Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 512 (2005) 165–172

www.elsevier.com/locate/ejphar

Extended blockade of the discriminative stimulus effects of nicotine with low doses of ethanol

Agnieszka Korkosz[a], Ewa Taracha[b], Adam Plaznik[b], Elzbieta Wrobel[a], Wojciech Kostowski[a], Przemyslaw Bienkowski[a,*]

[a]*Department of Pharmacology, Institute of Psychiatry and Neurology, 9 Sobieskiego St., PL-02957, Warsaw, Poland*
[b]*Department of Neurochemistry, Institute of Psychiatry and Neurology, PL-02957, Warsaw, Poland*

Received 8 February 2005; accepted 18 February 2005
Available online 29 March 2005

Abstract

The aim of the present study was to further evaluate effects of ethanol on nicotine discrimination and to correlate these effects with blood ethanol levels. Rats were trained to discriminate 0.3 mg/kg nicotine from its vehicle in the standard two-lever operant procedure. In antagonism tests, small doses of ethanol (0.25–0.5 g/kg) were injected either 5 or 50 min before nicotine. Both doses of ethanol partially antagonized the nicotine cue regardless of the pre-treatment time. Ethanol attenuated also inhibitory effects of nicotine on the rate of responding. Suppression of the cueing effects of nicotine was noted even 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. Ethanol-induced antagonism of the nicotine cue disappeared when longer time (110 min) was allowed to elapse between the ethanol (0.5 g/kg) and nicotine injection. Concluding, the present results may indicate that the effects of ethanol on nicotine discrimination are not primarily related to blood ethanol levels.

Keywords: Nicotine discrimination; Ethanol; Antagonism; Blood ethanol level

1. Introduction

Several human studies have demonstrated that a high correlation exists between ethyl alcohol (ethanol) consumption and tobacco smoking (Carmody et al., 1985; Bien and Burge, 1990; Dawson, 2000). Moreover, nicotine dependence increases the risk of developing dependence on alcohol and vice versa (Burling and Ziff, 1988; Bien and Burge, 1990; Di Franza and Guerrera, 1990; Miller and Gold, 1998). Although alcohol and nicotine dependence seems to be closely associated, exact mechanism(s) of the above correlation remains unknown.

Nicotine is a primary constituent of tobacco that reinforces cigarette smoking in humans (Stolerman and Jarvis, 1995; Malin, 2001; Groman and Fagerström, 2003) and many studies have confirmed that nicotine serves as a positive reinforcer in laboratory animals (Corrigall et al., 1994; Stolerman and Jarvis, 1995). It has been shown repeatedly that all major behavioural effects of nicotine, including its discriminative properties, are mediated through central nicotinic acetylcholine receptors and can be blocked by centrally active, but not by peripherally active, nicotinic acetylcholine receptor antagonists (Romano et al., 1981; Stolerman and Jarvis, 1995; Shoaib and Stolerman, 1996; Dani and De Biasi, 2001). Interestingly, brain nicotinic receptors may also be involved in some neurochemical and behavioural effects of ethanol (Blomqvist et al., 1996; Bienkowski et al., 1998; Larsson and Engel, 2004). Electrophysiological experiments confirmed that clinically relevant concentrations of ethanol modulate the function of different subtypes of nicotinic receptors (Cardoso et al., 1999; Marszalec et al., 1999). Depending on molecular composition of the nicotinic receptor subtype, acute ethanol exposure may either potentiate or inhibit the receptor function (for review, see Narahashi et al., 1999). Moreover, in some preparations, ethanol pre-treatment

* Corresponding author. Tel.: +48 22 4582676; fax: +48 22 8427644.
E-mail address: bienkow@ipin.edu.pl (P. Bienkowski).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.02.026

leads to increase in the nicotinic receptor-associated currents followed by prolonged desensitisation of nicotinic receptors (Wu et al., 1994; Nagata et al., 1996).

Both theory and empirical data support the belief that the drug discrimination procedure is especially useful for identifying receptor mechanisms that mediate the stimulus effects of psychoactive drugs (Järbe, 1987; Colpaert, 1999). In the discrimination procedure, animals learn a particular drug-induced interoceptive cue which can be tested for generalisation to other drugs or antagonised by still other compounds (Colpaert, 1986, 1999; Brioni et al., 1994, 1996; Mansbach et al., 1998). Studies on the discriminative stimulus effects of ethanol have indicated that nicotine may enhance the ethanol cue in rats (Signs and Schechter, 1986; Bienkowski and Kostowski, 1998). On the other hand, ethanol may rather antagonise the cueing properties of nicotine in rats trained to discriminate nicotine from its vehicle. Ethanol injected either 5 or 20 min before nicotine blocked the nicotine cue in the standard two-lever drug discrimination procedure (Kim and Brioni, 1995; McMillan et al., 1999). One may hypothesise that the effects of ethanol on nicotine discrimination are related to the above-mentioned interactions between ethanol and nicotinic acetylcholine receptors (Nagata et al., 1996; Narahashi et al., 1999).

