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Nicotine cue: lack of effect of the $\alpha 7$ nicotinic receptor antagonist methyllycaconitine

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Abstract

To assess the role of the $\alpha 7$ neuronal nicotinic acetylcholine receptor in the discriminative stimulus properties of (−)-nicotine, this study investigated the ability of the $\alpha 7$ receptor antagonist methyllycaconitine to modulate the nicotine cue. In rats trained to discriminate (−)-nicotine from saline, intraperitoneal injections of methyllycaconitine neither induced nor blocked the nicotine cue. Intracerebroventricular administration of methyllycaconitine, neither potentiated nor blocked the effect of (−)-nicotine. On the other hand, intracerebroventricular injections of mecamylamine blocked the nicotine cue. The available evidence indicate that the nicotinic acetylcholine receptors in the brain blocked by methyllycaconitine, those presumably containing $\alpha 7$ subunits, do not participate in the expression of the discriminative stimulus properties of (−)-nicotine.

Keywords: Nicotine; Drug discrimination; Mecamylamine; Methyllycaconitine

1. Introduction

The nicotine cue is one of the most robust behavioral effects of (−)-nicotine as animals can be trained to discriminate (−)-nicotine from saline after systemic administration. Although several studies have reported the effects of different nicotinic ligands like (+)-anatoxin-a, (−)-lobeline, (−)-cytisine and (±)-anabasine on nicotine-trained rats (Rosecrans and Chance, 1977; Stolerman, 1990; Brioni et al., 1996), the lack of selectivity of these ligands for the putative subunit combinations of the neuronal nicotinic acetylcholine receptor has precluded the identification of the subunits involved in the expression of the nicotine cue.

Neuronal nicotinic acetylcholine receptors are pentameric structures composed of different α and β subunits. Eleven gene products ($\alpha 2$–$\alpha 9$ and $\beta 2$–$\beta 4$) have been identified so far in different neuronal populations, and nine potential channels have been identified after expression of the combinations of these subunits in heterologous systems (Courturier et al., 1990; Sargent, 1993; Elgoyhen et al., 1994). In the brain, nicotinic acetylcholine receptors can be differentiated by labelling with [^{3}H]nicotine or [^{3}H]cytisine, a population that represent the binding to the $\alpha 4\beta 2$ subunit combination (Flores et al., 1992), and with [^{125}I]α-bungarotoxin, a population of receptors that show a good correlation with $\alpha 7$ mRNA distribution in the brain (Clarke, 1992).

Methyllycaconitine is the 2-methylsuccinylanthranilic acid ester of the norditerpenoid alkaloid lycoctonine. Methyllycaconitine is a selective probe for the neuronal α-bungarotoxin sites as it shows a 1000-fold higher potency to displace neuronal α-bungarotoxin sites (that represent the putative homo-oligomeric $\alpha 7$ channel) than to displace muscle α-bungarotoxin sites (the $\alpha 1\beta 1\delta\gamma$ channel) (Ward et al., 1990). Furthermore, in vertebrate brain preparations methyllycaconitine selectively binds to α-bungarotoxin sites (K_i between 1.4 and 4.0 nM) rather than to [^{3}H]nicotine sites ($K_i > 3700$ nM), and produces a reversible blockade of acetylcholine-activated currents in hippocampal preparations (Macallan et al., 1988; Alkondon et al., 1992). As methyllycaconitine is a selective antagonist of the $\alpha 7$ subtype, this compound was selected to determine the role of $\alpha 7$ subunits in the discriminative stimulus properties of (−)-nicotine.

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2. Materials and methods

2.1. Subjects

Male Wistar rats (weighing 200 g on arrival) supplied by Charles River (Portage, MI) were used. Animal studies were conducted according to the guidelines of the American Association for the Accreditation of Laboratory Animal Care (AAALAC) and according to protocols approved by the Abbott Institutional Animal Care and Use Committee. They were individually housed and given restricted amounts of food to maintain their body weights at about 80 ± 5% of those given free access to the food. Water was continuously available except during experimental sessions. Rats were maintained on a 12-h light-dark cycle.

2.2. Apparatus

Eight standard experimental chambers (Coulbourn Instruments, Lehigh Valley, PA, USA) contained within sound-attenuated enclosures were used. Each chamber contained two levers separated by a food receptacle in which 45 mg pellets (P.J. Noyes Co., Lancaster, NH, USA) could be presented by the dispenser. The chambers were controlled by a computerized system using the OPN software (Spencer and Emmett-Oglesby, 1985).

