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# Nicotine-induced conditioned taste aversion in the rat: Effects of ethanol

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**Abstract**

It has been shown that small doses of ethanol antagonise the discriminative stimulus properties of nicotine in the rat. The aim of the present study was to evaluate whether ethanol could antagonise the aversive stimulus effects of nicotine. Wistar rats were trained to associate nicotine injections with a novel tasting fluid (0.1% saccharin) in the conditioned taste aversion procedure. Nicotine (0.3 mg/kg, s.c.) was injected 5 min after the end of a 20-min exposure to the saccharin solution. Ethanol (0.25–0.5 g/kg, i.p.) was administered 5 or 50 min before nicotine. In general, ethanol did not inhibit nicotine-induced conditioned taste aversion. Contrary to the findings in drug discrimination studies, a slight but significant enhancement of nicotine-induced taste aversion conditioning was observed after ethanol pre-treatment. Blood ethanol levels were measured in a separate group of rats. Maximal blood ethanol levels after i.p. administration of 0.25 or 0.5 g/kg ethanol exceeded 20 and 80 mg%, respectively. Concluding, the present results may indicate that ethanol does not attenuate nicotine-induced conditioned taste aversion in the rat.


*Keywords:* Nicotine; Ethanol; Conditioned taste aversion; Blood ethanol levels; (Rat)

## 1. Introduction

Nicotine is a primary constituent of tobacco that reinforces cigarette smoking (Corrigall et al., 1994; Stolerman and Jarvis, 1995; Malin, 2001; Groman and Fagerström, 2003). It is widely accepted that all major behavioural effects of nicotine, including its rewarding, aversive and discriminative stimulus effects, are mediated through nicotinic acetylcholine receptors located within the brain (Romano et al., 1981; Stolerman and Jarvis, 1995; Dani and De Biasi, 2001).

Several human studies have demonstrated that a high correlation exists between tobacco smoking and ethyl alcohol (ethanol) consumption (Carmody et al., 1985; Bien and Burge, 1990; Mitchell et al., 1995; Dawson, 2000; Larsson and Engel, 2004). Moreover, nicotine dependence increases the risk of developing dependence on alcohol and vice versa (Burling and Ziff, 1988; Di Franza and Guerrera, 1990; Miller and Gold, 1998). Despite the profound clinical importance of the above association, its pharmacological and behavioural mechanisms remain poorly understood. For example, it is not known whether ethanol-induced increase in cigarette smoking is related to potentiation of the rewarding effects and/or to reduction of the aversive effects of nicotine.

Animal studies have indicated that clinically relevant concentrations of ethanol modulate the function of brain nicotinic receptors (Cardoso et al., 1999; Marszalec et al., 1999; Larsson and Engel, 2004). Depending on molecular composition of nicotinic receptor-associated channels, acute ethanol exposure may either enhance ($\alpha4\beta2$ heteropentamers) or inhibit ($\alpha7$ homopentamers) the receptor function (Covernton and Connolly, 1997; Narahashi et al., 1999; Zuo et al., 2004). In some preparations, ethanol pre-treatment led to increase in the nicotinic receptor-associated currents followed by prolonged receptor desensitisation (Wu et al., 1994; Nagata et al., 1996). The latter finding was supported by results of behavioural experiments. Ethanol antagonised the discriminative stimulus properties of nicotine in rats trained to discriminate nicotine from its vehicle (Kim and Brioni, 1995; McMillan et al., 1999). More recently, our group (Korkosz et al., 2005) have tried to correlate

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effects of ethanol on nicotine discrimination with blood ethanol levels. Ethanol (0.25 or 0.5 g/kg, i.p.) was injected 5 or 50 min before nicotine (15 or 60 min before a test session, respectively). Both doses of ethanol antagonised the nicotine cue regardless of the pre-treatment time. Interestingly, significant suppression of nicotine-appropriate responding was noted 60 min after the injection of 0.25 g/kg ethanol, i.e. at the time point when the blood ethanol level was close to zero. Both doses of ethanol attenuated also inhibitory effects of nicotine on the rate of operant responding (Korkosz et al., 2005). Our results might be related to the acute inhibitory effects of ethanol on brain nicotinic receptors (Nagata et al., 1996; Narahashi et al., 1999; De Fiebre And De Fiebre, 2005) and/or to ethanol-induced desensitisation of these receptors (De Fiebre And Collins, 1989; Wu et al., 1994; Nagata et al., 1996).

