Pharmacology, Biochemistry and Behavior 179 (2019) 27–33

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Discriminative stimulus effects of mecamylamine and nicotine in rhesus monkeys: Central and peripheral mechanisms

Colin S. Cunningham, Megan J. Moerke, Lance R. McMahon*

Department of Pharmacodynamics, The University of Florida, Gainesville, FL, USA

ABSTRACT

Mecamylamine is a non-competitive nicotinic acetylcholine receptor (nAChR) antagonist that has been prescribed for hypertension and as an off-label smoking cessation aid. Here, we examined pharmacological mechanisms underlying the interoceptive effects (i.e., discriminative stimulus effects) of mecamylamine (5.6 mg/kg s.c.) and compared the effects of nAChR antagonists in this discrimination assay to their capacity to block a nicotine discriminative stimulus (1.78 mg/kg s.c.) in rhesus monkeys. Central (pempidine) and peripherally restricted nAChR antagonists (pentolinium and chlorisondamine) dose-dependently substituted for the mecamylamine discriminative stimulus in the following rank order potency (pentolinium > pempidine > chlorisondamine > mecamylamine). In contrast, at equieffective doses based on substitution for mecamylamine, only mecamylamine antagonized the discriminative stimulus effects of nicotine, i.e., pentolinium, chlorisondamine, and pempidine did not. NMDA receptor antagonists produced dose-dependent substitution for mecamylamine with the following rank order potency (MK-801 > phencyclidine > ketamine). In contrast, behaviorally active doses of smoking cessation aids including nAChR agonists (nicotine, varenicline, and cytisine), the smoking cessation aid and antidepressant bupropion, and the benzodiazepine midazolam did not substitute for the discriminative stimulus effects of mecamylamine. These data suggest that peripheral nAChRs and NMDA receptors may contribute to the interoceptive stimulus effects produced by mecamylamine. Based on the current results, the therapeutic use of mecamylamine (i.e., for smoking or to alleviate green tobacco sickness) should be weighed against the potential for mecamylamine to produce interoceptive effects that overlap with another class of abused drugs (i.e., NMDA receptor agonists).

1. Introduction

Mecamylamine (3-methylaminoisocamphane hydrochloride or Inversine®) is a secondary amine that targets nicotinic acetylcholine receptors (nAChRs) in the CNS and the periphery. In the periphery it inhibits transmission of impulses across autonomic ganglia (Stone et al., 1956). Mecamylamine is currently available by prescription and has been used as an antihypertensive pharmacotherapy, although its use is limited by ganglionic side effects (Shytle et al., 2002). Despite long-standing use for hypertension, off-label use as a smoking cessation aid, and its potential to elicit CNS-mediated effects, the behavioral effects of mecamylamine have not been fully characterized. Mecamylamine was established as a discriminative stimulus in rats previously (Garcha and Stolerman, 1993), yet numerous pharmacological differences between rat and human nAChRs (Papke et al., 2001; Papke and Porter Papke, 2002) warrant cross-species comparisons. The effects of mecamylamine alone and in combination with nicotine have been examined previously in non-human primates (Preston et al., 1985; Katner et al., 2004). Here, drug discrimination methods were used to characterize the pharmacology of mecamylamine in non-human primates.

Mecamylamine is a prototypical nicotine antagonist (Garcha and Stolerman, 1993; Mariathasan and Stolerman, 1993; Webster et al., 1999). Mecamylamine acts at nAChRs, but not at the nicotine binding site. Mecamylamine blocks the effects of nicotine but does not displace nicotine binding (Collins et al., 1986; Banerjee et al., 1990), thereby producing non-competitive antagonism that is often insurmountable (Stolerman et al., 1983). The proposed binding site is located within the nAChR ion channel pore. Mecamylamine attenuates many behavioral effects of nicotine, including its positive reinforcing, aversive, and discriminative stimulus effects, as well as the effects of nicotine on schedule-controlled behavior and locomotor activity (Clarke and Kumar, 1983; Cunningham and McMahon, 2011; Fudala et al., 1985; Jutkiewicz et al., 2011; Reavill and Stolerman, 1990; Stolerman et al., 1999). Mecamylamine attenuates the discriminative stimulus effects of nicotine in mice, rats, and non-human primates across a range of training doses (Cunningham et al., 2012; Jutkiewicz et al., 2011; Stolerman et al., 1999).

Mecamylamine can serve as a discriminative stimulus; however, the dose of mecamylamine required to train a discrimination is much larger than the smallest doses of mecamylamine that reliably antagonize the behavioral effects of nicotine (Garcha and Stolerman, 1993). For example, in rats, the dose of mecamylamine (3.5 mg/kg) trained as a discriminative stimulus was 30 times larger than the dose of mecamylamine that antagonized the discriminative stimulus effects of nicotine (Stolerman et al., 1983; Garcha and Stolerman, 1993). In rats discriminating mecamylamine, various other ganglionic-blocking drugs

* Corresponding author.
E-mail address: lance.mcmahon@cop.ufl.edu (L.R. McMahon).

https://doi.org/10.1016/j.pbb.2019.02.002
Received 20 November 2018; Received in revised form 21 January 2019; Accepted 5 February 2019
Available online 06 February 2019
0091-3057/

shared effects with nicotine. Nicotine itself and muscarinic antagonists, however, failed to substitute for mecamylamine, and nicotine failed to antagonize the discriminative stimulus effects of mecamylamine (Garcha and Stolerman, 1993).

Ganglionic-blocking drugs inhibit the stimulating actions of acetylcholine at autonomic ganglia; these chemically diverse agents are divided into two groups. One group consists of quaternary compounds such as hexamethonium, chlorisondamine, and pentolinium, that have restricted access to the CNS due to charge that inhibits blood-brain-barrier penetration. The second group consists of amines, e.g., mecamylamine and pempidine, do cross the blood-brain-barrier (Sethi and Gulati, 1973). Hexamethonium (Bradley et al., 1966), pentolinium (Caulfield and Higgins, 1983), and chlorisondamine (Kumar et al., 1987) can antagonize some of the effects of nicotine. However, their limited penetration into the CNS requires intracerebroventricular administration to facilitate their ability to block the effects of nicotine mediated by brain nAChRs (Kumar et al., 1987). These two groups of antagonists can be used to differentiate the involvement of peripheral versus central nAChRs in the behavioral effects of nicotine and other nAChR agonists.

