Differences Between the Antinociceptive Effects of the Cholinergic Channel Activators A-85380 and (±)-Epibatidine in Rats

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ABSTRACT

(±)-Epibatidine (EPIB) and A-85380 are nicotinic acetylcholine receptor (nAChR) agonists that bind to the agonist ([3H]cytisine) binding site with 40 to 50 pM affinity but have different affinities in nAChR subtype selective functional receptor assays. In vivo EPIB was more (23-fold) potent than A-85380 in reducing open field activity and more (12-fold) potent in reducing nociception in the formalin test of persistent chemical pain. In the rat hot box test of thermal acute pain, both compounds produced antinociception, as indicated by an increase in the paw withdrawal latency, however EPIB was a ~33-fold more potent than A-85380 (ED50 = 0.004 and 0.11 μmol/kg, i.p., respectively). The systemic effects of both nAChR agonists were blocked by central (i.c.v.) administration of the nAChR antagonist chlorisondamine suggesting a central site of action for these compounds. Injections of EPIB (0.0013 to 0.013 nmol) and A-85380 (0.013 to 0.13 nmol) directly into the nucleus raphe magnus (NRM) were also effective in the hot box and could be blocked by coadministration of the nAChR antagonists chlorisondamine (0.23 nmol) or mecamylamine (0.8 nmol). The NRM was found to be critical for the antinociceptive effects of systemic EPIB but not for A-85380 in that NRM injections of either mecamylamine (0.8 nmol) or lidocaine (74 nmol) blocked the antinociceptive effects of systemic (i.p.) EPIB but not those of A-85380. These results suggest that A-85380 may act at multiple sites both within and outside the NRM, whereas EPIB acts largely via descending inhibitory pathways arising from the NRM.

EPIB, an nAChR ligand, is ~20 times more potent than (−)-nicotine in binding to the alpha-4 beta-2 nAChR subtype, the predominant high affinity binding site in the brain and elicits a variety of in vitro and in vivo effects (Damaj et al., 1994, 1996; Qian et al., 1993; Sullivan et al., 1994b), including effects on open field activity and antinociception. EPIB also potently interacts with several of the other major nAChR subtypes, including alpha-7, alpha-3 beta-4 and alpha beta delta gamma (Sullivan et al., 1994a, 1994b). This has led to the search of novel nAChR agonists that retain antinociceptive activity and are more subtype selective than EPIB. A-85380 (Abreo et al., 1996), which recently became commercially available (R.B.I.), binds with similar affinity to the nAChR alpha-4 beta-2 subtype as EPIB in the cytisine binding assay, is (2–7-fold) less potent than EPIB at the alpha-7 nAChR as identified by α-bungarotoxin binding in rat brain and K28 cell membranes and is far less potent (>100-fold) than EPIB in displacing α-bungarotoxin binding to muscular-type receptor in Torpedo californica electroplax (Sullivan et al., 1994b, 1996). The nAChR subtype responsible for the analgesic actions of nAChR agonists has not been identified; thus, the relative binding at different subtypes by EPIB and A-85380 at nAChRs may result in differential effects in behavioral measures, including locomotor activity and antinociception.

This series of experiments therefore was designed to compare the antinociceptive and motoric effects of EPIB and A-85380. The relative potency of these two nAChR agonists was examined in locomotor activity and two models of pain perception [i.e., the hot box (acute pain) and the formalin test (persistent pain)]. A more complete evaluation of the mechanism of action was accomplished in the hot box by establishing dose-response curves for both the systemic (i.p.) and central (NRM) effects of EPIB and A-85380. In centrally mediated pain perception, the pathway from the pedunculopontine tegmental nucleus to the NRM has been shown to be very sensitive to the actions of (−)-nicotine (Iwamoto, 1990). It has been postulated that these antinociceptive effects are the result of stimulation of presynaptic nAChRs in the NRM to release ACh. This released ACh then stimulates postsynaptic muscarinic receptors, which activate descending pathways to the dorsal horn of the spinal cord (Iwamoto, 1989, 1990; Rogers and Iwamoto, 1993). To establish a role for the NRM, the noncompetitive nAChR antagonist MEC was used.