In order to further characterise ethanol's actions on the stimulus properties of nicotine, we evaluated effects of ethanol on the aversive stimulus properties of the alkaloid. The conditioned taste aversion procedure was used to assess the aversive stimulus effects of nicotine. Given the above-mentioned data (Kim and Brioni, 1995; McMillan et al., 1999; Korkosz et al., 2005), we hypothesised that ethanol might inhibit nicotine-induced conditioned taste aversion.

Taste aversion conditioning is considered to be a special form of Pavlovian conditioning (Hunt and Amit, 1987; Cunningham et al., 2000). Animals are trained to associate drug effects (unconditioned stimuli) with a novel-tasting fluid (a conditioned stimulus). Conditioned taste aversion is assessed by measuring subsequent intake and/or preference of the fluid in the absence of drug injections (Kumar et al., 1983; Iwamoto and Williamson, 1984; Bienkowski et al., 1997b; Cunningham et al., 2000).

The range of ethanol doses (0.25–0.5 g/kg) and pre-treatment times were selected on the basis of our drug discrimination study (Korkosz et al., 2005). These doses of ethanol produced no sedative effects in Wistar rats (Bienkowski et al., 1997a; Korkosz et al., 2005) and were assumed to produce no taste aversion conditioning. A higher dose of ethanol (1.0 g/kg) induced significant conditioned taste aversion in our previous experiments (Piasecki et al., 2001) and thus higher doses of ethanol were not used in the present study.

Three separate experiments were done. In Experiment 1, a dose–response relationship was established for nicotine in the conditioned taste aversion procedure. In Experiment 2, the lower dose of ethanol (0.25 g/kg) was injected 5 min before nicotine. In Experiment 3, the higher dose of ethanol (0.5 g/kg) was administered either 5 or 50 min before nicotine. The dose of nicotine used in Experiments 2–3 was selected on the basis of results of Experiment 1.

## 2. Method

### 2.1. Subjects

Male Wistar rats (Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland) weighing 300–400 g at the start of the experiments were used. All subjects were housed individually in wire cages (20×25×28 cm, $W \times L \times H$) with removable, graduated 100-ml drinking tubes mounted at the front. Standard lab chow (Labofeed H, WPiK, Kcynia, Poland) was available ad libitum. Fluids available in the tubes are described below. The animals were housed in standard laboratory conditions at 22±1°C, ~60% humidity, and 12:12-h light: dark cycle (lights on at 06:00). All experiments were conducted between 12:00–15:00.

The study was performed in full accordance with respective Polish and European (Directive No. 86/609/EEC) regulations and was approved by a local Committee on Animal Studies.

### 2.2. Conditioned taste aversion procedure — general design

Typically, the conditioned taste aversion procedure consists of several one-bottle and two-bottle tests. Access to water is limited in order to increase motivation to drink during the tests (Kumar et al., 1983; Bienkowski et al., 1997b). In the one-bottle tests, one bottle filled with a novel-tasting fluid, e.g. saccharin solution, is presented to water-deprived animals. Drug injections are given after the end of exposure to saccharin solution. Accordingly, the tests following the first one-bottle test allow measurement of expression and facilitate acquisition of taste aversion conditioning.

The one-bottle and two-bottle tests which are not followed by drug injections allow measurement of expression of taste aversion conditioning. As a rule, no drug injections are given in the two-bottle choice tests. Animals are offered a choice between saccharin and water. Reduction of saccharin preference reflects conditioned taste aversion (Kumar et al., 1983; Hunt and Amit, 1987; Bienkowski et al., 1997b; Cunningham et al., 2000).

The two-bottle tests are a more sensitive measure of drug-induced conditioned taste aversion than the one-bottle tests (Hunt and Amit, 1987; Bienkowski et al., 1998). Conditioned avoidance of saccharin solution in the one-bottle tests must be associated with decrease in fluid intake. In the two-bottle tests, water is offered in the second bottle and rats may avoid saccharin solution without further dehydration.

#### 2.2.1. Experiment 1. Nicotine-induced conditioned taste aversion

The animals ($n$=29) were randomly assigned to 4 experimental groups ($n$=7–8 rats per group). The groups differed in treatment (saline, 0.3, 0.6 or 0.9 mg/kg of nicotine, s.c.) received during the one-bottle tests.

