Available online at www.sciencedirect.com
SCIENCE DIRECT
European Journal of Pharmacology 540 (2006) 96–106
www.elsevier.com/locate/ejphar

Evaluation of the role of nicotinic acetylcholine receptor subtypes and cannabinoid system in the discriminative stimulus effects of nicotine in rats

Magdalena Zaniewska [a], Andrew C. McCreary [b], Edmund Przegaliński [a], Małgorzata Filip [a,*]

[a] *Institute of Pharmacology, Polish Academy of Sciences, 31-343, Kraków, 12 Smętna, Poland*
[b] *Solvay Pharmaceutical Research, Weesp, The Netherlands*

Received 21 February 2006; received in revised form 12 April 2006; accepted 18 April 2006
Available online 3 May 2006

Abstract

Male Wistar rats were trained to discriminate (−)-nicotine (0.4 mg/kg) from saline under a two-lever, fixed-ratio 10 schedule of water reinforcement. During test sessions the following drugs were coadministered with saline (substitution studies) or nicotine (0.025–0.4 mg/kg; combination studies): the $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtype antagonist dihydro-β-erythroidine (DHβE), the non-selective nicotinic acetylcholine receptor subtype antagonist mecamylamine, the α_7 nicotinic acetylcholine receptor subtype antagonist methyllycaconitine (MLA), the $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtype agonist 5-iodo-3-(2(*S*)-azetidinylmethoxy)pyridine (5-IA), the cannabinoid CB_1 receptor antagonist/partial agonist rimonabant, the cannabinoid CB_2 receptor antagonist *N*-[(1*S*)-endo-1,3,3-trimethylbicyclo-[2.2.1]heptan-2-yl]5-(4-chloro-3-methyl-phenyl)-1-(4-methybenzyl)pyrazole-3-carboxamide (SR 144528), the cannabinoid $CB_{1/2}$ receptor agonists (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)cyclohexanol (CP 55,940) or *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl) methyl]-pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)-methanone mesylate (WIN 55,212-2), the endogenous cannabinoid agonist and non-competitive α_7 nicotinic acetylcholine receptor subtype antagonist anandamide, the anandamide uptake and fatty acid amide hydrolase inhibitor *N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (AM-404), the fatty acid amide hydrolase inhibitor cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB 597), AM-404+anandamide or URB 597+anandamide. 5-IA (0.01 mg/kg) fully substituted for nicotine, while other drugs were inactive. In combination studies, DHβE and mecamylamine dose-dependently attenuated the discriminative stimulus effects of nicotine and the full substitution of 5-IA, while MLA, rimonabant, SR 144528, CP 55,940, WIN 55,212-2, and URB 597 did not alter the nicotine cue. Pretreatment with AM-404+anandamide or URB 597+anandamide weakly enhanced nicotine-lever responding. Our pharmacological analyses demonstrates that the expression of nicotine discrimination is under the control of nicotinic acetylcholine receptor subtypes composed of $\alpha_4\beta_2$ (but not of α_7) subunits. Furthermore, we excluded the involvement of either cannabinoid CB_1 and CB_2 receptors or increases in the endocannabinoid tone in the nicotine discrimination.

Keywords: Cannabinoid system; Drug discrimination; Nicotine; Nicotinic acetylcholine receptor; Rat

1. Introduction

Nicotine is one of the main psychoactive constituents of tobacco smoke and studies with nicotine have demonstrated positive subjective effects in humans (Goldberg and Henningfield, 1988). In animal studies, exposure to nicotine alters locomotor activity and induces behavioral sensitization (Miller et al., 2001), conditioned place preference (Shoaib et al., 1994), intravenous self-administration (Corrigall and Coen, 1989) or can serve as a discriminative stimulus (Rosecrans and Villanueva, 1991).

It has been demonstrated that nicotine acetylcholine receptors identified in mammalian brain form pentameric, heteromeric or homomeric ion-channels that are composed of different (α_2–α_7, α_9–α_{10}, and β_2–β_4) transmembrane subunits (Millar, 2003). All these receptor subtypes are potential targets for pharmacologically administered nicotine, however, the most widely distributed in the brain are heteromeric $\alpha_4\beta_2$ or homomeric α_7 pentamers and it is thought that they mediate behavioral effects of nicotine (Gotti et al., 2006; Picciotto et al., 2000).

* Corresponding author. Tel.: +48 12 6623293; fax: +48 12 6374500.
E-mail address: filip@if-pan.krakow.pl (M. Filip).

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.04.034

It was established that the nicotine-mediated discriminative cue is primarily mediated through receptors located in the central nervous system since a peripherally acting nicotinic acetylcholine receptor agonist did not substitute for nicotine (Desai et al., 1999) and nicotine given into the brain substituted for the systemic drug injection (Ando et al., 1993; Miyata et al., 2002; Shoaib and Stolerman, 1996). Pharmacological blockade with the non-selective nicotinic acetylcholine receptor antagonist mecamylamine (Brioni et al., 1994; James et al., 1994; Stolerman et al., 1984; Zakharova et al., 2005) or with a blocker selective at $\alpha_4\beta_2$ receptor subtype (e.g. Shoaib et al., 2000; Stolerman et al., 1997) attenuated the nicotine-stimulus properties, while selective ligands of α_7 receptor subtype were inactive in this respect (Gommans et al., 2000; van Haaren et al., 1999). Conversely, nicotine analogues (Brioni et al., 1994; Pratt et al., 1983; Stolerman et al., 1984) or metabolites (Desai et al., 1999; Goldberg et al., 1989; Takada et al., 1989) fully or partially mimicked nicotine in substitution studies. Partly supporting pharmacological analyses, genetic studies showed a role of β_2 subunit in the nicotine discrimination since mutant mice failed to discriminate nicotine from its vehicle (Shoaib et al., 2002), while the α_7 subunit does not contribute to the discriminative stimulus of nicotine (Stolerman et al., 2004).

Recently, the existence of a specific functional interaction between nicotine and the cannabinoid system, composed of the endogenous substances that interact with at least two cannabinoid receptor subtypes, CB_1 and CB_2, has been demonstrated (for reviews see: Cohen et al., 2002; Mackie, 2006). Several studies point to a key role of cannabinoid CB_1 receptors in this interaction, since the cannabinoid CB_1 receptor antagonist rimonabant inhibited nicotine self-administration (Cohen et al., 2002), nicotine-evoked conditioned place preference (Le Foll and Goldberg, 2004) and the reinstatement of nicotine-seeking behavior induced by the drug-associated stimulus (Cohen et al., 2005b; De Vries et al., 2005), while genetic invalidation of cannabinoid CB_1 receptor resulted in alteration of the motivational effects of nicotine (Castane et al., 2002; Cossu et al., 2001). Contrary, activation of cannabinoid CB_1 receptors is critical for the short-term expression of nicotine-induced conditioned place preference (Forget et al., 2005).

