ELSEVIER

European Journal of Pharmacology 300 (1996) 51–57

ejp

Tolerance to the antinociceptive effect of epibatidine after acute and chronic administration in mice

M. Imad Damaj *, Billy R. Martin

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, P.O. Box 980613, Richmond, VA 23298-0613, USA

Received 26 October 1995; revised 27 November 1995; accepted 1 December 1995

Abstract

Epibatidine is a novel potent antinociceptive agent that acts through nicotinic receptors. In this study we investigated the development of tolerance to the antinociceptive effects of epibatidine enantiomers after acute and chronic administration in mice using the tail-flick test. Contrary to nicotine, mice treated with epibatidine enantiomers at different times and doses did not develop significant tolerance after s.c. acute nor after repeated injections. In mice that chronically received (+)-epibatidine, no significant tolerance was seen after acute challenge with (+)-epibatidine. However, a significant shift in (−)-epibatidine's dose-response curve was obtained in animals that received (−)-epibatidine. In nicotine-tolerant animals, no significant tolerance was seen after acute challenge with (+)-epibatidine. However, the animals were less sensitive to the acute (−)-epibatidine challenge. Our results show that development of tolerance to epibatidine antinociceptive effects has a different profile and characteristics than that found for nicotine.

Keywords: Nicotine; Epibatidine; Antinociception; Tolerance; (Mouse)

1. Introduction

Tobacco use, especially when smoked as cigarettes, remains a major avoidable cause of mortality and morbidity in the western world. Although many factors contribute to the overall effects of smoking, evidence is convincing that smoking behavior is maintained by the pharmacological effects of the tobacco alkaloid nicotine (Surgeon, 1988). Humans become physically dependent upon and tolerant to nicotine, and it has been suggested that these processes contribute to the difficulty smokers have in stopping cigarette use (Benowitz, 1992). Numerous studies have demonstrated that tolerance develops to the physiological and behavioral effects of nicotine in animals after acute and chronic administration of the drug, including its locomotor depressant effects (Stolerman et al., 1973), nicotine-induced hypothermia (Marks et al., 1993), nicotine-induced prolactin release (Hulihan-Giblin et al., 1990a, b), nicotine-induced seizures (Dunlop et al., 1960; Miner and Collins, 1988) and nicotine-stimulated $^{86}Rb^{+}$ efflux (Marks et al., 1994).

Chronic exposure to nicotine results in an increase in the number of nicotine receptor binding sites in rodent brain (reviewed by Wonnacott, 1990). This receptor up-regulation has been demonstrated to occur in a dose- and time-dependent manner, and to be reversible once nicotine is discontinued (Marks and Collins, 1985; Schwartz and Kellar, 1985). In addition, receptor up-regulation has been reported with several nicotinic receptor agonists (nicotine, cytisine, anabasine and methylcarbamylcholine) (Bhat et al., 1991; Flores et al., 1992; Marks and Collins, 1985; Schwartz and Kellar, 1985; Yang and Buccafusco, 1994) but not with lobeline which failed to elicit changes in nicotine binding after chronic infusion in mice (Bhat et al., 1991). To explain this pharmacological incongruity, it has been hypothesized that up-regulation occurs because of chronic agonist-induced desensitization or inactivation of the receptor (Marks and Collins, 1985; Schwartz and Kellar, 1985).

Recently, epibatidine, a compound found in trace amounts in the skin of the Ecuadorian poison frog (Spande et al., 1992), was reported to be the most potent neuronal nicotinic receptor ligand with an affinity in the picomolar range for the [^{3}H]nicotine and [^{3}H]cytisine binding sites in the rat brain (Damaj et al., 1994a; Qian et al., 1993;

* Corresponding author. Tel.: (804) 828-5596; fax: (804) 828-2117.

SSDI 0014-2999(95)00834-9

Sullivan et al., 1994). This agent is several hundred times more potent than morphine as an analgesic (Badio and Daly, 1994; Damaj et al., 1994a, b), and its effect is blocked by the nicotinic receptor channel blocker mecamylamine but not by the opiate antagonist naloxone (Damaj et al., 1994a; Qian et al., 1993). However, to our knowledge there are no reports of tolerance to the antinociceptive effects of epibatidine and its enantiomers.

The purpose of the current study was to investigate the development of tolerance to the antinociceptive effects of epibatidine enantiomers after acute and chronic administration in mice using the tail-flick test. Cross-tolerance to epibatidine was also assessed in mice rendered tolerant to (−)-nicotine.

2. Materials and methods

2.1. Animals

Male ICR mice (20–25 g) obtained from Harlan Laboratories (Indianapolis, IN, USA) were used throughout the study. The mice were housed in groups of six and had free access to food and water. Animals were housed in an American Association for Accreditation of Laboratory Animal Care approved facility and the study was approved by the Institutional Animal Care and Use Committee of Virginia Commomwealth University.

