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Involvement of adrenergic and cholinergic systems in nicotine-induced anxiogenesis in mice

Mohammad-Reza Zarrindast *, Houman Homayoun, Arash Babaie, Atoosa Etminani, Behdad Gharib

Department of Pharmacology, Medical School, Tehran University of Medical Sciences, PO Box 13145-784, Tehran, Iran

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Abstract

In the present study, the effect of α-adrenoceptor agents on response to nicotine in an anxiety model (elevated plus-maze) in mice was investigated. Administered nicotine reduced indices of anti-anxiety behaviour (percent open-arm time (%open-arm time) and percent open-arm entries (%open-arm entry)) and increased indices of anxiety behaviour (protected stretched attention posture and percent of protected head dipping (%protected dipping)), indicating that nicotine elicits an anxiogenic response. This response to the drug was obtained 7 min but not 30 min after drug injection and with doses of 0.25 and 0.5 mg/kg. Nicotinic receptor antagonists mecamylamine (0.5 and 1 mg/kg) and hexamethonium (5 and 10 mg/kg) reduced the response induced by nicotine (0.25 mg/kg). Mecamylamine (1 mg/kg; decreased %open-arm entry and increased protected stretched attention posture) and hexamethonium (10 mg/kg; decreased %open-arm time) showed an anxiogenic-like profile. A muscarinic receptor antagonist, atropine (2.5 and 5 mg/kg), did not alter the nicotine response but elicited an anxiogenic effect by itself. The α_1-adrenoceptor antagonist prazosin (0.25 and 0.5 mg/kg), but not the α_1-adrenoceptor agonist, phenylephrine (4 and 6 mg/kg), reversed the nicotine effect. Single administration of phenylephrine (6 mg/kg) increased %open-arm time, while prazosin did not alter the anxiety behaviour. The α_2-adrenoceptor agonist clonidine (0.001 and 0.01 mg/kg), induced complete immobility when administered in combination with nicotine. However, an α_2-adrenoceptor antagonist, yohimbine (0.5 and 1 mg/kg), appeared to reverse the nicotine response, but did not show interaction with nicotine's effect. Clonidine did not elicit any effect, but yohimbine (1 mg/kg) increased %open-arm entry and %open-arm time by itself. It can be concluded that certain doses of nicotine (0.25 and 0.5 mg/kg) 7 min after their injection induce an anxiogenic effect through nicotinic mechanism(s), and that involvement of α_1- but not α_2-adrenoceptors in the response to nicotine seems likely. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Nicotine; Anxiety; Elevated plus-maze; α-Adrenoceptor; (Mouse)

1. Introduction

It has been postulated that hippocampus and septohippocampal cholinergic (acetylcholine) system activation underlies anxiety (Belotti and Galey, 1996; Gray, 1987). The neuronal nicotinic acetylcholine receptor participates in the control of anxiety behaviour in rodents (O'Neill and Brioni, 1994). The acute administration of (−)-nicotine increases the number of transitions and reduces the responsiveness to the aversive properties of the white area in the light–dark transition test (Costall et al., 1989), and enhances the activity of mice in the mirrored chamber test (Cao et al., 1993). In the elevated plus-maze model of anxiety, intraperitoneal injections of the nicotinic acetylcholine receptor agonists, (−)-nicotine and (−)-lobeline, has been reported to increase the time spent by mice in the open-arms of the maze, and effect of (−)-nicotine was blocked by the centrally acting nicotinic channel blockers, mecamylamine and chlorisondamine, implying the involvement of central nicotinic receptors in anxiolytic effects of nicotine (Brioni et al., 1993).

Nicotine is known to mediate its actions through different neurotransmitters such as norepinephrine, acetylcholine, dopamine, and γ-aminobutyric acid (Wonnacott et al., 1987; O'Neill and Brioni, 1994; Fuxe et al., 1990; Balfour, 1991). It is presently unknown which nuerotransmitter systems are involved in the induction of the anxiolytic-like effect of (−)-nicotine beyond activation of the nicotinic acetylcholine receptor (O'Neill and Brioni, 1994).

* Corresponding author. Fax: +98-216-404-377.

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Meanwhile, nicotine has been shown to increase the turnover of norepinephrine (Mitchell et al., 1989; Brazell et al., 1991) and the release of catecholamines from central catecholaminergic neuron terminals (Arqueros et al., 1978; Snell and Johnson, 1989). It is also known that the α-adrenergic system modulates the effects of nicotine to increase the release of adrenocorticotropin hormone (Sharp and Shannon, 1995). Based on these data, in the present study, the probable interaction of the α-adrenergic system with the effect of nicotine on anxiety behaviour in mice was investigated using the elevated plus-maze. This test is one of the most commonly used animal models of anxiety (Hogg, 1996; Rodgers and Johnson, 1998; Rodgers and Dalvi, 1997) and has been validated in both rats and mice (Lister, 1987).

2. Materials and methods

2.1. Animals

Male albino NMRI mice, weighing 25–35 g were used; they were housed in groups of 10, and maintained on a 12-h light–dark cycle, at 22–24°C. Food and water were freely available except during the experiments.

2.2. Behavioural test

2.2.1. Elevated plus-maze

The methods were basically the same as those used in previous experiments (Lister, 1987; Wiley et al., 1995; Cao and Rodgers, 1997). The elevated plus-maze, a wooden, cross-shaped maze, consists of four arms arranged in the shape of a plus sign. Two of the arms have no side or end walls (open-arms; $30 \times 5 \times 0.25$ cm^3). The other two arms have side walls and end walls, but are open on top (closed arms; $30 \times 5 \times 15$ cm^3). The maze was elevated to a height of 50 cm. A relatively dark box was used to hold the mice in before exposure to the maze in order to increase their exploratory behaviour (Pellow and File, 1986). The tests were done in a silent environment under dim light. The animals were carried to the laboratory and left there undisturbed for 1 h before the experiment. Each mouse was individually placed on the central platform (5×5) facing toward an open-arm and was observed for 5 min by two observers sitting in the same room.

The animals' behaviour was evaluated as follows: (1) percent open-arm entries (%open-arm entry, number of open-arm entries to total arm entries. Entry was defined as all four paws in the arm); (2) percent open-arm time (%open-arm time, time spent in open-arm to total time spent in the maze); (3) protected stretched attention posture, (forward elongation of head and shoulders followed by retraction to original position occurring on a closed arm or central platform); (4) percent of protected head dipping (number of protected to total head dippings defined as exploratory head/shoulder movement over sides of the maze towards the floor); (5) unprotected head dipping (%protected dipping. number of head dippings occurring on an open-arm). The %open-arm entry, %open-arm time (also known as the classical parameters) and the unprotected dipping are indices of anxiolytic behaviour (Espejo, 1997). Protected stretched attention posture and %protected dipping are commonly used as indicators of anxiety (Espejo, 1997; Rodgers and Cole, 1994). The number of closed entries was also regarded as an index of locomotor activity (Espejo, 1997; Rodgers and Johnson, 1996; Cruz et al., 1994).