Beyond its use to control hypertension, mecamylamine has long been considered a viable candidate as a smoking cessation aid due to its effectiveness at blocking nicotine, the active alkaloid in tobacco that drives tobacco use. However, mecamylamine may have limited selectivity for nAChRs, i.e., off-target effects. Mecamylamine was reported to antagonize *N*-methyl-D-aspartate (NMDA) receptor mediated norepinephrine release (O'Dell and Christensen, 1988). PCP and MK-801 both have affinity for nAChRs, although at different binding sites than nicotine or acetylcholine, and can antagonize some effects mediated by peripheral and central nAChRs (Eldefrawi et al., 1980; Ramoa et al., 1990). However, in rats trained to discriminate mecamylamine (3.5 mg/kg), PCP produced predominantly saline-lever responding. MK-801, on the other hand, produced a partial effect, i.e., 34% mecamylamine-lever responding (Garcha and Stolerman, 1993).

One goal of the current study was to characterize the discriminative stimulus effects of mecamylamine in non-human primates by testing nAChR antagonists (dihydro-β-erythroidine, pempidine, chlorisondamine, pentolinium, and hexamethonium), a muscarinic acetylcholine receptor antagonist (atropine), NMDA antagonists (MK-801, PCP, and ketamine), smoking cessations aids (nicotine, varenicline, cytisine, and bupropion), and a benzodiazepine (midazolam). The NMDA antagonists were added to evaluate not only glutamate involvement in the effects of mecamylamine, but also potential NMDA antagonist abuse-related effects; the abuse-related effects were of interest insofar as mecamylamine remains a viable strategy for treating untoward effects of nicotine such as excessive use (i.e., cigarette smoking or vaping) and poisoning (i.e., green tobacco sickness; McMahon, 2019). A second goal was to identify the extent to which peripheral nAChRs might contribute to the discriminative stimulus effects of mecamylamine and nicotine. While discriminative stimulus effects are centrally mediated, actions of drugs outside the brain and detected by the peripheral nervous system could be relayed to the brain and contribute to discriminative stimulus effects. Four rhesus monkeys were trained to discriminate mecamylamine (5.6 mg/kg) from saline in a two-choice discrimination procedure. Five rhesus monkeys discriminated nicotine (1.78 mg/kg) from saline as previously described (Cunningham et al., 2012).

2. Materials and methods

2.1. Subjects

Nine adult rhesus monkeys (*Macaca mulatta*) were used. Five (three males and two females) that discriminated nicotine (1.78 mg/kg free base s.c.) from saline had been previously trained as described (Cunningham et al., 2012). Four (two males and two females) that discriminated mecamylamine (5.6 mg/kg s.c.) from saline had been previously trained as described (Cunningham et al., 2014). Monkeys were housed individually in stainless steel cages on a 14-h light/10-h dark schedule (lights on at 0600 h). They were maintained at 95% free-feeding weight (range 6–10.5 kg) with a diet consisting of primate chow (Harlan Teklad, High Protein Monkey Diet; Madison, WI), fresh fruit, and peanuts; water was continuously available in the home cage. Monkeys were maintained, and experiments were conducted in accordance with, the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio and the *National Institutes of Health's Guide for the Care and Use of Laboratory Animals* (Institute for Laboratory Animal Research, 2011).

2.2. Apparatus

Monkeys were seated individually in a chair (Model R001, Primate Products; Miami, FL) in front of a stainless-steel panel, two levers, and two lights (i.e., one above each lever) within ventilated chambers in the presence of continuous white noise. Feet were maintained in contact with brass electrodes to which a brief electric stimulus (3 mA, 250 ms) could be delivered from an a/c generator (Coulbourn Instruments; Whitehall, PA). An interface (MedAssociates) connected the chambers to a computer, which controlled and recorded lever responses with Med-PC software.

2.3. Discrimination training

Responding was maintained under an FR5 schedule of stimulus-shock termination. Illumination of the lights signaled that an electric stimulus was scheduled for delivery in 10 s; however, five consecutive responses on a lever extinguished the lights, prevented delivery of the electric stimulus, and postponed the schedule for 30 s. The schedule of stimulus-shock termination ended after 10 min.

Four rhesus monkeys were trained to discriminate mecamylamine (5.6 mg/kg) from saline. Experimental sessions began with a 30-min timeout followed by a 10-min schedule of stimulus-shock termination. The lever designated correct during training was assigned based on whether the monkey received saline or the training dose of mecamylamine (5.6 mg/kg) prior to the session. Mecamylamine lever assignments were balanced across the group for left and right levers and remained the same per monkey for the duration of the study. Five consecutive responses on the correct lever postponed the shock schedule; incorrect responses reset the response requirement. The first test was conducted when for five consecutive or six out of seven training sessions, at least 80% of the total responses occurred on the correct lever and fewer than five responses occurred on the incorrect lever prior to completion of the first fixed ratio on the correct lever.

Five rhesus monkeys were previously trained to discriminate nicotine (1.78 mg/kg) from saline (Cunningham et al., 2012). The nicotine versus saline training parameters were identical to those for monkeys discriminating mecamylamine, except the timeout was 10 min in duration; the period of stimulus-shock termination was 10 min. The lever designated correct during training was assigned based on whether the monkey received saline or the training dose of nicotine (1.78 mg/kg).

2.4. Discrimination testing

Test sessions were identical to training sessions except that five consecutive responses on either lever postponed the schedule of stimulus-shock termination and animals received saline or dose(s) of test compound. Further tests were conducted when performance for consecutive training sessions, including both saline and drug training sessions, satisfied the test criteria.

To characterize the discriminative stimulus effects mecamylamine (5.6 mg/kg), a dose of mecamylamine, pentolinium, chlorisondamine,

pempidine, hexamethonium, dihydro-beta-erythroidine, atropine, ketamine, phencyclidine, MK-801, nicotine, varenicline, cytisine, bupropion, or midazolam were administered at the beginning of the session. The effects of mecamylamine, pentolinium, chlorisondamine, pempidine, and hexamethonium were further examined alone and in combination with nicotine in the nicotine discrimination assay. For these tests in monkeys discriminating nicotine, a dose of antagonist that produced > 75% mecamylamine-lever responding was administered 10 min before a 10-min response period, or 20 min before nicotine, which was administered at the beginning of the 10-min timeout.