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ABBREVIATIONS: EPIB, (±)-epibatidine; CHLOR, chlorisondamine; i.c.v., intracerebral ventricular; LIDO, lidocaine; MEC, mecamylamine; nAChR, nicotinic acetylcholine receptor; NRM, nucleus raphe magnus.
to block the nAChRs in the NRM before a systemic injection of EPIB or A-85380. Also, LIDO, a local anesthetic, was used to block any involvement of other systems within the NRM before systemic injections of EPIB or A-85380.

Methods

Animals

Male Sprague-Dawley rats weighing 250 to 400 g (Charles River, Portage, MI) were used for all experiments. These animals were housed in AAALAC-approved facilities at Abbott Laboratories in a temperature-regulated environment with lights on between 7:00 a.m. and 8:00 p.m. All testing was conducted during the light portion of the cycle. For all experiments that used implanted cannula, animals were singly housed. In all other experiments, rats were group housed four to a cage. All testing was done following procedures outlined in protocols approved by Abbott’s Institutional Animal Care and Use Committee.

Behavioral Procedures

Open field activity. Vertical and horizontal activity was monitored in a 41 cm × 41 cm open field. After systemic injection of either EPIB or A-85380, animals were placed into activity chambers (Flex Field system; San Diego Instruments; San Diego, CA), and activity was monitored for 60 min after treatment.

Formalin test. This test has been previously described (Dubuisson and Dennis, 1977). Briefly, after a 20-min acclimation period to individual cages, rats were injected with either drug or vehicle. Five minutes later, 50 μl of a 5% formalin solution was injected subcutaneously into the dorsal aspect of one of the rear paws, and the rats were then returned to the clear observation cages suspended above mirror panels. Rats were observed for what has been defined as phase 2 (the 20-min period of time from 30 to 50 min after formalin injection). The investigator recorded nocifensive behaviors in the injected paw of four animals during the session by observing each animal for one 15-sec observation period during each 1-min interval. Nocifensive behaviors recorded included flinching, licking or biting the injected paw. Each response was recorded, and these were summed for statistical analysis.

Rat hot box. For assessing nociceptive responses to an acute thermal stimulus, a commercially available paw thermal stimulator (i.e., rat hot box) was used (Anesthesiology Research Laboratory, Department of Anesthesiology, University of California at San Diego, La Jolla, CA). This device and procedure have been detailed previously (Hargreaves et al., 1988). Briefly, rats were acclimated for 30 min in Plexiglas enclosures placed on a glass surface, the temperature of which is maintained at 30°C. Antinociception is measured as the latency (mean for left and right paws) for the rat to remove its paw when heated by light from a focused projector lamp (current set at 4.8 A). To avoid tissue injury, maximal response latency of the heat was set at 20.5 sec. Measurements of the response latency were made after drug treatment. For NRM administration, the response latencies were measured at 5, 15 and 30 min, and for systemic administration, measurements were taken at 15, 30 and 45 min after treatment.

Cannula Implantation and Lesion Procedure

Rats were anesthetized with sodium pentobarbital (55 mg/kg i.p.). Animals were placed in a David Kopf student stereotaxic instrument (Tujunga, CA) with the skull on an even horizontal plane. For central CHLOR administration, rats were injected with either PBS or CHLOR (23 nmol in 5 μl over 60 sec) using a 27-gauge injector. For these i.c.v. injections, the coordinates from bregma were 1.0 mm posterior, 1.5 mm lateral and 4.5 mm ventral from the skull, according to the atlas of Paxinos and Watson (1997). For NRM injections, the coordinates from intra-aural zero were 2.3 mm posterior, 0.0 mm lateral and 0.5 mm ventral, according to the atlas of Paxinos and Watson (1997). Animals were implanted with a C315G (26-gauge) guide cannula (Plastics One, Roanoke, VA) cut to 7 mm. The guide cannula was implanted so that the injector cannula (33 gauge) extended 4 mm beyond the tip of the guide. Animals recovered on a warming plate and were placed in single housing for ≥10 days before experimentation. For the NRM injections, 0.3 μl was delivered over 60 sec, and the cannula left in place for another 40 sec.

