Pharmacology of Lobeline, A Nicotinic Receptor Ligand

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ABSTRACT
In this study we investigated the pharmacology of lobeline, a high affinity nicotinic ligand with a unique pharmacological profile, in different in vitro and in vivo tests. Although lobeline displaced $[^3H]$-nicotine binding sites in the rat brain with a $K_i$ of 4.4 nM, it did not activate $\alpha 4\beta 2$ expressed receptors in frog oocytes. The in vivo pharmacological effects of lobeline were highly complex. Lobeline, at the time of maximal effect, dose-dependently produced motor impairment and decreased locomotor activity and body temperature in mice after s.c. treatment. However, antinociception was present after intrathecal but not after s.c. administration of lobeline in the tail-flick tests. The behavioral effects of lobeline were not blocked by pretreatment with either mecamylamine or dihydro-β-erythroidine. In addition, lobeline given s.c. enhanced nicotine-induced antinociception in a dose-related manner. No acute tolerance developed to either lobeline’s behavioral or antinociceptive effect after s.c. or intrathecal administration, respectively. However, tolerance developed to lobeline’s pharmacological effects after chronic treatment with the drug for 10 days (15 mg/kg, s.c. twice a day). Furthermore, cross-tolerance between lobeline and nicotine developed after chronic treatment with either drug. Although the $\alpha 4\beta 2$ receptor is unlikely to mediate the agonist effects of lobeline, our results indicate that lobeline does interact with the nicotinic receptor in a novel fashion.

Nicotine has diverse pharmacological effects on the CNS, many of which are marked by both stimulant and depressant phases of action. These effects include alterations in locomotor activity, hypothermia, convulsions, antinociception and others (for review see Martin, 1986). Nicotine also possesses anxiolytic effects (Brioni et al., 1994) and improves learning and memory in a variety of preclinical and clinical paradigms (Levin, 1992). These actions presumably occur as a result of nicotine’s interaction with its receptors in the CNS. However, activation of these receptors by different nicotinic ligands results in a complex pharmacological profile. For example, the behavioral and pharmacological effects of cytisine, a nicotinic agonist, do not correlate with its affinity for $[^3H]$-nicotine binding sites. Cytisine is about 5 times more potent than nicotine in binding assays but at least 10 times less potent in producing nicotine-like responding in drug discrimination (Reavill et al., 1988, 1990). However, the pharmacological effects of (+)-BN, a rigid analog of nicotine with little affinity to $[^3H]$-nicotine binding sites, were not blocked by nicotinic antagonists (Glassco et al., 1993). This pharmacological dilemma is probably not unique to cytisine and (+)-BN and may be related to the multiplicity of nicotinic receptors in the CNS. Indeed, recent molecular and electrophysiological studies suggest that these receptors are structurally and functionally diverse (for recent review, see Patrick and Luetje, 1993). However, specific ligands to explore their pharmacology are lacking.

Lobeline is another high affinity nicotinic ligand with a unique pharmacological profile. Indeed, it has been reported to displace brain $[^3H]$-nicotine binding with $K_i$s in the range of 5 to 30 nM (Lippiello and Fernandes, 1986; Reavill et al., 1990). Lobeline has been reported to have many nicotine-like effects including hypertension (Olin et al., 1995), bradycardia and hypotension in urethane-anesthetized rats (Sloan et al., 1988), anxiolytic effects in animals (Brioni et al., 1993) and enhancement of cognitive performance in rats (Decker et al., 1993). Moreover, lobeline has been used as a treatment for smoking cessation; however, its effectiveness after oral administration has not been well established (Olin et al., 1995). Furthermore, its usage as a smoking deterrent has been recommended for short-term periods (6 wk periods) due to gastrointestinal toxicity and to the fact that little information is available on its long-term usage (Olin et al., 1995). In contrast to nicotine, lobeline does not increase locomotor activity in rats (Stolerman et al., 1995) or produce conditioned place preference (Fudala and Iwamoto, 1986) and is unable to generate a discriminative stimulus in rats trained on nicotine (Reavill et al., 1990). Moreover, lobeline-induced dopamine release from rat and mouse striatal synaptosomes was mecamylamine insensitive and calcium independent (Clarke and Reuben, 1996; Grady et al., 1992). In addition,
chronic infusion of lobeline did not increase the number of
nicotinic receptors in the same brain regions that were shown
to have increased numbers after chronic nicotine exposure
(Bhat et al., 1991).

Therefore, the objective of our study was to establish a
more complete pharmacological profile of lobeline to deter-
mine whether it shares a common mechanism with nicotine.
For that we compared the effects of lobeline to those of
nicotine in different behavioral models (locomotor activity,
motor coordination, antinociception and body temperature
measurement), and tested lobeline’s sensitivity to different
nicotinic antagonists after s.c. and i.t. injections in mice. An
additional objective was to determine whether lobeline was
capable of modulating (enhancing or blocking) nicotine in
different pharmacological tests after acute administration in
mice. Although these behavioral models, coupled with recep-
tor binding, offer sufficient opportunity for ascertaining nic-
tinic effects, additional evidence for the mechanism of action
was obtained by assessing the activity of lobeline at the $\alpha_2\beta_2$
icotinic receptor expressed in oocytes. In addition, adapta-
tion of lobeline’s pharmacological effects was investigated
after acute and chronic administration of the drug.

