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Mecamylamine attenuates ephedrine-induced hyperactivity in rats

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Abstract

Ephedrine is a central nervous system stimulant that has a pharmacological profile similar to amphetamines. Ephedrine induces hyperactivity after acute administration to rats and locomotor sensitization develops to ephedrine with repeated administration. Recent research suggests that nicotinic receptors (nAChRs) play a role in the development of locomotor sensitization to D-amphetamine and the goal of the present study was to determine if nAChRs similarly mediate the effects of ephedrine after acute and repeated drug injection. On 12 consecutive days, rats were pretreated with the nAChR antagonist mecamylamine (0.3–3.0 mg/kg) or saline followed by (−)-ephedrine (10–30 mg/kg) or saline injection and locomotor activity was measured. Ephedrine produced a dose-dependent increase in locomotor activity, and sensitization to ephedrine developed with repeated injection. Mecamylamine pretreatment attenuated the hyperactivity and sensitization produced by repeated, but not acute, ephedrine (10 mg/kg) injection. The inhibitory effect of mecamylamine was overcome at the higher ephedrine dose (30 mg/kg). The present results indicate that nAChRs play a mediating role in the development of locomotor sensitization to ephedrine.

Keywords: Addiction; Amphetamine; Ephedra; Nicotine; Nicotinic acetylcholine receptor; Sensitization

1. Introduction

Dietary supplements that contain ephedrine (also known by its Chinese name, *ma huang*) have historically been consumed for purposes of weight reduction and enhanced athletic performance, as ephedrine increases "energy" (Shekelle et al., 2003). However, these ephedrine-containing supplements have been associated with increased risk of stroke, heart attack and hypertension (Haller and Benowitz, 2000), and the risk for these adverse effects may be potentiated with recurrent use (Haller and Benowitz, 2000). As such, the United States Food and Drug Administration removed ephedra-containing products from the market in 2004 (Food and Drug Administration HHS, 2004).

Ephedrine is an amphetamine-type central nervous system stimulant and has a pharmacological profile that is similar to D-amphetamine and methamphetamine (Rothman et al., 2001). In rodents, acute ephedrine injection produces hyperactivity (Wellman et al., 1998), and with repeated injection sensitization develops to ephedrine-induced hyperactivity (Miller et al., 1998). In rodent brain, ephedrine administration increases the extracellular concentration of norepinephrine and dopamine (Ruwe et al., 1985; Wellman et al., 1998), likely via an action as a substrate at the norepinephrine and dopamine transporters, respectively (Rothman et al., 2001). The effects of repeated ephedrine administration on dopamine and norepinephrine neurons and the extracellular catecholamine levels have not been reported.

Recent research has investigated the role of nicotinic acetylcholine receptors (nAChRs) in the development of sensitization to amphetamines. The nAChR antagonists mecamylamine and dihydro-β-erythroidine (DHβE) attenuated the expression of locomotor sensitization to D-amphetamine after repeated injection to rats and mice (Karler et al., 1996; Schoffelmeer et al., 2002). In these studies, the effect of the nAChR antagonist was not evident with acute amphetamine administration, but was only observed after

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repeated co-administration of the antagonist and amphetamine. These findings suggest that nAChRs mediate the neuronal adaptations in the nervous system that contribute to the development of sensitization. The behavioral and neuropharmacological similarities between D-amphetamine and ephedrine (Rothman et al., 2001) suggest a mediating role for nAChRs in the development of behavioral sensitization to ephedrine. The goal of the present study was to determine if nAChRs mediate sensitization to ephedrine, or if nAChRs selective interact with the neuroadaptions in sensitization to amphetamine (Karler et al., 1996; Schoffelmeer et al., 2002).

2. Methods

2.1. Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Missouri. The subjects were male Sprague–Dawley rats (175–200 g; Harlan, Indianapolis, IN) that were double-housed with ad libitum access to standard chow (LabDiet, Richmond, IN) and tap water.

2.2. Apparatus

Locomotor activity was monitored automatically using Med Associates' (St. Albans, VT) Open Field Test Environments (ENV-515), comprised of a 16×16 horizontal grid of infrared sensors and a bank of 16 vertical sensors. Each monitor surrounds an acrylic cage ($43.2 \times 43.2 \times 30.5$ cm), and each monitor and cage is housed in a large sound-resistant cubicle (ENV-017M). Data were collected in 5 min intervals using Med Associates' Open Field Activity Software (SOF-811) that records the number of sensor breaks and computes these data to measures of distance traveled (in centimeters).