2.3. Treatment

The animals in experiment 1 received different doses of nicotine (0.01, 0.025, 0.05, 0.1, 0.5 and 1 mg/kg) given 30 min before behavioural testing. As no significant effect on anxiety was observed in mice during pilot testing of the nicotine effect with different intervals, a 7-min interval was used (0.05, 0.1, 0.25, 0.5 and 1 mg/kg) and the time–response relationship was investigated for the most effective dose with a significant anxiogenic effect (0.25 mg/kg) 7, 15 and 30 min after drug injection. Based on this, the anxiogenic effect of nicotine 0.25 mg/kg 7 min after injection was chosen for subsequent experiments.

Animals in experiment 2 received the central nicotinic receptor antagonist, mecamylamine (0.5 and 1 mg/kg), peripheral nicotinic receptor antagonist, hexamethonium (5 and 10 mg/kg), or the antimuscarinic agent, atropine (2.5 and 5 mg/kg), 22 min before testing, along with either saline or nicotine which were injected 7 min before testing, in order to examine the involvement of cholinergenic receptor mechanism.

Animals in experiment 3 received the α_1-adrenoceptor agonist, phenylephrine (4 and 6 mg/kg), the α_1-adrenoceptor antagonist, prazosin (0.25 and 0.5 mg/kg), the α_2-adrenoceptor agonist, clonidine (0.001 and 0.1 mg/kg), or α_2-adrenoceptor antagonist, yohimbine (0.5 and 1 mg/kg), 22 min before testing, along with either saline or nicotine which were injected 7 min before, in order to evaluate the involvement of adrenoceptor mechanisms testing.

2.4. Drugs

The drugs used were (−)-nicotine hydrogen tartrate, mecamylamine, hexamethonium bromide, phenyleprine hydrochloride, prazosin hydrochloride, Clonidine hydrochloride and yohimbine (Sigma, Poole, UK) and atropine sulfate (Merck, Germany). Nicotine solutions were prepared in saline and the pH was adjusted between 7.2 and 7.4. Other drugs were dissolved in saline. All drugs were used intraperitoneally in volumes of 10 ml/kg body weight. The doses of drugs used had been shown to be active in previous studies (Zarrindast and Farzin, 1996; Zarrindast et al., 1996, 1999).

2.5. Statistical analysis

Analysis of variance (ANOVA) followed by least significant difference (LSD) test were used for data analysis. One-way ANOVA was used to evaluate the effect of different doses of nicotine. Two-way ANOVA was used to evaluate the influence of time on the nicotine response as well as interactions between nicotine and other agents. Differences with $P < 0.05$ between experimental groups at each point were considered statistically significant.

3. Results

3.1. Effects of different doses of nicotine on anxiety behaviour in plus-maze test

One-way ANOVA indicated that intraperitoneal (i.p) administration of different doses of nicotine (0.01, 0.025, 0.05, 0.1, 0.5 and 1 mg/kg) to groups of mice ($n = 10$), 30 min prior to test, induced no significant effects on

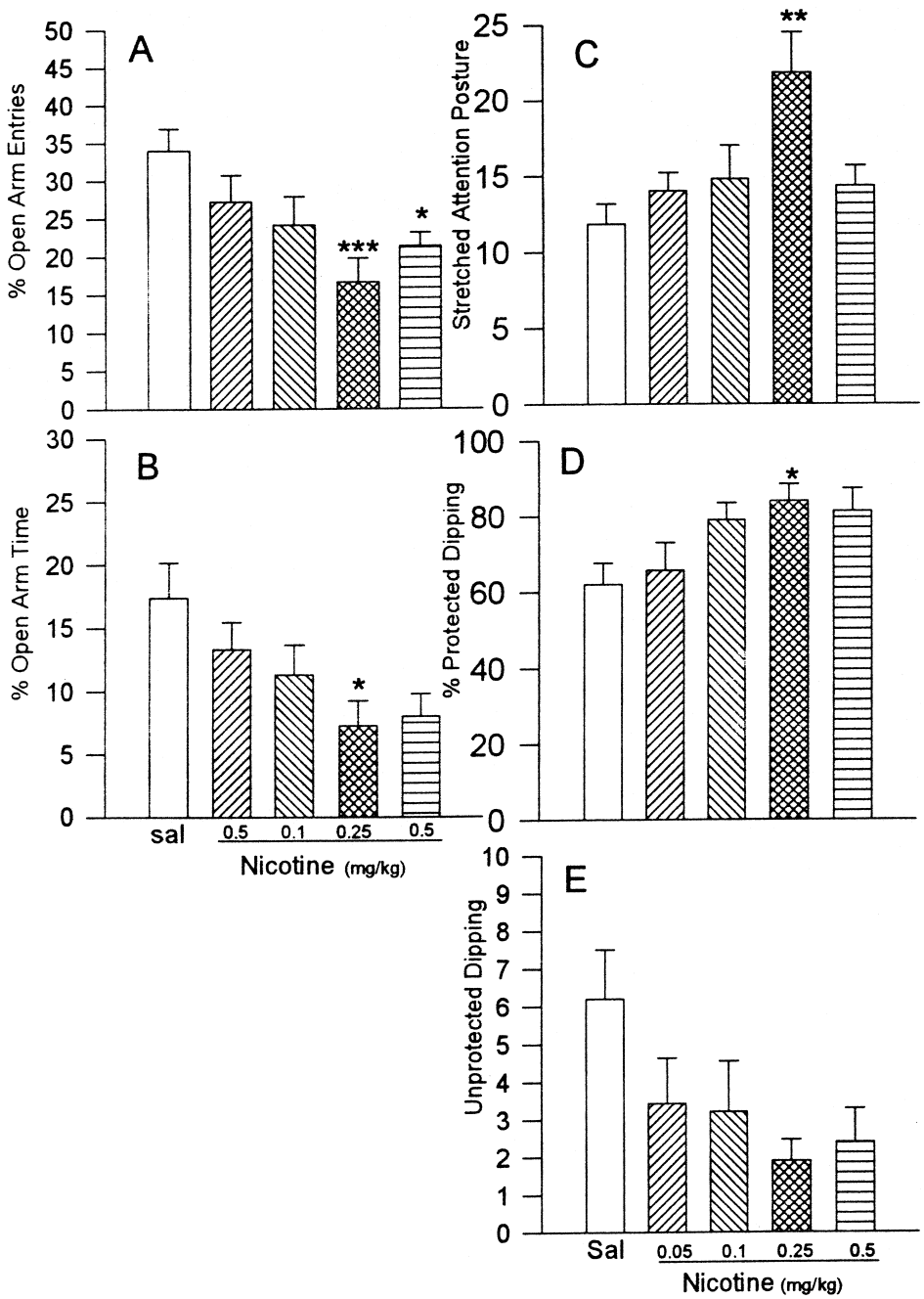

Fig. 1. Effect of different doses of nicotine (0.05, 0.1, 0.25 and 1 mg/kg, i.p.) administered 7 min before testing in the elevated plus-maze. Data are expressed as mean values ± S.E.M. Number of mice in treated groups = 10, control group = 12. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ vs. control group.