The present study focused on the effects of different cannabinoid ligands in the discriminative stimulus effects of nicotine in rats. Since the cannabinoid receptors can be engaged directly by antagonists and agonists, or indirectly by manipulating endocannabinoid metabolism, we used the cannabinoid CB_1 receptor antagonist/partial agonist rimonabant (Rinaldi-Carmona et al., 1995), the cannabinoid CB_2 receptor antagonist *N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]5-(4-chloro-3-methylphenyl)-1-(4-methybenzyl)pyrazole-3-carboxamide (SR 144528; Rinaldi-Carmona et al., 1998), the non-selective cannabinoid $CB_{1/2}$ receptor agonists (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol (CP 55,940; Thomas et al., 1998) and *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl) methanone mesylate (WIN 55,212-2; Herzberg et al., 1997), the endocannabinoid anandamide (Devane et al., 1992), the anandamide uptake and fatty acid amide hydrolase inhibitor *N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (AM-404; Beltramo et al., 1997; Lambert and Fowler, 2005) and the selective fatty acid amide hydrolase inhibitor cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB 597; Kathuria et al., 2003) and studied their ability to substitute for or alter the nicotine discrimination.

To link the nicotine interaction to a specific receptor subtype we used 5-iodo-3(2(*S*)-azetidinylmethoxy)pyridine (5-IA; Koren et al., 1998), a novel and preferential $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtype agonist (K_i=10–225 pM; Mogg et al., 2004; Mukhin et al., 2000) with 25000-fold higher selectivity for $\alpha_4\beta_2$ subunit vs. α_7 subunit and more than a 5000-fold selectivity for $\alpha_4\beta_2$ subunit vs. $\alpha_3\beta_4$ subunits (Mogg et al., 2004; Mukhin et al., 2000). Finally, in order to extend previous observations that nicotinic acetylcholine receptors modulate the discriminative stimulus effects of nicotine (e.g. Brioni et al., 1994; Gommans et al., 2000; James et al., 1994; Shoaib et al., 2000; Stolerman et al., 1984, 1997; van Haaren et al., 1999) we used the non-selective nicotinic acetylcholine receptor subtype antagonist mecamylamine (Varanda et al., 1985), the selective $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtype antagonist dihydro-β-erythroidine (DHβE; Reavill et al., 1988) and the α_7 nicotinic acetylcholine receptor subtype antagonist methyllycaconitine (MLA; Turek et al., 1995).

All doses and pretreatment times of the drugs used are in agreement with the previously published studies. Thus, 5-IA at a dose range of 0.001–0.01 mg/kg induced $\alpha_4\beta_2$-related hypothermic responses in rodents (Kleefstra et al., 2004) and is devoid of convulsive activity (A.C. McCreary, unpublished observations). MLA in the range 2.5–10 mg/kg was previously reported to selectively block the α_7 nicotinic acetylcholine receptor subtype-mediated responses (Turek et al., 1995). DHβE (1–4 mg/kg) and mecamylamine (0.25–1 mg/kg) were effective in blocking the discriminative stimulus effects of nicotine (Shoaib et al., 2000; Zakharova et al., 2005). AM-404 at a dose (10 mg/kg) was shown to prevent the apomorphine-induced stereotypic yawning (Beltramo et al., 2000), or induce anxiolytic-like activity in conditioned fear (Chhatwal et al., 2005), or enhance the hypothermic responses of the exogenously applied anandamide (Fegley et al., 2004), all these effects being blocked by a cannabinoid CB_1 receptor antagonist. Anandamide was tested at 2.5–5 mg/kg doses that were found to induce a cannabimimetic-related hypothermia in rats (Fegley et al., 2004; Kathuria et al., 2003), but not potent decreases in locomotor activation or catalepsy (Costa et al., 1999). URB 597 (0.3 mg/kg) was shown to elevate brain levels of the endogenous cannabinoid anandamide (Kathuria et al., 2003) and when used in combination with anandamide potentiated its several cannabimimetic responses (Fegley et al., 2005; Kathuria et al., 2003). CP 55,940 and WIN 55,212-2 share behavioral properties with naturally occurring cannabinoids such as Δ^9-tetrahydrocannabinoid (Compton et al., 1992; Little et al., 1988); CP 55,940 at a dose of 0.1 mg/kg served as a discriminative stimulus in rats and was blocked by a cannabinoid CB_1 receptor antagonist (Frankowska et al., 2005; Wiley et al., 1995), or fully substituted for Δ^9-tetrahydrocannabinoid (Gold

et al., 1992), while its higher doses > 0.2 mg/kg induced catalepsy in rats (Anderson et al., 1996). WIN 55,212-2 in a dose-range 3–10 mg/kg completely substituted for CP 55,940 (Wiley et al., 1995) and for Δ^9-tetrahydrocannabinoid discrimination (Compton et al., 1992), and in a dose of 6 mg/kg it was shown to attenuate the cocaine-evoked hyperactivity in rats without affecting the basal locomotor activity (Przegaliński et al., 2005). Rimonabant was used in doses ≥ 5 mg/kg, since 5 mg/kg effectively counteracted a cannabinoid CB_1 receptor agonist-mediated suppression of horizontal activity (Järbe et al., 2002; 2004) or CP 55,940-induced discrimination (Frankowska et al., 2005), and blocked the extinction of fear in rats (Chhatwal et al., 2005). SR 144528 was tested at the same dose (3 mg/kg) as used by Järbe et al. (2004, 2006) in drug discrimination model in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (derived from licensed animal breeder T. Górzkowska, Warszawa, Poland) weighing 280–320 g at the beginning of the experiment were used. The animals were housed in groups of 2/cage (38 × 25 × 15 cm) in a colony room maintained at 21 ± 1 °C and 40–50% humidity on a 12-h light–dark cycle (the lights on at 0600 h). Rodent chow was available ad libitum; the amount of water that an animal received was restricted to that given during daily training sessions (5–6 ml/rat/session), after test sessions (15 min) and on weekends (36 h). All the experiments were conducted during the light phase of the light–dark cycle (between 0800 and 1400 h), in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the approval of the Bioethics Commission (compliant with Polish Law (21 August 1997)).