The conditioned taste aversion procedure followed that described by Bienkowski et al. (1997b). The procedure consisted of 3 phases. In phase I, the animals received tap water for 20 min/24 h. Water intake (ml) was noted for each rat. After 5 daily sessions, the water intake stabilised and the one-bottle tests started (phase II). The one-bottle tests consisted of 20-min access to 0.1% saccharin (saccharin sodium salt dihydrate, Aldrich Chemical, Gillingham, UK). There were 5 one-bottle tests repeated every 48 h. Nicotine or its vehicle was injected 5 min after the end of one-bottle tests 1–4 (conditioning sessions). On the intervening days, the animals were allowed to drink water for 20 min and nicotine was not administered. No injections were given after one-bottle test 5 as this test was

thought to assess only expression of nicotine-induced conditioned taste aversion.

Forty eight hours after the last one-bottle test, the two-bottle tests started (phase III). In the two-bottle tests, the subjects had a choice between water and saccharin for 20 min. The 2 two-bottle tests were repeated every 48 h. On the intervening day, water was available for 20 min.

In order to control for differences in baseline saccharin consumption, results were expressed as a percentage of saccharin intake in one-bottle test 1 according to a formula: [saccharin intake in one-bottle test 2 (3, 4 or 5)/saccharin intake in one-bottle test 1]×100%. Preference (%) of saccharin in the two-bottle tests was calculated for each rat as a ratio of saccharin consumption to the total fluid intake: [saccharin/(saccharin+water)]×100%.

The 0.3 mg/kg dose of nicotine was chosen for Experiments 2–3 as this dose produced moderate level of taste aversion conditioning in Experiment 1 (see Results). Hence, both increase and decrease in the aversive stimulus effects of 0.3 mg/kg nicotine might be expected after ethanol pre-treatment. The dose of nicotine selected for Experiments 2–3 was identical to that used by Korkosz et al. (2005) in the study on ethanol's effects on nicotine discrimination.

#### 2.2.2. Experiment 2. Effects of 0.25 g/kg ethanol on nicotine-induced conditioned taste aversion

In Experiment 2, effects of the lower dose of ethanol (0.25 g/kg) on nicotine-induced conditioned taste aversion were assessed. Ethanol was injected i.p. immediately after the end of one-bottle tests 1–4. Nicotine was injected s.c. 5 min after the ethanol administration. The conditioned taste aversion procedure was identical to that described above, except that only 4 one-bottle tests were done. The animals ($n$=28) were randomly assigned to 4 groups ($n$=7 rats per group) differing in treatment received during the one-bottle tests: saline–saline (SAL–SAL), saline–0.3 mg/kg nicotine (SAL–NIC), 0.25 g/kg ethanol–saline (ET–SAL), and 0.25 g/kg ethanol–0.3 mg/kg nicotine (ET–NIC) group.

#### 2.2.3. Experiment 3. Effects of 0.5 g/kg ethanol on nicotine-induced conditioned taste aversion

In Experiment 3, effects of the higher dose of ethanol (0.5 g/kg) on nicotine-induced conditioned taste aversion were assessed. Nicotine was injected s.c. 5 min after the end of one-bottle tests 1–4. Ethanol was injected i.p. 5 or 50 min before the nicotine administration (Korkosz et al., 2005; see Introduction), i.e. immediately after the end of the test or 25 min before its start, respectively. Ethanol injected before the one-bottle tests (the 50-min inter-injection interval) could alter both expression and acquisition of nicotine-induced conditioned taste aversion.

The conditioned taste aversion procedure was identical to that used in Experiment 2. The animals ($n$=42) were randomly assigned to 8 experimental groups ($n$=4–7 rats per group) differing in treatment and inter-injection intervals: saline–5 min-saline (SAL–5-SAL; $n$=4 rats), saline–50 min-saline (SAL–50-SAL; $n$=4 rats), saline–5 min-0.3 mg/kg nicotine (SAL–5-NIC; $n$=4 rats), saline–50 min-nicotine (SAL–50-NIC; $n$=4 rats), 0.5 g/kg ethanol–5 min-saline (ET–5-SAL; $n$=6 rats), 0.5 g/kg ethanol–50 min-saline (ET–50-SAL; $n$=6 rats), 0.5 g/kg ethanol–5 min-0.3 mg/kg nicotine (ET–5-NIC; $n$=7 rats), and 0.5 g/kg ethanol–50 min-0.3 mg/kg nicotine (ET–50-NIC; $n$=7 rats) group.