Materials and Methods

Animals

Male ICR mice (20–25 g) obtained from Harlan Laboratories
(Indianapolis, IN) were used throughout the study. The mice were
housed in groups of six and had free access to food and water.

Drugs

(−)-Nicotine was obtained from Aldrich Chemical Company, Inc.
(Milwaukee, WI) and converted to the ditartrate salt as described
(Aceto et al., 1979). $[^3H]$(−)-Nicotine (80 Ci/mmol) was purchased
from New England Nuclear (Boston, MA). Mecamylamine hydrochlo-
ride and dihydro-β-erythroidine were gifts from Merck, Sharp and
Dohme & Co. (West Point, PA). $\alpha$-Lobeline HCl was purchased from
Sigma Chemical Co. (St. Louis, MO). All drugs were dissolved in
physiological saline (0.9% sodium chloride) and given in a total
volume of 1 ml/100 g body weight in mice for s.c. injections. All doses
are expressed as the free base of the drug.

Intrathecal Injections

Intrathecal injections were performed free-hand between the L5
and L6 lumbar space in unanesthetized male mice according to the
method of Hylden and Wilcox (1980). The injection was performed
using a 30-gauge needle attached to a glass microsyringe. The injec-
tion volume in all cases was 5 μl. The accurate placement of the
needle was evidenced by a quick “flick” of the mouse’s tail. In proto-
cols where two sequential injections were required in an animal, the
flicking motion of the tail could be elicited with each subsequent injection.

Behavioral and Pharmacological Assays in Mice

Locomotor activity. Mice were placed into individual Omnitech
photocell activity cages (28 × 16.5 cm) 5 min after s.c. administra-
tion of either 0.9% saline or lobeline. Interruptions of the photocell beams
(two banks of eight cells each) were then recorded for the next 10
min. Data were expressed as number of photocell interruptions. For
antagonism studies, the mice were pretreated s.c. with either saline or
mecamylamine 10 min before lobeline.

Antinociception. The tail-flick method of D’Amour and Smith
(1941) as modified by Dewey et al. (1970) was used. A control re-
sponse (2–4 sec) was determined for each animal before treatment,
and a test latency was determined after drug administration. To
minimize tissue damage, a maximum latency of 10 sec was imposed.
Antinociceptive response was calculated as percent maximum possi-
ble effect (% MPE), where %MPE = [(t-test-control)/(10-control)] ×
100. Groups of 8 to 12 animals were used for each dose and for each treatment.
For time course studies, separate groups of mice were tested
at the indicated times after drug administration. For nicotine-
lobeline interaction studies, mice were pretreated with different
doses of lobeline 10 min before nicotine. The animals were tested 5
min after administration of nicotine. For the intrathecal experi-
ments, the mice were tested at the indicated times after lobeline
administration. A dose-response curve was determined 5 min after
i.t. injection of lobeline. The antagonism studies involved i.t. pre-
treatment with either saline, mecamylamine or dihydro-β-erythroi-
dine 5 min before i.t. administration of lobeline.

Body temperature. Rectal temperature was measured by a ther-
mistor probe (inserted 24 mm) and digital thermometer (Yellow
Springs Instrument Co., Yellow Springs, OH). Readings were taken
just before and at different times after the s.c. injection of lobeline for
the time course determinations. In dose-response studies, the mice were
tested 20 min after treatment. For antagonism studies, mice were
pretreated with either saline, mecamylamine or dihydro-β-erythroi-
dine (s.c.) 10 min before lobeline. The difference in rectal tempera-
ture before and after treatment was calculated for each mouse. The ambient
temperature of the laboratory varied from 21 to 24°C from
day to day.

Motor coordination. To measure motor coordination, a wooden
rod 6 cm in diameter was partitioned into three compartments by
circular metal discs (28 cm in diameter) at 15-cm intervals. The rod
was attached to a motor and rotated at a rate of 4 rpm. Naive mice
were trained until they could remain on the rotarod for 3 min.
Animals that failed to meet this criterion within five trials were
discarded. This training took place no longer than 15 min before the
s.c. administration of lobeline. For time course studies, separate
groups of mice were tested at the indicated times after lobeline
administration. In the dose-response studies, 20 min after the injec-
tion, mice were placed on the rotarod for 5 min. The amount of time
the animals remained on the rotarod was recorded and percent
impairment was calculated as % Impairment = [(1-test time in
sec/300)] × 100. A value of 0% Impairment corresponds to subjects
that remained on the rotarod for 5 min (300 sec) and 100% Impair-
ment value corresponds to subjects that fell off the rotarod in less
than 1 sec.

To determine acute tolerance to lobeline-induced motor impair-
ment and hypothermia, mice were pretreated s.c. with different
doses of lobeline at different times before a second s.c. injection
of nicotine. The same protocol was followed to determine lobeline-in-
duced antinociception after i.t. administration.