%open-arm entry [$F(6,65) = 0.72$, $P > 0.05$], %open-arm time [$F(6,65) = 0.26$, $P > 0.05$], protected stretched attention posture [$F(6,65) = 2.154$, $P > 0.05$], %protected dipping [$F(6,65) = 0.62$, $P > 0.05$] and uprotected dipping [$F(6,65) = 0.76$, $P > 0.05$] as compared with the control group ($n = 12$) (data not shown). No difference was found between locomotor activity after different doses of the drug and that after saline [$F(6,65) = 0.65$, $P > 0.05$].

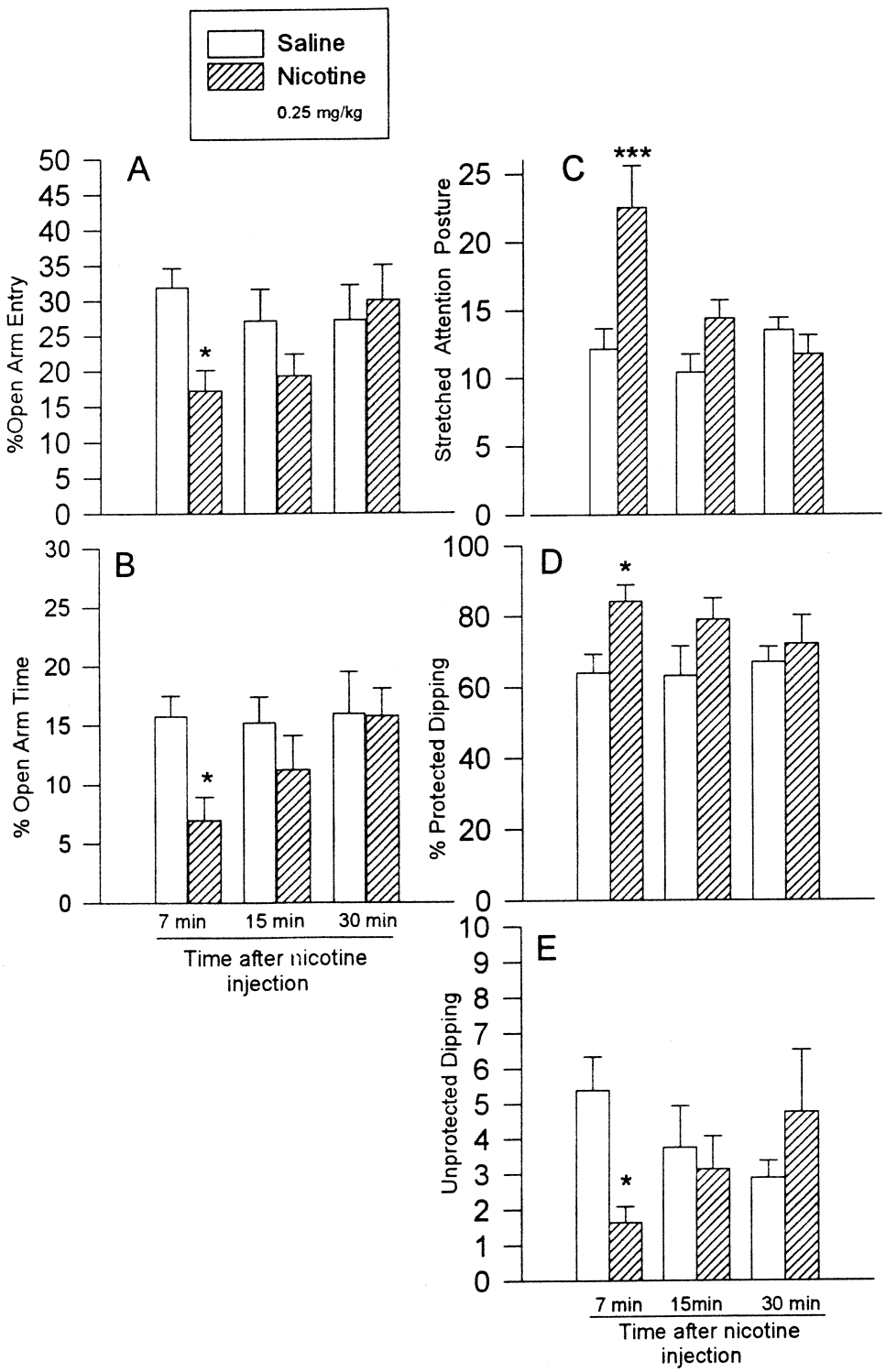

Fig. 2. Effect of nicotine (0.25 mg/kg) administered with different time intervals (7, 15, 30 min) before testing in the elevated plus-maze. Data are expressed as mean values ± S.E.M. of eight experiments. $^{*}P < 0.05$, $^{***}P < 0.005$ vs. respective control groups.

Fig. 1 shows the effect of different dose of nicotine (0.05, 0.1, 0.25 and 0.5 mg/kg), with a 7-min interval between injection and testing. One-way ANOVA indicated that nicotine altered %open-arm entry [$F(4,49) = 5.192$, $P < 0.005$], %open-arm time [$F(4,49) = 3.23$, $P < 0.05$], protected stretched attention posture [$F(4,49) = 4.57$, $P < 0.005$] and %protected dipping [$F(4,49) = 3.14$, $P < 0.05$], but not unprotected dipping [$F(4,49) = 0.75$, $P > 0.05$]. Further analysis showed that the doses of 0.25 and 0.5 mg/kg of nicotine decreased %open-arm entry and %open-arm time and increased protected stretched attention posture and %protected dipping. The results indicated that nicotine induced an anxiogenic response 7 min after nicotine administration. Different doses of nicotine did not

