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Is Epibatidine Really Analgesic? Dissociation of the Activity, Temperature, and Analgesic Effects of (±)-Epibatidine

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BANNON, A. W., K. L. GUNTHER AND M. W. DECKER. *Is epibatidine really analgesic? Dissociation of the activity, temperature, and analgesic effects of (±)-epibatidine.* PHARMACOL BIOCHEM BEHAV 51(4) 693-698, 1995.— The experiments in the present study were designed to determine if the activity, temperature, and analgesic effects of (±)-epibatidine treatment could be dissociated. Initially (i.e., 15 min) (±)-epibatidine treatment (0.1 μmol/kg = 28 μg/kg, IP) impaired rotorod performance, decreased activity, decreased temperature, and increased jump latency (e.g., analgesic effect). For the remaining time points measured (i.e., 30, 60, and 120 min), activity and temperature remained significantly reduced. In contrast, by 120 min (±)-epibatidine's effects on rotorod performance and analgesia (jump latency) were not observed. When administered after (±)-epibatidine (0.05 μmol/kg, IP), mecamylamine treatment (5 μmol/kg = 1 mg/kg, IP) produced a potentiation of analgesia. This potentiation effect was not observed on activity and temperature measures. The effect of (±)-epibatidine treatment (0.1 μmol/kg, IP) was also determined in mice with central nicotinic receptor blockade induced by treatment with chlorisondamine (23 μmol/kg = 10 mg/kg, IP). An (±)-epibatidine-induced reduction in activity was not attenuated in chlorisondamine-treated mice and only a minimal effect was observed on (±)-epibatidine-induced hypothermia in chlorisondamine-treated mice. In contrast, in chlorisondamine-treated mice (±)-epibatidine's analgesic effect was attenuated. Taken together, these data suggest that various centrally mediated effects of (±)-epibatidine can be dissociated.

Epibatidine Nicotinic acetylcholine receptor Analgesia Temperature Locomotor activity Chlorisondamine Mecamylamine

EPIBATIDINE is an alkaloid isolated from the skin of an Ecuadorian frog and has been described as a potent analgesic (3,14). The antinociceptive activity of epibatidine was reported to be 80 times more potent than morphine (15), but was not blocked by pretreatment with naloxone (12). Also, epibatidine-induced analgesia was attenuated by pretreatment with mecamylamine, but not hexamethonium, suggesting a central nicotinic mechanism of action.

Consistent with in vivo results, epibatidine has been reported to have little activity at opioid receptors (2). In vitro, epibatidine was found to be a potent inhibitor of binding of the nicotinic ligand [^{3}H]cytisine (2,12). In addition, using functional assays such as sodium influx (2) and $^{86}Rb^+$ efflux (15), epibatidine was shown to be a more potent agonist than (−)-nicotine. For other receptor types, including adrenergic (α_1 and α_2), BK-bradykinin, benzodiazepine, serotonergic, muscarinic (M_2) , and dopaminergic (D_1 and D_2), epibatidine has been reported to be ineffective in displacing ligands for each respective neurotransmitter receptor (2). Thus, these findings indicate that epibatidine can produce analgesia that is possibly mediated by activation of central nicotinic acetylcholine receptors (nAChRs).

In addition to analgesic effects, epibatidine reduces motor activity and temperature at doses lower than those producing antinociception (15). (−)-Nicotine, as well as other nAChR ligands, has also been shown to have effects on activity and temperature (10). Conceivably, activity and/or temperature effects of (±)-epibatidine could contribute to the apparent analgesic effect observed in experimental models. For example, in the hot plate paradigm, an animal is placed on a heated surface and the latency to make a response, such as a jump from the heated surface, is recorded. If a compound like epi-

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batidine decreases motor activity, an increase in jump latency could be erroneously interpreted as an analgesic effect. Likewise, it is plausible that a compound that decreases body temperature may reduce the animal's sensitivity to heat, producing an increase in jump latency. Thus, both factors may influence analgesia, as assessed by the hot plate paradigm.