Chronic Drug Treatment

Two groups of mice received a s.c. injection of either lobeline (15
mg/kg) or saline twice daily (0830 and 1630) for 10 days. Throughout
the period of treatment, body weight was recorded every other day.
At day 11, mice were challenged with different doses of lobeline for
determination of dose-response curves after s.c. and i.t. injections.
Injections and testing procedures were performed in the same room.

$[^3H]$(−)-Nicotine Binding in Vitro

$[^3H]$(−)-Nicotine binding assays in rat brain were performed in
vitro according to the method of Scimeca and Martin (1988) with
minor modifications. Tissue homogenate was prepared from whole
rat brain (minus cerebellum) in 10 volumes of ice-cold 0.05 M Na-K
phosphate buffer (pH 7.4) and centrifuged (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 then resus-
pended to a final tissue concentration of 10 mg/ml of buffer. Mem-
branes from whole brain (0.2 ml of final suspension) were incubated
at 4°C for 2 hr with phosphate buffer and \(^{3}H\)-nicotine (1.5 ng) in a total volume of 1 ml. Nonspecific binding was determined in the presence of 100 \(\mu\)M 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 to reduce radioligand binding to the filters). Filters were washed twice with 3 ml of the buffer, and radioactivity on the filters was measured using a liquid scintillation spectrometer. Displacement of 1.5 nM \(^{3}H\)-nicotine binding was determined in the presence of increasing concentrations of lobeline and nicotine.

**Oocyte Expression System**

**Injection of RNA into Xenopus oocytes.** Adult female *Xenopus laevis* frogs were anesthetized by partial immersion in a 0.2% solution of ethyl-M-amino benzoate for 30 to 60 min. Oocytes were surgically excised and defolliculated with collagenase type I (Sigma) treatment for 1 hr at room temperature. Diguanosine triphosphate-capped RNA was synthesized *in vitro* from linearized template DNA encoding for \(\alpha_4\) and \(\beta_2\) subunits using an RNA transcription kit (Ambion, Austin, TX). The mRNAs (79 nl) were injected into the oocytes (vegetal pole) under visual guidance using a Drummond microinjector (Broomal, PA) and glass micropipettes filled with 4 \(\mu\)l of the required mRNA mixture. Injected oocytes were incubated at 19°C in 0.5X L15 media (Sigma) for at least 3 days.

**Two-electrode voltage-clamp recordings.** Two-electrode voltage-clamp recordings were carried out on the 3rd day through the 7th day after injection. Recordings were performed on injected oocytes in a 300-\(\mu\)l chamber perfused with a saline solution containing 115 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl\(_2\), 10 mM HEPES (pH 7.2) and 1 \(\mu\)M atropine. The flow rate was approximately 10 to 15 ml/min. Oocytes were voltage-clamped at -70 mV using an Axoclamp-2A recording system. Microelectrodes were filled with 3 M KCl and had a resistance of 0.5 to 3 M\(\Omega\). Currents were filtered at 150 Hz with an 8-pole Bessel filter, and stored and subjected to analysis on a Macintosh Quadra 950 using Pulse Control data acquisition and analysis software. Drugs were superfused in various concentrations, and data for complete dose-response curves were obtained using maximal current response values. Each application was approximately 10 sec in duration and applications were separated by varying periods of wash-out (3–5 min). Switching between different superfusing solutions was controlled by solenoid switching valves. For each observation, data were acquired from a minimum of three separate oocytes obtained from at least two separate donor frogs.

**Statistical Analysis**

Data were analyzed statistically by an analysis of variance followed by the Fisher PLSD multiple comparison test. The null hypothesis was rejected at the 0.05 level. ED\(_{50}\) values with 95% CL for antinociception and motor impairment data were calculated by unweighted least-squares linear regression for log-doses vs. probits, as described by Tallarida and Murray (1987). The effects of drugs on rectal temperature and locomotor activity were calculated from double reciprocal analysis (1/\(\text{effect}\) vs. 1/dose) to yield a theoretical maximum effect (efficacy), as described by Tallarida and Murray (1987). The ED\(_{50}\) values were determined by calculating the functional response for each drug dose (based on the maximum effect being 1.0), converting the data to probit values and determining the unweighted least-squares linear regression for the log-dose vs. probit as described by Tallarida and Murray (1987).

**Results**

**In vitro experiments.** The Scatchard analysis of \(^{3}H\)-nicotine binding provided a \(K_d\) of 1.2 \(\pm\) 0.14 nM and a \(B_{max}\) of 253 \(\pm\) 56 fmol/mg protein. Both nicotine and lobeline displaced binding of \(^{3}H\)-nicotine to rat brain membranes. The \(K_d\) values were, respectively, 1.4 \(\pm\) 0.2 and 4.4 \(\pm\) 2.2 nM, which confirm that lobeline has high affinity for the nicotinic receptor. However, lobeline at 0.1 and 1 mM elicited little current when applied for 10 sec to oocytes expressing the \(\alpha_4\beta_2\) subunit combination. Indeed, at a concentration of 1 mM, lobeline’s response represents only 1.9 \(\pm\) 1.5% of 1 \(\mu\)M acetylcholine applied under the same conditions (fig. 1A). Although it did not activate \(\alpha_4\beta_2\) expressed receptor, lobeline antagonizes the effects of nicotine in oocyte. Indeed, the current-induced by 3 \(\mu\)M of nicotine is blocked by coapplication of lobeline and nicotine in a concentration-related manner (fig. 1B). The concentration of lobeline that blocked 50% of the nicotinic current is calculated to be 10 \(\mu\)M.