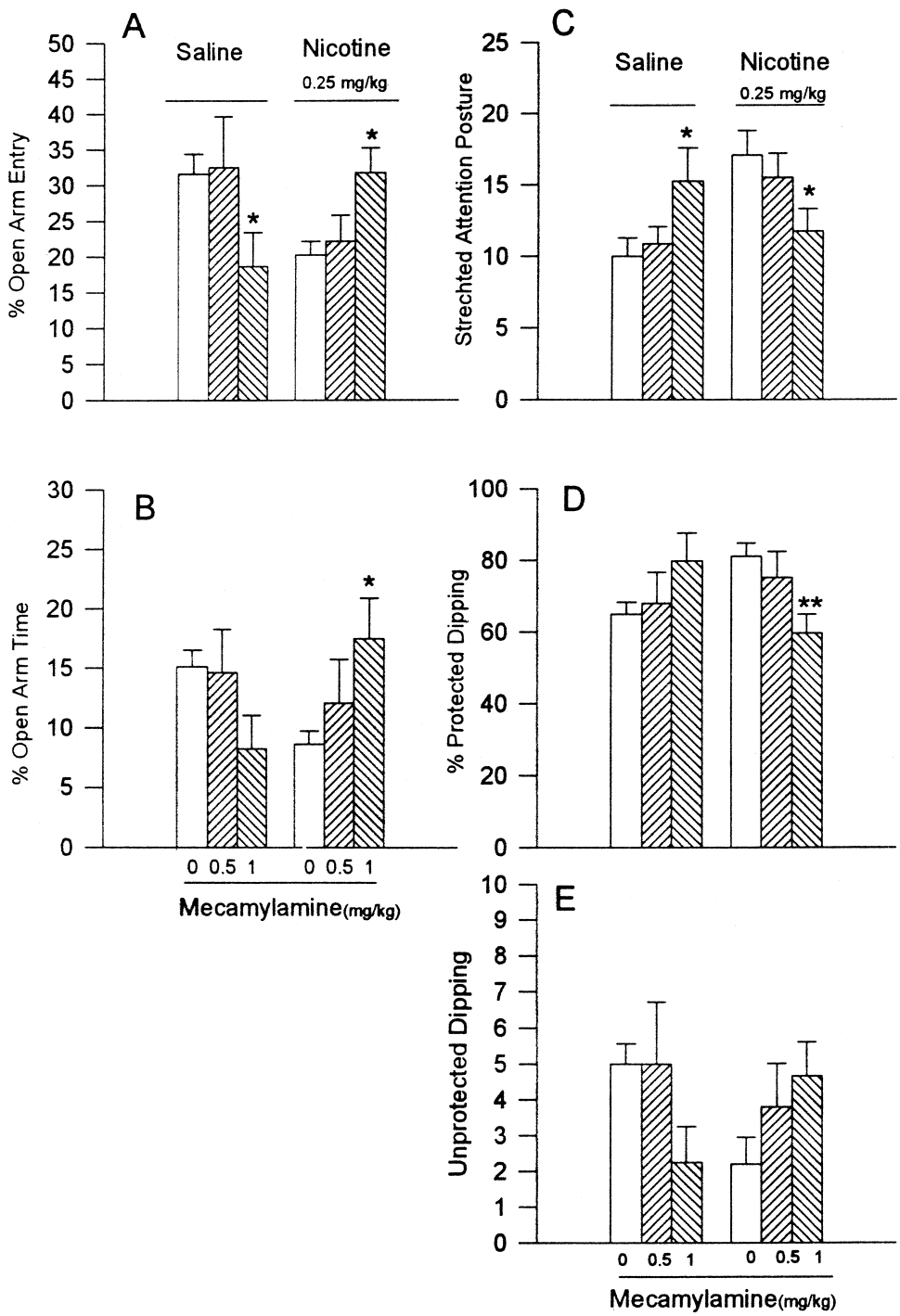

Fig. 3. Effect of mecamylamine on nicotine response. Mecamylamine (0.5 and 1 mg/kg) was administered 15 min before nicotine, and nicotine (0.25 mg/kg) was injected 7 min prior to testing. Data are expressed as mean values ± S.E.M. of 11 experiments (control group, $n = 13$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. respective control groups.

altered locomotion as compared with that of the saline control [$F(4,49) = 0.28$, $P > 0.05$].

In order to show possible differences between subcutaneous and intraperitoneal injection of nicotine, a dose of 0.25 mg/kg of nicotine was given subcutaneously. Subcutaneous administration of the drug induced a response similar to that with intraperitoneal injection, as compared with saline [%open-arm entry [$F(2,27) = 14$, $P < 0.01$], %open-arm time [$F(2,27) = 6$, $P < 0.01$], protected stretched attention posture [$F(2,27) = 18.3$, $P < 0.01$], %protected dipping [$F(2,27) = 4.4$, $P < 0.05$] and unprotected dipping [$F(2,27) = 5.9$, $P < 0.01$]. Pilot studies also showed that the doses of 0.001, 0.05 and 1 mg/kg of the drug when administered 15 min before testing, did not induce any response (data not shown).

Fig. 2 shows the response induced by a single dose of nicotine (0.25 mg/kg) with different time intervals (7, 15, 30 min) between injection and testing. Two-way ANOVA followed by LSD post-test indicated effects of nicotine (Factor 1), time (Factor 2) and interaction (Factor 3).