2.3. Training procedure

The training procedure has been described previously (Brioni et al., 1994; Brioni et al., 1995b). Briefly, rats were trained to press the levers to obtain food reinforcement under an autoshaping program. The animals were assigned randomly to one of the eight experimental chambers. A double alternation sequence of pretreatments followed the autoshaping routine. A pair of saline pretreatment sessions were followed by a pair of drug (1.9 μmol/kg (−)-nicotine, i.p.) pretreatment sessions. Saline and drug injections were given immediately before the 20-min session. The rats were placed inside the operant chambers for a 10-min time-out period (lights-off) during which no reinforcement was delivered, followed by a 10-min testing period (lights-on). Animals learned the discrimination procedure in approximately 40 days.

The testing sequence consisted of saline, drug and test sessions (i.e., S D T D S T etc.). Rats received the test drug injection immediately before the session. Responses were recorded during the 10 min lights-on period. The percent of the total responses that occurred on the drug appropriate-lever and the rate of responding on both levers during the test sessions were calculated for each rat. To avoid extinction of the discrimination during the test sessions, rats were rewarded on the lever where they reached 10 responses. Data were analyzed by the Kruskal-Wallis and Mann-Whitney U-tests.

2.4. Surgical and intracerebroventricular administration procedures

Chronic indwelling 22 gauge guide cannulas for intracerebroventricular (i.c.v.) injections were implanted under pentobarbital anesthesia (50 mg/kg, i.p.). Each cannula was implanted so that injections could be made directly into the lateral ventricle (AP: −0.8 from bregma; ML: 1.5; DV: −4.0, according to the Paxinos and Watson atlas). The cannulas were held in place by four screws attached to the skull and with dental acrylic. Injectors (28 gauge) were designed to protrude 0.5 mm beyond the tip of the permanent cannulas. Animals were allowed to recover on a warming plate, and testing started 48 h later. During the i.c.v. administration, rats were gently restrained by hand, the cap was withdrawn, and the injections were made slowly through an injecting cannula attached by polyethylene tubing to a 10 μl syringe and a motor-driven minipump. A total volume of 0.5 μl was injected over a 30 s period and the cannula was left in place another 30 s to allow diffusion of the drug. Following behavioral testing, the accuracy of the cannula placement was confirmed by a dye injection under anesthesia.

2.5. Drugs

(−)-Nicotine bitartrate (Sigma Chemical Co., St. Louis, MO, USA), mecamylamine (Sigma) and methyllycaconitine citrate (RBI, Natick, MA, USA) were dissolved in saline solution. Doses are expressed as μmol/kg. Drugs were injected i.p. in a volume of 1 ml/kg. During test sessions, methyllycaconitine was injected i.p. 5 min before, and saline or (−)-nicotine immediately before the test. Intracerebroventricular injections of methyllycaconitine or mecamylamine were made 5 min before, and saline or (−)-nicotine immediately before the test.

3. Results

3.1. Nicotine cue: lack of effect of i.p. injections of methyllycaconitine

Fig. 1 shows the lack of effect of i.p. injections of methyllycaconitine in rats trained to discriminate (−)-nicotine 1.9 μmol/kg from saline. Methyllycaconitine injections did not induce the nicotine cue ($H = 4.3$; NS) and did not block the cue ($H = 1.2$, NS). (−)-Nicotine was able to induce a full generalization in the presence of the α7 nicotinic receptor antagonist, methyllycaconitine. With regard to the response rates of the rats (lower graph), methyllycaconitine reduced operant rates of responding ($H = 17.3$, $P < 0.001$) with a significant effect at the 19 μmol/kg dose. Lower doses of methyllycaconitine (1.9 and 6.2 μmol/kg) did not affect the response rates of the rats.

3.2. Nicotine cue: lack of effect of i.c.v. injections of methyllycaconitine

In order to determine the dose range for the i.c.v. injections of methyllycaconitine, a preliminary experiment on the locomotor effects of 10, 30 and 100 nmol of methyllycaconitine was conducted in a group of naive male rats with chronically implanted cannulas. Animals were released in an open-field and the horizontal activity was automatically monitored in 10-min bins during 30 min. As methyllycaconitine significantly reduced locomotion only at the 100 nmol dose, the two lower doses were selected for further experimentation in nicotine-trained rats.

A group of rats trained to discriminate (−)-nicotine from saline was implanted with chronic cannulas as described in Methods. As shown in Fig. 2 (upper graph), a 1.9 μmol/kg dose of (−)-nicotine induced a full generalization in animals receiving either i.c.v. administration of saline or 10 nmol methyllycaconitine ($H = 13.9$, $P < 0.01$). Animals injected with 0.19 μmol/kg (−)-nicotine responded preferentially in the saline lever and the effect of 0.19 μmol/kg (−)-nicotine was not potentiated by the methyllycaconitine pretreatment. Response rates were similar between the different groups.