**In vivo pharmacology of lobeline.** Nicotine and lobeline at the time of maximal effect (time course not shown) dose-dependently produced motor impairment and decreased locomotor activity and body temperature in mice after s.c. treatment (fig. 2). However, contrary to nicotine, lobeline showed little antinociceptive activity (30% MPE at 20 mg/kg) in the tail-flick test after s.c. administration. Calculation of the ED\(_{50}\) values (table 1) showed that lobeline was two to six times less potent than nicotine in the different tests. Furthermore, pretreatment with mecamylamine and dihydro-\(\beta\)-erythroidine, at 1 and 2 mg/kg s.c. respectively, 10 min before lobeline did not significantly decrease lobeline-induced motor impairment, hypotemia and hypothermia (fig. 3, A–C). By themselves, mecamylamine and DH\(\beta\)E did not have a significant effect on test parameters measured.

In contrast to s.c. administration, lobeline injected i.t. elicited a dose-related antinociceptive effect (fig. 4A) with a similar potency to nicotine. Indeed, 5 min after injection the ED\(_{50}\) (CL) value for lobeline was 61.5 nmol/mouse or 23 \(\mu\)g/mouse, compared to that of 68 nmol/mouse or 12 \(\mu\)g/
mouse for nicotine. Furthermore, i.t. pretreatment with mecamylamine and dihydro-β-erythroidine, at 10 mg/mouse, 5 min before lobeline (40 mg/mouse) did not significantly decrease lobeline-induced antinociception (fig. 4B). Nicotine under the same experimental conditions, increased tail-flick latencies with an ED50 of 68 (60.0–105.5) nmol/mouse or 11 (9.5–17.0) mg/kg (see table 1) and was mecamylamine- and dihydro-β-erythroidine-sensitive (fig. 4B). By themselves, mecamylamine and DHβE did not induce hypothermia (control = 0.2 ± 0.1°C) or significant hypomotility (2 and 9% decrease compared to control; control = 2052 ± 342 interrupts). None of the animals fell after the administration of nicotinic antagonists in the rotarod test.

Although lobeline displaced [3H]-nicotine binding with high affinity, it failed to significantly increase tail-flick latencies after s.c. administration. Therefore, the interaction between lobeline and nicotine after s.c. administration was investigated for potential antagonistic properties. Surprisingly, lobeline enhanced nicotine-induced antinociception in a dose-dependent manner (table 2). Indeed, mice pretreated s.c. with lobeline (5 mg/kg, 10 min before nicotine) showed maximal antinociceptive activity after challenge with an inactive dose of nicotine (0.5 mg/kg). This enhancement was blocked by mecamylamine pretreatment in a dose-related manner (table 2). In addition, pretreatment with lobeline (5 mg/kg) shifted nicotine’s dose-response curve to the left (fig. 5) such that the ED50 value of nicotine (1.47 ± 0.8–2.6 mg/kg or 9 μmol/kg) was decreased nearly 6-fold to an ED50 of 0.25 (0.13–0.46) mg/kg or 1.5 μmol/kg.

Potentiation of nicotine’s effects was observed in the tail-flick test only, because lobeline at 1 mg/kg, did not significantly enhance nicotine-induced hypothermia and hypomotility (table 3). However, higher doses of lobeline could not be tested because they were active by themselves.

Acute tolerance to lobeline’s pharmacological effects. Contrary to what was reported with nicotine where a single pretreatment with the drug (4 mg/kg, s.c.) resulted in

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**TABLE 1**

<table>
<thead>
<tr>
<th>Pharmacological Effect</th>
<th>(−)-Nicotine (ED50 mg/kg)</th>
<th>Lobeline (ED50 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypomotility</td>
<td>0.4 (0.12–0.70)</td>
<td>5.4 (3.2–5.4)</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>1.2 (0.5–2.2)</td>
<td>5.7 (1.2–8.0)</td>
</tr>
<tr>
<td>Motor impairment</td>
<td>1.0 (0.5–2.1)</td>
<td>4.7 (2.9–7.5)</td>
</tr>
<tr>
<td>Antinociception after s.c.</td>
<td>1.3 (0.6–2.6)</td>
<td>30%@20</td>
</tr>
<tr>
<td>Antinociception after i.t.</td>
<td>12 (10.5–17)</td>
<td>23 (18–29)</td>
</tr>
</tbody>
</table>

Receptor affinity 1.4 4.4

ED50 values (± CL) were calculated from the dose-response curve of the respective compounds and expressed as mg/kg. Each dose group included six to eight animals.

* ED50 values (± CL) after i.t. administration are expressed as μg/animal.
the development of acute tolerance to a subsequent dose of nicotine (Damaj et al., 1996), no significant acute tolerance was seen to lobeline-induced hypothermia (fig. 6A) and motor impairment (fig. 6B) after s.c. administration. Indeed, in mice pretreated with lobeline (20 mg/kg, s.c.) and challenged with lobeline (5 mg/kg, s.c.) 0.5, 1, 3 or 24 hr later, no significant reduction was seen to either the hypothermic or motor impairment effects. Under the same experimental conditions, maximum acute tolerance developed to the same effects of nicotine 2 to 4 hr after the first injection (Damaj et al., 1996).