2.2. Drugs

The following drugs were used (supplier): anandamide (Tocris, UK) and *N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (AM-404; Tocris, UK), (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxypropyl)-cyclohexanol (CP 55,940; Tocris, UK), *R*(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-(1-naphthalenyl)methanone mesylate (WIN 55,212-2; Sigma Chemicals, St. Louis, USA), dihydro-β-erythroidine (DHβE; Solvay Pharmaceutical Research, Weesp, Netherlands), 5-iodo-3-(2(*S*)-azetidinylmethoxy)pyridine hydrochloride (5-IA; Solvay Pharmaceutical Research, Weesp, Netherlands), mecamylamine hydrochloride (Solvay Pharmaceutical Research, Weesp, Netherlands), methyllycaconitine citrate (MLA; Solvay Pharmaceutical Research, Weesp, Netherlands), (−)-nicotine bitartrate (Solvay Pharmaceutical Research, Weesp, Netherlands), rimonabant (Solvay Pharmaceutical Research, Weesp, Netherlands), *N*-[(1*S*)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]5-(4-chloro-3-methylphenyl)-1-(4-methybenzyl)pyrazole-3-carboxamide (SR 144528; Solvay Pharmaceutical Research, Weesp, Netherlands) and cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB 597; Solvay Pharmaceutical Research, Weesp, Netherlands). AM-404 and rimonabant were given at 60 min, CP 55,940 at 45 min, URB 597 at 40 min, mecamylamine, MLA, SR 144528 and WIN 55,212-2 at 30 min, DHβE at 20 min, anandamide at 16 min, and 5-IA and nicotine at 60, 30 or 15 min before tests. AM-404 and anandamide were dissolved in Tocrisolve 100 (Tocris, UK) and diluted to required dose in distilled water. CP 55,940 and WIN 55,212-2 were dissolved in 15% and 19% 2-hydroxypropyl-β-cyclodextrin (MP Biomedicals, France), respectively. DHβE, 5-IA, mecamylamine, MLA and nicotine were dissolved in 0.9% saline. Rimonabant was dissolved in a mixture of 1:1:10 ethanol, cremophor and 19% 2-hydroxypropyl-β-cyclodextrin. SR 144528 was dissolved in a mixture of 1:1:18 ethanol, cremophor and saline. URB 597 was dissolved in 2–3 drops of ethanol and diluted as required in 1% Tween 80 (Sigma-Aldrich, USA). In case of 5-IA, MLA, nicotine, rimonabant, URB 597 and WIN 55,212-2 pH was neutralized to 7.0 using 20% NaOH. All the drugs were administered in a volume of 1 ml/kg intraperitoneally (i.p.) except for DHβE, 5-IA, mecamylamine and nicotine that were administered subcutaneously (s.c.). The drug doses refer to the weight of the respective salts, while doses of nicotine are expressed as that of a free base.

2.3. Apparatus

Commercially available, two-lever operant chambers (MedAssociates, St. Albans, USA) were used. Each chamber was equipped with a water-filled dispenser mounted equidistantly between two response levers on the wall and housed in a light- and soundproof cubicle (MedAssociates). Illumination came from a 28-V house light; ventilation and masking noise were supplied with a blower. A computer with MedState software was used to program and record all the experimental events.

2.4. Discrimination procedure

Standard two-lever, water-reinforced drug discrimination procedures were utilized (Filip et al., 2006). A group of rats ($n = 16$) was trained to discriminate (−)-nicotine (0.4 mg/kg, s.c.) from saline (1 ml/kg). The training dose of (−)-nicotine (0.4 mg/kg) and the route of administration was chosen on the basis of the literature (Schechter, 1995). Daily sessions lasted 15 min and were conducted on Mondays through Fridays. In the initial "errorless training" phase, only the stimulus-appropriate (drug or saline) lever was present. Training began under a fixed ratio 1 (FR 1) schedule of water reinforcement and the FR requirement was incremented until all the animals were responding reliably under the FR 10 schedule for each experimental condition. For half of the rats, right-lever responses were reinforced after (−)-nicotine (0.4 mg/kg) administration, whereas left-lever responses were reinforced after saline administration; the conditions were reversed for the remaining rats. During the phase of training, (−)-nicotine and saline were administered irregularly with the restriction that neither condition prevailed for more than three consecutive sessions. After the responding was

stabilized, discrimination training was initiated and both levers were presented simultaneously during 15-min sessions. The rats were trained to respond on the stimulus-appropriate (correct) lever in order to obtain water reinforcement, and there were no programmed consequences of responding on the incorrect lever. That phase of training continued until the performance of all the trained rats met the criterion (defined as mean accuracies of at least 80% correct for 10 consecutive sessions).

When the rats achieved the criterion of accuracy, test sessions were initiated and conducted once or twice per week with training sessions intervening during the remaining days. The rats were required to maintain accuracies of at least 80% correct for the saline and nicotine maintenance sessions which immediately preceded a test. During test sessions, the animals were placed in the chambers and, upon completion of 10 responses on either lever, a single reinforcer was delivered and the house lights were turned off. The test sessions were terminated after 15 min if the rats did not complete 10 responses on either lever. Then the rats were removed from the chamber, returned to the colony and allowed free access to water for 15 min beginning 15–30 min after the end of each test.

Several pharmacological manipulations were performed during the test sessions. In substitution (generalization) tests, the rats were examined for lever responses after various doses of the training drug, AM-404+anandamide, anandamide, CP 55,940, DHβE, 5-IA, mecamylamine, MLA, rimonabant, SR 144528, URB 597, URB 597+anandamide, WIN 55,212-2. In combination (antagonism or potentiation) tests, doses of AM-404+anandamide, CP 55,940, DHβE, mecamylamine, MLA, rimonabant, SR 144528, URB 597, URB 597+anandamide or WIN 55,212-2 were administered prior to the different doses of nicotine (0.025–0.4 mg/kg).

2.5. Data analysis

During training sessions, accuracy was defined as the percentage of correct responses to total correct responses before the delivery of the first reinforcer; during test sessions, performance was expressed as the percentage of nicotine-appropriate responses to total responses before the delivery of the first reinforcer. Response rates (responses per second) were evaluated during training and test sessions (as a measure of behavioral disruption). For training sessions, the response rate was calculated as the total number of responses emitted on either lever before completion of the first FR 10 on the stimulus appropriate-lever divided by the number of seconds taken to complete that FR 10. During test sessions, the response rate was calculated as the total number of responses before the completion of 10 responses on either lever divided by the number of seconds necessary to complete the FR 10.

A drug was considered to substitute for nicotine if it evoked at least 80% till 100% (maximum) drug-appropriate lever responding; while an antagonism was claimed to occur when minimum (no more than 20%) drug-lever responding occurred after pretreatment with a dose of potential antagonist given in combination with nicotine (0.4 mg/kg). Student's *t*-test for repeated measures was used to compare the percentage of drug-lever responding and response rate during test sessions with the corresponding values for either the previous saline or nicotine session (substitution and antagonism tests). A two-way analysis of variance (ANOVA) for repeated measures was used to find out whether the percentage of nicotine-lever responding and response rates observed for several doses of nicotine differed in the presence vs. absence of a fixed dose of the test drug (combination tests); post hoc comparisons for each dose of nicotine with and without the test drug were made using Dunnett's test. Log-probit analyses were used to estimate the dose of nicotine predicted to elicit 50% drug-appropriate responding (ED_{50}) and 95% confidence limits (CL) for each treatment combination as well as the dose of nicotinic acetylcholine receptor antagonists predicted to reduce nicotine (0.4 mg/kg)-lever responding by 50% (ID_{50}) and 95% CL (Tallarida and Murray, 1987). All comparisons were made with an experimentwise type I error rate (α) set at 0.05.