Overall, the goal of this research was to provide evidence as to whether or not the analgesic effects of (±)-epibatidine could be separated from the activity and temperature effects. The studies conducted included determining the effectiveness of mecamylamine in reversing (±)-epibatidine-induced alterations of locomotor activity, temperature, and nociception. This was accomplished by administering mecamylamine after (±)-epibatidine treatment (i.e., posttreatment); comparing the time course of (±)-epibatidine's effects on analgesia, rotorod performance, locomotor activity, and temperature; and investigating the effects of (±)-epibatidine treatment on locomotor activity, temperature, and analgesia in mice with central nicotinic receptor blockade. The nicotinic antagonists utilized were mecamylamine and chlorisondamine. Both of these antagonists have been shown to differentially attenuate (−)-nicotine-induced behavioral effects (6,9,11).

METHOD

Subjects

Male CD-1 mice (Charles River, Portage, MI) weighing 30–35 g were used in this experiment. The mice were housed 14 to a cage and maintained in a climate-controlled facility with a 12L : 12D cycle. In all experiments, individual animals were only tested once.

Compounds

(±)-Epibatidine was purchased from Research Biochemicals International (Natick, MA). Mecamylamine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO). Chlorisondamine was obtained from Ciba Geigy (Summit, NJ). All drugs were dissolved in saline and the volume of injection was 10 ml/kg. Doses were expressed as μmol/kg (see abstract for mg/kg equivalent).

Analgesia Testing

Analgesia was measured using an automated hot plate analgesia monitor (model #AHP16AN, Omnitech Electronics, Inc., Columbus, OH). The temperature of the hot plate was maintained at 55°C. Mice were placed on the hot plate and the latency until the 10th jump was recorded. Jumps were recorded by disruption of a photocell beam located 12.5 cm above the surface of the hot plate. Mice were removed from the apparatus either after 10 jumps were made or 240 s (i.e., cut-off) elapsed, whichever occurred first. The latency (seconds) until the 10th jump was used for statistical analysis. Based on our preliminary studies, latency until the 10th jump provided a consistent measure in the hot plate paradigm.

Activity and Temperature Measurement

Open field activity was monitored using photobeam activity monitors (San Diego Instruments, San Diego, CA). Horizontal activity was recorded for 5 min. Following activity monitoring, body temperature was taken. Body temperature was measured using a rectal probe inserted 3 cm into the rectum (YSI Tele-Thermometer, Yellow Springs Instrument Co., Inc., Yellow Springs, OH.).

Rotorod Performance

Motor coordination was measured using an automated rotorod (Omnitech Electronics, Inc., Columbus, OH). Time spent on the accelerating rotorod (0–40 rpm over 120 s) was used for analysis.

Time Course Studies of (±)-Epibatidine's Effects on Analgesia, Activity, Temperature, and Rotorod

Separate studies were conducted for each of the measures (i.e., analgesia, activity/temperature, and rotorod) using separate groups of mice for each time point. The dose of (±)-epibatidine utilized was 0.1 μmol/kg (IP) and mice were tested 15, 30, 60, and 120 min following drug treatment. Two saline-reated mice were tested at each time point and data from these mice were pooled for statistical analysis.

Effect of Mecamylamine Posttreatment on (±)-Epibatidine-Induced Changes in Nociception, Activity, and Temperature

Separate experiments were conducted for activity/temperature measures and analgesia. In each study, mice were randomly divided into five groups: 1) saline/saline (sal/sal); 2) saline/mecamylamine (sal/mec); 3) epibatidine/saline (epib/sal); 4) epibatidine/mecamylamine (epib/mec); or 5) mecamylamine/epibatidine (mec/epib). The first and second treatments were separated by 15 min. Except for the mec/epib group, all groups were tested 30 min following the first treatment (i.e., saline or epibatidine). In the mec/epib group (i.e., pretreatment), the mice were tested 30 min following (±)-epibatidine treatment. The dose of (±)-epibatidine utilized was 0.05 μmol/kg, IP. This dose of (±)-epibatidine was chosen based on the results of earlier studies in this laboratory with (±)-epibatidine in the hot plate paradigm (15). The dose of mecamylamine utilized was 5 μmol/kg, IP. In preliminary experiments in this laboratory, this dose of mecamylamine was found to be effective in attenuating (±)-epibatidine's effects on activity, temperature, and analgesia (unpublished observations).