The SAL–5-SAL and SAL–50-SAL groups did not differ in terms of water intake [$F(1, 6)=0.63$, $P=0.46$], saccharin intake [$F(1, 6)=0.12$, $P=0.74$], and saccharin preference [$F(1, 6)=0.001$, $P=0.97$]. Thus, the two control groups were pooled before further analyses (a SAL–SAL group). Similarly, the SAL–5-NIC and SAL–50-NIC groups did not differ in terms of water intake [$F(1, 6)=0.36$, $P=0.57$], saccharin intake [$F(1, 6)=0.32$, $P=0.59$], and saccharin preference [$F(1, 6)=1.24$, $P=0.31$]. Thus, the two groups were pooled before further analyses (a SAL–NIC group).

### 2.3. Blood ethanol levels

A separate group of 16 male Wistar rats (Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland) was housed as described above. Silastic catheters (inner diameter: 0.5 mm, outer diameter: 1.0 mm) were inserted into the right jugular veins as described by Mierzejewski et al. (2003) and Korkosz et al. (2005). The rats were allowed 2 weeks for recovery. The catheters were flushed each day with a 0.2 ml sterile saline containing heparin (Polfa, Warsaw, Poland; 600 I.U./30 ml) and gentamicin (4 mg/30 ml; Krka, Novo Mesto, Slovenia).

The animals were administered i.p. with 0.25 or 0.5 g/kg ethanol. Blood samples (0.4 ml) were collected into Eppendorf tubes 5, 15, 30, 60 or 120 min after the ethanol injection. Three-four rats were tested at each time point. The samples were centrifuged for 5 min and serum (0.1 ml) was pipetted into 0.2-ml containers. Blood ethanol levels were determined as quickly as possible with the aid of commercially available REA enzymatic assays (Serrano et al., 1988; Slama et al., 1989) and Abbott TDx® autoanalyser (Abbott Laboratories, Abbott Park, IL, USA).

Blood ethanol concentrations were assessed in the subjects which did not undergo taste aversion conditioning with nicotine. Given the above, blood ethanol concentrations presented in Fig. 4 should be treated only as an approximation of ethanol levels achieved in Experiments 2 and 3.

### 2.4. Drugs

Nicotine di-tartrate (RBI, Natick, MA, USA) was dissolved in sterile physiological saline (0.9% NaCl, Polfa, Lublin, Poland) immediately prior to use. pH of nicotine solutions was adjusted to 7.3 with dilute NaOH. Nicotine was injected s.c. in a volume of 1 ml/kg. The doses referred to the free base.

Ethanol solutions (15% v/v) were prepared immediately prior to use from the 96% stock solution (Rectified Spirit, Polmos, Zielona Gora, Poland) and sterile saline. Ethanol (0.25 or 0.5 g/kg, i.p.) was administered in appropriate volumes to obtain a desired dose (2.15 or 4.3 ml/kg, respectively).

### 2.5. Statistics

All analyses were performed with the aid of the Statistica 5.0 software package for Windows (StatSoft, Inc., Tulsa, OK, USA).

Data from the one-bottle (changes in saccharin consumption) and two-bottle tests (saccharin preference) were compared by means of a two-way analysis of variance (ANOVA; Treatment × Test) with repeated measure on Test. Newman–Keuls test was used for individual post hoc comparisons. A probability level ($P$) less than 0.05 was considered significant.

## 3. Results

Body weights and water consumption did not differ significantly between the groups run in Experiment 1, 2 or 3 ($F$s < 0.3, $P$s > 0.9 for body weights, $F$s < 0.7, $P$s > 0.6 for water consumption).

### 3.1. Experiment 1. Nicotine-induced conditioned taste aversion

#### 3.1.1. One-bottle tests

The two-way ANOVA showed a non-significant effect of Treatment [$F(3, 25) = 2.94$, $P = 0.052$], a significant effect of Test [$F(3, 75) = 6.91$, $P < 0.001$], and a significant Treatment × Test interaction [$F(9, 75) = 6.17$, $P < 0.001$]. As expected, the group treated with saline tended to increase its saccharin intake during the consecutive tests (Fig. 1A). The lower doses of nicotine (0.3–0.6 mg/kg) did not induce any conditioned taste aversion.