3. Results

3.1. Nicotine (0.4 mg/kg)-saline discrimination

Acquisition of the nicotine (0.4 mg/kg) vs. saline discrimination was met in an average of 30 sessions (range: 25–36). Administration of nicotine (0.025–0.4 mg/kg) to rats produced a dose-dependent increase in the nicotine-appropriate responding, whereas saline administration resulted in <10% nicotine-lever responding (Fig. 1, left). The drug-lever responding after 0.025 and 0.05 mg/kg of nicotine was significantly different from the preceding nicotine training session ($P<0.05$). The

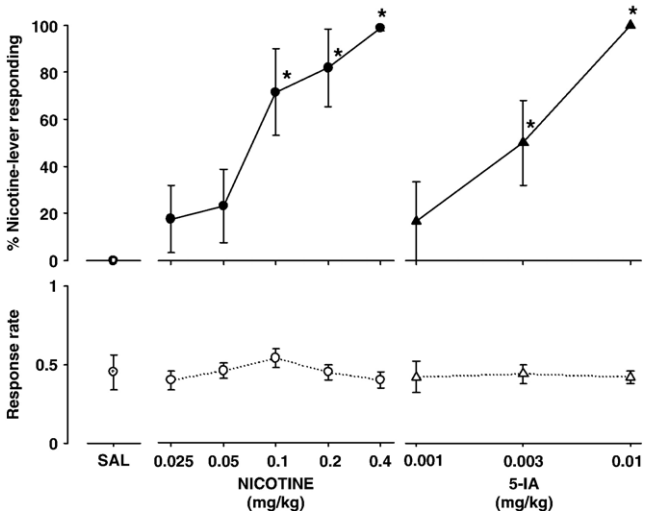

Fig. 1. Substitution studies with nicotine and 5-IA in rats trained to discriminate nicotine (0.4 mg/kg) from saline. Symbols show the mean percentage of nicotine-lever responses (±S.E.M.; closed symbols) and the mean number of responses/s (±S.E.M.; open symbols). Performance is shown after injection of saline (SAL; 1 mg/kg; circle dotted), nicotine (circles) or 5-IA (triangles). All the data points represent the means of data from 7–8/8 rats [*n*/*N*, number of rats (*n*) completing the FR 10 on either lever out of the number of rats tested (*N*)]. Asterisks denote performance during test sessions which was significantly different from that observed after the preceding maintenance training saline session ($P<0.05$).

dose of nicotine predicted to elicit 50% nicotine-lever responding (ED_{50}) in rats was 0.07 mg/kg (Table 2). Response rates for all the test doses of nicotine and saline did not differ from those obtained during the immediately preceding saline or nicotine maintenance session (Fig. 1, left).

The nicotine-lever responding after a training dose of a drug was time-dependent and 30 and 60 min after injection of nicotine the drug-lever responding significantly lowered in comparison with the preceding nicotine training session ($P<0.05$; Fig. 2, left). The response rates were unaltered except for the pre-treatment time 30 min ($P<0.05$; Fig. 2).

3.2. Substitution studies

The $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtype agonist 5-IA (0.001–0.01 mg/kg) caused substitution for nicotine (a maximum of 100% drug-lever responding at a dose of 0.01 mg/kg; Fig. 1, right). The drug-lever responding at 0.001 and 0.003 mg/kg, but not 0.01 mg/kg, of 5-IA was significantly different from the preceding nicotine maintenance session ($P<0.05$). The dose of 5-IA predicted to elicit 50% nicotine-lever responding in rats (ED_{50} 95% CL) was 0.0025 (0.0011–0.0058) mg/kg. Any dose of 5-IA did not affect the response rates (Fig. 1, right). The substitution of 5-IA (0.01 mg/kg) for nicotine was time-dependent and 60 min after drug injection nicotine-lever responding lowered in comparison with the preceding nicotine training session. The response rates did not change (Fig. 2, right).

AM-404+anandamide, CP 55,940, WIN 55,212-2, DHβE, mecamylamine, MLA, SR 144528, URB 597, or URB 597 +anandamide, at the doses tested evoked no more than 20% drug-lever responding when given alone; rimonabant (10 mg/kg) alone elicited ca. 35% nicotine-lever responding (Table 1).

Table 1
Substitution studies with AM-404, AM-404+anandamide, anandamide, CP 55,940, DHβE, mecamylamine, MLA, rimonabant, SR 144528, URB 597, URB 597+anandamide or WIN 55,212-2 in rats trained to discriminate nicotine (0.4 mg/kg) from saline

Drug (mg/kg)	% Nicotine-lever responding	Response rate responses/s
AM-404 (10)+ anandamide (2.5)	0±0	0.32±0.06
Anandamide (2.5)	0±0	0.47±0.11
CP 55,940 (0.1)	3±1.9	0.34±0.07
DHβE (4)	0±0	0.42±0.09
Mecamylamine (1)	20±20	0.45±0.13
MLA (5)	20.7±15.1	0.33±0.07
Rimonabant (10)	34.9±20.7	0.42±0.05
SR 144528 (3)	18.2±16.4	0.54±0.08
URB 597 (0.3)	0±0	0.45±0.11
URB 597 (0.3)+ anandamide (2.5)	6.6±4.3	0.23±0.04
WIN 55,212-2 (6)	0±0	0.45±0.07

Performance during test sessions were not significantly different from the preceding saline maintenance session.

None of the drugs used altered the response rates of animals as compared to previous saline and nicotine sessions (Table 1).

3.3. Combination studies

Pretreatment with DHβE (1–4 mg/kg) caused a dose-dependent attenuation in the nicotine (0.4 mg/kg)-lever responding (Fig. 3, left). A statistically significant reduction of nicotine discrimination was seen for a combination of DHβE (2 or 4 mg/kg) and nicotine ($P<0.05$), and these results did not differ from the previous saline maintenance session. The dose of DHβE predicted to reduce nicotine (0.4 mg/kg)-lever responding by

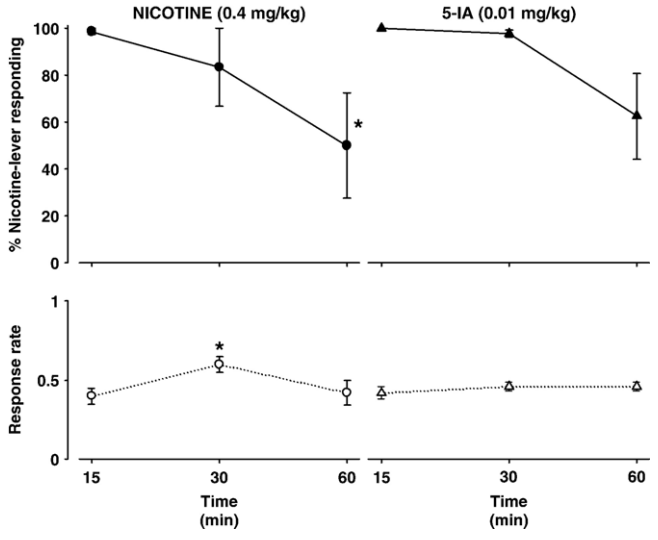

Fig. 2. Time-course effect of nicotine or 5-IA on the nicotine-lever responding. Performance is shown after injection of nicotine (0.4 mg/kg; circles) or 5-IA (0.01 mg/kg; triangles). All the data points represent the means of data from 8/8 rats. Asterisks denote performance during test sessions which was significantly different from that observed after the preceding maintenance training drug session ($P<0.05$). For more details see Fig. 1.