The purpose of the present study was two-fold. First, we decided to replicate the results of previous studies on the effects of ethanol on nicotine discrimination (Kim and Brioni, 1995; McMillan et al., 1999). For this aim, small doses of ethanol (0.25–0.5 g/kg) were injected 5 min before nicotine in rats learned to discriminate nicotine from its vehicle. Second, given the results of some in vitro and in vivo studies (De Fiebre and Collins, 1989; Wu et al., 1994; Nagata et al., 1996), we assumed that ethanol may desensitise nicotinic receptors and that this desensitisation may not depend on permanent presence of ethanol in the body. In order to test the above hypothesis, we administered the same doses of ethanol 50 min before nicotine injections. Blood ethanol levels were determined to analyse the relationship between ethanol-induced antagonism of the nicotine cue and ethanol concentration.

A training dose of nicotine (0.3 mg/kg) selected for the present study potentiated the discriminative stimulus effects of ethanol in our previous experiments (Bienkowski and Kostowski, 1998). The range of ethanol doses (0.25–0.5 g/kg) produced no sedative effects in the open field test (Bienkowski et al., 1997a) and did not alter response rates in rats trained to discriminate ethanol from its vehicle (Bienkowski et al., 1998).

2. Method

2.1. Nicotine discrimination

2.1.1. Animals

Eighteen male Wistar rats (Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland) weighing 300–330 g at the beginning of the experiments were kept in a room with constant environmental conditions: temperature of 22 ± 1 °C, 60% humidity and a 12-h light–dark cycle (light on at 6:00 a.m.). The subjects were maintained at ~80% of weight of the free-feeding control group (n=6 rats) by restricting daily food (Labofeed H, WPiK, Kcynia, Poland) to 13–17 g/day. Tap water was available ad libitum. All rats were trained and tested between 2:00 and 6:00 p.m.

Treatment of the rats in the whole study was in full accordance with the ethical standards laid down in respective European (directive no. 86/609) and Polish regulations. All procedures were reviewed and approved by an Ethics Committee on Animal Studies.

2.1.2. Apparatus

Nicotine discrimination was studied in four standard operant chambers (Coulbourn Instruments, Allentown, PA, USA). The chambers consisted of modular test cages (E10-18TC, Coulbourn) enclosed within sound-attenuating cubicles with fans for ventilation and background white noise (for details, see Bienkowski et al., 1996, 1997a,b). A white house light was centred near the top of the back wall of the chamber. The start of each session was signalled by turning the house light on. The test cages were also equipped with two response levers, separated by a liquid delivery system (a liquid dipper, E14-05, Coulbourn). The liquid delivery system presented 20% (w/v) sucrose solution in a 0.01-ml portion for 5 s. The availability of sucrose reward was signalled by a brief audible click and a small white light (4 W) located inside the dipper. Experimental sessions and data recording were accomplished using the L2T2 software package (Coulbourn) running on an IBM-compatible personal computer.

2.1.3. Discrimination training

The discrimination procedure followed that described by Bienkowski et al. (1996, 1997a,b, 1998). However, nicotine instead of ethanol served as the discriminative stimulus.

The animals were first trained to press both levers under a fixed-ratio 1 (FR1) schedule of sucrose delivery. Drug discrimination training began only after all of the animals responded reliably on both levers under the FR10 condition. The rats were trained to press one lever following s.c. (−)-nicotine injection (0.3 mg/kg) and to press the other lever following injection of sterile physiological saline (0.9% NaCl; Polfa, Lublin, Poland). All injections occurred 10 min prior to the start of 15-min training sessions. Nicotine (RBI, Natick, MA, USA) was dissolved in 0.9% NaCl and pH of nicotine solutions was adjusted to 7.3 with dilute NaOH. The nicotine dose referred to the free base.

The lever (left or right) corresponding with nicotine and saline treatment remained fixed for the duration of the study for a given animal and was counterbalanced across the group of rats. The responses emitted on the incorrect lever were recorded, but did not result in sucrose delivery. The sessions were conducted Monday through Saturday under

the alternating sequence: drug–vehicle–vehicle–drug–drug and vehicle–drug–drug–vehicle–vehicle. The animals continued to be trained under these conditions until they exhibited the discrimination criteria, which were defined as: (i) correct first-lever selection (≤1 incorrect response before selection of the correct lever), (ii) greater than 90% correct-lever responding during the entire session, for 9 out of 10 consecutive sessions. The animals were also required to complete at least one FR10 on the correct lever during each session.

2.1.4. Test sessions. Basic characteristics of nicotine discrimination

After the subjects had reached the discrimination criteria, test sessions were initiated. Typically, the 15-min test sessions were conducted twice per week with the training sessions intervening during the remaining days. In order to be tested in each subsequent test session, the rat must have reached the discrimination criteria for at least 2 days. During the test session, responding on either lever resulted in the delivery of sucrose reward. The percentage of nicotine-appropriate responding was calculated by dividing responses made on nicotine-appropriate lever by the total number of responses and multiplying the result by 100%. Only rats which completed at least one FR10 on either vehicle or nicotine lever were considered when nicotine-appropriate responding was calculated. Total number of responses emitted during the test session reflected response rate and was used as a measure of behavioural disruption. Data from all rats were included in statistical analyses of response rates.