2.5. Drugs

Drugs were administered s.c. in the midscapular region of the back in volumes of < 3 ml. The doses (mg/kg) of current test drugs were expressed as the weight of the base and salt, except for nicotine and pentolinium, which were expressed as the weight of the base. Nicotine hydrogen tartrate salt, atropine, hexamethonium bromide, 1,2,2,6,6-pentamethylpiperidine (pempidine), pentolinium tartrate, chlorisondamine diiodide, phencyclidine hydrochloride, (+)-MK-801 hydrogen maleate (Sigma-Aldrich; St. Louis, MO), mecamylamine hydrochloride (Waterstone Technology; Carmel, IN), bupropion hydrochloride, varenicline dihydrochloride (Research Triangle Institute, Research Triangle Park, NC), cytisine (Atomole Scientific, Hubei, China), and dihydro-beta-erythroidine hydrobromide (Tocris Bioscience; Bristol, United Kingdom) were dissolved in physiological saline. Ketamine (Butler Animal Health Supply, Dublin, OH) and midazolam (5 mg/ml in physiologic saline; Bedford Laboratories; Bedford, OH) were obtained in solution. The pH of all solutions was adjusted to 7.

2.6. Data analyses

Discrimination data were expressed as the mean of individual values from all four monkeys discriminating mecamylamine and five monkeys discriminating nicotine. Discrimination data were calculated as a percentage of responses on the drug lever out of total responses on the saline and drug levers, separately for the respective discriminations (mecamylamine or nicotine).

The potency to produce drug-lever responding was calculated by simultaneously fitting straight lines to individual dose-effect data by means of GraphPad Prism version 5.0 for Windows (San Diego, CA) with linear regression. Straight lines were fitted to the linear portion of dose-effect curves, defined by doses producing 20–80% drug-lever responding, including the largest dose that produced < 20% drug-lever responding and the smallest dose that produced > 80% drug-lever responding. Other doses were excluded from the analyses. Doses corresponding to the 50% level of the effect (ED_{50}) and 95% confidence limits were calculated by parallel line analyses of data from individual subjects (Tallarida, 2000). When an ED_{50} value could not be interpolated (i.e., maximum effect was not > 50%), an F-ratio test was used to compare the slope and intercept of functions with GraphPad.

Rate of responding on both levers (i.e., drug and saline) was calculated as responses per second, excluding responses during time-outs. Rate of responding during a test was expressed as the percentage of the control response rate for individual animals. The control was defined as the average response rate for all cycles during the five previous vehicle training sessions, excluding sessions during which the test criteria were not satisfied. A straight line was fitted to response rate data and the slope was obtained. A significant effect on response rate was evidenced by a slope that was significantly different from 0.

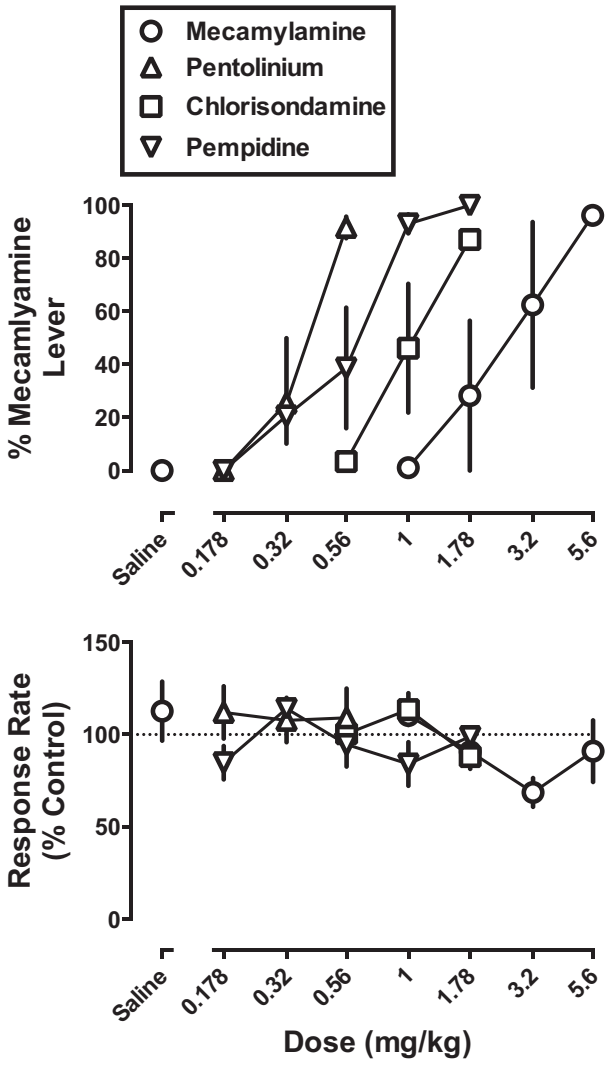

Fig. 1. Discriminative stimulus effects of mecamylamine, pentolinium, chlorisondamine, and pempidine in rhesus monkeys discriminating mecamylamine (5.6 mg/kg). Abscissae: Saline or dose in mg/kg body weight administered s.c. Ordinates: mean (± S.E.M.) percentage of responding on the mecamylamine lever (top) and mean (± S.E.M.) rate of responding expressed as a percentage of control (bottom).

3. Results

3.1. The discriminative stimulus effects of mecamylamine in rhesus monkeys

The criteria for conducting the first test in rhesus monkeys trained to discriminate mecamylamine (5.6 mg/kg) from saline had been satisfied as described previously (Cunningham et al., 2014). Here, mecamylamine dose-dependently increased the percentage of responses on the mecamylamine lever up to 98% at the training dose (5.6 mg/kg s.c.; Fig. 1, top, open circles), whereas saline produced no responses on the mecamylamine lever (Fig. 1, top). The ED_{50} value (95% confidence limits) of mecamylamine to produce discriminative stimulus effects was 2.60 (0.87–7.76) mg/kg. Mecamylamine (up to 5.6 mg/kg) did not significantly modify response rate (Fig. 1, bottom, open circles).