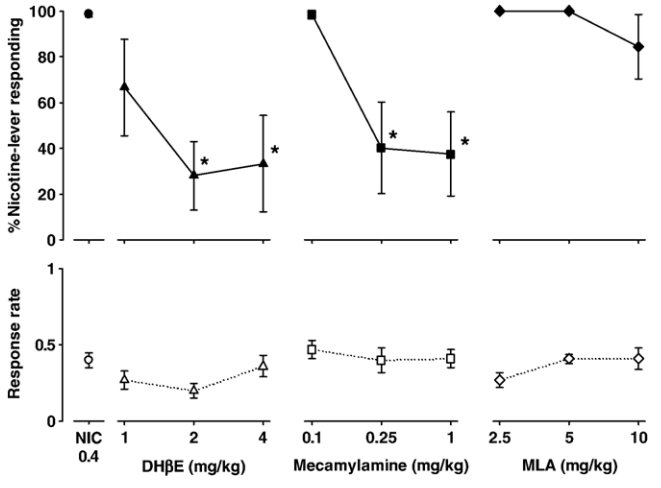

Fig. 3. Combination studies with DHβE, mecamylamine or MLA in rats trained to discriminate nicotine (0.4 mg/kg) from saline. Performance is shown after injection of nicotine alone (NIC; 0.4 mg/kg; circles), or preceded by injection of DHβE (1–4 mg/kg; triangles), or mecamylamine (0.1–1 mg/kg; squares), or MLA (2.5–10 mg/kg; diamonds). All the data points represent the means of data from 7–8/7–8 rats. Asterisks denote performance during test sessions which was significantly different from that observed after the preceding maintenance training drug session ($P<0.05$). For more details see Fig. 1.

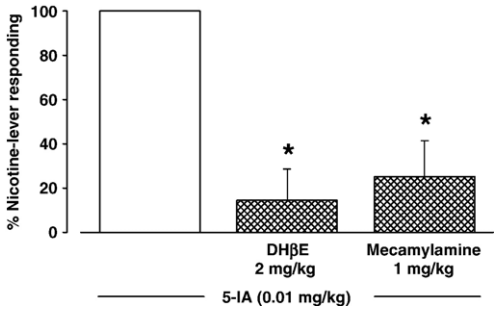

Fig. 4. Combination studies with DHβE or mecamylamine injected before 5-IA in rats trained to discriminate nicotine (0.4 mg/kg) from saline. Performance is shown after injection of 5-IA alone (5-IA; 0.01 mg/kg; white bar) or preceded by injection of DHβE (2 mg/kg; hatching bar) or mecamylamine (1 mg/kg; hatching bar). All the data points represent the means of data from 8/8 rats. Asterisks denote performance during test sessions which was significantly different ($P<0.05$) from that observed after 5-IA.

50% (ID_{50} 95% CL) was 1.46 (0.54–3.95) mg/kg. Response rates were not affected by any treatment combinations (Fig. 3, left).

Pretreatment with mecamylamine (0.1–1 mg/kg), in combination with nicotine (0.4 mg/kg), produced a dose-dependent attenuation in nicotine-lever responding (Fig. 3, center); a significant decrease in nicotine discriminability was observed at doses of mecamylamine 0.25 and 1 mg/kg ($P<0.05$) and these results did not differ from the previous saline maintenance session. The dose of mecamylamine predicted to reduce nicotine (0.4 mg/kg)-lever responding by 50% (ID_{50} 95%CL) was 0.37 (0.1–1.21) mg/kg. Following administration of all doses of mecamylamine together with nicotine, no alteration in the response rates was observed (Fig. 3, center).

Table 2
ED_{50} values for nicotine obtained in rats trained to discriminate nicotine (0.4 mg/kg) from vehicle and pretreated with cannabinoid drugs

Pretreatment and dose (mg/kg)	ED_{50} (95% CL) (mg/kg)
Vehicle	0.07 (0.04–0.12)
AM-404 (10)+anandamide (2.5)	0.04 (0.02–0.07)
CP 55,940 (0.1)	0.06 (0.03–0.12)
Rimonabant (5)	0.04 (0.02–0.09)
Rimonabant (10)	0.07 (0.02–0.27)
SR 144528 (3)	0.07 (0.03–0.15)
URB 597 (0.3)	0.09 (0.05–0.14)
URB 597 (0.3)+anandamide (2.5)	0.04 (0.02–0.08)
WIN 55,212-2 (6)	0.08 (0.05–0.12)

Combined administration of the fixed dose of DHβE (2 mg/kg) or mecamylamine (1 mg/kg) together with 5-IA (0.01 mg/kg), that alone evoked a 100% substitution for nicotine, produced a statistically significant reduction in drug-lever responding ($P<0.05$; Fig. 4) that was not different from the previous saline maintenance session. The response rates after combination of DHβE or mecamylamine with 5-IA were unaltered (5-IA: 0.42±0.04 responses/s; DHβE+5-IA: 0.4±0.08 responses/s; mecamylamine+5-IA: 0.48±0.1 responses/s).

Administration of MLA (2.5–10 mg/kg) together with nicotine (0.4 mg/kg) altered neither the nicotine-lever responding nor response rates (Fig. 3, right).

Pretreatment with AM-404 (10 mg/kg)+anandamide (2.5 mg/kg) with nicotine (0.025–0.1 mg/kg) neither altered the nicotine discrimination ($F(1,12)=1.36$) (Fig. 5, left) nor changed the ED_{50} value for nicotine (Table 2). The response rates were not altered after a combination of AM-404+ananadamide and nicotine ($F(1,12)=1.52$) (Fig. 5, left).