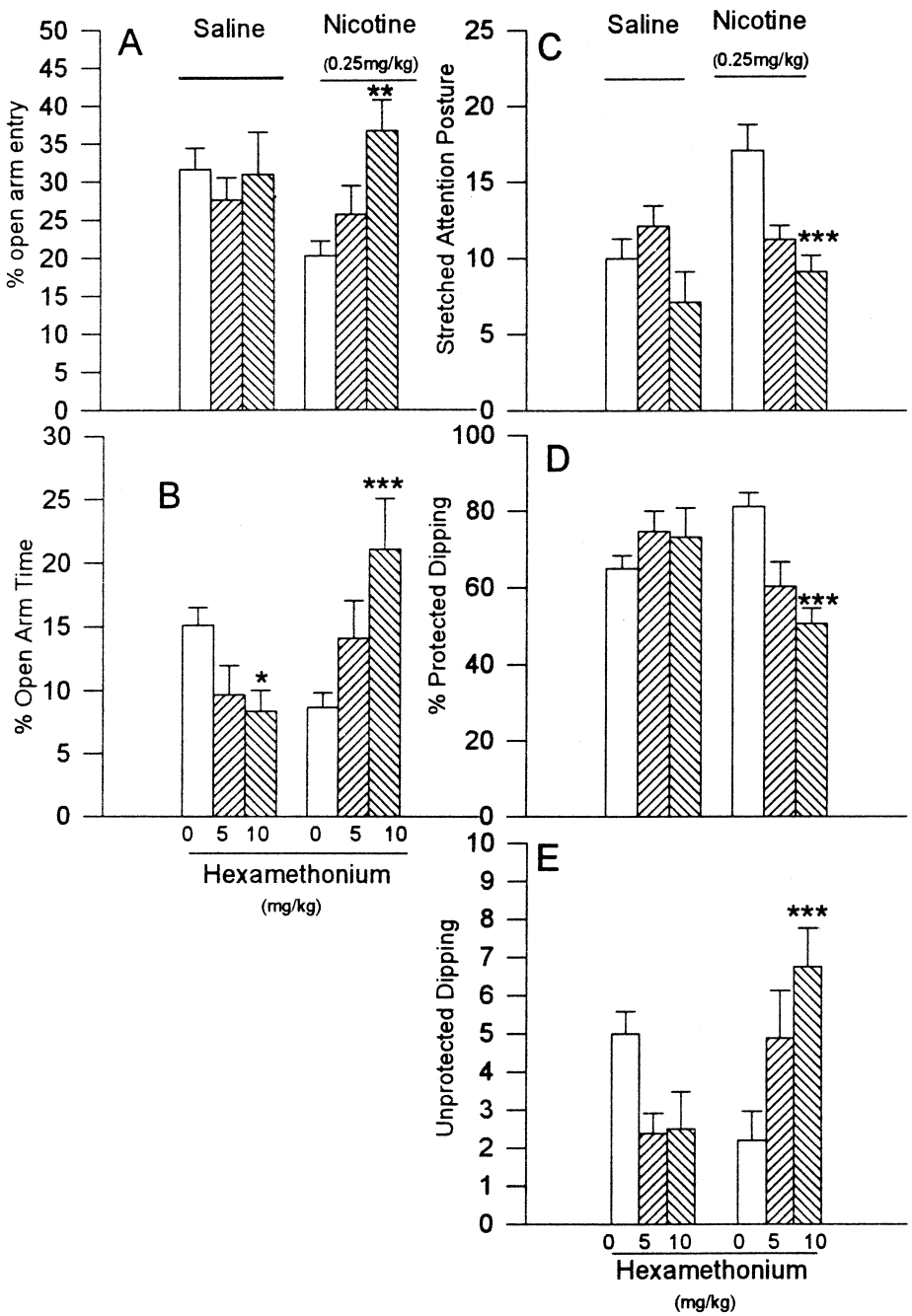

Fig. 4. Effect of hexamethonium on nicotine response. Hexamethonium (5 and 10 mg/kg) was administered 15 min before nicotine and nicotine (0.25 mg/kg) was injected 7 min prior to testing. Data are expressed as mean values ± S.E.M. of 9–11 experiments (control group, $n = 13$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ vs. respective control groups.

ANOVA showed that the drug (independent of time) decreased %open-arm entry [Factor 1, $F(1,42) = 4.04$, $P = 0.0508$; Factor 2, $F(2,42) = 0.99$, $P > 0.05$; Factor 3, $F(2,42) = 2.4$, $P > 0.05$] and %open-arm time [Factor 1, $F(1,42) = 4.37$, $P < 0.05$; Factor 2, $F(2,42) = 1.60$, $P > 0.05$; Factor 3, $F(2,42) = 1.44$, $P > 0.05$] and increased %protected dipping [Factor 1, $F(1,42) = 7.03$, $P < 0.05$; Factor 2, $F(2,42) = 0.27$, $P > 0.05$; Factor 3, $F(2,42) = 0.75$, $P > 0.05$]. Two-way ANOVA showed that nicotine increased protected stretched attention posture [Factor 1, $F(1,42) = 8.83$, $P < 0.005$; Factor 2, F2(2,42) = 5.14, $P < 0.05$; Factor 3, F3(2,42) = 6.11, $P < 0.005$] and decreased unprotected dipping [Factor 1, $F(1,42) = 0.92$, $P > 0.05$; Factor 2, $F(2,42) = 0.07$, $P > 0.05$; Factor 3, $F(2,42) = 3.54$, $P < 0.05$]. The results showed that nicotine elicited an anxiogenic effect 7 min after drug injection. Locomotor activity was not altered as compared to that with saline in different groups of animals [Factor 1, $F(2,42) = 3.36$, $P > 0.05$; Factor 2, $F(1,42) = 0.81$, $P > 0.05$; Factor 3, $F(2,42) = 0.01$, $P > 0.05$]. Based on dose and timing of assessments in the above results, a dose of 0.25 mg/kg of nicotine and 7-min interval were chosen

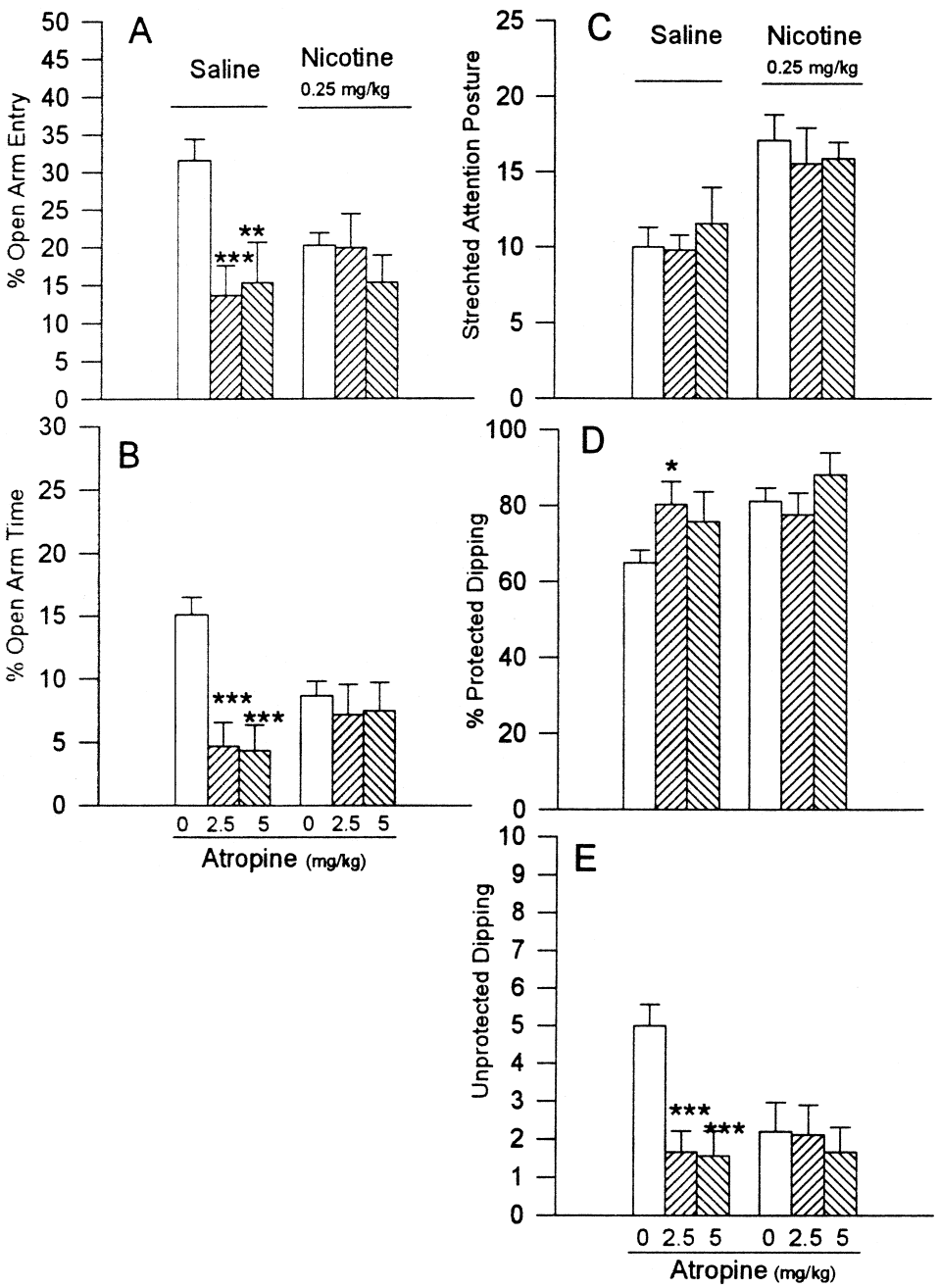

Fig. 5. Effect of atropine on nicotine response. Atropine (2.5 and 5 mg/kg) was administered 15 min before nicotine and nicotine (0.25 mg/kg) was injected 7 min prior to testing. Data are expressed as mean values ± S.E.M. of 10–11 experiments (control group, $n = 13$). $^{**}P < 0.01$, $^{***}P < 0.005$ vs. respective control groups.