2.2. Drugs

Nicotine was obtained from Aldrich Chemical Company, (Milwaukee, WI, USA) and converted to the ditartrate salt as described by Aceto et al. (1979). (+)- and (−)-epibatidine (hemi oxalate salt) were supplied by Dr. S. Fletcher (Merck Sharp and Dohme and Co, Essex, UK). 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. All doses were expressed as the free base of the drug. Natural epibatidine was found to be the (−)-enantiomer (negative rotation as free base, positive rotation as oxalate salt (Badio and Daly, 1994). The rotational notations in this manuscript refer to the free base. [^{3}H](−)-Nicotine (80 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). All doses are expressed as the free base of the drug.

2.3. Antinociceptive test

Antinociception was measured using the tail-flick method of D'Amour and Smith (1941) as modified by Dewey et al. (1970). A control response (2–4 s) was determined for each animal before treatment, and a test latency was determined after drug administration. A maximum latency of 10 s was imposed if no response occurred within that time. The antinociceptive response was calculated as percentage maximum possible effect (% MPE), where %MPE = [(test − control)/(10 − control)] × 100. Groups of 6–12 animals were used for each dose and for each treatment. The mice were tested 5 min after s.c. administration of nicotine or epibatidine isomers for dose-response evaluation.

2.4. Drug treatment paradigms

2.4.1. Acute tolerance

For one dose tolerance, mice were pretreated s.c. with different doses of drug (nicotine and epibatidine isomers) at different times prior to a second s.c. injection of nicotine or epibatidine isomers. Mice were tested 5 min later in the tail-flick test.

Tolerance after multiple injections was determined by injecting mice with s.c. nicotine or epibatidine with a maximum of four injections at 30-min intervals. Antinociceptive effects were measured 5 min after each injection. Separate groups of mice were used for each time point.

2.4.2. Chronic tolerance

Four groups of animals received a s.c. injection of either (−)-nicotine (2 mg/kg), (−)-epibatidine (10 μg/kg), (+)-epibatidine (10 μg/kg) or saline twice daily (08:30 and 16:30 h) for 10 days. During the treatment period the body weight was recorded every other day. At day 11, mice were challenged with different doses of the corresponding drug for determination of dose-response curves. Injections and testing procedures were performed in the same room.

2.5. [^{3}H](−)-Nicotine binding in vitro

Mice that were treated chronically as described above were decapitated 12 h after the last injection, and their brains were removed for tissue preparation.

[^{3}H](−)-Nicotine binding assays in mouse brain were performed in vitro according to the method of Scimeca and Martin (1988) with minor modifications. Tissue homogenate was prepared from whole mouse 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 resuspended to a final tissue concentration of 10 mg/ml of buffer. Membranes from whole brain (0.2 ml of final suspension) were incubated at 4°C for 2 h with phosphate buffer and [^{3}H]nicotine in a total volume of 1 ml. Nonspecific binding was determined in the presence of 100 μM unlabeled (−)-nicotine. The incubation was terminated by rapid filtration through a Whatman GF/C glass fiber filter (presoaked overnight in 0.1% poly-L-lysine 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.

2.6. Statistical analysis

Data were analyzed statistically by an analysis of variance followed by the Fisher least significance difference multiple comparison test. The null hypothesis was rejected at the 0.05 level. ED_{50} values with 95% confidence limits (CL) were calculated by unweighted least-squares linear regression for log-dose probits, as described by Tallarida and Murray (1987).

3. Results

3.1. Acute tolerance to nicotine- and epibatidine-induced antinociception after s.c. administration.

Acute tolerance to the effect of a challenge dose of nicotine (2 mg/kg, s.c.) occurred in mice pretreated with a single dose of nicotine (4 mg/kg, s.c.). As seen from the results presented in Fig. 1, maximum tolerance occurred between 0.5 and 1 h after nicotine pretreatment. Recovery of the pre-tolerance response was achieved after 6 h (data not shown). However, no significant acute tolerance was seen to epibatidine-induced antinociception (12 μg/kg) in mice pretreated with (+) or (−)-epibatidine (12 μg/kg, s.c.) at different times (Fig. 1). Higher doses of epibatidine failed to induce acute tolerance (Table 1). Indeed, mice pretreated with 30 μg/kg did not exhibit significant acute tolerance to a subsequent dose of 12 μg/kg of (−)- or (+)-epibatidine, 1 h later (time of maximum tolerance to nicotine).