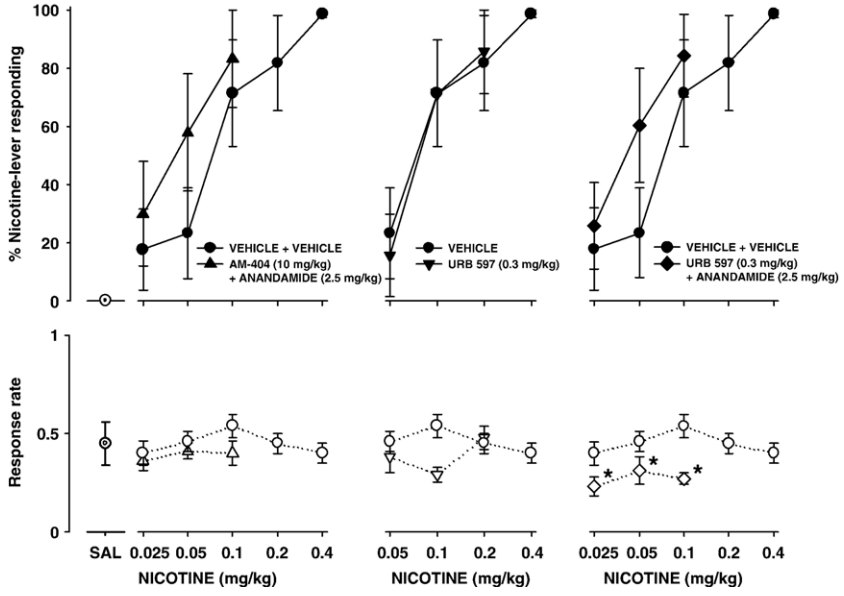

Fig. 5. Combination studies with AM-404+anandamide, URB 597 or URB 597+anandamide in rats trained to discriminate nicotine (0.4 mg/kg) from saline. Performance is shown after injection of vehicle (circles), or AM-404 (10 mg/kg)+anandamide (2.5 mg/kg; triangles), or URB 597 (0.3 mg/kg; reversed triangles), or URB 597 (0.3 mg/kg)+anandamide (2.5 mg/kg; diamonds) in combination with nicotine (0.025–0.2 mg/kg). For comparison, performance after saline (SAL) alone is shown (circle dotted). The nicotine dose–response curve has been replotted from Fig. 1. All the data points represent the means of data from 7/7 rats. Asterisks denote performance during test sessions which was significantly different from that observed after the appropriate dose of nicotine ($P<0.05$). For more details see Fig. 1.

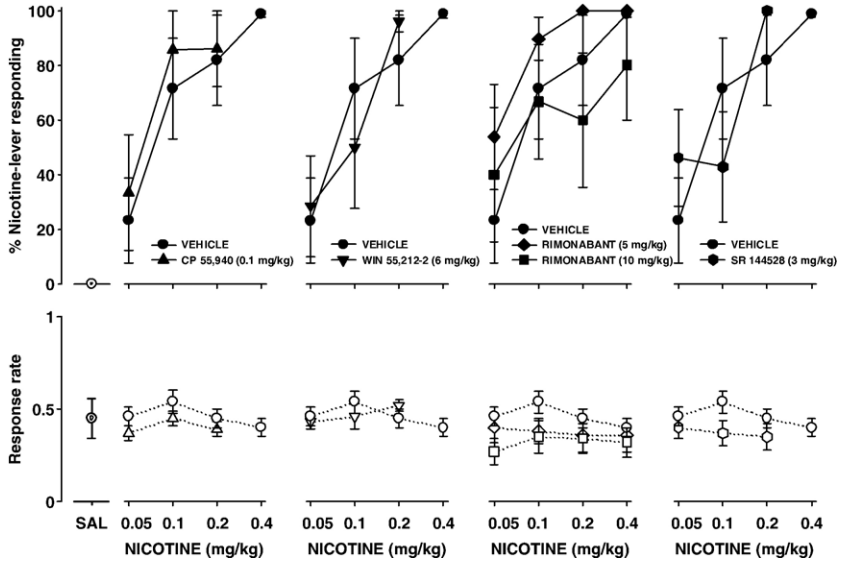

Fig. 6. Combination studies with CP 55,940, or WIN 55,212-2, or rimonabant, or SR 144528 in rats trained to discriminate nicotine (0.4 mg/kg) from saline. Performance is shown after injection of vehicle (circles), or CP 55,940 (0.1 mg/kg; triangles), or WIN 55,212-2 (6 mg/kg; reversed triangles), or rimonabant (5 mg/kg; diamonds), or rimonabant (10 mg/kg; squares), or SR 144528 (3 mg/kg; hexagons) in combination with nicotine (0.05–0.4 mg/kg). For comparison, performance after saline (SAL) alone is shown (circle dotted). All the data points represent the means of data from 7/7 rats. For more details see Fig. 1.

The fixed dose of URB 597 (0.3 mg/kg) in combination with various doses of nicotine (0.05–0.2 mg/kg) did not alter the nicotine-lever responding ($F(1,12)=0.006$) (Fig. 5, center) or the ED_{50} value for nicotine (Table 2). The combination of URB 597 and nicotine (0.1 mg/kg) did not change significantly the response rates ($F(1,12)=2.78$) (Fig. 5, center).

Pretreatment with URB 597 (0.3 mg/kg) +anandamide (2.5 mg/kg) did not alter the nicotine (0.025–0.1 mg/kg) discriminative stimulus effects ($F(1,12)=1.93$) (Fig. 5, right) or the ED_{50} value for nicotine (Table 2); however, following a combination of URB597+anandamide+nicotine (0.05 mg/kg) a weak enhancement in nicotine-lever responding was observed. The combinations of URB 597+anandamide with nicotine significantly reduced the response rates ($F(1,12)=9.72$, $P=0.008$); the decreases were seen for low doses of nicotine, 0.025–0.1 mg/kg (Fig. 5, right).

Administration of the fixed dose of CP 55,940, 0.1 mg/kg (Fig. 6, left), or WIN 55,212-2, 6 mg/kg (Fig. 6, center left) with various doses of nicotine (0.05–0.2 mg/kg) did not alter the discriminability of nicotine ($F(1,12)=0.33$ or $F(1,12)=0.001$, respectively), nor did it significantly change the ED_{50} value for nicotine (Table 2). The response rates were not altered after a combination of CP 55,940 (Fig. 6, left) or WIN 55,212-2 (Fig. 6, center left) and nicotine ($F(1,12)=2.11$ or $F(1,12)=0.06$, respectively).

Pretreatment with rimonabant (5–10 mg/kg) before nicotine (0.05–0.4 mg/kg) did not alter the nicotine-lever responding ($F(2,18)=1.55$) (Fig. 6, center right), nor did it change the ED_{50} value for nicotine (Table 2). The response rates were not significantly altered after rimonabant (5–10 mg/kg) and nicotine ($F(2,18)=3.17$) (Fig. 6, center right).

Pretreatment with SR 144528 (3 mg/kg) before nicotine (0.05–0.2 mg/kg) did not change the nicotine-lever responding ($F(1,12)=0.07$) (Fig. 6, right) or the ED_{50} value for nicotine (Table 2). The response rates were not significantly altered after a combination of SR 144528 and nicotine (($F(1,12)=3.03$) (Fig. 6, right).