3.2. The discriminative stimulus effects of peripherally restricted nAChR antagonists in rhesus monkeys discriminating mecamylamine

Antagonists of nAChRs that are thought not to penetrate the CNS were also examined for the ability to produce mecamylamine-like

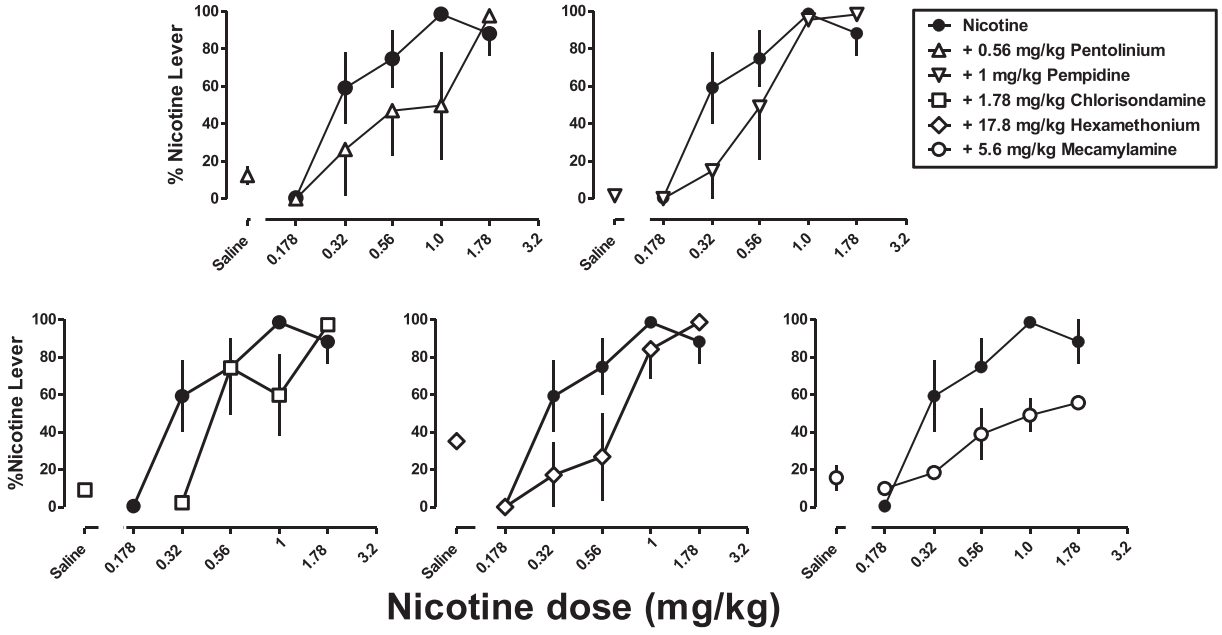

Fig. 2. Discriminative stimulus effects of nicotine alone and in combination with pentolinium (0.56 mg/kg), pempidine (1 mg/kg), chlorisondamine (1.78 mg/kg), hexamethonium (17.8 mg/kg), and mecamylamine (5.6 mg/kg). Abscissae: Dose of nicotine free base in mg/kg body weight administered s.c. Ordinates: mean (± S.E.M.) percentage of responding on the nicotine lever (top) and mean (± S.E.M.) rate of responding expressed as a percentage of control (bottom). Points above Saline are the effects of the dose of the nAChR antagonist alone in combination with saline. Points above nicotine dose are the effects of nicotine alone (closed circles) or the effects of the same nicotine dose in the presence of the nAChR antagonist (open symbols).

discriminative stimulus effects. Pentolinium and chlorisondamine dose-dependently increased mecamylamine-lever responding; the maximum effects were 92% and 87%, respectively (Fig. 1, top, triangles and squares, respectively). The ED_{50} values (95% confidence limits) were 0.37 (0.21–0.67) for pentolinium, and 1.07 (0.62–1.84) for chlorisondamine. The ED_{50} values for pentolinium and chlorisondamine were compared to mecamylamine by calculation of potency ratios. Pentolinium was significantly more potent than mecamylamine, with a potency ratio (95% confidence limits) of 7.0 (2.6–19.1), whereas chlorisondamine was equipotent with mecamylamine based on confidence limits (0.9–6.4 mg/kg) of the ED_{50} value (2.4 mg/kg) including 1.

3.3. The discriminative stimulus effects of pentolinium, pempidine, chlorisondamine, hexamethonium, and mecamylamine alone and in combination with nicotine in rhesus monkeys discriminating nicotine

Pentolinium (0.56 mg/kg), pempidine (1 mg/kg), chlorisondamine (1.78 mg/kg), and mecamylamine (5.6 mg/kg) produced a maximum of 15.6% nicotine-lever responding (Fig. 2, open circles above saline). Hexamethonium (17.8 mg/kg), produced on average 35% nicotine-lever responding. When the ganglionic-blocking drugs were combined with nicotine, there was no significant shift in the nicotine dose-response curve except for mecamylamine; the potency ratio values (95% confidence limits) were 1.9 (0.5–6.5) for pentolinium, 1.3 (0.6–2.9) for pempidine, 1.5 (0.7–3.6) for chlorisondamine, 1.6 (0.7–3.7) for hexamethonium. For mecamylamine in combination with nicotine, the slope and intercept were significantly different from those calculated for nicotine alone ($p > 0.05$). Relative to the saline control, response rate was not significantly altered by the antagonists alone, nicotine alone, or by their combination at the doses studied (data not shown).