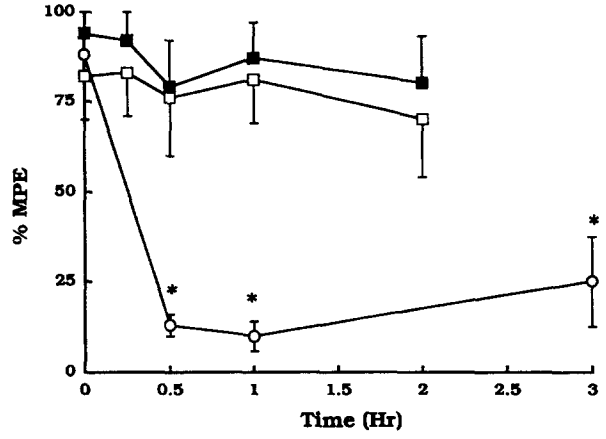

Fig. 1. Acute tolerance to nicotine- and epibatidine-induced antinociception after s.c. administration in mice using the tail-flick test. Mice were pretreated s.c. with 4 mg/kg of nicotine and then challenged at different times with nicotine 2 mg/kg s.c. (○). Other groups of mice were pretreated s.c. with epibatidine isomers (12 μg/kg) and then challenged at different times with 12 μg/kg of the respective isomer [■ for the (+) isomer and □ for the (−) isomer]. Antinociception was measured 5 min after the last injection. The time 0 h represents mice receiving a saline injection 1 h before nicotine (2 mg/kg) or epibatidine isomers (12 μg/kg) injection. Each point represents the mean ± S.E. of 6–12 mice. * Statistically different from nicotine (2 mg/kg) at zero time at $P < 0.05$.

Cross-tolerance studies were conducted after acute injections of epibatidine isomers and nicotine. Pretreatment of mice with a high dose of nicotine (4 mg/kg, s.c.) produced a pronounced acute tolerance to the antinociceptive effects of a subsequent dose of nicotine. When nicotine-tolerant mice were challenged with epibatidine isomers (12 μg/kg), a significant reduction of (−)-epibatidine's effect but not that of (+)-epibatidine was seen (Fig. 2A). However, in mice pretreated with the epibatidine isomers (30 μg/kg) and challenged 1 h later with an active dose of nicotine (2 mg/kg, s.c.), no significant reduction was seen in nicotine-induced antinociception (Fig. 2B).

3.2. Tolerance to nicotine- and epibatidine-induced antinociception after repeated s.c. injections

Tolerance to nicotine's effect in the tail-flick test was seen in mice injected repeatedly with s.c. nicotine (2 mg/kg) every 30 min. Indeed, nicotine's effect was attenuated after the second injection and was decreased significantly after the 4th injection (Fig. 3). However, no reduction (if not a slight increase) was seen to the antinociceptive effects of epibatidine isomers after repeated injections (12 μg/kg every 30 min).

3.3. Tolerance to nicotine- and epibatidine-induced antinociception after chronic administration

The effect of chronic treatment with nicotine and epibatidine (an ED_{84} dose twice a day for 10 days) on mouse body weight is shown in Fig. 4. The gain in body weight was significantly smaller in the nicotine-treated animals than in the saline-treated controls. However, chronic treatment with epibatidine isomers (12 μg/kg twice daily) did not significantly reduce the animals body weight.

Dose-response curves for the nicotine-induced antinociception in chronic nicotine- and saline-treated animals are presented in Fig. 5A. Animals that received chronic nicotine (2 mg/kg twice a day) were less sensitive to the acute nicotine challenge in the tail-flick test and nicotine's dose-response curve was shifted to the right. The ED_{50} values (and 95% CL) for saline-treated and nicotine-treated animals were 1.05 (0.5–2.0) and 4.0 (2.6–6.0) mg/kg, respectively.

In animals that received (+)-epibatidine (12 μg/kg twice daily), no significant tolerance was seen after acute challenge with (+)-epibatidine in the tail-flick test (Fig. 5B). The ED_{50} values (and 95% CL) for saline-treated and (+)-epibatidine-treated animals were 8.6 (6.6–11.3) and 12.1 (8.1–18.2) μg/kg, respectively. However, a significant shift in (−)-epibatidine's dose-response curve was obtained in animals that received (−)-epibatidine (12