for subsequent studies. In all these studies (Figs. 4–9), there was a significant nicotine effect ($P < 0.05$, LSD test) on all five factors.

3.2. Effects of central and peripheral nicotinic receptor antagonists on nicotine-induced anxiety behaviour

Effects of the central nicotinic receptor antagonist, mecamylamine, and peripheral nicotinic receptor antagonist, hexamethonium, are shown in Figs. 3 and 4. Two-way ANOVA showed significant interactions between the effect of mecamylamine and nicotine on all five factors assessed [%open-arm entry, $F(2,62) = 5.49$, $P < 0.01$; %open-arm time, $F(2,62) = 4.94$, $P < 0.05$; protected stretched attention posture, $F(2,62) = 5.91$, $P < 0.005$; %protected dipping, $F(2,62) = 5.92$, $P < 0.005$; unprotected dipping, $F(2,62) = 3.63$, $P < 0.05$]. Post-analysis showed that mecamylamine alone (1 mg/kg) had an anx-

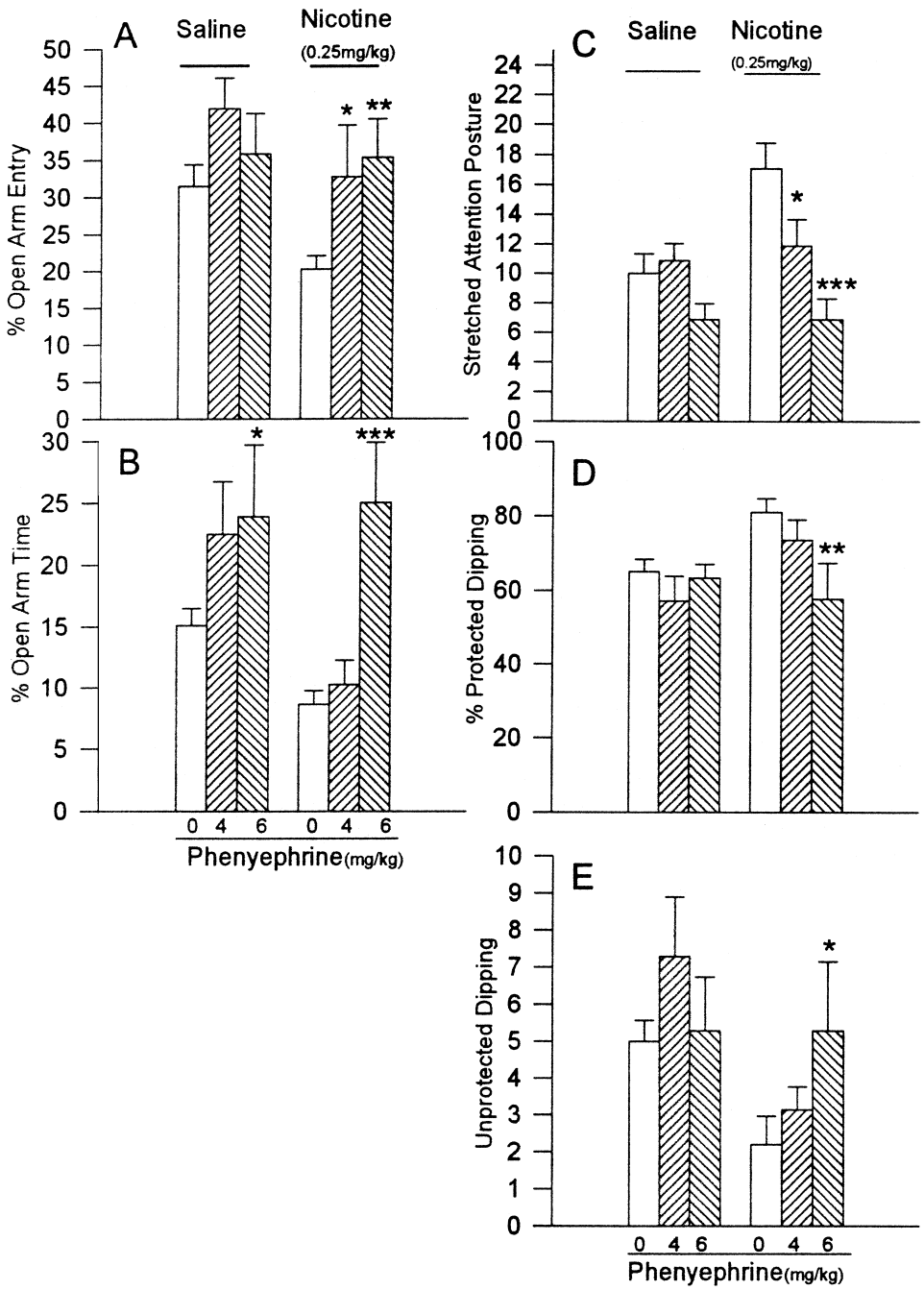

Fig. 6. Effect of phenylephrine on nicotine response. phenylephrine (4 and 6 mg/kg) was administered 15 min before nicotine and nicotine (0.25 mg/kg) was injected 7 min prior to testing. Data are expressed as mean values ± S.E.M. of nine experiments (control group, $n = 13$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ vs. respective control groups.

iogenic-like profile [%open-arm entry, $P < 0.05$; protected stretched attention posture, $P < 0.05$, %protected dipping and unprotected dipping, $P < 0.07$]. Locomotor activity was not altered significantly with mecamylamine [$F(2,62) = 0.3$, $P > 0.05$]. Two-way ANOVA also indicated that hexamethonium had a significant interaction with nicotine [%open-arm entry, $F(2,56) = 3.25$, $P < 0.05$; %open-arm time, $F(2,56) = 11.25$, $P < 0.0001$; protected stretched attention posture, $F(2,56) = 4.14$, $P < 0.05$; %protected dipping, $F(2,56) = 10.91$, $P < 0.0001$; unprotected dipping, $F(2,56) = 10.63$, $P < 0.0005$]. Hexamethonium (10 mg/kg) alone showed a significant anxiogenic effect (%open-arm time and unprotected dipping, $P < 0.05$ LSD test). The drug by itself significantly decreased locomotor activity [$F(2,56) = 3.7$, $P < 0.05$] but this effect was not seen after the combination with nicotine [$F(2,56) = 0.7$,