3.4. The discriminative stimulus effects N-methyl-D-aspartate (NMDA) receptor antagonists in rhesus monkeys discriminating mecamylamine

MK-801, PCP, and ketamine dose-dependently increased responding on the mecamylamine lever (Fig. 3, top); the maximum effects were 87%, 97%, and 84%, respectively. The slopes obtained from dose-response functions of mecamylamine, MK-801, PCP and ketamine were not significantly different from one another. The ED_{50} values (95% confidence limits) determined from the common slope were 0.019 (0.008–0.046) for MK-801, 0.031 (0.022–0.044) for PCP, and 1.97 (1.19–3.26) for ketamine. Based on potency ratio values (95% confidence limits), MK-801 and PCP were 136.2 (37.9–489.6) and 86.6 (35.7–210) fold more potent than mecamylamine, respectively. In contrast, the potencies for ketamine and mecamylamine were not significantly different according to potency ratios (95% confidence limits), i.e., 1.3 (0.5–3.6).

3.5. The discriminative stimulus effects of the muscarinic acetylcholine receptor antagonist atropine in rhesus monkeys discriminating mecamylamine

Atropine (0.1–1.78 mg/kg) produced no greater than a mean of 5% mecamylamine-lever responding; there was no effect on response rate. Atropine (1 and 1.78 mg/kg) produced increased vocalizations throughout the discrimination session.

3.6. Tests with the smoking cessation aids nicotine, varenicline, bupropion, and cytisine and the benzodiazepine midazolam

Nicotine (1.78–3.2 mg/kg), varenicline (1–3.2 mg/kg), bupropion (5.6 and 10 mg/kg), and cytisine (17.8–56 mg/kg) produced a maximum of 7% mecamylamine-lever responding (Fig. 4, top). Although none of these doses significantly decreased response rate to < 50% control (Fig. 4, bottom), and the slopes of their dose-response functions were not significantly different from 0, these are behaviorally active doses as evidenced by their ability to produce discriminative stimulus effects, or to substitute for or antagonize the discriminative stimulus effects of nicotine in rhesus monkeys (Cunningham et al., 2012). Midazolam produced a maximum of 47% mecamylamine-lever responding (Fig. 4, top); however, this was accompanied by marked a decrease in

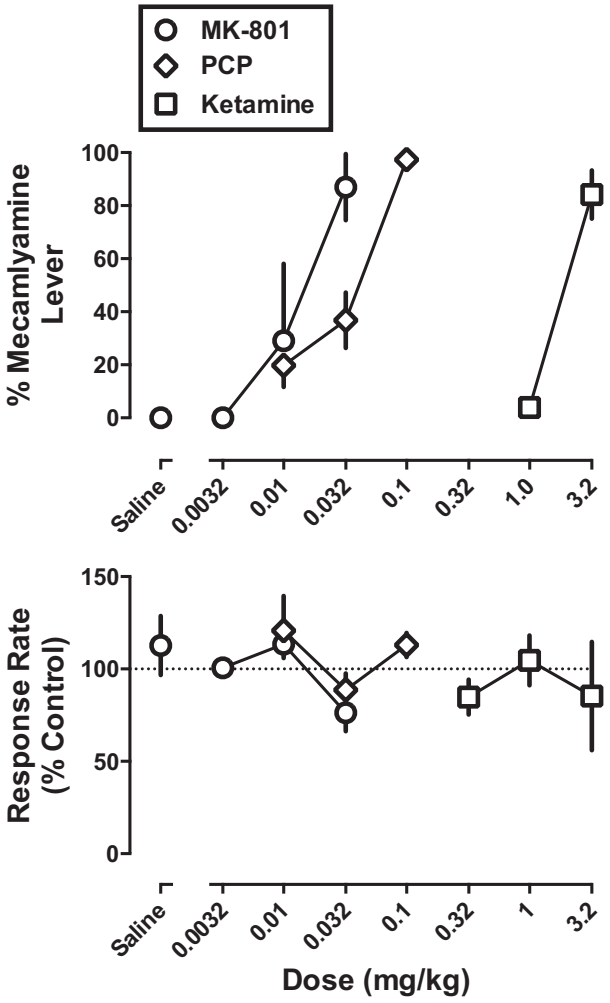

Fig. 3. Discriminative stimulus effects of MK-801, PCP, and ketamine in rhesus monkeys discriminating mecamylamine (5.6 mg/kg). Abscissae: Dose in mg/kg body weight administered s.c. Ordinates: mean (± S.E.M.) percentage of responding on the mecamylamine lever (top) and mean (± S.E.M.) rate of responding expressed as a percentage of control (bottom).

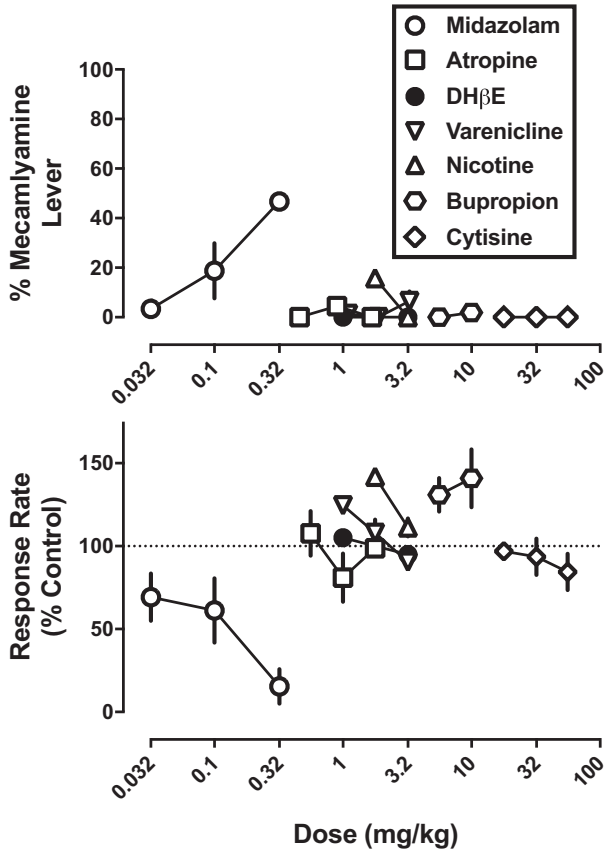

Fig. 4. Effects of smoking cessation aids (nicotine, varenicline, bupropion, and cytisine), DHβE, and a benzodiazepine (midazolam) in rhesus monkeys discriminating mecamylamine (5.6 mg/kg). Abscissae: Dose in mg/kg body weight administered s.c. Ordinates: mean (± S.E.M.) percentage of responding on the mecamylamine lever (top) and mean (± S.E.M.) rate of responding expressed as a percentage of control (bottom).

response rate, i.e., to 15% of control (Fig. 4, bottom).