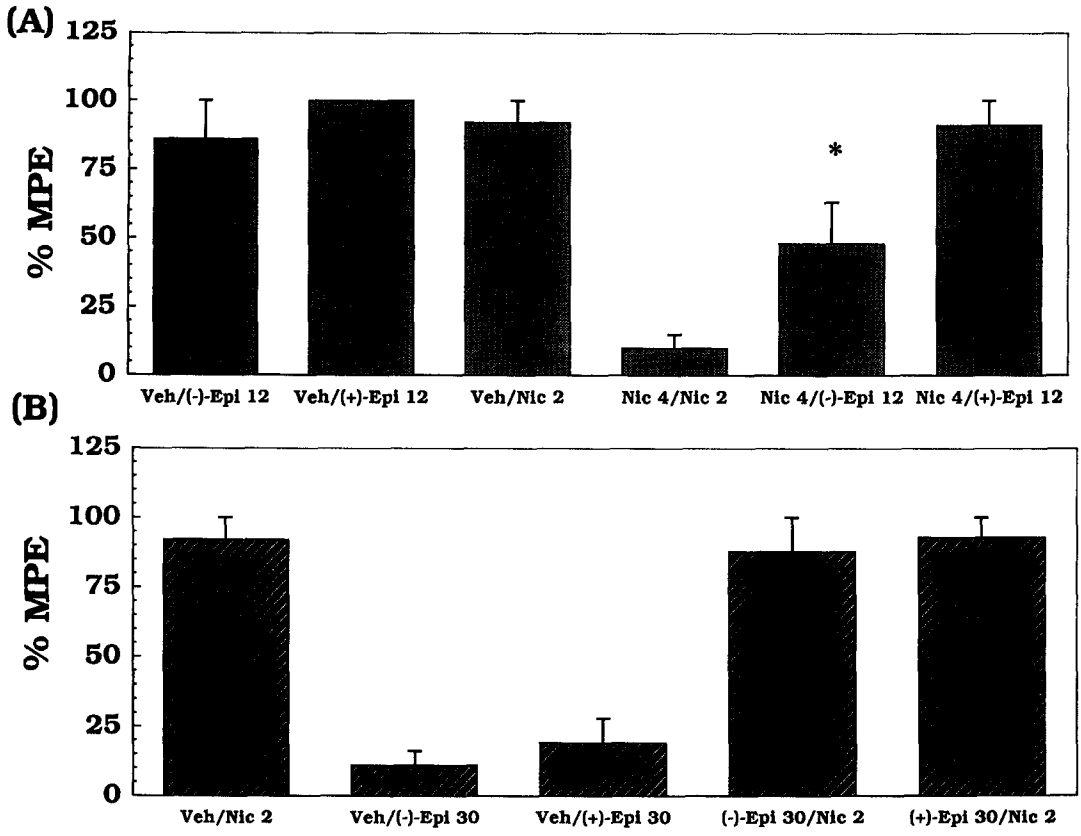

Fig. 2. Cross-tolerance studies after acute s.c. injection in mice using the tail-flick test. (A) Mice were pretreated with either vehicle or nicotine (4 mg/kg) and then challenged 1 h later with either nicotine (2 mg/kg), (+)-epibatidine (12 μg/kg) or (−)-epibatidine (12 μg/kg). * Statistically different from veh/(−)-epi 12 at $P < 0.05$. (B) Mice were pretreated with (+)-epibatidine (30 μg/kg) or (−)-epibatidine (30 μg/kg) and then challenged 1 h later with nicotine 2 mg/kg. Antinociception was measured 5 min after the last injection. Each point represents the mean ± S.E. of 6–12 mice. Veh = Vehicle; (+)-Epi 12 = (+)-epibatidine at 12 μg/kg, s.c.; (−)-Epi 12 = (−)-epibatidine at 12 μg/kg, s.c.; (−)-Epi 30 = (−)-epibatidine at 30 μg/kg, s.c.; (+)-Epi 30 = (+)-epibatidine at 30 μg/kg, s.c.; Nic 4 = nicotine at 4 mg/kg, s.c.; Nic 2 = nicotine at 2 mg/kg, s.c.

μg/kg twice daily) (Fig. 5C). This shift, although modest (2.7-fold shift), was significant and the ED_{50} values (and 95% CL) for saline-treated and (−)-epibatidine-treated animals were 7.3 (5.2–10.3) and 19.5 (17.8–21.3) μg/kg, respectively.

3.4. Antinociceptive effects of epibatidine isomers in animals rendered tolerant to nicotine

Dose-response curves for the (+)-epibatidine-induced antinociception in chronic nicotine- and saline-treated animals are presented in Fig. 6A. In animals that received chronic nicotine (2 mg/kg twice daily) no significant tolerance was seen after acute challenge with (+)-epibatidine in the tail-flick test. The ED_{50} values (and 95% CL) for saline-treated and nicotine-treated animals were 11.5 (6.0–23.0) and 13.8 (9.2–20.7) μg/kg, respectively.

Table 1
Effect of dose on rapid tolerance to epibatidine enantiomers in the tail-flick test

Pretreatment dose [(+) or (−)-epibatidine]	Challenge [a] [(−)-Epibatidine 12 μg/kg]	[(+)-Epibatidine 12 μg/kg]
Saline	82 ± 12	77 ± 15
12 μg/kg	90 ± 10	87 ± 10
30 μg/kg	100 ± 0	74 ± 16

Mice were pretreated with different doses of epibatidine isomers and then challenged 1 h later with a subsequent dose (12 μg/kg, s.c.) of the appropriate isomer. Each dose group included 8–12 animals. [a] Data are expressed as %MPE (means with S.E.M.).

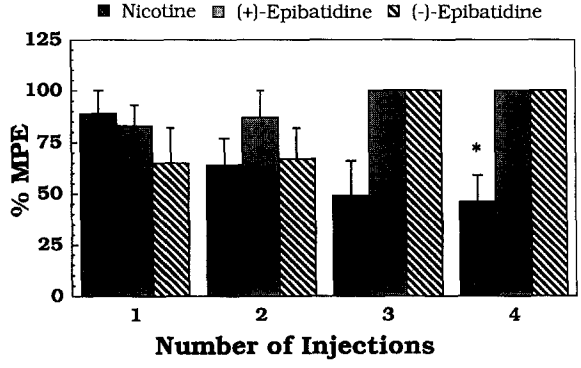

Fig. 3. Tolerance to nicotine- and epibatidine-induced antinociception after repeated s.c. injections in mice using the tail-flick test. Mice were injected with nicotine (2 mg/kg) or epibatidine isomers (12 μg/kg) every 30 min. A maximum of 4 injections was given and antinociception was measured 5 min after each injection. * Statistically different from nicotine first injection 1 at $P < 0.05$. Each point represents the mean ± S.E. of 6–12 mice.