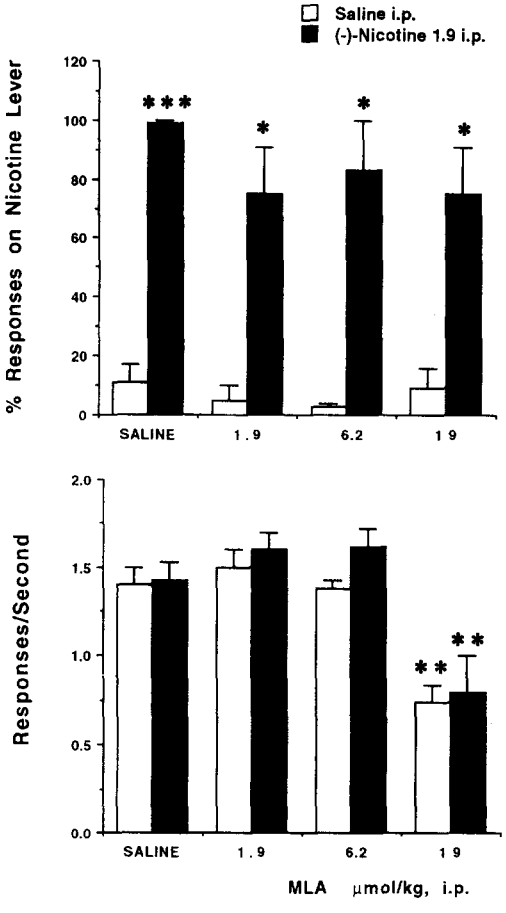

Fig. 1. Lack of effect of i.p. injections of methyllycaconitine (MLA) in animals trained to discriminate 1.9 μmol/kg (−)-nicotine from saline. Data represent the mean ± S.E.M. percentage of responses on the (−)-nicotine lever (upper graph) and the response rate (lower graph) of 4–5 rats. Animals were injected with methyllycaconitine i.p., and 5 min later followed by an i.p. injection of (−)-nicotine 1.9 μmol/kg or saline. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared to the saline/saline group.

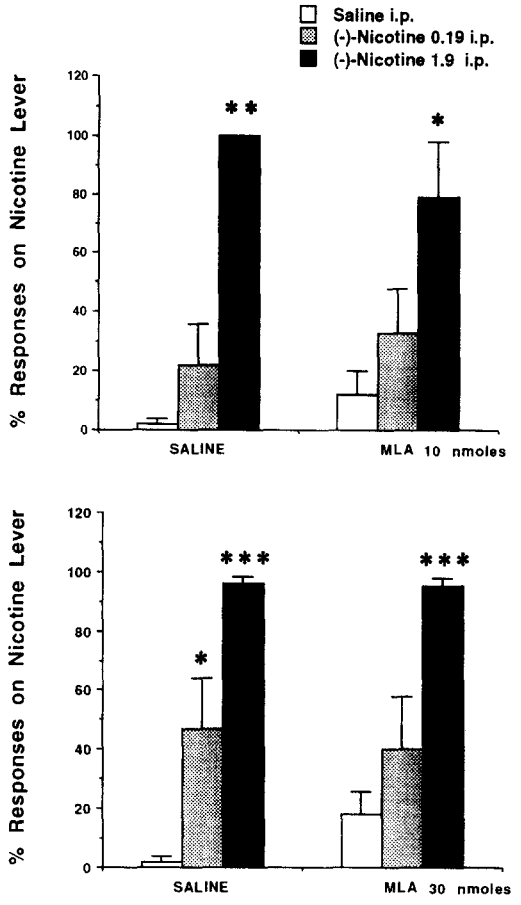

Fig. 2. Intracerebroventricular injections of methyllycaconitine (10 and 30 nmol) did not affect the nicotine cue. Data represent the mean ± S.E.M. percentage of responses of 5–8 rats. Animals trained to discriminate 1.9 μmol/kg (−)-nicotine from saline were injected with methyllycaconitine and 5 min later with i.p. saline or (−)-nicotine. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ as compared to the saline/saline group.

In a similar experiment (−)-nicotine induced a full generalization in animals receiving i.c.v. administration of saline or 30 nmol methyllycaconitine ($H = 22.8$, $P < 0.001$) as shown in Fig. 2 (lower graph). A partial generalization to the 0.19 μmol/kg (−)-nicotine dose was observed in this experiment, but this partial generalization was not potentiated by the methyllycaconitine pretreatment. Response rates were similar between the different groups. A higher dose of 100 nmol methyllycaconitine was tested in two rats but the animals were not motivated to lever-press, in agreement with the data on the locomotor effects at this dose.