4. Discussion

In this study nicotine served as a discriminative stimulus in rats, as has been demonstrated previously (e.g. Pratt et al., 1983; Schechter, 1995). The calculated ED_{50} value for nicotine ($ED_{50}=0.07$ mg/kg) was in a similar dose-range to that determined by Schechter (1995) when using the same training dose and route of administration ($ED_{50}=0.11$ mg/kg). Our present pharmacological analyses using several nicotinic acetylcholine receptor and cannabinoid ligands provide new insights into the mechanism of the discriminative stimulus effects of nicotine. To summarize, we extended and confirmed previous observations that nicotinic acetylcholine receptors composed of $\alpha_4\beta_2$ (but not α_7) subtypes are important for the expression of the subjective effects of nicotine. Additionally, we showed that neither cannabinoid CB_1 and CB_2 receptors, nor their pharmacological stimulation, nor increases in the endocannabinoid tone exhibited significant interactions with the nicotine discrimination.

Stimulation of any one or a combination of nicotinic acetylcholine receptor subtypes could elicit the discriminative stimulus effects of nicotine. Accordingly with some previous findings (e.g. Brioni et al., 1994; Stolerman et al., 1984, 1997; Zakharova et al., 2005), the present study shows that blockade with mecamylamine (a non-selective nicotinic acetylcholine receptor antagonist; Khan et al., 1994) or DHβE (a selective antagonist of $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtypes; Reavill et al., 1988) attenuated the stimulus effects of nicotine. To further support the role of $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtype in the nicotine subjective effects we employed 5-IA, a high affinity and potent agonist (Koren et al., 1998;

Mukhin et al., 2000) and used at doses that induced $\alpha_4\beta_2$-related hypothermic responses (Kleefstra et al., 2004). In substitution studies 5-IA engendered a dose-dependent and complete generalization for nicotine with a high potency (ED_{50}=0.0025 mg/kg). Furthermore, the 5-IA-evoked substitution for nicotine was potently blocked by both mecamylamine and the selective $\alpha_4\beta_2$ nicotinic acetylcholine receptor subtype antagonist DHβE. It should be also mentioned that in an in vitro functional assay 5-IA activated $\alpha_6\beta_2$ nicotinic acetylcholine receptors (Mogg et al., 2004), the response abolished by MLA, a selective α_7 nicotinic acetylcholine receptor antagonist (Turek et al., 1995) which in vitro possessed a relatively potent antagonistic activity toward $\alpha_6\beta_2$ subunits (Mogg et al., 2002). However, in the present behavioral study the role of $\alpha_6\beta_2$ nicotinic acetylcholine receptors in the effects of 5-IA should be excluded since this agonist completely generalized for nicotine, the nicotine cue was not altered by MLA (see below), and nicotine alone has at least 13-fold higher affinity for $\alpha_4\beta_2$ than for $\alpha_6\beta_2$ subunits (Vailati et al., 2003; Wei et al., 2003). Apart from nicotine discrimination, other behavioral responses of this drug are correlated with the selective stimulation of heterodimeric nicotinic acetylcholine receptors composed of $\alpha_4\beta_2$ subunits. In fact, chronic administration of both nicotine and the $\alpha_4\beta_2$ subtype selective agonist SIB 1765F resulted in an enhanced locomotor response to acute challenge with either nicotine or SIB 1765F (cross-sensitization) (Grottick et al., 2000). On the other hand, selective blockade of $\alpha_4\beta_2$ nicotinic acetylcholine receptors by DHβE (Grottick et al., 2000; Watkins et al., 1999) or erysodine (Mansbach et al., 2000), or genetic depletion of β_2 subunit (Picciotto et al., 1998) reduced nicotine self-administration. Moreover, in mice with a deletion of the β_2 subunit reinstatement of operant responding was seen after intrategmental lentiviral administration β_2 subunit mRNA (Maskos et al., 2005). Recent studies also demonstrated an alleviation of nicotine sensitization following DHβE (Grottick et al., 2000) or point mutation of the α_4 subunit (Tapper et al., 2004).

The investigations with MLA, a selective antagonist at α_7 nicotinic acetylcholine receptor subtype, pointed no role of this subtype for the expression of the nicotine discrimination (Grottick et al., 2000; present study). Such observations are further supported by nicotine's low affinity to α_7 receptor subtype (Briggs et al., 1995) and the lack of generalization for nicotine following a selective α_7 nicotinic acetylcholine receptor subtype agonist (van Haaren et al., 1999). Furthermore, additional findings show a negligible role of α_7 subunit in nicotine-induced hyperlocomotion and reward in the rat (Grottick et al., 2000; Liu et al., 2003). Moreover, pharmacological stimulation of α_7 homomeric receptors failed to alter both the initiation and expression of nicotine sensitization (Grottick et al., 2000).

Several recent findings indicate bi-directional interactions between nicotine and cannabinoid system. Thus, nicotinic acetylcholine receptors colocalize with cannabinoid CB_1 receptors within certain brain regions (Herkenham et al., 1990; Picciotto et al., 2000; Tsou et al., 1998), nicotine evoked an increase of endogenous cannabinoids (Cohen et al., 2005a; Gonzalez et al., 2002) and the activation of cannabinoid CB_1 receptors augmented the rewarding effects of nicotine (Cohen et al., 2005a). Moreover, rimonabant (0.3–3 mg/kg) blocked the rewarding effects of nicotine (Cohen et al., 2002; Rinaldi-Carmona et al., 1995) and nicotine-seeking behavior induced by conditioned cues in rats (Cohen et al., 2005b; De Vries et al., 2005). Recently rimonabant was postulated as a potential smoking cessation therapy (Cohen et al., 2005a) and clinical studies with this drug are encouraging (Ducobu, 2005).

Taken together the above findings prompted us to determine potential interaction between cannabinoid drugs and the nicotine discrimination. Contrary to the nicotine rewarding effects in a self-administration model (see above), we observed no role of cannabinoid CB_1 receptors in the discriminative stimulus effects of nicotine since rimonabant (5 and 10 mg/kg) failed to substitute for nicotine or antagonize the nicotine-lever responding. It should be added that, when administered alone at a dose of 10 mg/kg, rimonabant produced a weak (ca. 35% drug-lever responding) substitution for nicotine, the effect probably reflecting its potential "partial agonist-like" profile in vivo (cf. De Vry and Jentzsch, 2004). Our results with rimonabant showing that it neither substituted for nicotine nor antagonized the nicotine cue support some previous studies (Cohen et al., 2002; Le Foll and Goldberg, 2004), although those authors examined much lower doses of rimonabant (0.3–3 mg/kg) in the nicotine discrimination.