3.7. The discriminative stimulus effects of other CNS penetrating nAChR antagonists in rhesus monkeys discriminating mecamylamine

Pempidine dose-dependently increased mecamylamine-lever responding; the maximum effect was 100% (Fig. 1, top, inverted triangles). The ED_{50} value (95% confidence limits) of pempidine to produce mecamylamine-like discriminative stimulus effects was 0.52 (0.37–0.73). Dihydro-β-erythroidine, up to a dose (3.2 mg/kg) that is toxic in rhesus monkeys (tremor and ataxia), produced 0% mecamylamine-lever responding and did not significantly modify response rate (Fig. 4).

4. Discussion

In rhesus monkeys discriminating the non-competitive nAChR antagonist mecamylamine (5.6 mg/kg), another CNS penetrating ganglionic-blocking drug (i.e., pempidine) produced mecamylamine-like effects. The peripherally restricted nAChR antagonists pentolinium and chlorisondamine substituted for the mecamylamine discriminative stimulus (current results), as did hexamethonium as reported previously (Cunningham et al., 2014). Nicotine did not produce mecamylamine-like effects as reported previously (Cunningham et al., 2014), and was unable to attenuate the discriminative stimulus effects of mecamylamine (5.6 mg/kg). Other smoking cessation aids (varenicline, cytisine, and bupropion) and a benzodiazepine (midazolam) did not substitute for the discriminative stimulus effects of mecamylamine at doses that were reported to be behaviorally active in rhesus monkeys (Cunningham et al., 2012), indicating that the mecamylamine discriminative stimulus was pharmacologically selective. None of the ganglionic-blocking drugs (i.e. pentolinium, pempidine, chlorisondamine, and hexamethonium) antagonized the discriminative stimulus effects of nicotine at a training dose of 1.78 mg/kg in rhesus monkeys. The NMDA receptor antagonists (ketamine, PCP, and MK-801) reliably substituted for the discriminative stimulus effects of mecamylamine at doses that did not alter rate of responding. These data suggest that the discriminative stimulus effects of mecamylamine in primates are mediated by both nAChR and NMDA receptors, and that the discrimination is mediated at least in part by peripheral nAChRs. It should be noted that the doses of mecamylamine and nicotine used for training were relatively high; one consequence of this might be loss of pharmacological selectivity at nAChRs.

Drug discrimination is centrally mediated; typically, that mediation is thought to reflect actions on receptors in the brain. Data supporting an essential role for the CNS in drug discrimination has been obtained with various training drugs including nicotine (Perkins et al., 1999; Stolerman et al., 1984), apomorphine (Woolverton et al., 1987), and morphine (France et al., 1987). Consistent with these findings, drugs like mecamylamine and pempidine that readily penetrate the blood-brain barrier shared discriminative stimulus effects with

mecamylamine. However, drugs that do not readily penetrate the blood-brain barrier (i.e., pentolinium, chlorisondamine, and hexamethonium) also substituted for a mecamylamine discriminative stimulus, suggesting that drug actions at peripheral nAChR can be discriminated. There are species differences in the maximum effects produced by hexamethonium and chlorisondamine, which fully generalized to a mecamylamine discriminative stimulus in rhesus monkeys, but only produced partial effects in rats (Garcha and Stolerman, 1993). Chlorisondamine in the previous study was not tested up to doses that eliminated responding, leaving open the possibility for greater mecamylamine-lever responding at larger doses. The difference in effects produced by hexamethonium across the two experiments could be due the training dose of mecamylamine, or to species differences such as nAChR subtype expression and function (Papke and Porter Papke, 2002) and pharmacokinetics.

There are two possible interpretations of a presumed peripherally restricted antagonist producing mecamylamine-like effects in rhesus monkeys. First, it is possible that the peripherally restricted nAChR antagonists are being administered in large enough doses that enough reaches the CNS to stimulate nAChRs and exert mecamylamine-like discriminative stimulus effects. This interpretation is unlikely because peripherally restricted nAChR antagonists like hexamethonium have consistently failed to antagonize the centrally mediated effects of nicotine when administered systemically (current study; Hazell et al., 1978; Stolerman et al., 1984; Besheer et al., 2004). However, when administered directly into brain ventricles, thereby circumventing the blood-brain barrier, hexamethonium was reported to antagonize the effects of nicotine (Kumar et al., 1987). The second interpretation is that the discriminative stimulus effects of a relatively large dose of mecamylamine are mediated, at least partially, by actions of the ganglionic-blocking drugs outside the brain.

The ganglionic-blocking drugs that produced mecamylamine-like discriminative stimulus effects in rhesus monkeys, pentolinium, pempidine, chlorisondamine, and hexamethonium, were examined for the ability to antagonize a nicotine discriminative stimulus (1.78 mg/kg). The doses tested were the smallest doses of antagonist that produced > 75% mecamylamine-lever responding. In previous studies mecamylamine (1 mg/kg) significantly antagonized the discriminative stimulus effects of nicotine in rhesus monkeys and shifted the nicotine dose-response curve 3.2 (1.3–8.0)-fold (Cunningham et al., 2012). Here, 5.6 mg/kg of mecamylamine significantly antagonized the discriminative stimulus effects of nicotine as well, in a manner that appeared insurmountable. While there was a trend for there to be a rightward shift of the nicotine dose-response function in the presence of some of the other antagonists, none of them significantly antagonized nicotine.

The discriminative stimulus effects of nicotine are thought to be mediated by nAChRs in the CNS (Stolerman et al., 1984). This hypothesis was to some extent confirmed in rhesus monkeys inasmuch as pentolinium, chlorisondamine, and hexamethonium, at doses that produced mecamylamine-like discriminative stimulus effects, failed to antagonize the discriminative stimulus effects of nicotine. It was somewhat surprising that pempidine, a tertiary amine that readily penetrates the CNS, did not significantly antagonize nicotine. In other studies, pempidine has been shown to antagonize several effects of nicotine in rats including depression of spontaneous activity, discriminative stimulus effects, and antinociceptive effects (Martin et al., 1990; Romano et al., 1981). The muscarinic antagonist atropine did not produce mecamylamine-like effects, indicating that muscarinic receptors are not involved in the mecamylamine discriminative stimulus.