In dose–response tests, which were performed in the beginning of the study, the rats were injected with various doses of nicotine (0.035, 0.075, 0.15, 0.3 or 0.6 mg/kg, s.c.) or its vehicle 10 min before the start of the test session. A non-competitive nicotinic acetylcholine receptor antagonist, mecamylamine (0.1 or 1.0 mg/kg, i.p.), was tested in antagonism and substitution tests in order to confirm pharmacological specificity of nicotine discrimination (Romano et al., 1981). In the antagonism tests, mecamylamine was injected 15 min before 0.3 mg/kg nicotine, i.e. 25 min before the start of the session. In the substitution tests, mecamylamine was administered in combination with saline.

Mecamylamine (Sigma, Poznan, Poland) was dissolved in sterile physiological saline. Both nicotine and mecamylamine were administered in a volume of 1.0 ml/kg. All solutions were prepared immediately prior to use and protected from direct exposure to light.

2.1.5. Antagonism tests with ethanol

Ethanol was injected 5 or 50 min before 0.3 mg/kg nicotine, i.e. 15 or 60 min before the start of the test session. Ethanol solutions (15% v/v) were prepared immediately prior to use from the 96% stock solution (Rectified Spirit, Polmos, Zielona Gora, Poland) and sterile physiological saline. Ethanol (0.25 or 0.5 g/kg) was administered i.p. in appropriate volumes to obtain a desired dose (2.15 ml/kg or 4.3 ml/kg, respectively).

2.1.6. Control experiments

After completion of the antagonism tests with ethanol, further experiments were run to assess whether: (i) 0.5 g/kg ethanol might antagonise the nicotine cue after longer pre-treatment interval (110 min), (ii) higher doses of ethanol (0.75–1.0 g/kg) might fully block the nicotine cue after the 5-min pre-treatment interval and (iii) 0.5 g/kg ethanol injected 15, 60 or 120 min before the session might substitute for the nicotine cue.

2.2. Blood ethanol levels

2.2.1. Animals

Blood ethanol levels were determined after the discrimination procedure had been completed. For this aim, a separate group of 14 male Wistar rats (Medical Research Centre, Polish Academy of Sciences, Warsaw, Poland) was used. The rats (300–330 g) were housed as described above. The subjects were tested between 2:00 and 6:00 p.m.

2.2.2. Collection of blood samples and determination of blood ethanol levels

Silastic catheters (inner diameter: 0.5 mm, outer diameter: 1.0 mm) were constructed as described by Mierzejewski et al. (2003). The rats were deeply anaesthetised with diazepam (Polfa, Warsaw, Poland; 5 mg/kg, i.p.) and ketamine (Richter Gedeon, Budapest, Hungary; 50 mg/kg, i.p.). The catheter was inserted into the right jugular vein and anchored into the neck muscles by sutures (for details, see Mierzejewski et al., 2003). The other end of the catheter was threaded s.c. around the animal's back and exited the skin through a small opening near the midscapular region. An obturator was inserted into the distal end of the catheter to prevent clogging and infection. 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 IU/30 ml) and gentamicin (4 mg/30 ml; Krka, Novo Mesto, Slovenia).

On a test day, the animals were administered i.p. with 0.25 or 0.5 g/kg ethanol (15% v/v). Blood samples (0.4 ml) were collected into Eppendorf tubes 15 or 60 min after the ethanol injection. 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® autoanalyzer (Abbott Laboratories, Abbott Park, IL, USA).

2.3. Statistics

Student's *t*-test was used to analyse basic parameters of nicotine discrimination. A two-way analysis of variance (ANOVA) (mecamylamine dose × nicotine dose) was

Table 1
Mean (± S.E.M.) percentage of nicotine-appropriate responding and mean (± S.E.M.) response rate following increasing doses of nicotine in rats trained to discriminate 0.3 mg/kg nicotine from its vehicle

Nicotine dose (mg/kg)	Nicotine-appropriate responding (%), $F(5,30)=8.53$, $P=0.0001$[a]	Total number of responses/15 min, $F(5,30)=1.89$, $P=0.13$	N responding[b]/N tested
Saline	3.4 ± 1.9	599.7 ± 173.8	6/6
0.035	26.7 ± 11.3	689.8 ± 183.4	6/6
0.075	61.1 ± 18.0	367.7 ± 161.3	6/6
0.15	66.6 ± 14.9	331.3 ± 101.4	6/6
0.3	83.9 ± 9.7	368.8 ± 111.1	8/8
0.6	99.8 ± 0.3	84.5 ± 17.7	4/4

[a] Summary of the one-way ANOVA.
[b] N responding=the number of rats which completed at least one FR10 on the nicotine- or saline-appropriate lever during the 15-min test session.

employed to evaluate effects of mecamylamine on nicotine discrimination. The two-way ANOVA (ethanol dose × pre-treatment time) was used to evaluate effects of ethanol on the nicotine cue. Student's t-test was used for individual post hoc comparisons. Arbitrary criteria of partial and complete substitution for nicotine were set at 40–79% and ≥80% of responding on the nicotine lever, respectively (Bienkowski et al., 1996).

A probability level (P) less than 0.05 was considered significant. The Statistica software package (StatSoft, Tulsa, OK., USA) was used to analyse all data.

3. Results

3.1. Nicotine discrimination

3.1.1. Basic characteristics

Sixteen out of 18 rats reached the discrimination criteria and the mean (± S.E.M.) number of sessions-to-criterion was 39.0 ± 1.5. The two rats, which did not reach the criteria within 80 training sessions, were eliminated from further experiments.

