, 23 mL) was warmed at 45−50 °C for 22 h. The reaction mixture was taken up in H_2O (100 mL) and made basic with solid K_2CO_3 (pH ∼ 8). The pH was adjusted by the addition of solid NaOH (pH ∼ 10) and the aqueous solution was extracted with EtOAc (3 × 75 mL). The combined organic portion was dried (K_2CO_3) and solvent was removed under reduced pressure to give a solid. The solid was recrystallized from hexane to give **18** (0.06 g, 75%) as colorless crystals: mp 82−24 °C; $[\alpha]^{25}{}_D$ = −28.3; ^{1}H NMR ($CDCl_3$) δ 1.3−1.9 (m, 6H, CH), 2.27 (s, 3H, CH_3), 2.70−3.00 (m, 3H, CH), 3.47 (dd, *J* = 6.45, 16.23, 1H, CH), 6.18 (dd, *J* = 8.67, 15.93, 1H, C═C*H*), 6.50 (d, *J* = 15.87, 1H, C═C*H*), 7.2−7.61 (m, 8H, ArH), 7.99 (d, *J* = 6.99, 2H, ArH); IR (KBr) 1687, 2775, 2943 cm^{-1}. Anal. Calcd for ($C_{22}H_{25}NO$) C, H. N.

(−)*cis*-*N*-Methyl-2-(2-hydroxy-2-phenylethyl)-6-(2-phenylethen-1-yl)piperidine (19). (−)Lobeline HCl (**1**) (0.50 g, 1.34 mmol) was added to a suspension of amalgamated zinc and aqueous 5% HCl (20 mL) and heated at reflux for 15 min. The mixture was made basic by the addition of aqueous NaOH (1 N, 25 mL) followed by solid NaOH to a pH = 10 and extracted with Et_2O (3 × 50 mL). The combined organic portion was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting oil was purified by column chromatography (silica gel, 30 g) using EtOAc as eluent. The oil was taken up in hot hexane and a precipitate formed on cooling. The precipitate was collected and recrystallized from hexane to give **19** (0.20 g, 46%) as a white solid: mp 109−111 °C; $[\alpha]_D^{25}$ = −45.7; ^{1}H NMR ($CDCl_3$) δ 1.4−1.7 (m, 6H, CH), 1.8−2.1 (m, 2H, CH), 2.32 (s, 3H, CH_3), 2.9−3.0 (m, 1H, CH), 3.22−3.38 (m, 1H, CH), 4.9 (d, 1H, C*H*OH), 6.23 (dd, *J* = 5.28, 16.11, 1H, CH), 6.46 (d, *J* = 16.35, 1H, CH), 7.2−7.4 (m, 10H, ArH); IR (KBr) 1457, 1600, 2937, 3552 cm^{-1}. Anal. Calcd for ($C_{22}H_{27}$-NO) C, H, N.

(−)cis-*N*-Methyl-2-(2-chloro-2-phenylethyl)-6-(2-oxo-2-phenylethyl)piperidine Hydrochloride (20). Thionyl chloride (0.24 g, 2.01 mmol) was added to a solution of (−)lobeline HCl (**1**) (0.50 g, 1.34 mmol) in $CHCl_3$ (30 mL). The mixture was stirred in a sealed tube at room temperature. After 24 h the solvent was removed under reduced pressure and the resulting oil was taken up in hot EtOAc (50 mL). Upon cooling, a precipitate formed and was collected by filtration. The precipitate was recrystallized from absolute EtOH/anhydrous Et_2O to give **20** (0.42 g, 80%) as colorless crystals: mp 184−186 °C (lit.[26] mp 172−174 °C); $[\alpha]_D^{25}$ = −38.4; ^{1}H NMR ($CDCl_3$) δ 1.4−2.4 (m, 7H, CH), 2.72 (m, 5H, CH_3), 3.3−3.5 (m, 1H, CH), 3.8−4.0 (m, 1H, CH), 4.1−4.3 (m, 1H, CH), 4.97 (s, 1H, C*H*OH), 7.30−7.7 (m, 8H, ArH), 8.03 (d, *J* = 7.92, 2H, ArH); IR (KBr) 1681 cm^{-1}. Anal. Calcd for ($C_{22}H_{26}ClNO$·HCl) C, H, N.