Dose-Response Effect of EPIB and A-85380 and Central Blockade

A dose-response curve was analyzed after systemic treatment with either A-85380 (0.1–3.0 μmol/kg) or EPIB (0.0016–0.05 μmol/kg) using a quantal measure ED50 and ED84 values (Litchfield and Wilcoxon, 1948). To determine whether the antinociception for A-85380 and EPIB was mediated via central nAChRs, the ED84 dose for systemic treatment for each drug was tested in rats previously injected (5 days prior) with either CHLOR or PBS (vehicle) into the cerebral ventricles. A within subject testing design was used. Each rat was tested with EPIB (0.011 μmol/kg), A-85380 (0.56 μmol/kg) and vehicle (1.0 ml/kg). A counterbalanced order of presentation of treatment was used with 2 days off between treatments.

Central Effects of EPIB and A-85380 and Central Blockade

A dose-response curve was generated for centrally administered A-85380 (0.013–0.13 nmol) or EPIB (0.0013–0.013 nmol) into the NRM. The central blockade of this response with coinjection of CHLOR (0.23 nmol) or MEC (0.8 nmol) with A-85380 (0.04 nmol) or EPIB (0.013 nmol) was conducted to establish that antinociception via neurons within the NRM were mediated by nAChRs. However, brain areas other than the NRM may be responsible for the effects of systemic administration of A-85380 or EPIB. Thus, to investigate whether nAChR receptors in the NRM alone are responsible for antinociception after systemic EPIB and A-85380, the nAChR channel blocker MEC (0.8 nmol) was injected into the NRM 2 min before the systemic administration of EPIB (0.011 μmol/kg) or A-85380 (0.56 μmol/kg). The systemic administration of a higher dose of EPIB (0.017 μmol/kg) was also tested with an NRM injection of MEC (0.8 nmol).

Effect of NRM Inactivation on EPIB and A-85380 Antinociception

The role of the NRM was further characterized by examining systemic antinociception with A-85380 and EPIB after deactivation of the neurons within the NRM, through the use of a microinjection of LIDO (74 nmol/rat) (Pertovaara et al., 1996). LIDO (0.3 μl) was injected 2 min before the systemic administration of EPIB and A-85380.

Compounds

A-85380 [3-(2S)-azetidinylmethoxy]pyridine dihydrochloride, and (z)-EPIB were obtained from R.B.I. (Natick, MA). LIDO and mecamylamine hydrochloride were obtained from Sigma Chemical (St. Louis, MO). Chlorisondamine was obtained from Ciba-Geigy.

Data Analysis

Data were analyzed by ANOVA and subsequent post hoc analysis with Fisher’s Protected Least Significant Difference test performed where appropriate. Quantal analysis of the data (using an effects criterion of a score beyond the extreme control scores) was used to calculate an ED50 and ED84 by the method of Litchfield and Wilcoxon (1948) (Pharm/PCS version 4.2; MicroComputer Specialists, Philadelphia, PA).

Results

Locomotor activity. The effects of systemic treatment with EPIB (0.0017, 0.0054 and 0.017 μmol/kg) and A-85380...
(0.03, 0.1 and 0.3 \mu mol/kg) on vertical activity for 60 min after treatment are shown in 15-min blocks in figure 1, A and B, respectively. Vehicle control rats showed a typical habituation effect over the 60-min period. As can be seen in figure 1C during the first 15 min, the period of maximal drug effect, the potency ratio shows a 24-fold (95% CI = 6.27–90.17) difference in the A-85380 dose-response curve from that of EPIB. There was a dose-response effect on vertical activity for both EPIB \[ ED_{50} = 0.0018 \mu mol/kg \ (95\% \ CI = 0.0006–0.0051) \] and A-85380 \[ ED_{50} = 0.042 \mu mol/kg \ (95\% \ CI = 0.019–0.096) \]. The effect on horizontal activity paralleled the vertical activity (data not shown). Only the highest dose of EPIB remained significantly lower than control for the complete 60-min session.

**Formalin test.** There was a dose-dependent effect of EPIB \[ (0.0053–0.053 \mu mol/kg) \ \[ ED_{50} = 0.017 \mu mol \ (95\% \ CI = 0.005–0.058) \] treatment to decrease the nocifensive responses after a formalin injection into the hind paw (fig. 2) with the two highest doses of EPIB \[ (0.017 and 0.053 \mu mol/kg) \] different from control \( (P < .01) \). There was a similar effect for A-85380 \[ (0.1–1.0 \mu mol/kg) \ \[ ED_{50} = 0.21 \mu mol/kg \ (95\% \ CI = 0.08–0.57) \] and 0.3 and 1.0 \mu mol/kg were different \( (P < .05) \) from control. There was a potency ratio difference of 12.3 \( (95\% \ CI = 2.5–61) \) between A-85380 and EPIB.