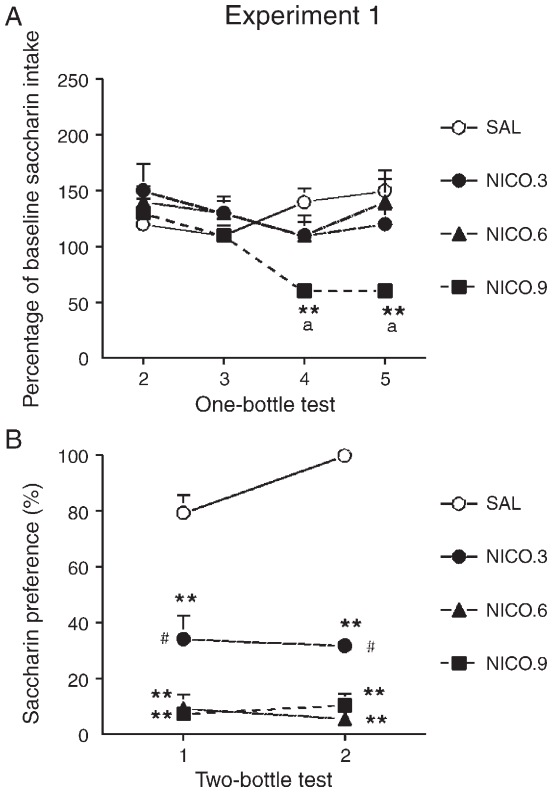


Fig. 1. Nicotine-induced conditioned taste aversion in Experiment 1. (A) Mean (±S.E.M.) saccharin consumption in one-bottle tests 2–5 expressed as a percentage of saccharin intake in one-bottle test 1. (B) Mean (±S.E.M.) saccharin preference in two-bottle tests. Groups are labelled according to the treatment received during conditioning sessions (one-bottle tests 1–4). **$P$<0.01 vs. the SAL group; [a]$P$<0.01 vs. the NIC0.3 and NIC0.6 group, [#]$P$<0.01 vs. the NIC0.6 and NIC0.9 group; $n$=7–8 rats. SAL=saline, NIC=nicotine.

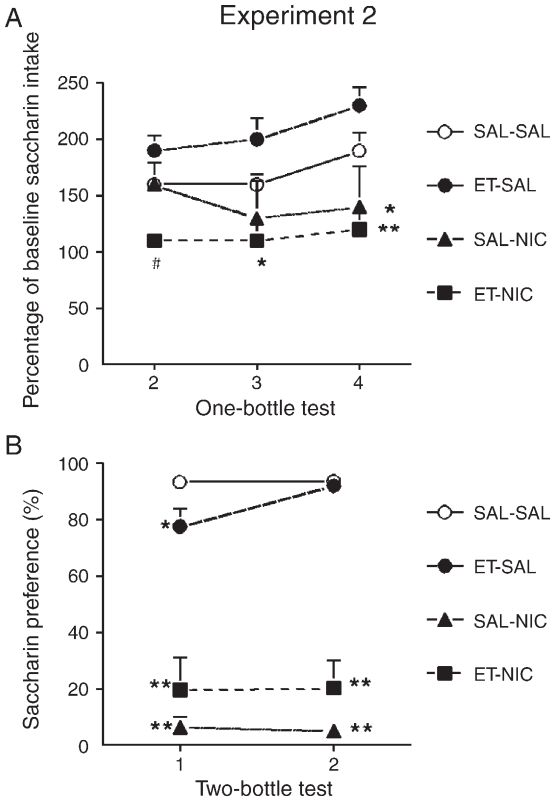


Fig. 2. Effects of 0.25 g/kg ethanol on nicotine-induced conditioned taste aversion in Experiment 2. (A) Mean (±S.E.M.) saccharin consumption in one-bottle tests 2–4 expressed as a percentage of saccharin intake in one-bottle test 1. (B) Mean (±S.E.M.) saccharin preference in two-bottle tests. Groups are labelled according to the treatment received during conditioning sessions (one-bottle tests 1–4). *$P$<0.05, **$P$<0.01 vs. the SAL–SAL group, [#]$P$<0.01 vs. the SAL–NIC group; $n$=7 rats. ET=ethanol, SAL=saline, NIC=nicotine.