Similarly, no significant acute tolerance was seen to lobeline-induced antinociception (40 μg/mouse, i.t.) in mice pretreated with lobeline (10 μg/mouse, i.t.) at different times after i.t. administration (fig. 7A). Furthermore, higher doses of lobeline failed to induce acute tolerance (fig. 7B), because mice pretreated with 80 μg/animal did not exhibit significant acute tolerance to a subsequent dose of 40 μg/animal of lobeline, 10 min later (time of maximum tolerance to nicotine after i.t. injection).

In addition, no acute cross-tolerance developed to either lobeline-induced hypothermia after s.c. administration (table 4A), or to lobeline-induced antinociception after i.t. injection (table 4B) in mice pretreated with nicotine at different doses. Indeed, animals pretreated with nicotine developed acute tolerance to a subsequent dose of nicotine, but not lobeline in either response.

The lack of tolerance to lobeline’s effects was also seen in mice receiving injections s.c. or i.t. repeatedly in the different pharmacological tests. Indeed, no significant reduction was seen to either the hypothermic effect of lobeline (5 mg/kg) (fig. 8A), or to lobeline-induced motor impairment (20 mg/kg) (fig. 8B) after repeated s.c. injections (one s.c. injection every 30 min, total of four injections). Similarly, no tolerance developed to lobeline-induced antinociception after i.t. injection (40 μg/animal every 10 min, total of three injections) in mice (fig. 8C).

**Table 2**

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Challenge</th>
<th>% MPE ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Saline</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Saline</td>
<td>lobeline (5)</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>Saline + saline</td>
<td>Nicotine (0.5)</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Saline + lobeline (1)</td>
<td>Nicotine (0.5)</td>
<td>31 ± 15</td>
</tr>
<tr>
<td>Saline + lobeline (5)</td>
<td>Nicotine (0.5)</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>Mecamylamine (0.05) + lobeline (5)</td>
<td>Nicotine (0.5)</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>Mecamylamine (0.5) + lobeline (5)</td>
<td>Nicotine (0.5)</td>
<td>5 ± 2</td>
</tr>
</tbody>
</table>

* Doses are expressed in mg/kg in parentheses.

**Fig. 4.** Antinociceptive effect of lobeline after i.t. administration in mice. (A) Dose-response curve of nicotine (C) and lobeline (●) induced antinociception in mice after i.t. injection in the tail-flick test. The mice were then tested 5 min after injection. Each point represents the mean ± S.E. of 8 to 12 mice. (B) Effect of i.t. mecamylamine and dihydro-β-erythroidine on lobeline-induced antinociception after i.t. injection. Mice were pretreated with nicotinic antagonists 5 min before lobeline. Results are expressed as mean ± S.E. with 6 to 12 mice/group. Lob 40 = lobeline at 40 μg/animal; Meca 10 = mecamylamine at 10 μg/animal; DBE 10 = dihydro-β-erythroidine at 10 μg/animal.

**Fig. 5.** Potentiation of nicotine-induced antinociception and by lobeline. Lobeline (5 mg/kg) (●) or saline (□) were injected s.c. 10 min before nicotine (s.c.). The mice were tested 5 min after nicotine in the tail-flick test. Each point represents the average %MPE for six to eight mice.

**Table 3**

Interaction of lobeline and nicotine in different pharmacological tests after s.c. administration in mice (effects on body temperature and locomotor activity)

<table>
<thead>
<tr>
<th>Pretreatment Dosea</th>
<th>Challenge Dosea</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Body Temperatureb Δt°C (mean ± S.E.)</td>
</tr>
<tr>
<td>Saline</td>
<td>Saline</td>
<td>-0.3 ± 0.2</td>
</tr>
<tr>
<td>Saline</td>
<td>Nicotine (0.25)</td>
<td>-0.6 ± 0.1</td>
</tr>
<tr>
<td>Lobeline (1)</td>
<td>Saline</td>
<td>-1.0 ± 0.1</td>
</tr>
<tr>
<td>Lobeline (1)</td>
<td>Nicotine (0.25)</td>
<td>-1.0 ± 0.1</td>
</tr>
<tr>
<td>Saline</td>
<td>Nicotine (0.1)</td>
<td>1625 ± 194</td>
</tr>
<tr>
<td>Lobeline (1)</td>
<td>Saline</td>
<td>1547 ± 71</td>
</tr>
<tr>
<td>Lobeline (1)</td>
<td>Nicotine (0.1)</td>
<td>1854 ± 248</td>
</tr>
</tbody>
</table>

* Doses are expressed in mg/kg in parentheses.
  b Mice were challenged with nicotine 20 min after lobeline pretreatment.
  c Mice were challenged with nicotine 10 min after lobeline pretreatment.
Tolerance to lobeline’s pharmacological effects after chronic administration. Chronic treatment with lobeline (15 mg/kg twice a day for 10 days) did not produce any overt toxicity in that neither lethality, nor significant reduction in body weight, was observed (data not shown).