2.3. Procedures

Testing was conducted during the light phase of the light/dark cycle. For the first week after arrival, rats were handled and weighed daily. For 2 acclimation days, rats were transported to the laboratory in their home cage, weighed, injected (sc) with saline, returned to the home cage for 20 min, injected (ip) with saline, and placed in the activity monitor for 60 min. The subsequent 12 days (Days 1–12) followed a similar procedure, with the exception that rats were injected (sc) with mecamylamine (0.3, 1.0 or 3.0 mg/kg) or saline, followed 20 min later by injection (ip) with ephedrine (10 or 30 mg/kg) or saline. Thus, in the design of the study Mecamylamine Dose (0.3, 1.0 or 3.0 mg/kg mecamylamine or saline) and Ephedrine Dose (10 or 30 mg/kg ephedrine or saline) were between-group factors ($n=6$ rats/group).

2.4. Drugs

(−)-Ephedrine anhydrous and (±)-mecamylamine HCl were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO) and dissolved in saline (0.9% w/v) vehicle. Drug doses represent the free base weight and the injection volume was 1 ml solution/kg body weight. The (−)-ephedrine enantiomer was selected because it is more potent than (+)- or (±)-ephedrine to increase locomotor activity (Wellman et al., 1998) and the (−)-ephedrine doses (10–30 mg/kg) produce hyperactivity and sensitization under a regimen of 1 injection/day for 12 days (Miller et al., 1998). Mecamylamine (0.3–3.0 mg/kg) was used because it inhibits a range of nAChR subtypes (Papke et al., 2001) and the doses are within the range that attenuated locomotor sensitization to nicotine and to D-amphetamine (Stolerman et al., 1995; Schoffelmeer et al., 2002).

2.5. Data analysis

Distance traveled (in centimeters) was the primary dependent measure, consistent with published studies on ephedrine-induced hyperactivity (Miller et al., 1998). Data were analyzed via 4-way repeated measures analysis of variance with Mecamylamine Dose and Ephedrine Dose as between-group factors and Test Day (Day 1–12) and Session Time (5–60 min) as within-subject factors. Differences were considered statistically significant at $P<.05$, and simple main effect analyses and Tukey post hoc tests were performed when appropriate.

3. Results

The effect of acute mecamylamine and ephedrine injection was determined on Day 1 (Fig. 1, left panel). Analysis of total distance traveled data revealed a significant main effect of Ephedrine Dose [$F(2,60)=13.06$, $P<.001$] and analysis of the time course revealed a significant main effect of Session Time [$F(11,660)=135.53$, $P<.001$] and an Ephedrine Dose × Session Time interaction [$F(22,660)=15.34$, $P<.001$]. The main effect of Mecamylamine Dose, the Mecamylamine Dose × Ephedrine Dose interaction and the Mecamylamine Dose × Session Time interaction were not found to be significant. Regarding the time course, rats injected with 30 mg/kg of ephedrine were hyperactive from 30 to 60 min in the session (Fig. 1, left panel). Rats injected with 10 mg/kg of ephedrine were hyperactive from 35 to 50 min (Fig. 1, left panel). Thus, acute ephedrine injection produced dose-dependent hyperactivity and pretreatment with mecamylamine did not inhibit ephedrine-induced hyperactivity.

Rats were injected with mecamylamine and ephedrine daily for 12 consecutive days to determine the effect of repeated drug injection on locomotor activity (Fig. 2). A