Nicotine- and saline-lever selections as well as overall response rates in the last 10 training sessions were analysed for the 16 rats, which reached the discrimination criteria. The mean (±S.E.M.) percentage of nicotine-appropriate lever selection (96.6 ± 1.2%) did not differ from the mean percentage of saline-appropriate lever selection (95.4 ± 1.0%, $P=0.45$, Student's t-test). Nicotine tended to suppress operant responding as compared to saline administration ($P=0.07$).

The discriminative stimulus effects of nicotine were dose-dependent (see Table 1 for details). ED_{50} value calculated with the aid of linear regression was 0.06 mg/kg. The discriminative stimulus effects of nicotine were completely blocked by 1.0 mg/kg mecamylamine (Table 2). As expected, mecamylamine (0.1–1.0 mg/kg) given in combination with saline did not substitute for the nicotine cue. These observations were confirmed by the two-way ANOVA, which indicated a significant effect of mecamylamine dose: $F(2,25)=18.20$, $P<0.001$, a significant effect of nicotine dose: $F(1,25)=79.50$, $P<0.001$, and a significant mecamylamine dose × nicotine dose interaction: $F(2,25)=32.40$, $P<0.001$.

Mecamylamine did not alter the rate of operant behaviour but tended to increase lever pressing suppressed by nicotine (Table 2). The ANOVA showed a non-significant effect of mecamylamine dose: $F(2,25)=3.10$, $P=0.06$, a significant effect of nicotine dose: $F(1,25)=4.60$, $P=0.04$, and a non-significant mecamylamine dose × nicotine dose interaction: $F(2,25)=0.90$, $P=0.4$.

3.1.2. Antagonism tests with ethanol

Ethanol (0.25–0.5 g/kg) partially antagonised the discriminative stimulus effects of 0.3 mg/kg nicotine regardless of the pre-treatment time (Table 3). The two-way ANOVA indicated a significant effect of ethanol dose: $F(2,43)=5.32$, $P<0.01$, a non-significant effect of pre-treatment time: $F(1,43)=0.41$, $P=0.53$, and a non-significant ethanol dose × pre-treatment time interaction: $F(2,43)=0.34$, $P=0.71$.

Ethanol blocked the effects of nicotine on the rate of operant responding (Table 3). The ANOVA revealed a significant effect of ethanol dose: $F(2,45)=4.22$, $P<0.05$. An effect of pre-treatment time: $F(1,45)=0.33$, $P=0.57$, and an ethanol dose × pre-treatment time interaction: $F(2,45)=0.2$, $P=0.8$ was not significant.

Table 2
Effects of mecamylamine on the discriminative stimulus effects of 0.3 mg/kg nicotine

Mecamylamine dose (mg/kg)	Nicotine dose (mg/kg)	Nicotine-appropriate responding (%)	Total number of responses/15 min	N responding[a]/N tested
Substitution tests				
Saline	saline	3.4 ± 1.9	599.7 ± 173.8	6/6
0.1	saline	1.2 ± 0.6	537.4 ± 198.3	5/5
1.0	saline	14.8 ± 14.1	722.6 ± 160.9	5/5
Antagonism tests				
Saline	0.3	89.8 ± 4.6	210.8 ± 52.5	5/5
0.1	0.3	80.0 ± 9.1	152.0 ± 108.1	5/5
1.0	0.3	0.7 ± 0.3[b]	691.4 ± 164.3	5/5

[a] N responding=the number of rats which completed at least one FR10 on the nicotine- or saline-appropriate lever.
[b] $P<0.01$ vs. the saline/0.3 mg/kg nicotine group.

Table 3
Effects of ethanol on the discriminative stimulus effects of 0.3 mg/kg nicotine

Ethanol dose (g/kg)	Pre-treatment time (min)[a]	Nicotine-appropriate responding (%)	Total number of responses/15 min	*N* responding[b]/*N* tested
Saline	15	92.0 ± 2.7	254.3 ± 53.2	7/7
Saline	60	93.0 ± 3.4	285.3 ± 84.8	7/7
0.25	15	71.9 ± 13.4	591.9 ± 166.2[c]	8/9
0.25	60	54.2 ± 18.9[c]	487.3 ± 170.0	6/6
0.5	15	58.8 ± 14.1[c]	684.2 ± 152.0[c]	8/9
0.5	60	56.4 ± 16.1[c]	571.5 ± 146.8	8/8

[a] Before the start of the 15-min antagonism test. Nicotine was always injected 10 min before the test session.
[b] *N* responding=the number of rats which completed at least one FR10 on the nicotine- or saline-appropriate lever.
[c] $P<0.05$ vs. the respective saline/nicotine controls.

3.1.3. Control experiments

The cueing properties of nicotine remained unaffected when the rats were tested 120 min after the injection of 0.5 g/kg ethanol. The percentage of nicotine-appropriate responding after ethanol pre-treatment (80.4 ± 12.1%) did not differ from that noted after saline pre-treatment (92.3 ± 3.4%, $P=0.35$, Student's *t*-test).

The higher doses of ethanol (0.75–1.0 g/kg) administered in combination with 0.3 mg/kg nicotine completely eliminated operant responding and thus discrimination behaviour could not be assessed.