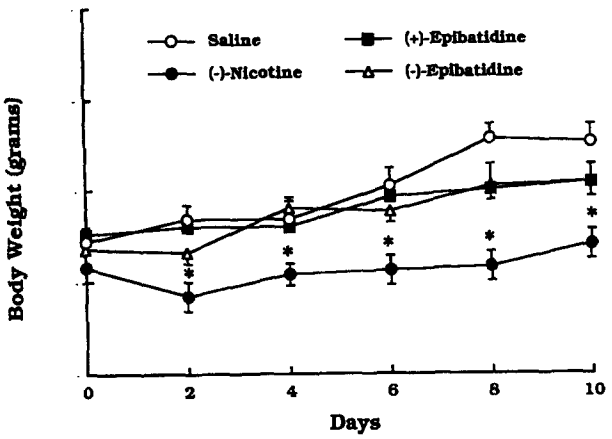

Fig. 4. Effects of chronic treatment with nicotine (2 mg/kg twice daily) and epibatidine (12 μg/kg twice daily) isomer treatment on body weight. Each points represents the mean ± S.E. of 6–12 mice. * Statistically different from control at $P < 0.05$.

However, animals that received nicotine (2 mg/kg twice daily) were less sensitive to the acute (−)-epibatidine challenge in the tail-flick test (Fig. 6B) and (−)-epibatidine's dose-response curve was shifted to the right. The ED_{50} values (and 95% CL) for saline-treated and nicotine-treated animals were 13.2 (11.0–17.0) and 23.5 (18.0–39.0) μg/kg, respectively.

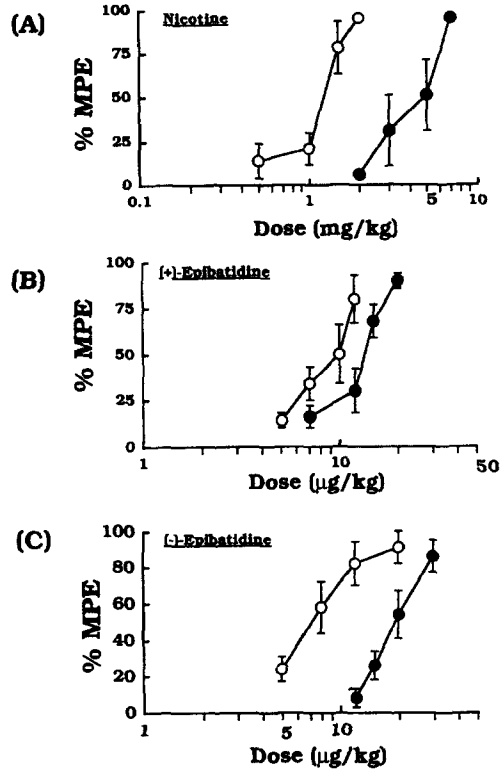

Fig. 5. Dose-response curves for: (A) nicotine, (B) (+)-epibatidine and (C) (−)-epibatidine antinociceptive effects in mice that were chronically injected twice daily for 10 days with either saline (○) or nicotine (2 mg/kg), (+)- epibatidine (12 μg/kg), (−)-epibatidine (12 μg/kg) (●). Each points represents the mean ± S.E. of 6–12 mice.

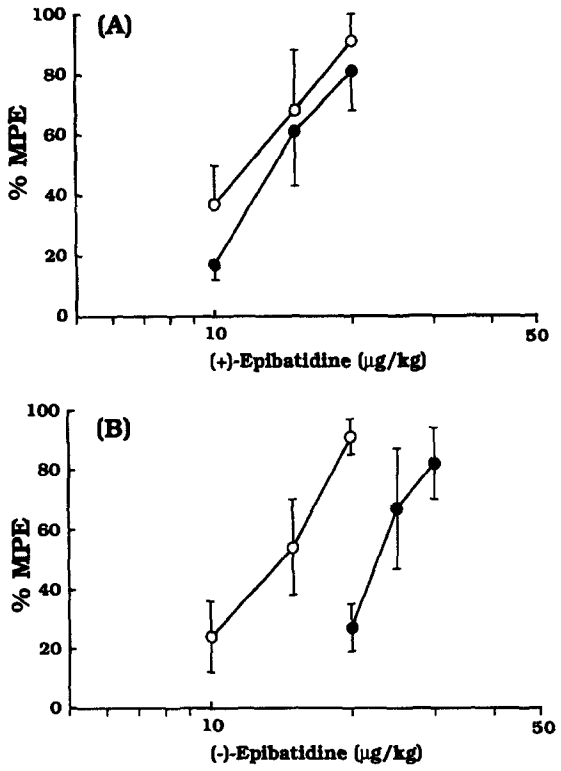

Fig. 6. Dose-response curves for: (A) (+)-epibatidine and (B) (−)-epibatidine antinociceptive effects in mice that were chronically injected twice daily for 10 days with either saline (○) or nicotine (2 mg/kg) (●). Each points represents the mean ± S.E. of 6–12 mice.