(−)Lobeline Methiodide (21). Methyl iodide (0.38 g, 2.67 mmol) was added to a stirred mixture of (−)lobeline (**1**) (0.5 g, 1.34 mmol) and EtOAc (100 mL) and heated at reflux. After 3 h the solvent was removed under reduced pressure and the remaining oil was purified by column chromatography (silica gel, 60 g) using EtOAc and MeOH (9:1) as eluent. The most polar fraction was dissolved in EtOAc and the solution stored in a refrigerator overnight. The hygroscopic precipitate was collected dried (33 °C, 0.5 mmHg) to give **21** (0.03 g, 5%) as colorless crystals: mp 86−88 °C; $[\alpha]_D^{25}$ = −9.9; ^{1}H NMR ($CDCl_3$) δ 1.71 (s, 6H, CH), 1.78−1.85 (m, 1H, CH), 2.18−2.2 (m, 1H, CH), 2.3−2.6 (m, 2H, CH), 3.30 (s, 6H, CH_3), 4.2−4.4 (m, 2H, CH), 4.85 (d, *J* = 7.7, 1H, C*H*OH), 6.96−7.04 (m, 2H, ArH), 7.33(t, *J* = 7.14 2H, ArH), 7.42 (d, *J* = 7.83, 2H, ArH), 7.48 (d, *J* = 7.2, 1H, ArH), 7.56 (t, *J* = 6.18, 1H, ArH), 7.96 (d, *J* = 7.8, 2H, ArH); IR (KBr) 1668, 3422 cm^{-1}. Anal. Calcd for ($C_{23}H_{29}INO_2$) C, H, N.

Radioligand Binding Assay. The [^{3}H]nicotine binding assay using rat brain homogenates followed the method of Scimeca and Martin.[36] Briefly, tissue homogenates were prepared from whole rats brain (minus cerebellum) in 10 volumes of ice-cold 0.05 M Na-K phosphate buffer (pH 7.4) and centrifuged at 17 500*g* (4 °C) for 30 min. The pellet was then

resuspended in 20 volumes of ice-cold glass-distilled water and allowed to remain on ice for 60 min before being centrifuged as before. The resulting pellet was resuspended to a final tissue concentration of 10 mg/mL of buffer. Membranes from whole brain (0.2 mL of final suspension) were incubated at 4 °C for 2 h with phosphate buffer and [^{3}H](−)nicotine (1.5 ng, 80 Ci/mmol) (New England Nuclear, Boston, MA) in a total volume of 1 mL. Nonspecific binding was determined in the presence of 100 μM unlabeled nicotine. The incubation was terminated by rapid filtration through a Whatman GF-C glass fiber filter (presoaked overnight in 0.1% poly-L-lysine). Filters were washed twice with 3 mL of buffer and radioactivity on the filters was measured using a liquid scintillation spectrometer. Values represent triplicate determinations.

Analgesic Activity. The procedure used was the tail-flick method of D'Amour and Smith[37] as modified by Dewey et al.[38] A control response (2−4 s) was determined for each animal before treatment, and a test of latency was determined after drug administration. A maximum latency of 10 s was used to avoid tissue damage. The antinociceptive response was calculated as percent maximal possible effect (%MPE) where %MPE = [(test − control)/(10 − control)] × 100. Groups of 8−12 male ICR mice (20−25 g; Harlan Laboratories, Indianapolis, IN) were used to examine each dose of compound. Agents were administered via intrathecal injections performed free-hand between the L5 and L6 lumbar space in unanesthetized animals according to the method of Hylden and Wilcox.[39] The injection was performed using a 30-gauge needle attached to a glass microsyringe. An injection volume of 5 μL was used and animals were tested 5 min postinjection. We have previously described these techniques in further detail.[1,8]

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