Effect of Mecamylamine Pretreatment on (±)-Epibatidine-Induced Changes in Nociception, Temperature/Activity, and Rotorod Performance

Separate studies were conducted for each of the measures (i.e., analgesia, activity/temperature, and rotorod). For each of the studies, the first treatment, mecamylamine (5 μmol/kg, IP) or saline, was administered 15 min prior to the second treatment, (±)-epibatidine (0.1 μmol/kg, IP) or saline. Mice were tested 30 min later.

Effect of (±)-Epibatidine on Activity and Temperature in Chlorisondamine-Treated Mice

Mice were dosed with chlorisondamine (23 μmol/kg, IP) and maintained in their home cages for 5 days prior to testing. This treatment paradigm has been shown to produce a persistent blockade of central nAChRs with minimal effects on peripheral nAChRs (6). In addition, this type of chlorisondamine treatment has been shown to attenuate activity and temperature effects of (−)-nicotine (10). On the test day, control mice and chlorisondamine-treated mice were dosed with either saline or (±)-epibatidine (0.1 μmol/kg, IP). Activity measures and temperature measures were recorded 30 min later (see protocol above). For analgesia testing, control mice

and chlorisondamine treated-mice were dosed with either saline or (±)-epibatidine (0.1 μmol/kg, IP). Mice were tested for analgesia 30 min later.

Statistics

Statistical analyses were conducted utilizing StatView (Abacus Concepts, Inc., Berkeley, CA) on a Macintosh computer. All measures were analyzed using ANOVA followed by Fisher's Protected Least Significant Difference where appropriate. Differences were considered significant when $p < 0.05$.

RESULTS

The effects of (±)-epibatidine treatment (0.1 μmol/kg, IP) on locomotor activity, body temperature, rotorod performance, and analgesia are presented in Table 1. Using separate groups of animals, measures were taken 15, 30, 60, and 120 min following (±)-epibatidine treatment. (±)-Epibatidine produced significant reductions in both activity and temperature at all of the time points examined. The activity measures did recover slightly, but remained significantly decreased (64%) compared to control at the final time point (i.e., 120 min). Rotorod performance was impaired at 15 and 30 min following (±)-epibatidine administration, but reached control levels at 60 and 120 min. At 15, 30, and 60 min following (±)-epibatidine dosing, a significant increase in jump latency was found, with the maximal effect being at 30 min (160% increase). By 120 min, the analgesic effect was not observed. Thus, (±)-epibatidine's maximal effects on all parameters except activity was at 30 min. The maximum effect on activity was observed at the 15-min time point.

Figure 1 shows the effects of pre- and posttreatment with mecamylamine (5 μmol/kg, IP) on (±)-epibatidine-induced (0.05 μmol/kg, IP) changes in locomotor activity, temperature, and analgesia. For each of the parameters, an overall significant group effect was observed. Alone, mecamylamine treatment (i.e., sal/mec) produced no statistically significant effects on any of the parameters analyzed. (±)-Epibatidine treatment (i.e., epib/sal) produced a statistically significant 91% reduction in activity and significantly reduced temperature to 34.6 ± 0.2°C compared to 39.0 ± 0.2°C in saline-treated mice (i.e., sal/sal). A 90% increase in jump latency was observed in mice treated with (±)-epibatidine (0.05 μmol/kg, IP), but this increase did not reach statistical significance ($p < 0.07$). With mecamylamine posttreatment (i.e., epib/mec), an attenuation by 42% and 40% was observed for (±)-epibatidine-induced reductions of activity and temperature, respectively. In contrast, mecamylamine posttreatment (i.e., epib/mec) significantly potentiated the analgesic effect of (±)-epibatidine by 144%. Pretreatment with mecamylamine (i.e., mec/epib) significantly blocked (±)-epibatidine's effects on activity and temperature (96% and 89%, respectively) with a similar trend noted for (±)-epibatidine's effect on analgesia (78% attenuation).

Using a 0.1-μmol/kg dose (0.05 μmol/kg utilized in first mecamylamine experiment) of (±)-epibatidine, effects on locomotor activity, temperature, and analgesia were all significantly attenuated by pretreatment with mecamylamine (5 μmol/kg, IP). Alone, mecamylamine treatment showed no statistically significant effects on any of the parameters measured (data not shown).