3.5. Effects of nicotine and epibatidine chronic treatment on [^{3}H]nicotine binding sites in the brain

The binding of [^{3}H]nicotine to whole brain membranes from animals that received chronic nicotine or saline treatment for 10 days was investigated. Chronic nicotine injections did not alter [^{3}H]nicotine binding sites in the brain. Scatchard analysis confirmed that neither the B_{max} (253 ± 54 fmol/mg in chronic saline-injected animals versus 268 ± 87 fmol/mg in chronic nicotine-injected animals) nor the K_D (3.8 ± 1.3 nM in chronic saline-injected animals versus 4.5 ± 3.3 nM in chronic nicotine-injected animals) was altered by chronic nicotine treatment. Consequently, binding experiments were not performed in epibatidine-treated animals.

4. Discussion

Tolerance to the pharmacological and behavioral effects of several nicotinic receptor agonists (including nicotine, acetylcholine, methylcarbamylcholine, cytisine, anabasine and dimethylpiperazonium) after acute and chronic systemic administration in animals as measured by hormonal responses in vivo (Balfour and Benwell, 1981; Hulihan-Giblin et al., 1990a, b; Matta et al., 1993; Sharp and Beyer, 1986), by dopamine release in vivo (Benwell et al.,

1995) and by locomotor depressant effects (Stolerman et al., 1973) is well documented. In this study, experimental paradigms were established in which tolerance to nicotinic compounds were investigated after a single injection, repeated injections during a 2-h period or chronic administration for 10 days. Tolerance to epibatidine, a potent nicotinic receptor agonist recently discovered, was investigated and results yielded some unexpected findings.

A major finding of this study was the lack of development of significant tolerance to the antinociceptive effects of the epibatidine isomers following acute administration of single and repeated injections. However, cross-tolerance to nicotine occurs only with (−)-epibatidine. The findings that epibatidine isomers do not show in vivo desensitization is particularly intriguing in light of a large body of evidence indicating that acute or repeated administration of nicotine can produce desensitization to nicotine-induced effects in in vitro and in vivo experiments (for review see Ochoa et al., 1990). In addition, our results indicate that cross-tolerance between nicotine and epibatidine is asymmetrical after acute injection. Interestingly, Loring et al. (1994) reported that (±)-epibatidine is 100-fold more potent than nicotine and selectively desensitizes functional nicotinic receptors in intact chick retina. The reasons behind these discrepancies are still unclear but may lie in the kinetics of desensitization at distinct nicotinic receptor subtypes expressed in different brain areas.

The second question addressed by these experiments was whether tolerance develops after chronic injection of epibatidine. Given that the two isomers display similar affinities for [^{3}H]nicotine and [^{3}H]cytisine binding sites and demonstrate similar pharmacological effects (Badio and Daly, 1994; Damaj et al., 1994a), it was anticipated that tolerance would develop to the enantiomers in a fashion similar to that seen for nicotine. However, the observation that chronic epibatidine injection failed to elicit a degree of tolerance (in particular the (+)-enantiomer) similar to that observed with nicotine is somewhat surprising given the fact that an equipotent dose (an $ED_{84\%}$ dose) for both epibatidine an nicotine was given. There are several possible explanations for the difference observed including: a difference in the pharmacokinetic profile of nicotine and epibatidine; and a possible difference between epibatidine and nicotine as stress inducers at the doses used. Indeed, it was reported that chronic nicotine injection is a stressor and leads to increases in pre-injection levels of plasma corticosterone that exceed those seen in saline-injected mice (Pauly et al., 1992). Since stress elicits a decreased response to nicotine in mice (Pauly et al., 1990, 1992), it may be that the failure in developing a substantial tolerance to epibatidine after chronic injection is due to a difference in inducing stress between nicotine and epibatidine. Presently, it is not known whether a higher degree of tolerance to epibatidine-induced antinociception occurs when injections of the drug are given at shorter time intervals or given as chronic infusion. Further experiments are needed to clarify this issue, especially in the absence of any pharmacokinetic data on these compounds.

After 10 days of twice daily injections of nicotine, mice expressed differential cross-tolerance with the epibatidine isomers and differences in the ability to induce tolerance. For instance the dose-response curve for nicotine antinociception could be shifted a 4-fold shift to the right by chronic nicotine injections, whereas an equivalent nicotine treatment produced only a 1.2-fold and a 1.8-fold shift of the (−)- and (+)-epibatidine dose-response curves, respectively. One explanation for unequal cross-tolerance is that tolerance is determined by dispositional factors. Intrinsic activity could also be a factor, particularly in case of the epibatidine enantiomers, which have a high affinity (picomolar range) for [^{3}H]nicotine binding sites. Clearly, more studies are needed to explore these different explanations.