**Hot box.** It was observed that the effects of EPIB were of short duration in hot box test in contrast to the longer-lasting effects of A-85380. For systemic administration of EPIB, the antinociceptive effect was maximal at 15 min, reduced at 30 min and absent at 45 min (fig. 3B). For NRM administration, the effect was maximal at 5 min, reduced at 15 min and at 30 min (fig. 4B). Therefore, to make a true comparison of these compounds, data for only the first 15 min after systemic administration and 5 min after NRM administration are presented.

**Hot box and systemic administration.** Systemic administration of either EPIB or A-85380 increased paw withdrawal latencies in the hot box. The dose response curves are shown in figure 3A, where the data are presented as difference from saline control values. There was an overall effect of treatment \( F(4,36) = 33.814, P = .0001 \), and post hoc analysis revealed differences \( (P < .05) \) at doses of 0.0053 \mu mol/kg and higher. The ED\(_{50}\) value was 0.003 \mu mol/kg \( (95\% \ CI = 0.0015–0.07) \), and the ED\(_{84}\) value was 0.01 \mu mol/kg \( (95\% \ CI = 0.006–0.18) \). For A-85380, there was an overall significant effect of treatment \( F(4,39) = 10.908, P = .0001 \), and post hoc analysis revealed differences \( (P < .05) \) at doses of 0.3 \mu mol/kg and higher. The ED\(_{50}\) value was 0.11 \mu mol/kg \( (95\% \ CI = 0.036–0.326) \), and the ED\(_{84}\) value was 0.56 \mu mol/kg \( (95\% \ CI = 0.11–2.89) \). There was a significant difference in the dose-response curves with a potency ratio of 33 \( (95\% \ CI = 8.5–130.2) \), with EPIB therefore being 30 times more potent than A-85380.

**Hot box and central blockade.** The effects of pretreatment with CHLOR (23 nmol) or PBS into the cerebral ventricles on the antinociceptive effects of the ED\(_{84}\) dose of either A-85380 \( (0.56 \mu mol/kg) \) or EPIB \( (0.011 \mu mol/kg) \) are shown in Fig. 1.
There were significant effects of CHLOR treatment \( F(1,14) = 54.308, P < .0001 \) and a CHLOR treatment interaction \( F(2,14) = 5.736, P = .0082 \). There were significant effects of EPIB and A-85380 \( F(1,14) = 19.286, P = .0001 \). Significant differences \((P = .0001)\) from vehicle control were produced by both EPIB and A-85380 in PBS-treated rats, but this effect was blocked by CHLOR treatment.

**Hot box and intra-NRM nAChR agonist injection.** Dose-response curves for the effect on paw withdrawal latency after central administration into the NRM for both EPIB (0.0013–0.013 nmol) and A-85380 (0.04 nmol/rat) are shown in figure 4A. The data are presented as differences from a saline control value \((\pm \text{S.E.M.)}}\). B, Time course effects for similarly effective doses for EPIB (0.013 nmol/rat) and A-85380 (0.04 nmol/rat) in the first 5 min. \(*, P < .05 \) difference from control, \( n = 8 \)/group.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>EPIB (0.011 ( \mu )mol/kg)</th>
<th>A-85380 (0.05 ( \mu )mol/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9.0 ± 0.6</td>
<td>14.6 ± 0.8 (^a)</td>
<td>16.2 ± 1.2 (^a)</td>
</tr>
<tr>
<td>CHLOR</td>
<td>7.4 ± 0.7</td>
<td>9.5 ± 0.6</td>
<td>9.3 ± 0.8</td>
</tr>
</tbody>
</table>

\(^a\) \( P < .05 \) difference from the saline-treated rats, \( n = 8 \)/group.

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**Fig. 2.** The dose-response curves after i.p. administration of EPIB and A-85380 in the formalin test. The data are represented as the difference from the saline control nocifensive score \((\pm \text{S.E.M.)}}\). \( *, P < .05 \) difference from control, \( n = 5–7 \)/group.