In contrast, the post hoc analysis revealed that the group treated with 0.9 mg/kg nicotine reduced its saccharin intake as compared to the other groups (Fig. 1A). This latter effect was significant for one-bottle-tests 4–5 ($P$<0.01).

#### 3.1.2. Two-bottle tests

The ANOVA indicated a significant effect of Treatment [$F(3, 25) = 49.22$, $P < 0.001$], a non-significant effect of Test [$F(1, 25) = 2.17$, $P = 0.15$], and a significant Treatment × Test interaction [$F(3, 25) = 3.37$, $P < 0.05$]. The post hoc analysis revealed that all doses of nicotine induced conditioned taste aversion ($P$s < 0.01; Fig. 1B). The effects of nicotine were dose-dependent as the two higher doses (0.6–0.9 mg/kg) induced stronger conditioned taste aversion than 0.3 mg/kg nicotine ($P$s < 0.01).

### 3.2. Experiment 2. Effects of 0.25 g/kg ethanol on nicotine-induced conditioned taste aversion

#### 3.2.1. One-bottle tests

The ANOVA showed a significant effect of Treatment [$F(3, 24) = 5.76$, $P < 0.01$], a significant effect of Test [$F(2, 48) = 3.53$, $P < 0.05$], and a non-significant Treatment × Test interaction [$F(6, 48) = 2.09$, $P = 0.7$; Fig. 2A]. The SAL–NIC group decreased its saccharin intake in one-bottle test 4 as compared to the SAL–

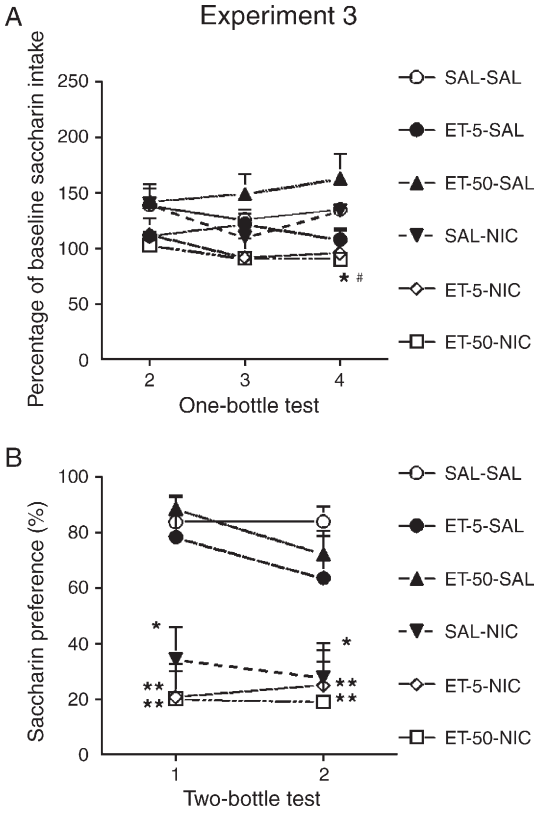


Fig. 3. Effects of 0.5 g/kg ethanol on nicotine-induced conditioned taste aversion in Experiment 3. (A) Mean (±S.E.M.) saccharin consumption in one-bottle tests 2–4 expressed as a percentage of saccharin intake in one-bottle test 1. (B) Mean (±S.E.M.) saccharin preference in two-bottle tests. Groups are labelled according to the treatment received during conditioning sessions (one-bottle tests 1–4) and inter-injection interval (5 or 50 min). *$P<0.05$, **$P<0.01$ vs. the SAL–SAL group, $^{\#}P<0.05$ vs. the SAL–NIC group; $n=6$–8 rats. ET=ethanol, SAL=saline, NIC=nicotine, 5=5 min, 50=50 min.

SAL group ($P<0.05$). Ethanol given in combination with saline (the ET–SAL group) did not induce any conditioned taste aversion.

Ethanol pre-treatment tended to increase nicotine-induced conditioned taste aversion. The ET–NIC group showed significantly lower relative saccharin intake than the SAL–NIC group in one-bottle test 2 ($P<0.05$; Fig. 2A).