Ethanol (0.5 g/kg) did not substitute for nicotine cue (Table 4) regardless of the pre-treatment time (15, 60 or 120 min).

3.2. Blood ethanol levels

Table 5 shows ethanol concentrations achieved in the blood after the single injection of 0.25 or 0.5 g/kg ethanol. As expected, the blood ethanol level was dose-and time-dependent. In general, the blood ethanol level after the 60-min pre-treatment interval was lower than that achieved after the 15-min interval. It is worthy to note that 60 min after the administration of 0.25 g/kg ethanol the blood ethanol level was close to zero.

4. Discussion

As expected, most subjects (89.9%) quickly and reliably discriminated nicotine from its vehicle. The cueing properties of nicotine were dose-dependent and were completely blocked by the non-competitive nicotinic acetylcholine receptor antagonist, mecamylamine. In this respect, our findings fit well to the several previous reports that nicotine may be reliably discriminated by various strains of rats (Romano et al., 1981; Shoaib and Stolerman, 1996).

The basic finding of the present study is that the low doses of ethanol (0.25–0.5 g/kg) partially antagonise the nicotine cue in the rat. Our results replicate the observation by Kim and Brioni (1995) that 1.0 g/kg ethanol attenuated nicotine cue in male Wistar rats trained to discriminate 0.3 mg/kg nicotine from saline. Similarly, ethanol (0.6–0.8 g/kg) blocked the discriminative stimulus effects of nicotine in genetically selected alcohol preferring P rats (McMillan et al., 1999). In line with our findings, only partial antagonism of the nicotine cue was observed in this latter study when 0.6 g/kg ethanol was combined with the training dose of nicotine (0.6 mg/kg).

In the present study, ethanol decreased nicotine-appropriate responding to the minimal level of 54%. Bearing in mind the well-known effects of ethanol on memory processes (e.g. Popke et al., 2000), one may consider random responding on both levers as an explanation for the above finding. This possibility seems to be rather unlikely for two reasons. First, ethanol (given in combination with saline) did not alter discrimination between the levers. The rats administered with 0.5 g/kg ethanol responded almost exclusively on the saline-appropriate lever (82.1%–97.8%). Second, ethanol antagonised not only the nicotine cue but also the effects of nicotine on the rate of operant responding. It is worthy to note that our attempts to fully antagonise the nicotine cue with the higher doses of ethanol (>0.5 g/kg) were unsuccessful. These doses given in combination with

Table 4
Mean (±S.E.M.) percentage of nicotine-appropriate responding and mean (±S.E.M.) response rate following pre-treatment with 0.5 g/kg ethanol

Pre-treatment time (min)[a]	Nicotine-appropriate responding (%)	Total number of responses/15 min	*N* responding[b]/*N* tested
15	2.2 ± 1.6	666.2 ± 209.5	6/6
60	6.9 ± 4.5	738.7 ± 236.9	6/6
120	17.9 ± 13.0	843.3 ± 227.0	6/6

[a] Before the start of the 15-min substitution test.
[b] *N* responding=the number of rats which completed at least one FR10 on the nicotine- or saline-appropriate lever.

Table 5
Mean (±S.E.M.) blood ethanol levels achieved after i.p. ethanol administration

Ethanol dose (g/kg)	Pre-treatment time (min)	Ethanol concentration (mg%)	*N* tested
0.25	15	18.35 ± 4.64	4
0.25	60	4.33 ± 2.12	4
0.5	15	86.36 ± 11.86	3
0.5	60	29.73 ± 9.83	3

nicotine completely suppressed operant behaviour (see also Gatch et al., 2003).

Surprisingly, the effect of ethanol on the discriminative stimulus effects of nicotine was relatively long-lasting and was not simply related to the blood ethanol level. Partial antagonism of the nicotine cue was observed regardless of whether ethanol was injected 5 or 50 min before nicotine (15 or 60 min before the start of the test session, respectively). However, the effect of ethanol on nicotine discrimination disappeared when the pre-treatment time was extended to 110 min. Notably, 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.

There are several possible explanations for the above findings. At the pharmacokinetic level, long-lasting blockade of the nicotine cue could be related to ethanol-induced decrease of the nicotine level in the rat's body. Although ethanol (0.75 g/kg) did not change blood and brain levels of nicotine in mice (De Fiebre and Collins, 1989), one may not completely rule out this possibility as nicotine concentrations were not assessed in our study. Considering pharmacokinetic properties of ethanol, one may suggest that it was not ethanol but rather its primary metabolite, acetaldehyde, which was responsible for the effects observed in the present study. Recently, Kunin et al. (2000) have suggested that acetaldehyde plays a role in behavioural interactions between nicotine and ethanol in the conditioned taste aversion procedure. Further experiments with specific inhibitors of ethanol metabolism should be performed to verify the above hypotheses.