In Figure 2, data are presented showing the effects of (±)-epibatidine (0.1 μmol/kg, IP) in mice pretreated with chlorisondamine (23 μmol/kg, IP). Compared to control mice, chlorisondamine treatment alone produced a modest 26% increase in locomotor activity, but had no effect on temperature. Following (±)-epibatidine administration, locomotor activity was significantly reduced by 90% in control mice and 85% in chlorisondamine-pretreated mice compared to saline-treated mice. (±)-Epibatidine treatment significantly reduced temperature to 34.4 ± 0.2°C in control mice compared to 39.3 ± 0.1°C in saline-treated mice, but a modest attenuation of this effect was observed in chlorisondamine-pretreated mice (35.3 ± 0.4°C). Following (±)-epibatidine treatment, a significant 153% increase in jump latency was observed in control mice. In chlorisondamine-pretreated mice, the analgesic effect was significantly attenuated by 61%.

DISCUSSION

Although there are several reports describing the analgesic effect of epibatidine, there are few studies that have investigated other in vivo effects (e.g., motor activity) of this compound. In particular, further consideration of epibatidine's effects on locomotor activity and temperature seems necessary because alteration of either of these parameters could potentially produce an analgesic response as assessed by most

TABLE 1

EFFECT OF (±)-EPIBATIDINE (0.1 μmol/kg, IP) ON LOCOMOTOR ACTIVITY, TEMPERATURE, ROTOROD PERFORMANCE, AND ANALGESIA 15, 30, 60, AND 120 MIN AFTER INJECTION

		(±) Epibatidine			
	Control	15 Min	30 Min	60 Min	120 Min
Activity # (counts)	910.8 ± 29.1	7.6 ± 4.3*	54.6 ± 20.4*	345.3 ± 115.6*	325.1 ± 109.9*
Temperature # (°C)	38.6 ± 0.1	33.4 ± 0.1*	32.4 ± 0.2*	34.3 ± 0.9*	33.6 ± 1.4*
Rotorod # (s)	102.0 ± 9.6	59.4 ± 14.4*	46.8 ± 7.0*	85.8 ± 11.7	81.7 ± 13.1
Jump latency # (s)	54.4 ± 5.1	130.9 ± 17.7*	141.3 ± 18.8*	124.5 ± 16*	88.8 ± 16.4

Values are expressed as mean ± SEM. Data for control values were pooled from saline-treated mice tested at each time point. Overall, (±)-epibatidine significantly affected all parameters: Activity ($n = 5$), $F(4, 27) = 30.61$, $p < 0.0001$; temperature ($n = 5$), $F(4, 26) = 26.05$, $p = 0.0001$; rotorod performance ($n = 7$), $F(5, 42) = 5.44$, $p < 0.0006$; and jump latency ($n = 14$), $F(4, 65) = 4.861$, $p < 0.0017$. At 60 min, the analgesic effect was dissociated from (±)-epibatidine's effect on rotorod performance. By 120 min, there was an apparent dissociation between analgesia and (±)-epibatidine's effects on activity and temperature.

*Significantly different from control ($p < 0.01$).