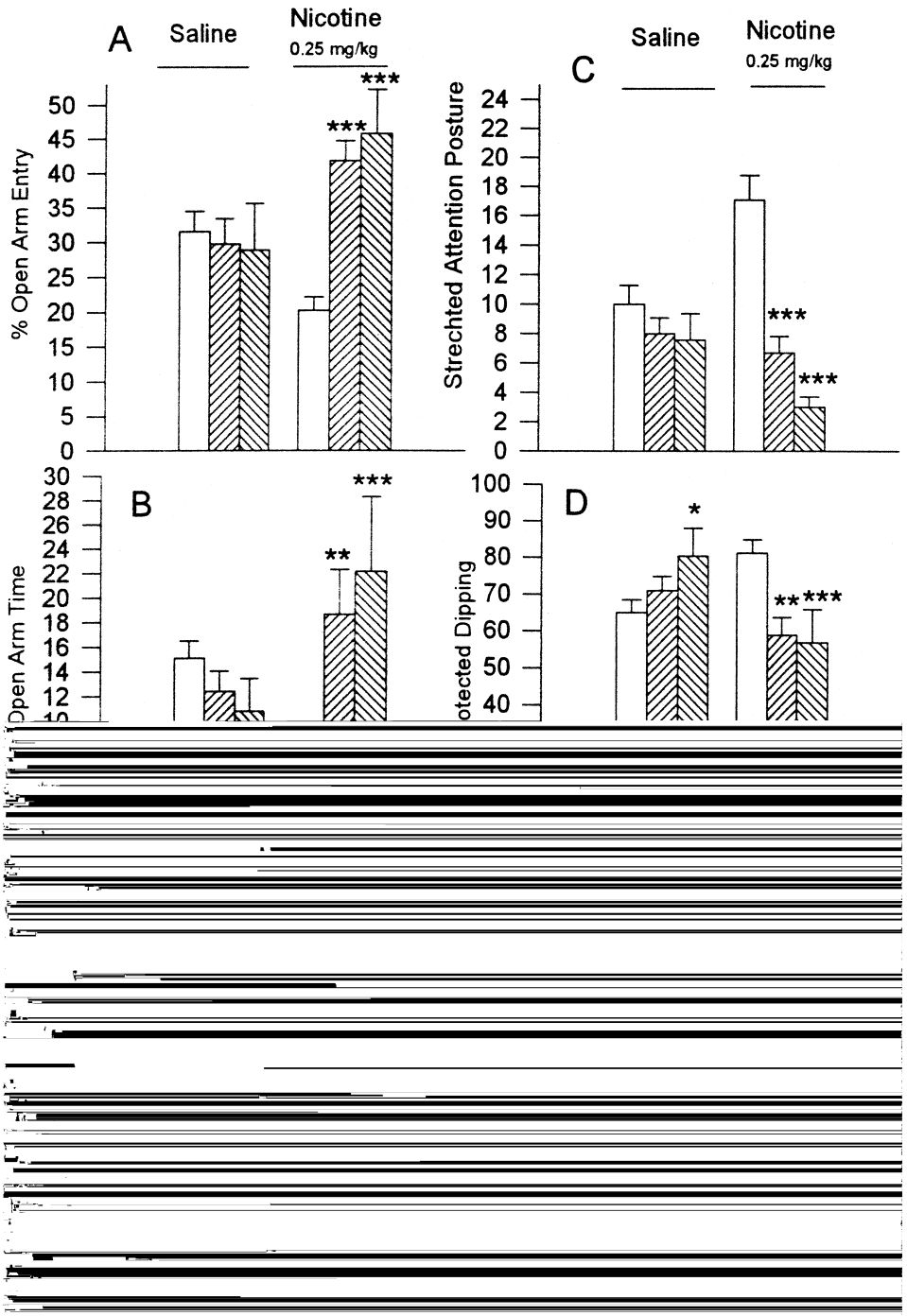

Fig. 7. Effect of prazosin on nicotine response. Prazosin (0.25 and 0.5 mg/kg) was administered 15 min before nicotine and nicotine (0.25 mg/kg) was injected 7 min prior to testing. Data are expressed as mean values ± S.E.M. of nine experiments (control group, $n = 13$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ vs. respective control groups.

1.50, $P > 0.05$] (Fig. 6). Analysis showed that phenylephrine alone elicited no change in anxiety behaviour except for an anxiolytic effect of a dose of 6 mg/kg of the drug on %open-arm time [$P < 0.05$]. Phenylephrine did not induce any significant change in locomotor activity [$F(2,52) = 2.3$, $P > 0.05$].

Prazosin alone had no significant effect except an increase in %protected dipping associated with a decrease in unprotected dipping induced by a 0.5 mg/kg dose [two-way ANOVA; $P < 0.05$]. It showed significant interaction with the effect of nicotine on all five factors [%open-arm entry, $F(2,52) = 9.04$, $P < 0.0005$; %open-arm time, $F(2,52) = 7.13$, $P < 0.005$; protected stretched attention posture, $F(2,52) = 9.40$, $P < 0.0005$; %protected dipping, $F(2,52) = 9.53$, $P < 0.0005$; unprotected dipping, $F(2,52) = 6.69$, $P < 0.005$] (Fig. 7). Furthermore, the interaction of prazosin (0.5 mg/kg) and nicotine induced an anxiolytic profile [LSD post-test; %open-arm entry, $P < 0.01$; %open-arm time, $P = 0.056$; protected stretched attention posture, $P < 0.005$]. Prazosin by itself did not produce a significant reduction in locomotor activity [$F(2,52) = 2.5$, $P > 0.05$]. Clonidine alone did not have any significant effect on anxiety behaviour [one-way ANOVA with LSD post-test; %open-arm entry, $F(2,29) = 0.44$, $P > 0.05$; %open-arm time, $F(2,29) = 0.94$, $P > 0.05$; protected stretched attention posture, $F(2,29) = 0.04$, $P > 0.05$; %protected dipping, $F(2,29) = 1.99$, $P > 0.05$; unprotected dipping, $F(2,29) = 15.72$, $P < 0.05$]. The drug did not by itself alter locomotor activity [$F(2,29) = 1.23$, $P > 0.05$]. Clonidine (0.001 and 0.01 mg/kg) associated with nicotine induced a sedative effects without locomotor activity, making it impossible to check for anxiety-related behaviour (Fig. 8).