**Fig. 3.** A, The dose-response curves 15 min after i.p. administration of EPIB and A-85380 on paw withdrawal latency in the rat hot box. The data are represented as the difference from a saline control value \((\pm \text{S.E.M.)}}; \( n = 7–8 \)/group for EPIB and A-85380 treatment, \( n = 11 \) for vehicle control. B. Time course effects for similarly effective doses for EPIB (0.017 \( \mu \)mol/kg) and A-85380 (1.0 \( \mu \)mol/kg) in the first 15 min, \( n = 8 \)/group. \( *, P < .05 \) difference from control.

**Fig. 4.** A, The dose-response curves 5 min after NRM administration of EPIB and A-85380 on paw withdrawal latency in the rat hot box. The data are represented as differences from a saline control value \((\pm \text{S.E.M.)}}. B. Time course effects of similarly effective doses for EPIB (0.013 nmol/rat) and A-85380 (0.04 nmol/rat) in the first 5 min. \(*, P < .05 \) difference from control, \( n = 8 \)/group.
**TABLE 2**
The effect of NRM injection of either (A and C) EPIB or (B and C) A-85380 on paw withdrawal latency (±S.E.M.) in the rat hot box 5 min after coadministration of (A and B) CHLOR (0.23 nmol) or (C) MEC (0.8 nmol).

<table>
<thead>
<tr>
<th>NRM Injection</th>
<th>Latency (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>8.4 ± 0.3</td>
</tr>
<tr>
<td>CHLOR</td>
<td>10.2 ± 1.3</td>
</tr>
<tr>
<td>EPIB</td>
<td>13.2 ± 0.9</td>
</tr>
<tr>
<td>CHLOR/EPIB</td>
<td>9.1 ± 0.6</td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td>CHLOR</td>
<td>8.5 ± 0.4</td>
</tr>
<tr>
<td>A-85380</td>
<td>12.5 ± 1.0</td>
</tr>
<tr>
<td>CHLOR/A-85380</td>
<td>9.4 ± 0.8</td>
</tr>
<tr>
<td>C</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>8.9 ± 0.69</td>
</tr>
<tr>
<td>MEC</td>
<td>8.2 ± 0.81</td>
</tr>
<tr>
<td>EPIB</td>
<td>14.7 ± 1.17</td>
</tr>
<tr>
<td>MEC/EPIB</td>
<td>9.1 ± 0.56</td>
</tr>
<tr>
<td>A-85380</td>
<td>14.7 ± 1.23</td>
</tr>
<tr>
<td>MEC/85380</td>
<td>8.2 ± 0.36</td>
</tr>
</tbody>
</table>

The same controls were used in (C) for both A-85380 and EPIB. *P < .05 difference from PBS control, n = 7–8/group.

**Discussion**

The acute effects of (-)-nicotine (Clarke and Kumar, 1983a, 1983b; Decker and Majchrzak, 1993; Martin et al., 1990) and EPIB (Damaj et al., 1996; Mennzagi et al., 1996) on locomotor activity have been well documented; there is initial depression of horizontal and vertical activity. Similarly, in mice, EPIB has been shown to reduce locomotor activity (Damaj et al., 1994; Sullivan et al., 1994b). A dose-dependent decrease in vertical activity was observed with both EPIB and A-85380. The effects at the highest dose of EPIB (0.017 μmol/kg) did not recover over the full 60 min of testing. The potency difference for vertical activity in the first 15 min between EPIB and A-85380 was ~23-fold.

In the formalin test, a model of persistent pain, EPIB and A-85380 reduced the number of nocifensive responses after formalin injection in the hind paw. There was a 12-fold shift...
similar to that seen in locomotor activity for the dose-response curve of A-85380 relative to EPIB. In the rat hot box test, for A-85380 or EPIB there was a dose-dependent increase in paw withdrawal latencies. Comparison of the dose response curves indicates that EPIB was ~33-fold more potent than A-85380.

It is evident that both EPIB and A-85380 reduce locomotor activity at doses that produce antinociception, potentially interfering with the ability of the animal to respond to the nociceptive stimulus. However, A-85380 antinociception is still present in the hot box and in the formalin test at a time (30–50 min after injection) when the effects on locomotor activity are no longer evident. At least for A-85380, there appears to be a dissociation between the effects on locomotor activity and antinociception.