#### 3.2.2. Two-bottle tests

The ANOVA indicated a significant effect of Treatment [$F(3, 24)=57.24$, $P<0.001$], a non-significant effect of Test [$F(1, 24)=3.35$, $P=0.08$] and a significant Treatment × Test interaction [$F(3, 24)=3.54$, $P<0.05$; Fig. 2B]. As expected, the SAL–NIC group showed significantly lower saccharin preference as compared to the SAL–SAL group ($P<0.01$). The ET–SAL group reduced its saccharin preference in comparison to the SAL–SAL group in two-bottle test 1 ($P<0.05$) but not in two-bottle test 2.

The ET–NIC group showed significantly lower saccharin preference than the SAL–SAL group ($P<0.01$). Ethanol did not alter nicotine-induced conditioned taste aversion as differences in saccharin preference between the SAL–NIC and ET–NIC group were not significant ($P>0.1$; Fig. 2B).

### 3.3. Experiment 3. Effects of 0.5 g/kg ethanol on nicotine-induced conditioned taste aversion

#### 3.3.1. One-bottle tests

The ANOVA showed a significant effect of Treatment [$F(5, 36)=3.98$, $P<0.001$], a non-significant effect of Test [$F(2, 72)=1.66$, $P=0.2$], and a non-significant Treatment × Test interaction [$F(10, 72)=1.10$, $P=0.38$; Fig. 3A]. Neither nicotine nor ethanol when given in combination with saline induced any conditioned taste aversion. The SAL–NIC, ET–5-SAL and ET–50-SAL group did not differ from the SAL–SAL group in terms of relative saccharin intake ($P>0.05$).

The combination of ethanol and nicotine tended to produce conditioned taste aversion in the one-bottle tests. The ET–50-NIC group reduced its saccharin consumption in one-bottle test 4 as compared to the SAL–SAL and SAL–NIC group ($P<0.05$). A similar trend for the ET-5-NIC group did not reach significance (Fig. 3A).

#### 3.3.2. Two-bottle tests

The ANOVA indicated a significant effect of Treatment [$F(5, 36)=14.34$, $P<0.001$], a non-significant effect of Test [$F(1, 36)=0.92$, $P=0.34$] and a non-significant Treatment × Test interaction [$F(5, 36)=0.31$, $P=0.9$; Fig. 3B]. As expected, the SAL–NIC group showed significantly lower saccharin preference in comparison to the SAL–SAL group ($P<0.05$). Ethanol given in combination with saline did not produce conditioned taste aversion ($P>0.05$).

The combination of ethanol and nicotine induced significant conditioned taste aversion regardless of the inter-injection interval ($Ps<0.01$). Ethanol did not alter nicotine-induced conditioned taste aversion as differences in saccharin preference between the SAL–NIC, ET-5-NIC, and ET-50-NIC group were not significant ($P>0.8$; Fig. 3B).

### 3.4. Blood ethanol levels

Fig. 4 shows blood ethanol concentrations after the single injection of ethanol. As expected, ethanol levels were dose- and time-dependent. Maximal blood ethanol levels after i.p. administration of 0.25 and 0.5 g/kg ethanol exceeded 20 and 80 mg%, respectively.

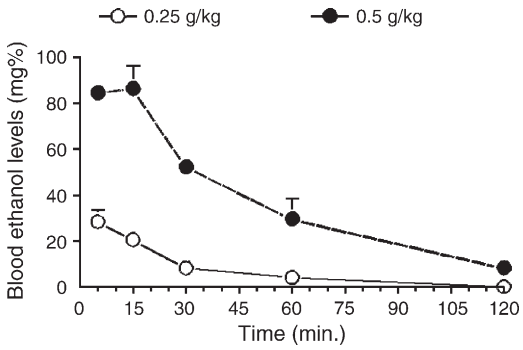


Fig. 4. Mean (±S.E.M.) blood ethanol levels achieved after ethanol administration (0.25 or 0.5 g/kg, i.p.). $n=3$–4 rats for each time point.

## 4. Discussion

Nicotine produced dose-dependent conditioned taste aversion in Experiment 1. In this respect, the present results fit well to several previous reports on nicotine-induced conditioned taste aversion (e.g. Kumar et al., 1983; Iwamoto and Williamson, 1984). Ethanol induced marginal, if any, conditioned taste aversion when given in combination with saline. The latter finding was in agreement with our previous observation that higher ethanol doses ($\geq 1$ g/kg) were needed to induce conditioned taste aversion in Wistar rats (Bienkowski et al., 1997b; Piasecki et al., 2001).