Dose-response curves for lobeline-induced hypothermia in chronic lobeline and saline-treated animals after s.c. injection are presented in figure 9A. Animals that received chronic lobeline (15 mg/kg, s.c. twice a day) were less sensitive to the acute lobeline challenge in decreasing body temperature as evidenced by the rightward shift of lobeline’s dose-response curve. The ED$_{50}$ values (± 95% CL) for saline-treated and lobeline-treated animals were 4.7 (3.5–7.1) and 16.0 (8.1–34.5) mg/kg, respectively. Similarly, tolerance developed to lobeline-induced hypomotility after chronic treatment and lobeline’s dose-response curve was shifted to the right (fig. 9B). The ED$_{50}$ values (and 95% CL) for saline-treated and lobeline-treated animals were 4.0 (1.2–13.2) and 13.0 (4.7–35.6) mg/kg, respectively. In addition, mice chronically treated s.c. with either saline or lobeline were challenged with different doses of lobeline given i.t., and their antinociceptive action was measured. Mice became tolerant to the antinociceptive effects of lobeline as shown by the rightward shift of the dose-response curve after chronic treatment with lobeline. The degree of tolerance to nicotine’s effects seen after chronic treatment with lobeline was almost similar to the one observed after chronic administration of nicotine (2 mg/kg, twice a day for 10 days). Indeed, dose-response curves observed after chronic lobeline or nicotine overlapped (fig. 10, A and B), and the ED$_{50}$ values were similar (table 5), except for nicotine-induced antinociception after i.t. injection, where a bigger tolerance to nicotine was seen in nicotine-treated animals.

Cross-tolerance after chronic administration of lobeline and nicotine. To evaluate cross-tolerance to nicotine in lobeline-tolerant mice, several groups of mice chronically treated with either saline, lobeline or nicotine were challenged with different doses of nicotine given s.c. or i.t., and their hypothermic, hypomotilic and antinociceptive actions were measured. Mice became tolerant to the hypothermic (fig. 10A), hypomotilic (fig. 10B) and antinociceptive effects (fig. 10C) of nicotine as shown by the rightward shift of the dose-response curve after chronic treatment with lobeline.

The cross-tolerance observed was bidirectional, because animals treated chronically with nicotine (2 mg/kg, twice a day for 10 days) became tolerant to the hypothermic (fig. 11A) and hypomotilic (fig. 11B) effects of lobeline as shown by the rightward shift of the dose-response curves and the increase in the respective ED$_{50}$ values (table 5). Animals that received lobeline chronically (15 mg/kg, s.c. twice a day) in the same experimental conditions were less sensitive to the
The antagonist, chlorisondamine (Decker lobeline, was reduced by pretreatment with the nicotinic and rearing activity in mice produced by nicotine, but not by consistent with the report that decreased body temperature discrimination task (Terry Reuben, 1996) and memory enhancement in the rat stimulus.

These findings are consistent with the fact that mecamylamine failed to block lobeline-induced depression in oocytes. These findings are consistent with the report that decreased body temperature discrimination task (Terry Reuben, 1996) and memory enhancement in the rat stimulus.

acute lobeline challenge in decreasing body temperature and locomotor activity and lobeline's dose-response curve was shifted to the right to the same extent as observed with nicotine-treated animals (fig. 11, A and B; and table 5).

### Discussion

Results from our study demonstrate that lobeline evokes some behavioral changes in mice that are similar to those produced by nicotine. Contrary to nicotine, lobeline-induced antinociception was observed only after i.t. but not s.c. administration, suggesting that the spinal cord as a main site for the antinociceptive action of this drug. With the exception of antinociception, the difference in pharmacological potency between the two drugs correlates well with their affinity to [3H]-nicotine binding sites. Although lobeline is thought to be a nicotinic agonist, our results suggests that the acute effects of lobeline are not mediated by the α4β2 receptor subunit combination. Contrary to nicotine, lobeline's agonist effects in mice were mecamylamine and dihydro-β-erythroidine-insensitive. Furthermore, in contrast to acetylcholine and nicotine, lobeline did not activate α4β2 receptor subtype expressed in oocytes. These findings are consistent with the fact that mecamylamine failed to block lobeline-induced dopamine release from rat striatal synaptosomes (Clarke and Reuben, 1996) and memory enhancement in the rat stimulus discrimination task (Terry et al., 1996). Furthermore, it is consistent with the report that decreased body temperature and rearing activity in mice produced by nicotine, but not by lobeline, was reduced by pretreatment with the nicotinic antagonist, chlorisondamine (Decker et al., 1994).

The lack of agonist effect on the α4β2 receptor subtype, raises the possibility of the involvement of other nicotinic subunits in lobeline's actions. However, lobeline was reported to be 30-fold less potent than nicotine in its ability to activate currents in a sympathetic ganglion preparation (a preparation that contains α3 subunits), and 6-fold less potent at activating α3β4 receptors in oocytes (Kojima et al., 1994). It is also unlikely that α7 subunits mediate the effects of lobeline, because its affinity to neuronal [125I]-αBGTX binding sites is in the higher micromolar range (Marks et al., 1986). Other nicotinic receptor subtypes remain as possible candidates.