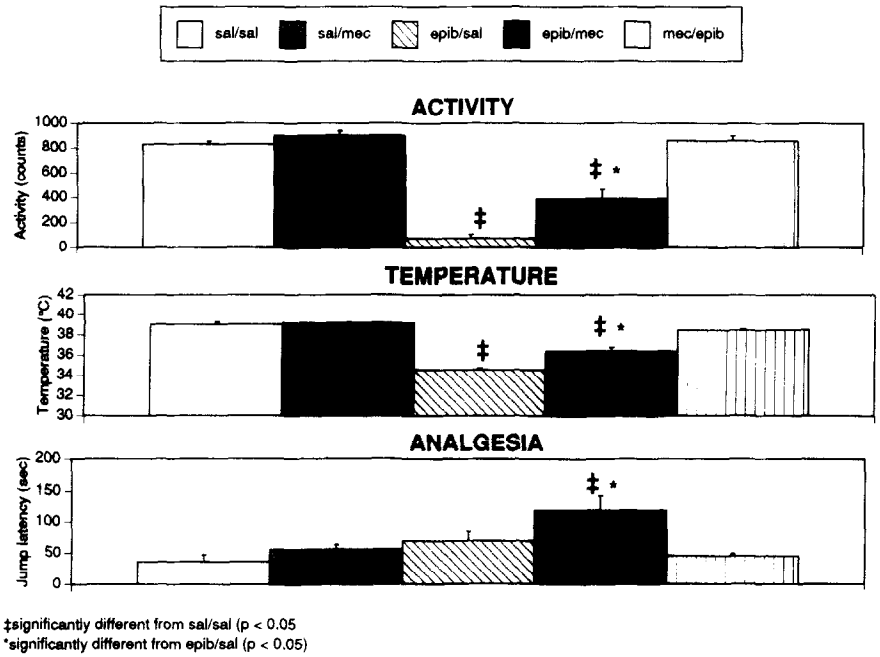

FIG. 1. Values are expressed as group means ± SEM (n = 8). Injections were separated by 15 min, and analgesia testing was conducted 30 min after the first treatment in all groups except in the mec/epib group. In the mec/epib group, analgesia testing was conducted 30 min following (±)-epibatidine treatment (5 μmol/kg, IP). ANOVA indicated a significant group effect for activity, $F(4, 35) = 61.49$, $p < 0.0001$, temperature, $F(4, 35) = 69.25$, $p < 0.0001$, and analgesia, $F(7, 48) = 4.046$, $p < 0.0015$. Mecamylamine pretreatment (5 μmol/kg, IP) blocked (±)-epibatidine's effects on all parameters. In contrast, posttreatment with mecamylamine had differential effects on (±)-epibatidine-induced analgesia and (±)-epibatidine's effects on activity and temperature. *Significantly different from epib/sal ($p < 0.05$); ‡significantly different from sal/sal ($p < 0.05$).

rodent models (e.g., hot plate). Clarke has speculated that (−)-nicotine's apparent analgesic effect observed in rodent models (e.g., tail flick) may actually be a function of other effects of (−)-nicotine such as inhibition of spinal reflexes (5). In the current study, separation of the activity, temperature, and analgesic effects was attempted using pharmacological manipulations. The nicotinic antagonists mecamylamine and chlorisondamine were utilized to produce nAChR blockade. In addition to differences with regard to the persistence of neuronal nAChR blockade, these two compounds have also been found to have divergent effects on attenuation of (−)-nicotine-induced behavioral effects [see (9)]. Use of these compounds in the present study helped to demonstrate that the analgesic effects of (±)-epibatidine could be separated, in part, from the various other effects of (±)-epibatidine.

Epibatidine (0.1 μmol/kg, IP) clearly produced an analgesic effect (i.e., increase in jump latency) in mice as well as an impairment in rotorod performance, reduction in locomotor activity, and reduction in temperature (Table 1). However, the duration of these effects was dissimilar. For example, unlike the analgesic response that returned to control values by 120 min after (±)-epibatidine treatment, locomotor activity and temperature remained significantly reduced at 120 min after (±)-epibatidine administration. It could be argued that the increase in jump latency (i.e., analgesia) observed on the hot plate was due to the inability of the animal to jump. This seems unlikely based on the finding that locomotor activity was significantly reduced at all points measured but rotorod performance was only impaired at 15 and 30 min following (±)-epibatidine treatment. Thus, although locomotor activity was reduced, the ability of the mice treated with (±)-epibatidine to ambulate (i.e., rotorod performance) was similar to saline-treated mice at 60 min, a time when analgesia can be observed. (±)-Epibatidine treatment significantly reduced temperature at all times measured. Analgesia, however, was not observed at the 120-min time point. Clearly, this experiment did not address to what degree these factors could have contributed to the observed analgesic response. However, these findings suggested that the analgesic effects of (±)-epibatidine were not entirely a function of changes in locomotor activity and/or temperature.