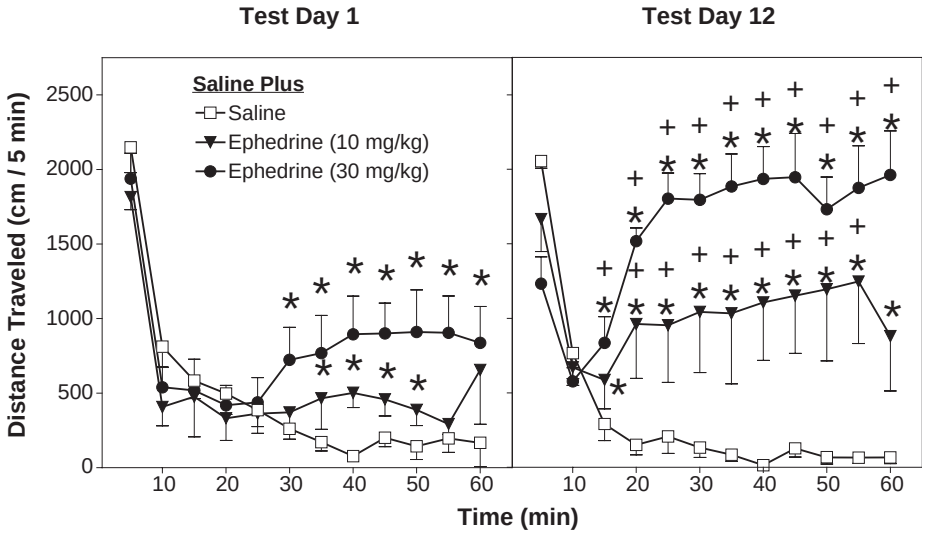

Fig. 1. Ephedrine produces dose-dependent hyperactivity after acute (Day 1, left panel) and repeated administration (Day 12, right panel). Rats were injected (sc) with saline followed 20 min later by injection (ip) with ephedrine (10 or 30 mg/kg) or saline and placement in a locomotor activity monitor for 60 min. Data represent mean (±SEM) distance traveled (in centimeters) in 5-min intervals after the second injection. *=significant ($P<.05$) between-group comparison from the Saline-Plus-Saline group at each time point. +=significant ($P<.05$) within-group comparison from the corresponding time point on Day 1.

significant Mecamylamine Dose × Ephedrine Dose × Test Day interaction was found [$F(66,660)=1.39$, $P<.05$].

Rats injected twice with saline did not significantly differ from rats injected with any dose of mecamylamine (0.3–3.0 mg/kg) followed by saline (Fig. 2, top panel). For all 4 of these groups, locomotor activity did not significantly differ across the 12 test days. Thus, mecamylamine did not alter basal locomotor activity after acute or repeated injection.

For rats injected with saline followed by 10 mg/kg of ephedrine [Fig. 2, middle panel; $F(11,55)=2.02$, $P<.05$] and for rats injected with saline followed by 30 mg/kg of ephedrine [Fig. 2, bottom panel; $F(11,55)=4.81$, $P<.05$] locomotor activity was greater on Days 2–12 than on Day 1. On Days 5–12, activity was greater for rats injected with 30 mg/kg of ephedrine than for rats injected with 10 mg/kg ephedrine (compare middle and bottom panels of Fig. 2).

Analysis of the distance traveled time course on Day 12 (Fig. 1, right panel) revealed a significant main effect of Session Time [$F(11,660)=40.87$, $P<.001$] and a significant Session Time × Ephedrine Dose interaction [$F(22,660)=33.72$, $P<.001$]. Neither the Mecamylamine Dose × Session Time interaction nor the Mecamylamine Dose × Ephedrine Dose × Session Time interaction was significant for Day 12. On Day 12, rats injected saline followed by 10 mg/kg or 30 mg/kg of ephedrine showed greater activity than rats injected twice with saline. Within-group comparisons for the rats injected with saline followed by 10 or 30 mg/kg of ephedrine revealed significant interactions of Test Day × Session Time [$F(121,605)=1.36$, $P<.05$ and $F(121,605)=1.94$, $P<.01$; respectively]. Post hoc comparisons revealed that for the rats injected with saline followed by 10 mg/kg of ephedrine, locomotor activity was greater at the 20–55 min time points on Day 12 than at the corresponding time points on Day 1 (compare left and right panels of Fig. 1). For the rats injected with saline followed by 30 mg/kg of ephedrine, activity was greater at the 15–60 min time points on Day 12 than on the corresponding time points on Day 1 (compare left and right panels of Fig. 1). Thus, after repeated administration, dose-dependent sensitization developed to ephedrine.

Further analyses were performed to determine the effect of mecamylamine on 10 mg/kg ephedrine-induced hyperactivity and sensitization on Days 2–12. Rats injected with mecamylamine (0.3–3.0 mg/kg) followed by 10 mg/kg of ephedrine were less active than rats injected with saline followed by this dose of ephedrine (Fig. 2, middle panel). However, this effect of mecamylamine was not dose-dependent. Mecamylamine did not completely prevent ephedrine (10 mg/kg)-induced hyperactivity, as rats injected with 0.3 or 1.0 mg/kg of mecamylamine followed by ephedrine (10 mg/kg) were more active on Days 2–12 than rats injected with 0.3 or 1.0 mg/kg of mecamylamine followed by saline (compare middle and top panels of Fig. 2). Rats injected with 3.0 mg/kg of mecamylamine followed by ephedrine (10 mg/kg) were more active than rats injected with mecamylamine followed by saline on Days 5, 8, 9 and 12 only (compare middle and top panels of Fig. 2). Within-group analyses compared the change in activity for each group across the 12 test days. For rats injected with 0.3, 1.0 and 3.0 mg/kg of mecamylamine followed by 10 mg/kg of ephedrine, there were no significant differences in distance traveled across Days 1–12 (Fig. 2, middle panel). Thus, mecamylamine attenuated hyperactivity induced by and prevented the development of sensitization to the low (10 mg/kg) dose of ephedrine.