In the present study three different NMDA antagonists, ketamine, PCP, and MK-801, produced high levels of mecamylamine-lever responding at doses that did not decrease the rate of responding in rhesus monkeys. The substitution of NMDA antagonists for a mecamylamine cue is at odds with data reported in rats (Garcha and Stolerman, 1993). However, the current data are consistent with in vitro data showing that mecamylamine has NMDA receptor antagonist activity. Mecamylamine can antagonize NMDA stimulated 3H-norepinephrine release (Snell and Johnson, 1989). Mecamylamine has also been shown to antagonize NMDA induced currents in catfish retina (O'Dell and Christensen, 1988). Lastly, the NMDA receptor antagonist MK-801 interacts with nAChRs as a low-affinity noncompetitive antagonist (Pessôa et al., 2005). Furthermore, the rank order potency of the NMDA receptor antagonists to produce the discriminative stimulus effects of mecamylamine falls in line with the rank order potency of these drugs to produce NMDA-receptor mediated effects (Koek et al., 1990). These data suggest that NMDA receptors mediate the discriminative stimulus effects of mecamylamine in rhesus monkeys. Although the mecamylamine-like effects of MK-801, PCP, and ketamine could be mediated by neuronal NMDA receptors, peripherally restricted nAChR antagonists produced a similar effect. These data might suggest that the discriminative stimulus effects of peripheral nAChRs antagonists are qualitatively similar to those produced by NMDA receptor antagonists. Alternatively, the ganglionic-blocking drugs could have non-selective actions at peripheral NMDA receptors.

It is currently unclear whether the discriminative stimulus effects of mecamylamine (5.6 mg/kg) in rhesus monkeys are mediated by peripheral NMDA receptors or peripheral nAChRs. If peripheral nAChRs are mediating the discriminative stimulus effects of mecamylamine, then antagonism of endogenous acetylcholine tone is likely mediating the effect. This hypothesis can be tested by artificially manipulating endogenous acetylcholine tone, perhaps with acetylcholinesterase inhibitors. Alternatively, to test the hypothesis that peripheral NMDA receptors mediate the discriminative stimulus effects of mecamylamine (5.6 mg/kg) in rhesus monkeys, the ganglionic-blocking drugs used in the current study can be examined for the ability to produce NMDA-receptor antagonist-like effects in animals trained to discriminate an NMDA-antagonist (like PCP or MK-801). Regardless of whether the effects originate peripherally, centrally, or some combination of both, these findings suggest caution is warranted over the continued therapeutic use of mecamylamine inasmuch as overlapping discriminative stimulus effects with NMDA receptor antagonists indicate that mecamylamine has NMDA receptor antagonist-like abuse liability.

Acknowledgements

Supported by USPHS grants DA25267 and DA48353.

References

Banerjee, S., Punzi, J.S., Kreilick, K., Abood, L.G., 1990. [3H]mecamylamine binding to rat brain membranes. Studies with mecamylamine and nicotine analogues. Biochem. Pharmacol. 40, 2105–2110.

Besheer, J., Palmatier, M.I., Metschke, D.M., Bevins, R.A., 2004. Nicotine as a signal for the presence or absence of sucrose reward: a Pavlovian drug appetitive conditioning preparation in rats. Psychopharmacology 172, 108–117.

Bradley, P.B., Dhawan, B.N., Wolstencroft, J.H., 1966. Pharmacological properties of cholinoceptive neurones in the medulla and pons of the cat. J. Physiol. 183, 658–674.

Caulfield, M.P., Higgins, G.A., 1983. Mediation of nicotine-induced convulsions by central nicotinic receptors of the 'C6' type. Neuropharmacology 22, 347–351.

Clarke, P.B., Kumar, R., 1983. The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br. J. Pharmacol. 78, 329–337.

Collins, A.C., Evans, C.B., Miner, L.L., Marks, M.J., 1986. Mecamylamine blockade of nicotine responses: evidence for two brain nicotinic receptors. Pharmacol. Biochem. Behav. 24, 1767–1773.

Cunningham, C.S., McMahon, L.R., 2011. The effects of nicotine, varenicline, and cytisine on schedule-controlled responding in mice: differences in alpha4beta2 nicotinic receptor activation. Eur. J. Pharmacol. 654, 47–52.

Cunningham, C.S., Javors, M.A., McMahon, L.R., 2012. Pharmacologic characterization of a nicotine-discriminative stimulus in rhesus monkeys. J. Pharmacol. Exp. Ther. 341, 840–849.

Cunningham, C.S., Moerke, M.J., McMahon, L.R., 2014. The discriminative stimulus effects of mecamylamine in nicotine-treated and untreated rhesus monkeys. Behav. Pharmacol. 25, 296–305.

Eldefrawi, M.E., Eldefrawi, A.T., Aronstam, R.S., Maleque, M.A., Warnick, J.E., Albuquerque, E.X., 1980. [3H]Phencyclidine: a probe for the ionic channel of the nicotinic receptor. Proc. Natl. Acad. Sci. U. S. A. 77, 7458–7462.