As previously reported (Benowitz, 1992; Grunberg et al., 1984), chronic nicotine administration decreased body weight gain. The loss in body weight was evident within 2 days of injection. However, no significant decrease was found after chronic injection of the epibatidine isomers. Recently, Qian et al. (1994) reported that chronic injection of (−)-epibatidine at 2.8 μg/kg/day for 9 days, resulted in a pronounced body weight loss (6.9%) in CD-1 (equivalent to ICR mice used in our studies) and obese Ob/Ob mice. Although the reported decrease in body weight was significant (Qian et al., 1994), the dose-response curve for (−)-epibatidine-induced body weight loss was inverted at higher doses and a decrease of only 3.5% was observed (statistical significance not reported) with a dose (28 μg/kg/day) similar to the one used in our studies.

The observation that chronic nicotine injection failed to elicit increases in mouse brain [^{3}H]nicotine binding sites was previously reported (Grun et al., 1992; Pauly et al., 1992). The difference with previous reports where chronic nicotine administration up-regulates [^{3}H]nicotine binding sites (Marks and Collins, 1985; Schwartz and Kellar, 1985) is most probably due to a species difference (mice versus rat) or to the mode of administration (chronic s.c. injections versus chronic infusion) (Marks et al., 1991). Due to an unchanged nicotinic receptors number after chronic nicotine administration and a lack of a pronounced tolerance after chronic epibatidine, studies examining epibatidine's effects on [^{3}H]nicotine binding sites were not performed.

In summary, our results show that development of tolerance to epibatidine antinociceptive effects has a different profile and characteristics than that found for nicotine.

Acknowledgements

The authors greatly appreciate the technical assistance of Kim Creasy and Bin Yang. This work was supported by the National Institute on Drug Abuse grant No. DA-05274.

References

Aceto, M.D., B.R. Martin, I.M. Uwaydah, E.L. May, L.S. Harris, C. Izazola-Conde, W.L. Dewey and W.C. Vincek, 1979, Optically pure (+)-nicotine from (±)-nicotine and biological comparisons with (−)-nicotine, J. Med. Chem. 22, 174.

Badio, B. and J. Daly, 1994, Epibatidine, a potent analgetic and nicotinic agonist, Mol. Pharmacol. 45, 563.

Balfour, D.J.K. and M.E.M. Benwell, 1981, Effects of chronic nicotine administration on plasma corticosterone and brain 5-hydroxyindoles in stressed rats, Br. J. Pharmacol. 1–3, 212.

Benowitz, N.L., 1992, Cigarette smoking and nicotine addiction, Med. Clin. N. Am. 76, 415.

Benwell, M., D. Balfour and C. Birell, 1995, Desensitization of the nicotine-induced mesolimbic dopamine responses during constant infusion with nicotine, Br. J. Pharmacol. 114, 454.

Bhat, R.V., S.L. Turner, S.R. Selvaag, M.J. Marks and A.C. Collins, 1991, Regulation of brain nicotinic receptors by chronic agonist infusion, J. Neurochem. 56, 1932.

D'Amour, F.E. and D.L. Smith, 1941, A method for determining loss of pain sensation, J. Pharmacol. Exp. Ther. 72, 74.

Damaj, M.I., K.R. Creasy, A.D. Grove, J.A. Rosecrans and B.R. Martin, 1994a, Pharmacological effects of epibatidine optical enantiomers, Brain Res. 664, 34.

Damaj, M.I., S. Tucker, M. Aceto and B.R. Martin, 1994b, Analgesic activity of epibatidine and its enantiomers in a chemical model of pain in mice, Med. Chem. Res. 4, 483.

Dewey, W.L., L.S. Harris, J.S. Howes and J.A. Nuite, 1970, The effect of various neurohormonal modulations on the activity of morphine and the narcotic antagonists in taik-flick and phenylquinone test, J. Pharmacol. Exp. Ther. 175, 435.

Dunlop, C.W., C. Stumpf, D.S. Maxwell and W. Schindler, 1960, Modification of cortical, reticular and hippocampal unit activity by nicotine in the rabbit, Am. J. Physiol. 198, 515.

Flores, C.M., S.W. Rogers, L.A. Pabreza, B.B. Wolfe and K.J. Kellar, 1992, A subtype of nicotinic cholinergic receptor in the rat brain is composed of alpha-4 and beta-2 subunits and is up-regulated by chronic nicotine treatment, Mol. Pharmacol. 41, 31.

Grun, E.A., J.R. Pauly and A.C. Collins, 1992, Adrenalectomy reverses chronic injection-induced tolerance to nicotine, Psychopharmacology 109, 299.

Grunberg, N.E., D.J. Bowen and D.E. Morse, 1984, Effects of nicotine on body weight and food consumption in rats, Psychopharmacology 83, 93.

Hulihan-Giblin, B.A., M.D. Lumpkin and K.J. Kellar, 1990a, Acute effects of nicotine on prolactin release in the rat: agonist and antagonist effects of a single injection of nicotine, J. Pharmacol. Exp. Ther. 252, 15.