It is well established that nicotine elicits its discriminative stimulus effects through interactions with brain nicotinic acetylcholine receptors (Romano et al., 1981; Shoaib and Stolerman, 1996). Brain nicotinic receptors are also highly sensitive to physiologically relevant concentrations of ethanol (Nagata et al., 1996; Narahashi et al., 1999) and thus one may assume that the interaction between ethanol and nicotine observed in the present study was pharmacodynamic in nature. Multiple subtypes of the nicotinic acetylcholine receptor are expressed in the central nervous system and molecular composition of the receptor may determine its sensitivity to ethanol. Studies on nicotinic acetylcholine receptor subtypes expressed in *Xenopus* oocytes have indicated that ethanol enhances the function of the $\alpha4\beta2$ and $\alpha2\beta4$ receptor subtype (Cardoso et al., 1999; Marszalec et al., 1999). On the other hand, ethanol inhibits ion conductance through the $\alpha7$ subtype (Yu et al., 1996; Covernton and Connolly, 1997; Cardoso et al., 1999). Unfortunately, relative contribution of these molecular variants to the discriminative stimulus properties of nicotine remains unknown. Brioni et al. (1996) have shown that an $\alpha7$ receptor antagonist, methyllycaconitine, did not alter the cueing properties of nicotine in the rat. Given the above, one may tentatively hypothesise that ethanol attenuates the nicotine cue through interactions with non-$\alpha7$ nicotinic acetylcholine receptors.

Nagata et al. (1996) have reported that ethanol accelerated desensitisation of neuronal-type nicotinic receptors expressed by PC12 cells. (Composition of nicotinic receptors was not determined in this latter study.) Regarding the present results, one should stress that the acceleration of whole-cell current decay was greater when exposure to ethanol occurred a few minutes before acetylcholine application (Nagata et al., 1996). Wu et al. (1994) have shown that ethanol increased desensitisation of the *Torpedo* nicotinic acetylcholine receptors and stabilised the receptor in a desensitised state. Pre-treatment with a small dose of ethanol that was not anticonvulsant enhanced nicotine-induced behavioural desensitisation to seizures evoked by high doses of nicotine (De Fiebre and Collins, 1989). Further behavioural and biochemical studies in our laboratories will address this problem in more detail.

Considering mechanisms of any interaction between ethanol and nicotine, one should bear in mind the fact that both drugs modify activity of many neurotransmitter systems (Uzbay et al., 1998; Di Chiara, 2000; Dani and De Biasi, 2001; Tizabi et al., 2002; George and O'Malley, 2004). Consequently, apart from direct interactions at the nicotinic acetylcholine receptor level, ethanol might have influenced the cueing effects of nicotine through interactions with dopaminergic (Di Chiara, 2000; Tizabi et al., 2002), serotonergic (Jang et al., 2002), glutamatergic and/or GABAergic pathways (Kim and Brioni, 1995; Bienkowski and Kostowski, 1998; Kostowski and Bienkowski, 1999).

One may also try to explain our data in the context of perceptual masking of one drug stimulus by another (for discussion, see Gauvin and Young, 1989). It has been shown that ethanol may produce a compound interoceptive cue mediated by a number of receptor systems (Kostowski and Bienkowski, 1999). Thus, one could hypothesise that in the present study non-specific interoceptive stimuli produced by ethanol masked relatively specific cueing effects of nicotine. However, this hypothesis seems to be rather unlikely for two reasons. First, ethanol-induced blockade of the nicotine cue was not simply related to blood ethanol concentrations. Second, ethanol attenuated also the inhibitory effects of nicotine on the rate of responding and this latter action may not be easily explained by perceptual masking.

Although clinical studies have shown that alcohol use is associated with increased nicotine consumption and vice versa (Griffiths et al., 1976; Burling and Ziff, 1988; Bien and Burge, 1990), ethanol and nicotine interact asymmetrically in the drug discrimination procedure. While nicotine enhances the interoceptive cue produced by ethanol (Signs and Schechter, 1986; Bienkowski and Kostowski, 1998), ethanol may rather antagonise the nicotine cue in rats (Kim and Brioni, 1995; McMillan et al., 1999; the present study). Taken together, these results may indicate that people consume more cigarettes when they are drinking alcohol (Griffiths et al., 1976; Henningfield et al., 1984; Mitchell et

al., 1995; Zacny et al., 1996) because more nicotine is needed to stimulate desensitised (or blocked) nicotinic acetylcholine receptors and to achieve desired levels of subjective effects.

Acknowledgements

This study was supported by the State Committee for Scientific Research (grant no. PBZ-KBN-033/P05/2000).

References

Bien, T.H., Burge, R., 1990. Smoking and drinking: a review of the literature. Int. J. Addict. 25, 1429–1454.

Bienkowski, P., Kostowski, W., 1998. Discrimination of ethanol in rats: effects of nicotine, diazepam, CGP 40116, and 1-(*m*-chlorophenyl)-biguanide. Pharmacol. Biochem. Behav. 60, 61–69.

Bienkowski, P., Stefanski, R., Kostowski, W., 1996. Competitive NMDA receptor antagonist, CGP 40116, substitutes for the discriminative stimulus effects of ethanol. Eur. J. Pharmacol. 314, 277–280.

Bienkowski, P., Koros, E., Piasecki, J., Kostowski, W., 1997a. Interactions of ethanol with nicotine, dizocilpine, CGP 40116, and 1-(*m*-chlorophenyl)-biguanide in rats. Pharmacol. Biochem. Behav. 58, 1159–1165.

Bienkowski, P., Stefanski, R., Kostowski, W., 1997b. Discriminative stimulus effects of ethanol: lack of antagonism with *N*-methyl-D-aspartate and D-cycloserine. Alcohol 14, 345–350.