3.3. Blockade of the nicotine cue with i.c.v. injections of mecamylamine

As it has been previously demonstrated that the nicotine cue can be blocked with the channel blocker mecamyl-

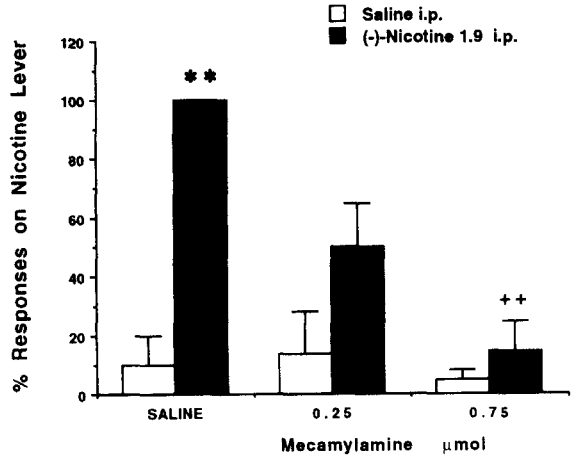

Fig. 3. Blockade of the stimulus properties of i.p. 1.9 μmol/kg (−)-nicotine by i.c.v. injections of mecamylamine (0.75 μmol). Data represent the mean ± S.E.M. percentage of responses of 6 rats. Animals were injected with mecamylamine i.c.v., followed 5 min later by an i.p. injection of (−)-nicotine 1.9 μmol/kg or saline. ** $P < 0.01$ as compared to the saline/saline group; $^{++}$ $P < 0.01$ as compared to the saline/(−)-nicotine 1.9 group.

amine, we investigated the effect of mecamylamine after i.c.v. injections to validate our experimental conditions. Fig. 3 shows that mecamylamine did not induce the nicotine cue by itself ($H = 1.6$, NS), but significantly blocked the effect of (−)-nicotine ($H = 7.5$, $P < 0.05$) with a significant effect at the 0.75 μmol dose. Response rates were similar between the different groups.

4. Discussion

In animals trained to discriminate (−)-nicotine from saline, the i.p. and i.c.v. injections of the selective α7 receptor antagonist methyllycaconitine did not induce the nicotine cue by itself and did not block the effect of (−)-nicotine. However, the nicotine cue was blocked by the i.c.v. injections of the non-selective cholinergic channel blocker mecamylamine. These data suggest that the nicotinic acetylcholine receptors composed of α7 subunits do not participate in the expression of the discriminative stimulus properties of (−)-nicotine.

The major fractions of brain nicotinic acetylcholine receptors can be labelled at the present time with [^{3}H]cytisine and [^{125}I]α-bungarotoxin. These ligands bind to two different populations of nicotinic receptors that represent the putative α4β2 and the putative α7 subunit population, respectively. Binding sites for α-bungarotoxin can be detected in the neuromuscular junction, autonomic ganglia, spinal cord and brain (Clarke, 1992). While the binding at the level of the neuromuscular junction or to Torpedo membranes reflects binding to the α1 subunit, the binding to brain membrane preparation reflects its affinity to the α7 subunit (Courturier et al., 1990). Despite extensive research in this field, the physiological role of the nicotinic acetylcholine receptors of the α7 subtype is still unknown (Clarke, 1992). The demonstration that methyllycaconitine is a selective α7 receptor antagonist (Macallan et al., 1988; Ward et al., 1990), provides a unique opportunity to evaluate the role of the α7 subtype in the expression of the nicotine cue.

Systemic injections of methyllycaconitine did not generalize to (−)-nicotine in animals trained to discriminate (−)-nicotine from saline, and methyllycaconitine was unable to block the nicotine cue. In (−)-nicotine-trained rats with chronically implanted cannulas, methyllycaconitine injections in the lateral ventricle did not potentiate or block the nicotine cue, while i.c.v. injections of mecamylamine significantly blocked the nicotine cue.

Pharmacological studies have revealed that nicotinic receptor agonists can induce the nicotine cue with different potency and efficacy in comparison to (−)-nicotine (Rosecrans and Chance, 1977; Stolerman, 1990). However, available nicotinic acetylcholine receptor agonists do not display the desired selectivity to the different nicotinic acetylcholine receptor subtypes expressed in the brain (Brioni et al., 1996) in order to causally relate this behavioral effect of (−)-nicotine with specific subunits forming the nicotinic acetylcholine receptors. For example, (±)-epibatidine can induce a full generalization in nicotine-trained rats at a very low dose (0.01 μmol/kg), but as (±)-epibatidine is a potent activator of channels composed of α1, α3, α4 and α7 subunits it is unclear at the present time which subunit is activated to induce such a potent behavioral effect (Decker et al., 1995; Brioni et al., 1996). With regards to the participation of α7 subunits, the partial α7 receptor agonist GTS-21 has been reported to induce a saline response in nicotine-trained rats (Brioni et al., 1995a). These data are in agreement with the lack of behavioral effects of methyllycaconitine on the nicotine cue.

In summary, the available experimental evidence indicate that the nicotinic acetylcholine receptors in the brain blocked by methyllycaconitine, those presumably composed of α7 subunits, do not participate in the expression of the discriminative stimulus properties of (−)-nicotine.

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