Although the α4β2 receptor is unlikely to mediate the agonist effects of lobeline, our data suggest that this receptor can be modulated by this drug. Indeed, lobeline enhancement of nicotine-induced antinociception was dose-dependent and mecamylamine-sensitive evoking a receptor-mediated process. The α4β2 receptor is a possible candidate, because lobeline binds with high affinity to [3H]-nicotine sites. The mechanism of their interaction is still unknown, but lobeline may be potentiating the effects of nicotine by acting as a “coagonist” on the nicotinic receptor by an allosteric modulation of the protein. Potentiation of nicotine’s effects has been reported in rat cultured hippocampal neurons with compounds such as physostigmine and galanthamine that enhanced nicotinic channel activation by acetylcholine (Pereira et al., 1994; Schrattenholz et al., 1996). These substances were described by the authors as noncompetitive nicotinic agonists.

Separate mechanisms may underlie differences between

### TABLE 4

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Challenge</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>saline</td>
<td>-0.3 ± 0.1</td>
</tr>
<tr>
<td>saline</td>
<td>nicotine (1.5)</td>
<td>-4.1 ± 0.3</td>
</tr>
<tr>
<td>saline</td>
<td>lobeline (5)</td>
<td>-3.4 ± 0.2</td>
</tr>
<tr>
<td>nicotine (4)</td>
<td>nicotinic (1.5)</td>
<td>-1.2 ± 0.1b</td>
</tr>
<tr>
<td>nicotine (4)</td>
<td>lobeline (5)</td>
<td>-3.4 ± 0.2</td>
</tr>
</tbody>
</table>

Tail-flick test

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Challenge</th>
<th>% MPE ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>saline</td>
<td>saline</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>saline</td>
<td>nicotine (20)</td>
<td>75 ± 17</td>
</tr>
<tr>
<td>saline</td>
<td>lobeline (40)</td>
<td>75 ± 18</td>
</tr>
<tr>
<td>nicotine (1)</td>
<td>nicotine (40)</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>nicotine (1)</td>
<td>lobeline (40)</td>
<td>82 ± 12</td>
</tr>
<tr>
<td>nicotine (5)</td>
<td>lobeline (40)</td>
<td>90 ± 10</td>
</tr>
<tr>
<td>nicotine (20)</td>
<td>lobeline (40)</td>
<td>100 ± 0</td>
</tr>
</tbody>
</table>

* Doses are expressed in mg/kg in parentheses—mice were challenged with either s.c. nicotine or lobeline 3 hr after nicotine pretreatment at 4 mg/kg.

** Statistically different from saline-nicotine 20 at P < .05.

*** Doses are expressed in μg/animal—mice were challenged with either i.t. nicotine or lobeline 10 min after nicotine pretreatment at 1 μg/animal.

**** Statistically different from saline-nicotine 20 at P < .05.

Fig. 8. Evaluation of tolerance to lobeline-induced (A) hypothermia, (B) motor impairment and (C) antinociception after repeated s.c. or i.t. injections in mice. For lobeline-induced hypothermia and motor impairment, mice received injections with s.c. or i.t. lobeline every 30 min. A maximum of four injections was given and the effect was measured 20 min after each injection. For lobeline-induced antinociception after i.t. injection, mice received injections with i.t. lobeline (40 μg/animal) every 10 min. A total of three injections was given and the effect was measured 5 min after each injection. Results are expressed as mean (± S.E.) with 6 to 12 mice/group.
the effects of nicotine and lobeline such as their actions on catecholamines, in particular with respect to its hypothermic and hypomotilic actions. For instance, contrary to nicotine, lobeline-evoked striatal dopamine and hippocampal norepinephrine release were calcium-independent (Clarke and Reuben, 1996; Grady et al., 1992). In addition, lobeline potently inhibits dopamine uptake into synaptic vesicles (L. Dwoskin, personal communication). These reports suggest that different mechanisms are responsible for the effects of nicotine and lobeline on neurotransmitter release. In addition, lobeline has been reported to displace $[^{3}H]$MK-801 binding from cortical membranes with an IC$_{50}$ of 25 $\mu$M (Aizenman et al., 1991), leading to speculations that NMDA receptors may be involved in lobeline-induced pharmacological effects. However, the fact that MK-801-like compounds produce a different behavioral profile than lobeline and that mecamylamine which displaced $[^{3}H]$MK-801 binding (Court et al., 1990) failed to block lobeline's effects argues against an NMDA-receptor mediated mechanisms of action.

Although it is possible that lobeline-induced in vivo desensitization in the different pharmacological measures would have been observed at different doses or at different time points, we obtained no evidence that revealed nicotine-induced desensitization after systemic and spinal administration (Damaj et al., 1996). The failure to observe lobeline-induced in vivo desensitization contrasts with the desensitization that has been reported with this compound in the nicotinic cholinergic receptor at the neuromuscular junction (Volle and Reynolds, 1973), but is consistent with a report that acute tolerance did not develop to lobeline-induced hypothermia in mice after s.c. administration (Decker et al., 1994). Furthermore, the lack of desensitization could account for the absence of up-regulation of $[^{3}H]$-nicotine and $[^{125}I]$-a-BGTX binding sites in mouse brain after chronic infusion of lobeline (Bhat et al., 1991). These results should be interpreted cautiously because very little is known about the pharmacokinetic properties of lobeline in animals (such as distribution and accumulation particularly after repeated injections). Overall our findings suggest that nicotine and lobeline differ in their ability to desensitize central nicotinic receptors.