Hulihan-Giblin, B.A., M.D. Lumpkin and K.J. Kellar, 1990b, Effects of chronic administration of nicotine on prolactin release in the rat: inactivation of prolactin response by repeated injections of nicotine, J. Pharmacol. Exp. Ther. 252, 21.

Loring, R.H., T. McHugh, J. McKay and X.G. Zhang, 1994, Epibatidine: a potent desensitizing agonist of neuronal nicotinic receptors in the chick nernous system, Med. Chem. Res. 4, 517.

Marks, M.J. and A.C. Collins, 1985, Tolerance, cross-tolerance, and receptors after chronic nicotine or oxotremorine, Pharmacol. Biochem. Behav. 22, 283.

Marks, M.J., S.M. Campbell, E. Romm and A.C. Collins, 1991, Genotype influences the development of tolerance to nicotine in the mouse, J. Pharmacol. Exp. Ther. 259, 392.

Marks, M., S. Grady and A. Collins, 1993, Down-regulation of nicotinic receptor function after chronic nicotine infusion, J. Pharmacol. Exp. Ther. 266, 1268.

Marks, M., S. Grady, J.M. Yang, P. Lippiello and A. Collins, 1994, Desensitization of nicotine-stimulated $^{86}Rb^{+}$ efflux from mouse brain synaptosomes, J. Neurochem. 63, 2125.

Matta, S.G., C.A. Foster and B.M. Sharp, 1993, Nicotine stimulates the expression of cFos protein in the parvocellular paraventricular nucleus and brainstem catecholaminergic regions, Endocrinology 132, 2149.

Miner, L.L. and A.C. Collins, 1988, Effect of nicotine pretreatment on nicotine-induced seizures, Pharmacol. Biochem. Behav. 29, 375.

Ochoa, E.L.M., L. Li and M.G. McNamee, 1990, Desensitization of central cholinergic mechanisms and neuroadaption to nicotine, Mol. Neurobiol. 4, 251.

Pauly, J.R., E.U. Grun and A.C. Collins, 1990, Chronic corticosterone administration modulates nicotine sensitivity and brain nicotinic receptor binding in C3H mice, Psychopharmacology 101, 310.

Pauly, J.R., E.U. Grun and A.C. Collins, 1992, Tolerance to nicotine following chronic treatment by injections: A potential role for corticosterone, Psychopharmacology 108, 33.

Qian, C., T. Li, T. Shen, L. Libertine-Graham, J. Eckman, T. Biftu and S. Ip, 1993, Epibatidine is a nicotinic analgesic, Eur. J. Pharmacol. 250, R13.

Qian, C., S. Chen, L. Libertine-Graham, D.M. Wypij, P. Yao, F. Zuo and J. Eckman, 1994, Effects of epibatidine on body weight in mice, Med. Chem. Res. 4, 493.

Schwartz, R.D. and K.J. Kellar, 1985, In vivo regulation of [^{3}H]acetylcholine recognition sites in brain by nicotinic cholinergic drugs, J. Neurochem. 45, 427.

Scimeca, J.A. and B.R. Martin, 1988, The effect of acute dyflos (DFP) treatment on [^{3}H]nicotine binding to mouse brain homogenate, J. Pharm. Pharmacol. 40, 793.

Sharp, B.M. and H.S. Beyer, 1986, Rapid desensitization of the acute stimulatory effects of nicotine on rat plasma adrenocorticotropin and prolactin, J. Pharmacol. Exp. Ther. 238, 486.

Spande, T., H. Garraffo, M. Edwards, H. Yeh, L. Pannell and J. Daly, 1992, Epibatidine: a novel (chloropyridyl) azabicycloheptane with potent anlgesic activity from an ecuadoran poison frog, J. Am. Chem. Soc. 114, 3475.

Stolerman, I.P., R. Fink and M.E. Jarvik, 1973, Acute and chronic tolerance to nicotine measured by activity in rats, Psychopharmacology 30, 329.

Sullivan, J.P., M.W. Decker, J. Brioni, D. Donnelly-Roberts, D.J. Anderson, A.W. Bannon, C. Kang, P. Adams, M. Piattoni-Kaplan, M. Buckley, M. Gopalakrishnan, M. Williams and S.P. Arneric, 1994, (±)Epibatidine elicits a diversity of in vitro and in vivo effects mediated by nicotinic acetylcholine receptors, J. Pharmacol. Exp. Ther. 271, 624.

Surgeon, G., 1988, The Health Consequences of Smoking: Nicotine Addiction. A Report of the Surgeon General (U.S. Gov. Printing Office, Washington, D.C.).

Tallarida, R.J. and R.B. Murray, 1987, Manual of Pharmacological Calculations with Computer Programs (Springer-Verlag, New York) p. 293.

Wonnacott, S., 1990, The paradox of nicotinic acetylcholine receptor upregulation, Trends Pharmacol. Sci. 11, 216.

Yang, X. and J. Buccafusco, 1994, Effect of chronic central treatment with the acetylcholine analog methylcarbamylcholine on cortical nicotinic receptors: correlation between receptor changes and behavioral function, J. Pharmacol. Exp. Ther. 271, 651.