The dose of nicotine selected for Experiments 2–3 produced moderate taste aversion conditioning in Experiment 1. Thus, both increase and decrease in the aversive effects of nicotine after ethanol pre-treatment could have been expected in the present study. Contrary to our hypothesis, ethanol did not antagonise nicotine-induced conditioned taste aversion. In fact, ethanol enhanced taste aversion conditioning produced by 0.3 m/kg nicotine in the one-bottle tests. Given blood ethanol levels achieved in the separate experiment, one may conclude that ethanol concentrations up to 80 mg% do not block nicotine-induced conditioned taste aversion in Wistar rats.

Blood ethanol concentrations were assessed in the group of rats which did not undergo the conditioned taste aversion procedure. Thus, effects of water deprivation, saccharin drinking and nicotine administration on blood ethanol levels could not be excluded. Nicotine did not change blood ethanol concentrations in mice and rats (Hisaoka and Levy, 1985; De Fiebre And Collins, 1989). Effects of saccharin drinking and water deprivation on blood ethanol levels have not been described. Accordingly, blood ethanol concentrations reported in Fig. 4 should be treated as an approximation of ethanol levels achieved in Experiments 2–3.

The same doses of ethanol (0.25–0.5 g/kg) were sufficient to antagonise the cueing properties of 0.3 mg/kg nicotine in the drug discrimination procedure (Korkosz et al., 2005). Taken together, the present results and previous reports may indicate that low doses of ethanol block the discriminative stimulus effects (Kim and Brioni, 1995; McMillan et al., 1999; Korkosz et al., 2005) but not the aversive stimulus effects of nicotine in the rat. Our data provide also indirect evidence that the discriminative stimulus properties of nicotine may not be related to its aversive stimulus effects.

In a single study on a similar topic, Kunin et al. (1999) have analysed effects of 1.2 g/kg ethanol on nicotine-induced conditioned taste aversion in the rat. The latter study included a conditioning and expression phase both consisting of 3 one-bottle tests with 0.1% saccharin. In the conditioning phase, nicotine di-tartrate (1.0 mg/kg of the salt form, i.p.) was administered twice, 30 min apart, immediately following 20-min saccharin presentation. Ethanol was injected 80 min before the alkaloid. Ethanol did not block acquisition of nicotine-induced conditioned taste aversion in the conditioning phase. Similarly, no difference was noted between vehicle-treated and ethanol-treated rats in expression session 3. Ethanol pre-treatment in the conditioning phase slightly attenuated expression of nicotine-induced conditioned taste aversion in expression sessions 1–2 (Kunin et al., 1999). One may interpret the latter findings as enhancement of extinction of nicotine-induced conditioned taste aversion rather than true antagonism. Different doses of ethanol and nicotine, different pre-treatment times and different route of nicotine administration (s.c. vs. i.p.) were used in the present and previous study. The above differences make direct comparisons between the two studies difficult.

It has been shown repeatedly that ethanol promotes cigarette smoking in humans (Griffiths et al., 1976; Henningfield et al., 1984; Mitchell et al., 1995). However, it is not known whether ethanol-induced increase in smoking behaviour might be related to potentiation of the rewarding and/or to reduction of the aversive effects of nicotine. Zacny et al. (1997) have studied effects of ethanol on cigarette preference in nicotine-dependent volunteers. The authors analysed the number of responses emitted and reinforcers earned on a series of concurrent random-ratio schedules that yielded tobacco and money reinforcers. Although ethanol (0.2–0.6 g/kg) produced dose-related alterations in mood and psychomotor performance, preference for cigarettes remained unaffected. More recently, Rose et al. (2004) have reported that administration of 0.5 g/kg ethanol to heavy smokers (leading to maximal ethanol concentrations in the range 20 to 40 mg%) slightly enhanced the rewarding effects of nicotine. In the same study, ethanol tended to enhance the aversive effects of nicotine (nausea/dizziness) but results of respective statistical analyses were not shown. In contrast to the previous reports (Griffiths et al., 1976; Henningfield et al., 1984; Mitchell et al., 1995), ethanol pre-treatment did not modify smoking behaviour in the latter study.

Concluding, the present results may indicate that ethanol does not attenuate nicotine-induced conditioned taste aversion in rats. More studies are needed to assess effects of ethanol on the aversive stimulus properties of nicotine in humans.

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