Bienkowski, P., Piasecki, J., Koros, E., Stefanski, R., Kostowski, W., 1998. Studies on the role of nicotinic acetylcholine receptors in the discriminative and aversive stimulus properties of ethanol in the rat. Eur. Neuropsychopharmacol. 8, 79–87.

Blomqvist, O., Ericson, M., Johnson, D.H., Engel, J.A., Soderpalm, B., 1996. Voluntary ethanol intake in the rat: effects of nicotinic acetylcholine receptor blockade or subchronic nicotine. Eur. J. Pharmacol. 314, 257–267.

Brioni, J.D., Kim, D.J.B., O'Neill, A.B., Williams, J.E.G., Decker, M.W., 1994. Clozapine attenuates the discriminative stimulus properties of (−)-nicotine. Brain Res. 643, 1–9.

Brioni, J.D., Kim, D.J.B., O'Neill, A.B., 1996. Nicotine cue: lack of effect of the α7 nicotinic receptor antagonist methyllycaconitine. Eur. J. Pharmacol. 301, 1–5.

Burling, T.A., Ziff, D.C., 1988. Tobacco smoking: a comparison between alcohol and drug abuse inpatients. Addict. Behav. 13, 185–190.

Cardoso, R.A., Brozowski, S.J., Chavez-Noriega, L.E., Harpold, M., Valenzuela, C.F., Harris, R.A., 1999. Effects of ethanol on recombinant human neuronal nicotinic acetylcholine receptors expressed in *Xenopus* oocytes. J. Pharmacol. Exp. Ther. 289, 774–780.

Carmody, T.P., Brischetto, C.S., Matarazzo, J.D., O'Donnell, R.P., Connor, W.E., 1985. Co-occurent use of cigarettes, alcohol, and coffee in healthy, community-living men and women. Health Psychol. 4, 323–335.

Colpaert, F., 1986. Drug discrimination: behavioral, pharmacological and molecular mechanisms of discriminative drug effects. In: Goldberg, S.R., Stolerman, I.P. (Eds.), Behavioral Analysis of Drug Dependence. Academic Press, New York, pp. 161–193.

Colpaert, F., 1999. Drug discrimination in neurobiology. Pharmacol. Biochem. Behav. 64, 337–345.

Corrigall, W.A., Coen, K.M., Adamson, K.L., 1994. Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res. 653, 278–284.

Covernton, P.J., Connolly, J.G., 1997. Differential modulation of rat neuronal nicotinic receptor subtypes by acute application of ethanol. Br. J. Pharmacol. 122, 1661–1668.

Dani, J.A., De Biasi, M., 2001. Cellular mechanisms of nicotine addiction. Pharmacol. Biochem. Behav. 70, 439–446.

Dawson, D.A., 2000. Drinking as a risk factor for sustained smoking. Drug Alcohol Depend. 59, 235–249.

De Fiebre, C.M., Collins, A., 1989. Behavioral desensitization to nicotine is enhanced differentially by ethanol in long-sleep and short-sleep mice. Alcohol 6, 45–51.

Di Chiara, G., 2000. Role of dopamine in the behavioural actions of nicotine related to addiction. Eur. J. Pharmacol. 393, 295–314.

Di Franza, J.R., Guerrera, M.P., 1990. Alcoholism and smoking. J. Stud. Alcohol 51, 130–135.

Gatch, M.B., Youngblood, B.D., Forster, M.J., 2003. Effects of ethanol on cocaine discrimination in rats. Pharmacol. Biochem. Behav. 75, 837–844.

Gauvin, D.V., Young, A.M., 1989. Evidence for perceptual masking of the discriminative morphine stimulus. Psychopharmacology 98, 212–221.

George, T.P., O'Malley, S.S., 2004. Current pharmacological treatments for nicotine dependence. Trends Pharmacol. Sci. 25, 42–48.

Griffiths, R.R., Bigelow, G.E., Liebson, I., 1976. Facilitation of human tobacco self-administration by ethanol: a behavioral analysis. J. Exp. Anal. Behav. 25, 279–292.

Groman, E., Fagerström, K., 2003. Nicotine dependence: development, mechanisms, individual differences and links to possible neurophysiological correlates. Wien. Klin. Wochenschr. 115, 155–160.

Henningfield, J.E., Chait, L.D., Griffiths, R.R., 1984. Effects of ethanol on cigarette smoking by volunteers without histories of alcoholism. Psychopharmacology 82, 1–5.

Jang, M.H., Shin, M.C., Lee, T.H., Kim, Y.P., Jung, S.B., Shin, D.H., Kim, H., Kim, S.S., Kim, E.H., Kim, C.J., 2002. Alcohol and nicotine administration inhibits serotonin synthesis and tryptophan hydroxylase expression in dorsal and median raphe of young rats. Neurosci. Lett. 329, 141–144.

Järbe, T.U.C., 1987. Drug discrimination learning. In: Greenshaw, A.J., Dourish, C.T. (Eds.), Experimental Psychopharmacology. Humana Press, Clifton, NJ, pp. 433–450.

Kim, D.J.B., Brioni, J.D., 1995. Modulation of the discriminative stimulus properties of (−)-nicotine by diazepam and ethanol. Drug Dev. Res. 34, 47–54.