To our surprise, the blockade of nicotine’s effects by lobeline observed at the $\alpha_{4}\beta_{2}$ expressed receptor was not mimicked by an antagonism of nicotine’s behavioral actions in vivo. The discrepancy between the in vitro and the in vivo results is difficult to explain. Although, pretreatment with lobeline was reported to cause an attenuation of some of the effects of nicotine (lethality, seizures, cardiovascular effects) after i.c.v. and i.t. administration (Abood et al., 1988; Khan et al., 1994), different lobeline responses were measured in our tests. In addition, lobeline failed to block nicotine discriminative stimulus after s.c. administration in rats (Reavill et al., 1990). It also should be noted that the interaction of lobeline with nicotine seen in the oocyte system, may not be of the same nature at the neuronal receptor which contains $\alpha_{4}\beta_{2}$ subunits. Further investigations are needed to probe
the mechanisms of the antagonism observed in the oocyte expressed receptor.

In contrast to acute administration, tolerance developed to lobeline after chronic s.c. injections. Lobeline’s dose-response curves evaluated in all tests were shifted to the right by a factor of 3- to 4-fold, comparable to that observed with nicotine. One would conclude that the nicotinic receptor subtype that mediates lobeline’s actions is undergoing adaptation after chronic but not after short-term exposure in a separate fashion from nicotine. However, the fact that cross-tolerance between nicotine and lobeline develops, supports the idea of a common mechanism of adaptation of the two drugs. In general, adaptation after chronic exposure of a drug occurs usually at the receptor level and/or postreceptor events such as second and third messengers systems. In the case of lobeline, chronic infusion of the drug neither altered the number of nicotinic receptors (labeled by [125I]-αBGTX and [3H]-nicotine) nor their binding affinities in mouse brain areas (Bhat et al., 1991). However, after chronic exposure to lobeline, one may speculate that the nicotinic ion channel may be allosterically modified resulting in a nonfunctional receptor that may not be detectable by conventional binding techniques. Moreover, an adaptation of the signal transduction systems linked to nicotinic receptors after chronic treatment may explain the apparent tolerance observed in our studies. However, it should be noted that such adaptation may not be receptor mediated. Indeed, tolerance to the behavioral effects of nicotine has been shown to be influenced by both pharmacological (nonassociative) and learning (associative) processes. It is reported that tolerance to nicotine-induced antinociception in rats may be influenced by learning (Epstein et al., 1989) and that the release of corticosterone could contribute to the development to some of nicotine’s effects after chronic injection of the drug (Caggiula et al., 1991, 1993). Therefore, then cross-tolerance between nicotine and lobeline may have arisen from a shared reduction in response generated by environmental cues and hormonal effects.

Our overall results demonstrate the complexity of the interaction between nicotinic ligands with their receptors. Such interaction seems to be ligand-dependent that may depend on several factors such as receptor localization, subtype specificity, antagonist sensitivity, the capacity of the ligand to desensitize or to produce tolerance. In this context, lobeline is a unique ligand that interacts with nicotinic receptors in a manner distinct from nicotine. Lobeline may be binding to [3H]-nicotine binding sites in a similar fashion to nicotine with different consequences, or it is interacting with mecamylamine-insensitive nicotinic sites. Clearly, lobeline along with other ligands such as cytisine, may serve as unique tools for unraveling the complexities of neuronal nicotinic receptors. Clearly, understanding the mechanisms of action of nicotinic ligands has a significant impact on their clinical potential because preclinical and clinical evidence support the potential role of nicotinic receptors in smoking cessation therapy and in a number of CNS disorders.

Acknowledgments

The authors greatly appreciate the technical assistance of Ming-Fei Yin.

References


Table 5

Summary of the potency of lobeline and nicotine in cross-tolerance experiments*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Challenge</th>
<th>Body Temperature</th>
<th>Locomotor Activity</th>
<th>Tail-Flick</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-tolerance to nicotine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>Nicotine</td>
<td>0.85 (0.5–1.5)</td>
<td>0.40 (0.2–1.0)</td>
<td>0.85 (0.4–1.5)</td>
</tr>
<tr>
<td>Lobeline (15)</td>
<td>Nicotine</td>
<td>2.8 (1.8–4.2)</td>
<td>1.2 (0.9–1.5)</td>
<td>1.8 (1.2–2.7)</td>
</tr>
<tr>
<td>Nicotine (2)</td>
<td>Nicotine</td>
<td>3.0 (1.9–4.8)</td>
<td>1 (0.4–2.4)</td>
<td>2.9 (2.0–3.9)</td>
</tr>
</tbody>
</table>

*ED50 (±CL) values were calculated from the dose-response curve of the respective compounds and are expressed in mg/kg. Each dose group included six to eight animals.


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