France, C.P., Adams, J.U., Woods, J.H., 1987. Comparison of naltrexone and quaternary

naltrexone after systemic and intracerebroventricular administration in pigeons. Neuropharmacology 26, 541–548.
Fudala, P.J., Teoh, K.W., Iwamot, E.T., 1985. Pharmacologic characterization of nicotine-induced conditioned place preference. Pharmacol. Biochem. Behav. 22, 237–241.
Garcha, H.S., Stolerman, I.P., 1993. Discriminative stimulus effects of the nicotine antagonist mecamylamine in rats. J. Psychopharmacol. 7, 43–51.
Hazell, P., Peterson, D.W., Laverty, R., 1978. Brief communication. Inability of hexamethonium to block the discriminative stimulus (SD) property of nicotine. Pharmacol. Biochem. Behav. 9, 137–140.
Institute of Laboratory Animal Resources, 2011. Guide for the Care and Use of Laboratory Animals, 8th ed. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, Washington, DC.
Jutkiewicz, E.M., Brooks, E.A., Kynaston, A.D., Rice, K.C., Woods, J.H., 2011. Patterns of nicotinic receptor antagonism: nicotine discrimination studies. J. Pharmacol. Exp. Ther. 339, 194–202.
Katner, S.N., Davis, S.A., Kirsten, A.J., Taffe, M.A., 2004. Effects of nicotine and mecamylamine on cognition in rhesus monkeys. Psychopharmacology 175, 225–240.
Koek, W., Woods, J.H., Colpaert, F.C., 1990. N-methyl-D-aspartate antagonism and phencyclidine-like activity: a drug discrimination analysis. J. Pharmacol. Exp. Ther. 253, 1017–1025.
Kumar, R., Reavill, C., Stolerman, I.P., 1987. Nicotine cue in rats: effects of central administration of ganglion-blocking drugs. Br. J. Pharmacol. 90, 239–246.
Mariathasan, E.A., Stolerman, I.P., 1993. Discrimination of agonist-antagonist mixtures: experiments with nicotine plus mecamylamine. Behav. Pharmacol. 4, 555–561.
Martin, T.J., Suchocki, J., May, E.L., Martin, B.R., 1990. Pharmacological evaluation of the antagonism of nicotine's central effects by mecamylamine and pempidine. J. Pharmacol. Exp. Ther. 254, 45–51.
McMahon, L.R., 2019. Green tobacco sickness: mecamylamine, varenicline, and nicotine vaccine as clinical research tools and potential therapeutics. Expert. Rev. Clin. Pharmacol (PMID: 30650314).
O'Dell, T.J., Christensen, B.N., 1988. Mecamylamine is a selective non-competitive antagonist of N-methyl-D-aspartate- and aspartate-induced currents in horizontal cells dissociated from the catfish retina. Neurosci. Lett. 94, 93–98.
Papke, R.L., Porter Papke, J.K., 2002. Comparative pharmacology of rat and human alpha7 nAChR conducted with net charge analysis. Br. J. Pharmacol. 137, 49–61.
Papke, R.L., Sanberg, P.R., Shytle, R.D., 2001. Analysis of mecamylamine stereoisomers on human nicotinic receptor subtypes. J. Pharmacol. Exp. Ther. 297, 646–656.
Perkins, K.A., Sanders, M., Fonte, C., Wilson, A.S., White, W., Stiller, R., McNamara, D., 1999. Effects of central and peripheral nicotinic blockade on human nicotine discrimination. Psychopharmacology 142, 158–164.
Pessôa, R.F., Castro, N.G., Noël, F., 2005. Binding of [3H]MK-801 in subcellular fractions of Schistosoma mansoni: evidence for interaction with nicotinic receptors. Biochem. Pharmacol. 69, 1509–1516.
Preston, K.L., Schuster, C.R., Seiden, L.S., 1985. Methamphetamine, physostigmine, atropine and mecamylamine: effects on force lever performance. Pharmacol. Biochem. Behav. 23, 781–788.
Ramoa, A.S., Alkondon, M., Aracava, Y., Irons, J., Lunt, G.G., Deshpande, S.S., Wonnacott, S., Aronstam, R.S., Albuquerque, E.X., 1990. The anticonvulsant MK-801 interacts with peripheral and central nicotinic acetylcholine receptor ion channels. J. Pharmacol. Exp. Ther. 254, 71–82.
Reavill, C., Stolerman, I.P., 1990. Locomotor activity in rats after administration of nicotinic agonists intracerebrally. Br. J. Pharmacol. 99, 273–278.
Romano, C., Goldstein, A., Jewell, N.P., 1981. Characterization of the receptor mediating the nicotine discriminative stimulus. Psychopharmacology 74, 310–315.
Sethi, O.P., Gulati, O.D., 1973. Analysis of mode of action of some nicotinic blocking drugs. Jpn. J. Pharmacol. 23, 437–451.
Shytle, R.D., Penny, E., Silver, A.A., Goldman, J., Sanberg, P.R., 2002. Mecamylamine (Inversine): an old antihypertensive with new research directions. J. Hum. Hypertens. 16, 453–457.
Snell, L.D, Johnson, K.M., 1989. Effects of nicotinic agonists and antagonists on N-methyl-D-aspartate-induced 3H-norepinephrine release and 3H-(1-[1-(2-thienyl)cyclohexyl]-piperidine) binding in rat hippocampus. Synapse 3, 129–135.
Stolerman, I.P., Pratt, J.A., Garcha, H.S., Giardini, V., Kumar, R., 1983. Nicotine cue in rats analysed with drugs acting on cholinergic and 5-hydroxytryptamine mechanisms. Neuropharmacology 22, 1029–1037.
Stolerman, I.P., Garcha, H.S., Pratt, J.A., Kumar, R., 1984. Role of training dose in discrimination of nicotine and related compounds by rats. Psychopharmacology 84, 413–419.
Stolerman, I.P., Naylor, C., Elmer, G.I., Goldberg, S.R., 1999. Discrimination and self-administration of nicotine by inbred strains of mice. Psychopharmacology 141, 297–306.
Stone, C.A., Torchiana, M.L., Navarro, A., Beyer, K.H., 1956. Ganglionic blocking properties of 3-methylaminoisocamphane hydrochloride (mecamylamine): a secondary amine. J. Pharmacol. Exp. Ther. 117, 169–183.
Tallarida, R.J., 2000. Drug Synergism and Dose-Effect Data Analysis. Chapman & Hall/CRC, Boca Raton.
Webster, J.C., Francis, M.M., Porter, J.K., Robinson, G., Stokes, C., Horenstein, B., Papke, R.L., 1999. Antagonist activities of mecamylamine and nicotine show reciprocal dependence on beta subunit sequence in the second transmembrane domain. Br. J. Pharmacol. 127, 1337–1348.
Woolverton, W.L., Kamien, J.B., Goldberg, L.I., 1987. Pharmacological analysis of the apomorphine discriminative stimulus in rhesus monkeys. J. Pharmacol. Exp. Ther. 241, 213–217.