An additional series of analyses were performed to assess the effect of mecamylamine (0.3–3.0 mg/kg) pretreatment on activity induced by 30 mg/kg of ephedrine (Fig. 2,

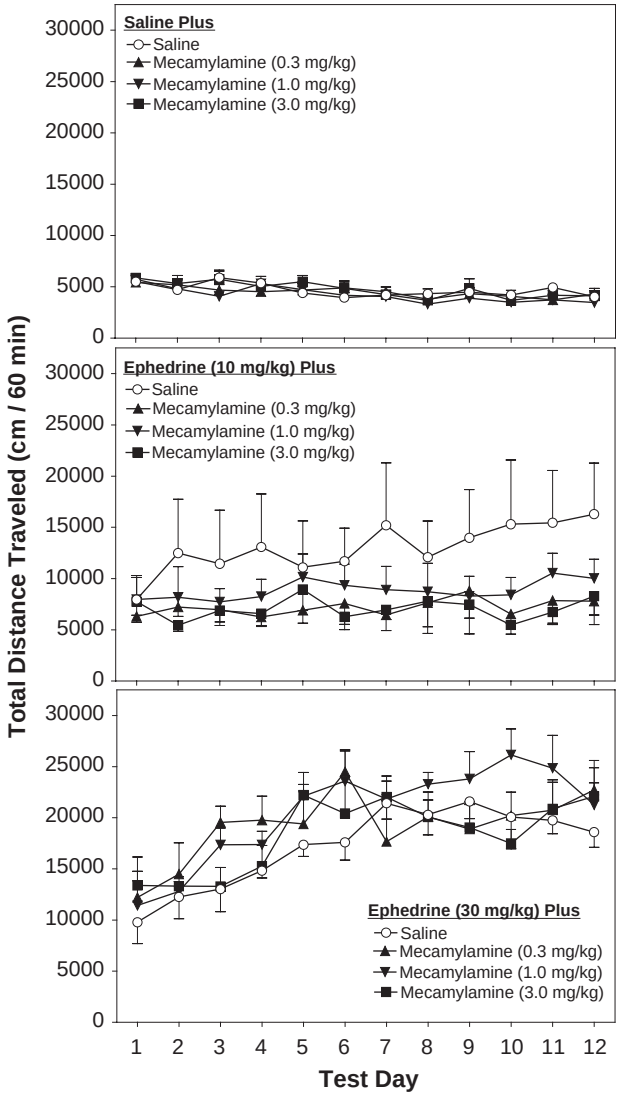

Fig. 2. Mecamylamine inhibits hyperactivity induced by 10 mg/kg, but not 30 mg/kg of ephedrine after repeated administration. Once daily for 12 consecutive days, rats were injected (sc) with mecamylamine (0.3–3.0 mg/kg) or saline followed 20 min later by injection (ip) with ephedrine (10–30 mg/kg) or saline and placement in a locomotor activity monitor for 60 min. Data represent mean (±SEM) total distance traveled (in centimeters) after the second injection on each of 12 days.

bottom panel). Rats injected with mecamylamine (0.3–3.0 mg/kg) followed by 30 mg/kg of ephedrine were more active than rats injected with mecamylamine followed by saline on Days 2–12, indicating that mecamylamine did not prevent ephedrine-induced hyperactivity (compare top and bottom panels of Fig. 2). Moreover, between-group analyses revealed that there were no differences in activity among rats injected with mecamylamine (0.3–3.0 mg/kg) followed by 30 mg/kg of ephedrine and rats injected with saline followed by 30 mg/kg of ephedrine (Fig. 2, bottom panel). Within-group analyses compared the change in activity for each group across the 12 test days. Activity was greater on Days 2–12 than on Day 1 for rats injected with 0.3, 1.0 and 3.0 mg/kg of mecamylamine followed by 30 mg/kg ephedrine [$F(11,55)=3.55$, $P<.01$; $F(11,55)=7.87$, $P<.001$; $F(11,55)=3.52$, $P<.01$; respectively; Fig. 2, bottom panel]. Thus, mecamylamine did not prevent ephedrine (30 mg/kg)-induced hyperactivity or the development of sensitization to the higher dose of ephedrine.