Kostowski, W., Bienkowski, P., 1999. Discriminative stimulus effects of ethanol: neuropharmacological characterization. Alcohol 17, 63–80.

Kunin, D., Latendresse, M.W., Gaskin, S., Smith, B.R., Amit, Z., 2000. Preexposure effects of nicotine and acetaldehyde on conditioned taste aversion induced by both drugs. Pharmacol. Biochem. Behav. 66, 695–699.

Larsson, A., Engel, J.A., 2004. Neurochemical and behavioral studies on ethanol and nicotine interactions. Neurosci. Biobehav. Rev. 27, 713–720.

Malin, D.H., 2001. Nicotine dependence: studies with a laboratory model. Pharmacol. Biochem. Behav. 70, 551–559.

Mansbach, R.S., Rovetti, C.C., Freedland, C.S., 1998. The role of monoamine neurotransmitter systems in the nicotine discriminative stimulus. Drug Alcohol Depend. 52, 125–134.

Marszalec, W., Aistrup, G.L., Narahashi, T., 1999. Ethanol-nicotine interactions at α-bungarotoxin-insensitive nicotinic acetylcholine receptors in rat cortical neurons. Alcohol., Clin. Exp. Res. 23, 439–445.

McMillan, D.E., Li, M., Shide, D.J., 1999. Differences between alcohol-preferring and alcohol-nonpreferring rats in ethanol generalization. Pharmacol. Biochem. Behav. 64, 415–419.

Mierzejewski, P., Rogowski, A., Stefanski, R., Goldberg, S.R., Kostowski, W., Bienkowski, P., 2003. Ethanol-reinforced behaviour predicts acquisition but not extinction of cocaine self-administration in the rat. Alcohol Alcohol. 38, 543–549.

Miller, N.S., Gold, M.S., 1998. Comorbid cigarette and alcohol addiction: epidemiology and treatment. J. Addict. Dis. 17, 55–66.

Mitchell, S.H., de Wit, H., Zacny, J.P., 1995. Effects of varying ethanol dose on cigarette consumption in healthy normal volunteers. Behav. Pharmacol. 6, 359–365.

Nagata, K., Aistrup, G.L., Huang, C.-S., Marszalec, W., Song, J.-H., Yeh, J.Z., Narahashi, T., 1996. Potent modulation of neuronal nicotinic acetylcholine receptor-channel by ethanol. Neurosci. Lett. 217, 189–193.

Narahashi, T., Aistrup, G.L., Marszalec, W., Nagata, K., 1999. Neuronal nicotinic acetylcholine receptors: a new target site of ethanol. Neurochem. Int. 35, 131–141.

Popke, E.J., Allen, S.R., Paule, M.G., 2000. Effects of acute ethanol on indices of cognitive-behavioral performance in rats. Alcohol 20, 187–192.

Romano, C., Goldstein, A., Jewell, N.P., 1981. Characterization of the receptor mediating the nicotine discriminative stimulus. Psychopharmacology 74, 310–315.

Serrano, L., Capshaw, B., Quattrocchi, F., 1988. A comparison of a microdiffusion method for ethanol and the Abbott TDx ethanol assay. J. Anal. Toxicol. 12, 348–349.

Shoaib, M., Stolerman, I.P., 1996. Brain sites mediating the discriminative stimulus effects of nicotine in rats. Behav. Brain Res. 78, 183–188.

Signs, S.A., Schechter, M.D., 1986. Nicotine-induced potentiation of the ethanol discrimination. Pharmacol. Biochem. Behav. 24, 769–771.

Slama, G., Bruzzo, F., Dupeyron, J.P., Lassechere, M., Dauchy, F., 1989. Keton bodies do not give falsely positive alcohol tests. Diabet. Med. 6, 142–143.

Stolerman, I.P., Jarvis, M.J., 1995. The scientific case that nicotine is addictive. Psychopharmacology 117, 2–10.

Tizabi, Y., Copeland, R.L., Louis, V.A., Taylor, R.E., 2002. Effects of combined systemic alcohol and central nicotine administration into ventral tegmental area on dopamine release in the nucleus accumbens. Alcohol., Clin. Exp. Res. 26, 394–399.

Uzbay, I.T., Usanmaz, S.E., Tapanyigit, E.E., Aynacioglu, S., Akarsu, E.S., 1998. Dopaminergic and serotonergic alterations in the rat brain during ethanol withdrawal: association with behavioral signs. Drug Alcohol Depend. 53, 39–47.

Wu, G., Tonner, P.H., Miller, K.W., 1994. Ethanol stabilizes the open channel state of the *Torpedo* nicotinic acetylcholine receptor. Mol. Pharmacol. 45, 102–108.

Yu, D., Zhang, L., Eisele, J.L., Bertrand, D., Changeux, J.P., Weight, F.F., 1996. Ethanol inhibition of nicotinic acetylcholine type $\alpha 7$ receptors involves the amino-terminal domain of the receptor. Mol. Pharmacol. 50, 1010–1016.

Zacny, J.P., Mitchell, S.H., Cramblett, M.J., de Wit, H., 1996. The effects of ethanol on the relative preference for cigarettes. Behav. Pharmacol. 7, 75–81.