A 0.05-μmol/kg dose of (±)-epibatidine did not produce reliable analgesia, although significant reductions in activity and temperature were observed in separate groups of mice given the same treatment. These findings were consistent with an earlier report indicating that activity and temperature effects were found at doses that do not produce analgesia (15) and indicate that decreases in activity and temperature do not necessarily result in an increase in jump latency in the hot plate paradigm. Surprisingly, in the current investigation, when mecamylamine (5 μmol/kg, IP) was given after (±)-epibatidine (0.05 μmol/kg, IP), a significant enhancement was seen with regard to analgesia. The enhancement of (±)-epibatidine's effect was not found with the activity and

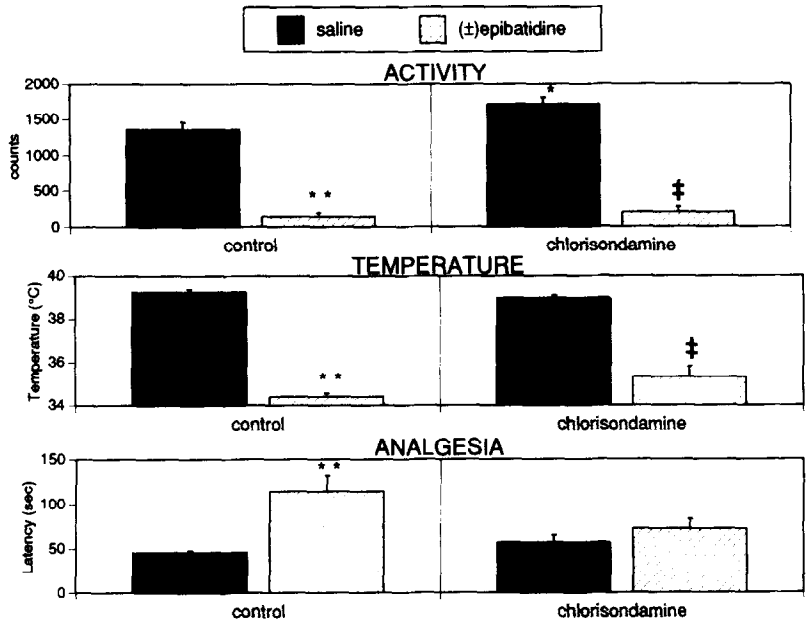

FIG. 2. Values are expressed as group means ± SEM (n = 8). Five days after chlorisondamine treatment (23 μmol/kg, IP) mice were challenged with either saline or (±)-epibatidine (0.1 μmol/kg, IP). As in control mice (i.e., saline treated), in chlorisondamine-treated mice (±)-epibatidine treatment significantly reduced both activity, $F(3, 19) = 85.47$, $p < 0.0001$, and temperature, $F(3, 19) = 105.72$, $p < 0.0001$. In contrast, (±)-epibatidine-induced analgesia was attenuated in chlorisondamine-treated mice. *,**Significantly different from control mice challenged with saline (*$p < 0.05$, **$p < 0.001$); ‡significantly different from chloirsondamine mice challenged with saline ($p < 0.01$).

temperature measures. In fact, mecamylamine posttreatment attenuated the activity and temperature effects. Thus, administration of mecamylamine after (±)-epibatidine treatment produced a dissociation between activity/temperature effects and the analgesia response. In the current study, as expected, mecamylamine pretreatment blocked epibatidine-induced reductions in activity and temperature, with a similar trend noted for analgesia.

The apparent enhancement of (±)-epibatidine's analgesic effect observed with mecamylamine posttreatment could have resulted from additive effects of epibatidine and mecamylamine, because alone both compounds produced slight increases in jump latency (Fig. 1). There has also been speculation that mecamylamine's nAChR antagonism may reflect an action on some intracellular calcium-dependent mechanism (8). Potentiation of (−)-nicotine's analgesic effect and locomotor effects have also been reported with pretreatment with (±)-Bay K 8644, a calcium channel activator, implicating involvement of calcium in neuronal nicotinic acetylcholine receptor (nAChR) functioning (7). In our laboratory, investigations are currently being conducted to determine the influence of calcium dynamics on (±)-epibatidine's in vivo effects.