The 10- to 30-fold greater potency of EPIB relative to A-85380 in measures of activity and antinociception is consistent with the 10-fold difference between EPIB and A-85380 also observed in rats trained to discriminate (−)-nicotine from saline in a drug discrimination paradigm (Brioni et al., 1996). These 10- to 30-fold in vivo potency differences contrast with the similar 40 to 50 pM binding of these compounds at the high affinity cytisine site predominately in rat brain (probably alpha-4 beta-2; Flores et al., 1992) and to the 150-fold difference in affinity at the neuromuscular type nAChR (Sullivan et al., 1994b, 1996). However, functional in vitro data indicate a different pattern of results with these compounds. Despite similar affinity at alpha-4 beta-2 subtype expressed in K-177 cells in binding assays, EPIB is ~40-fold more potent than A-85380 in a functional assay in this cell line (Gopalakrishnan et al., 1995; Sullivan et al., 1994b, 1996). This might explain why EPIB is more potent than A-85380 in vivo. Similarly, EPIB is ~7-fold more potent than A-85380 in activating the alpha-7 subtype and >100-fold more potent in activating the ganglionic like nAChR expressed in IMR-32 cells (Briggs et al., 1995; Sullivan et al., 1994b, 1996). Comparison of these in vitro and in vivo data suggest that it is not likely that the effects on activity or antinociception or the discriminative stimulus properties of these compounds are the result of activation of the striated muscle or autonomic ganglion type receptors. However, further studies are required to clarify the subtype(s) involved.

Systemic antinociceptive effects of both EPIB and A-85380 are blocked by i.c.v. pretreatment with the nAChR antagonist CHLOR. After a single i.c.v. treatment, CHLOR has long-lasting effects (weeks) at central nAChRs (Decker and Majchrzak, 1993; El-Bizri and Clarke, 1994; Kumar et al., 1987), suggesting a central nAChR site of action for the antinociceptive effects of these two compounds.

When EPIB and A-85380 were injected into the NRM, there again was a similar difference in the dose-response curve, with minimally effective doses of 0.0013 and 0.04 nmol, respectively. However, with the NRM injections, there was a reduced effect with the highest dose (0.13 nmol) of A-85380 tested. Thus, the potency difference between these two compounds observed with systemic administration is largely maintained with direct central administration, suggesting that pharmacokinetic differences may not account for the potency differences observed with systemic injections. The effect of NRM EPIB and A-85380 injections was also blocked by a coinjection of the nAChR antagonist CHLOR as well as the noncompetitive nAChR antagonist MEC, again showing an nAChR-mediated antinociceptive response. This is similar to a report of antinociceptive effects of (−)-nicotine (Iwamoto, 1990) injected into the NRM that can be blocked with the coadministration of MEC. Coinjection of the nicotinic competitive antagonist dihydro-β-erythroidine (ED50 0.35 nmol) also blocks centrally administered A-85380 (Curzon et al., 1998).

When MEC was injected into the NRM just before the systemic administration of either EPIB or A-85380, only the effect of EPIB was blocked. The reason A-85380 was not blocked by NRM MEC does not appear to be simply that the dose of EPIB was too low because antinociception after a higher dose of EPIB was also blocked by the same dose of MEC. A similar profile was obtained for the NRM blockade by the local anesthetic LIDO in that antinociceptive effects of systemic EPIB were blocked, whereas those of systemic A-85380 were not. These data suggest that although antinociception after systemic (−)-nicotine or EPIB is mediated in large part within the NRM, the antinociception after the novel azetidine nAChR agonist A-85380 involves systems in addition to the NRM.

It is evident from these experiments that EPIB and A-85380 are very potent nAChR agonists that produced similar effects in rats on locomotor activity and in two models of antinociception. However, even though A-85380 and EPIB bind with equal affinity to the [3H]cytisine binding site, they have different potencies in vitro assays of nAChR subtype function that could account for the difference in the potency between EPIB and A-85380 in these in vivo experiments. In addition to differences in potency, antinociception in the hot box after systemically administered EPIB, but not A-85380, requires activation of the NRM. Thus, the nAChR-mediated antinociceptive effects of A-85380 involve other central pathways yet to be identified.
The authors would like to thank Dr. M. W. Holladay for confirming the purity of A-85380 obtained from R.B.I.

References


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