4. Discussion

Acute injection of ephedrine produced dose-dependent hyperactivity and with repeated drug injection sensitization developed to this effect of ephedrine, results consistent with published research (Miller et al., 1998). This pattern of behavior is similar to that reported for other amphetamines (Wise and Bozarth, 1987) and the mechanism for the development of sensitization to amphetamines has been well studied (Pierce and Kalivas, 1997). Multiple neurotransmitters and receptors, including nAChRs, have important roles in the development of behavioral and neurochemical sensitization to amphetamines (Bardo, 1998). The mechanism responsible for ephedrine sensitization has not been determined; however, it is likely to be similar to that for other amphetamines. Like D-amphetamine, ephedrine evokes catecholamine release via action as a substrate for dopamine and norepinephrine transporters (Rothman et al., 2001), indicating a similar neuropharmacological profile.

In the present experiment, mecamylamine did not alter ephedrine-induced hyperactivity after acute injection of either the low (10 mg/kg) or high (30 mg/kg) dose of ephedrine. The doses of mecamylamine (0.3–3.0 mg/kg) used in the present experiment block nicotine-induced changes in activity (Stolerman et al., 1995), demonstrating the efficacy of these mecamylamine doses to determine if a behavior is nAChR-mediated. This indicates that acute ephedrine effects on activity are not nAChR-mediated, a finding that is consistent with the previous research with mecamylamine and D-amphetamine (Karler et al., 1996; Schoffelmeer et al., 2002). This also suggests that mecamylamine does not directly compete with ephedrine at ephedrine's primary sites of action, likely the dopamine and norepinephrine transporters (Rothman et al., 2001). This proposition, that mecamylamine does not competitively interact with catecholamine transporters, is consistent with a recent study demonstrating that mecamylamine did not alter dopamine clearance in rat striata or prefrontal cortex using in vivo voltammetry (Middleton et al., 2004) and are also consistent with emerging research that nAChRs *indirectly* interact with the function of catecholamine transporters. For example, superfusion with mecamylamine and DHβE, did not inhibit D-amphetamine-evoked [^{3}H]dopamine release in prefrontal cortex slices; however, when nicotine was included in buffer, the nAChR antagonists inhibited D-amphetamine-evoked [^{3}H]dopamine release (Drew et al., 2000).

In the present study, mecamylamine prevented the development of locomotor sensitization to ephedrine,

results consistent with previous studies using mecamylamine and D-amphetamine (Karler et al., 1996; Schoffelmeer et al., 2002). The present study suggests that nAChRs mediate the adaptations that occur during the development of sensitization to ephedrine. NAChRs are located throughout the brain, particularly on neurons in the mesocorticolimbic pathway (Mansvelder et al., 2002) which is believed to be critical in the development of sensitization to psychostimulants (Pierce and Kalivas, 1997). The mechanism described for the effect of ephedrine on behavior after acute and repeated injection is based within the context of a specific centrally-mediated drug effect. Although mecamylamine is an antagonist at central nAChRs, it is also a potent blocker of both parasympathetic and sympathetic ganglia (Papke et al., 2001). Follow-up experiments must be conducted to determine if the phenomenon observed is mediated in the central nervous system, peripheral nervous system or both.

The inhibitory effect of mecamylamine on the low (10 mg/kg) ephedrine dose was overcome with the high (30 mg/kg) ephedrine dose, as mecamylamine did not attenuate hyperactivity or prevent the development of sensitization to 30 mg/kg of ephedrine. This finding, that the inhibitory effect of the antagonist (mecamylamine) was overcome with increasing doses of an agonist (ephedrine), implies a competitive interaction between the drugs. However, previous studies indicate that mecamylamine does not directly interact with catecholamine transporters (Drew et al., 2000; Middleton et al., 2004), and in the present experiment the inhibition of ephedrine was not mecamylamine dose-dependent. Thus, the findings indicate that the inhibitory effect of mecamylamine on ephedrine is reversible, and suggest that nAChRs play a mediating, rather than a direct, role in sensitization to ephedrine.

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