Yohimbine alone (1 mg/kg) showed an anxiolytic effect [two-way ANOVA with LSD post-test; %open-arm entry, $P < 0.05$; %open-arm time, $P = 0.0502$]. The antagonist showed no significant interaction with nicotine according to two-way ANOVA [%open-arm entry, $F(2,52) = 1.61$, $P > 0.05$; %open-arm time, $F(2,52) = 0.75$, $P > 0.05$; protected stretched attention posture, $F(2,52 = 1.68$, $P > 0.05$; %PD, $F(2,52) = 2.30$, $P > 0.05$; UPD, $F(2,52) = 0.72$, $P > 0.05$] though the higher dose of the drug (1 mg/kg) reversed the anxiogenic effect of nicotine on all five factors ($P < 0.05$). Yohimbine by itself induced a significant reduction in locomotor activity [$F(2,52) = 7.6$, $P < 0.005$] (Fig. 9).

4. Discussion

The elevated plus-maze test is a useful model for the selective identification of anxiolytic and anxiogenic drug effects in rodents (Pellow et al., 1985; Pellow and File, 1986; Menard and Treit, 1996; Treit et al., 1998). Various parameters are considered as measures of anxiety (see Section 2). The %open-arm entry, %open-arm time and unprotected dipping are indices of anxiolytic, while protected stretched attention posture and %protected dipping are commonly used as indicators of anxiety behaviour.

It has been reported that nicotine (0.3 mg/kg) induces anxiolytic-like effect int he elevated plus-maze in CD1 mice (Brioni et al., 1993). The biochemical mechanisms underlying nicotine's antiaversive actions are unknown (O'Neill and Brioni, 1994; Fuxe et al., 1990). The aim of this study was to investigate the role of nicotine in anxiety behaviour and the influence of adrenoceptor agents on the response to nicotine, by using the elevated plus-maze with mice.

In the present study, nicotine showed an anxiogenic profile. This response to the drug was prominent 7 min after drug administration. Although a similar response was observed after 15 min, it was not significant. Furthermore, no effect was observed 30 min after injection, indicating that the duration of nicotine's response is short, with an early onset. There is a report that, in adult mice, the peak levels of brain nicotine occur at 10 min after administration (Manser and Mattila, 1977). This confirms the early onset of action of the drug in our experiments. Our results did not agree with those obtained by other investigators who had shown anti-anxiety effects for nicotine (O'Neill and Brioni, 1994). However, a recent study has revealed bimodal effects of nicotine in the elevated plus-maze 30 min after drug administration. The lower doses (0.01 and 0.1 mg/kg) of nicotine show anxiolytic effects while the higher doses (0.05 and 1 mg/kg) are anxiogenic (Ouagazzal et al., 1999). The bimodal effect of nicotine has also been shown in the light–dark transition test of anxiety (File et al., 1998). In the present study, different doses of nicotine (0.01–1 mg/kg) administered 30 min before testing showed no effect on anxiety, while certain doses of the drug (0.25 and 0.5 mg/kg) caused obvious anxiogenic effects when administered 7 min before testing. Whether the bimodal effects of the drug depend on time and/or drug dose should be examined. Moreover, nicotine has been shown to cause very different responses in different strains of mice (Stitzel et al., 1995; Marks et al., 1991). This may be because of the different number of brain nicotinic receptors among strains (Marks et al., 1989). This may also explain inconsistencies observed in different studies. It should be considered that we used intraperitoneal injection of nicotine. We also used subcutaneous administration of nicotine to show the possible influence of route of drug injection and/or local effect of the drug within the gut. The response obtained 7 min after the drug injection was similar with both methods. Moreover, some effects of nicotine can be mediated through increase locomotion. Since our results did not indicate any difference in locomotor activity among groups, the involvement of locomotion is not included.

The antimuscarinic receptor antagonist, atropine, did not prevent the present effect. However, the antagonist is itself anxiogenic. Therefore, an involvement of a mus-

carinic mecahnism in nicotine's present effect is unlikely. Meanwhile, the central nicotinic receptor antagonist, mecamylamine, reduced the nicotine-induced anxiety profile which may lead to the conclusion that central nicotinic mechanisms may mediate the response to nicotine. However, the peripheral nicotinic receptor antagonist, hexamethonium, also reduced nicotine's effect. Thus, the results of studies with the nicotinic receptor antagonists may suggest that the response to nicotine is mediated by receptors not well protected by the blood-brain barrier (e.g. medulla), and/or peripheral nicotinic receptors. The present data showed that hexamethonium decreased locomotion. To clarify whether this effect and/or possible decrease in blood pressure accounts for the response to hexamethonium, more experiments may be required. It should be considered that both mecamylamine and hexamethonium themselves are able to cause an axiogenic-like behaviour, indicating that a brain nicotinic receptor mechanism may express an anxiolytic tone. One may speculate that there is a modulating system, which is influenced by nicotine receptor mechanism(s). For example, anxiogenic response to nicotine by itself in the present study, maybe mediated through an indirect mechanism. Sytematically administered nicotine is known to release dopamine, noradrenaline (Balfour, 1982) and serotonin (Takahashi et al., 1998). All these monoamines have been implicated in anxiety. There are reports indicating that dopamine D_1 and D_2 receptors induced angiogenic-like effects (Simon et al., 1994) and mice without functional D_3 dopamine receptors showed reduced anxiety (Steiner et al., 1997). The 5-HT receptor agonist, *m*-chlorophenylpiperazine, has also shown anxiogenic effects in the both healthy subjects and patients with panic disorder (Charrey et al., 1987). Therefore, the involvement of dopamine and/or serotonin may be another explanation for nicotine response.

In the present study, the combination of nicotine with the α_2-adrenoceptor agonist clonidine, induced immobilization which made it impossible to evaluate interactions between two drugs. This response should be studied further. Since the α_2-adrenoceptor antagonist, yohimbine, had no significant interaction with the nicotine response, an α_2-adrenoceptor mechanism cannot be involved in the angiogenic-like effect of nicotine. Yohimbine, by itself, exerts an anxiolytic effect (increased %open-arm time and %open-arm entry) that is in accordance with previous studies. However, it has been suggested that an α_2-adrenoceptor mechanism maybe little involved in the anxiolytic action of yohimbine and that other receptor mechanisms such as 5-HT_{1A} may be involved (Cole et al., 1995). Moreover, the drug decreased locomotion, which cannot explain the anxiolytic effect of yohimbine. The α_1-adrenoceptor agonist, phenylephrine, did not show a significant interaction with the nicotine effect. However, a higher dose of the drug increased %open-arm time by itself, while there was no significant change in locomotor activity of the animals. Therefore, the decrease in nicotine effect caused by phenylephrine may be due to phenylephrine's own effect.

The α_1-adrenoceptor antagonist, prazosin, by itself did not influence anxiety, but reversed the nicotine effect. Considering the release of monoamines by nicotine, the involvement of an α_1-adrenoceptor mechanism may be likely. Prazosin has also been shown to inhibit the release of a serotonin (Rouquier et al., 1994), therefore, the involvement of serotonergenic mechanism in the nicotine's effect should be considered.

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