In the current study, analgesic effects, locomotor effects, and temperature effects of (±)-epibatidine (0.1 μmol/kg, IP) were significantly attenuated with mecamylamine (5 μmol/kg, IP) pretreatment (data not shown). Unlike mecamylamine pretreatment, chlorisondamine (23 μmol/kg, IP) pretreatment had no effect on (±)-epibatidine-induced changes in activity, and only a small effect on (±)-epibatidine-induced changes in temperature. However, chlorisondamine pretreatment appeared as effective as mecamylamine pretreatment in attenuating (±)-epibatidine-induced analgesia. Both mecamylamine and chlorisondamine are described as producing central nicotinic antagonism, as evidenced by their ability to block behavioral effects of nicotine (4,11). However, central nicotinic antagonism produced by mecamylamine and chlorisondamine may occur by different mechanisms. For example, unlike mecamylamine treatment, high-dose, systemic chlorisondamine treatment, as utilized in the current study, produces long-term central nicotinic blockade without affecting peripheral nAChRs (4). Acute treatment with mecamylamine may antagonize peripheral as well as central nAChRs. These two anatagonists have also been found to have distinct behavioral effects that may possibly reflect regional and/or neuronal nAChR subtype specificity (9). Because only a single dose of mecamylamine and chlorisondamine was tested in the current investigation, a dose effect cannot be ruled out as an explanation for the different effects observed with these compounds. However, this does not invalidate the basic finding with chlorisondamine in which an attenuation of (±)-epibatidine's analgesic effect was observed, with no effect on locomotor activity and a minimal effect on temperature.

There has been speculation that the diverse central effects produced by nAChR ligands (e.g., nicotine) may be mediated

by activity at specific neuronal nAChR subtype(s) (1,9). This postulation has been supported with data showing distinctive binding profiles for various nicotinic ligands at neuronal nAChR subtypes, dissimilar activities in functional assays associated with neuronal nAChR subtypes, and the diverse behavioral effects associated with different nAChR ligands. (±)-Epibatidine has been shown to differentiate between two of the major classes of nAChR binding sites in rodent brain, with higher potency at sites labeled by [^{3}H](−)-nicotine or [^{3}H](−)-cytisine than those labeled with [^{125}I]α-bungarotoxin (α-BgT) (15). The neuronal nAChR site labeled with high affinity by (−)-nicotine has been associated with nAChRs comprised primarily of α4β2 subunits (16) whereas that labeled by α-BgT has been correlated with the distribution of the α7 gene (13). nAChR antagonists have also been found to have different activities at nAChR subtypes. For example, methyllycaconitine (MLA) differentiates between α-BgT-sensitive sites on neuronal and muscle nAChRs (1). In vivo, (±)-epibatidine is several-fold more potent than (−)-nicotine in producing a variety of centrally mediated nAChR effects, but it lacks anxiolytic activity, an effect observed with (−)-nicotine treatment (15). In the present study, different effects were found with mecamylamine and chlorisondamine with regard to modulation of (±)-epibatidine's effects on activity, temperature, and analgesia, providing additional evidence for possible regional or nAChR subtype selectivity for these compounds. Also, the results obtained in this study with mecamylamine and chlorisondamine demonstrated that the activity, temperature, and analgesic effects of (±)-epibatidine can, in part, be separated.

In conclusion, this investigation demonstrated that although (±)-epibatidine produces effects on motor activity and temperature, these effects were not entirely responsible for the analgesic effect induced by (±)-epibatidine treatment. Interestingly, mecamylamine given after (±)-epibatidine produced an analgesic effect at a dose of epibatidine (0.05 μmol/kg) that was not effective alone. This effect of mecamylamine posttreatment on analgesia was in contrast to its effects on activity and temperature where partial attenuation was observed. Differences were also observed between (±)-epibatidine's effects on analgesia and effects seen on rotorod performance, locomotor activity, temperature with regard to duration. Finally, clorisondamine treatment effectively attenuated (±)-epibatidine-induced analgesia, but was ineffective in blocking (±)-epibatidine-induced reduction of activity, and only moderately effective in attenuating the reduction in temperature produced by (±)-epibatidine treatment. Overall, these data provide evidence that the analgesic effects of (±)-epibatidine can be dissociated, in part, from (±)-epibatidine's effects on locomotor activity and temperature.

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