Recently, using immunohistochemical analyses brain localization of cannabinoid CB_2 receptors has been reported in rat (Gong et al., 2006). Therefore, to study the involvement of cannabinoid CB_2 receptors in nicotine discrimination we used the cannabinoid CB_2 receptor antagonist SR 144528 (Pertwee, 2005). SR 144528 (3 mg/kg) failed to antagonize the nicotine cue, suggesting that cannabinoid CB_2 receptors do not modulate the discriminative stimulus properties of nicotine. Furthermore, we found that direct stimulation of cannabinoid receptors by synthetic or endogenous agonists did not mimic nicotine or alter the dose–response curve for nicotine. In fact, neither the CP 55,940 nor WIN 55,212, the non-selective cannabinoid receptor agonists showing high intrinsic activities at cannabinoid $CB_{1/2}$ receptors (Pertwee, 2005) substituted for nicotine when given alone or altered the nicotine dose–response curve. Similarly no effects in substitution and combinations experiments toward the nicotine discrimination were demonstrated after administration of URB 597, an irreversible inhibitor of fatty acid amide hydrolase (for review: Pertwee, 2005) that acts rapidly (<15 min) and persistently (>6 h), URB 597 (0.3 mg/kg) was shown to elevate brain levels of the endogenous cannabinoid anandamide (Kathuria et al., 2003).

When used in combination with anandamide URB 597 potentiated the cannabinomimetic-related responses (Fegley et al., 2005; Kathuria et al., 2003). In the present study we demonstrated that the combination of URB 597 or AM-404 (anandamide uptake and fatty acid amide hydrolase inhibitors; Lambert and Fowler, 2005) with exogenously applied anandamide did not evoke substitution for nicotine, extending our findings with direct cannabinoid $CB_{1/2}$ receptor agonists about no role of cannabinoid receptors in the discriminative stimulus effects of nicotine. However, the above treatment combinations produced a weak (nonsignificant) enhancement of nicotine-

lever responding. An upward shift was seen especially following URB 597+anandamide or AM-404+anandamide with a low dose of nicotine (0.05 mg/kg). The use of higher dose of anandamide (5 mg/kg) together with URB 597 (0.3 mg/kg) resulted in a potent reduction of response rates (when combined with 0.1 mg/kg of nicotine) or even total disruption of animals' behavior (when combined with 0.2 mg/kg of nicotine) (Zaniewska et al., unpublished observations). The present pharmacological manipulations lead to the conclusion that endocannabinoid tone only weakly, if at all, modulates the nicotine discrimination. It should be added that anandamide, apart from being a cannabinoid agonist, interacts with α_7 nicotinic acetylcholine receptors (Oz et al., 2005) and is regarded as an non-competitive antagonist of this subtype (Oz et al., 2003). However, the interaction between anandamide and nicotine in our drug discrimination model via α_7 nicotinic acetylcholine receptors seems to be excluded due to the fact that the selective α_7 nicotinic acetylcholine receptor subtype antagonist MLA did not alter the discriminative stimulus effects of nicotine (Grottick et al., 2000; present study).

In conclusion, our detailed pharmacological analyses demonstrate that the expression of nicotine discrimination is under control of nicotinic acetylcholine receptor subtypes composed of $\alpha_4\beta_2$ (but not of α_7) subunits. Furthermore, we excluded the involvement of either cannabinoid CB_1 or CB_2 receptors, their pharmacological stimulation or increases in the endocannabinoid tone in the nicotine discrimination.

Acknowledgements

This research was supported by the statutory funds from the Department of Pharmacology, Institute of Pharmacology Polish Academy of Sciences (Kraków). Conflict of interest. None.

References

Anderson, J.J., Kask, A.M., Chase, T.N., 1996. Effects of cannabinoid receptor stimulation and blockade on catalepsy produced by dopamine receptor antagonists. Eur. J. Pharmacol. 295, 163–168.

Ando, K., Miyata, H., Hironaka, N., Tsuda, T., Yanagita, T., 1993. The discriminative effects of nicotine and their central sites in rats. Yakubutsu, Seishin, Kodo 13, 129–136.

Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., Piomelli, D., 1997. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. Science 277, 1094–1097.

Beltramo, M., de Fonseca, F.R., Navarro, M., Calignano, A., Gorriti, M.A., Grammatikopoulos, G., Sadile, A.G., Giuffrida, A., Piomelli, D., 2000. Reversal of dopamine D(2) receptor responses by an anandamide transport inhibitor. J. Neurosci. 20, 3401–3407.

Briggs, C.A., McKenna, D.G., Piattoni-Kaplan, M., 1995. Human alpha 7 nicotinic acetylcholine receptor responses to novel ligands. Neuropharmacology 34, 583–590.

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

Castane, A., Valjent, E., Ledent, C., Parmentier, M., Maldonado, R., Valverde, O., 2002. Lack of CB1 cannabinoid receptors modifies nicotine behavioural responses, but not nicotine abstinence. Neuropharmacology 43, 857–867.

Chhatwal, J.P., Davis, M., Maguschak, K.A., Ressler, K.J., 2005. Enhancing cannabinoid neurotransmission augments the extinction of conditioned fear. Neuropsychopharmacology 30, 516–524.

Cohen, C., Perrault, G., Voltz, C., Steinberg, R., Soubrie, P., 2002. SR141716, a central cannabinoid (CB(1)) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. Behav. Pharmacol. 13, 451–463.

Cohen, C., Kodas, E., Griebel, G., 2005a. CB1 receptor antagonists for the treatment of nicotine addiction. Pharmacol. Biochem. Behav. 81, 387–395.

Cohen, C., Perrault, G., Griebel, G., Soubrie, P., 2005b. Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). Neuropsychopharmacology 30, 145–155.

Compton, D.R., Gold, L.H., Ward, S.J., Balster, R.L., Martin, B.R., 1992. Aminoalkylindole analogs: cannabimimetic activity of a class of compounds structurally distinct from delta 9-tetrahydrocannabinol. J. Pharmacol. Exp. Ther. 263, 1118–1126.

Corrigall, W.A., Coen, K.M., 1989. Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology 99, 473–478.

Cossu, G., Ledent, C., Fattore, L., Imperato, A., Bohme, G.A., Parmentier, M., Fratta, W., 2001. Cannabinoid CB1 receptor knockout mice fail to self-administer morphine but not other drugs of abuse. Behav. Brain Res. 118, 61–65.

Costa, B., Vailati, S., Colleoni, M., 1999. SR 141716A, a cannabinoid receptor antagonist, reverses the behavioural effects of anandamide-treated rats. Behav. Pharmacol. 10, 327–331.

De Vries, T.J., de Vries, W., Janssen, M.C., Schoffelmeer, A.N., 2005. Suppression of conditioned nicotine and sucrose seeking by the cannabinoid-1 receptor antagonist SR141716A. Behav. Brain Res. 161, 164–168.

De Vry, J., Jentzsch, K.R., 2004. Partial agonist-like profile of the cannabinoid receptor antagonist SR141716A in a food-reinforced operant paradigm. Behav. Pharmacol. 15, 13–20.

Desai, R.I., Barber, D.J., Terry, P., 1999. Asymmetric generalization between the discriminative stimulus effects of nicotine and cocaine. Behav. Pharmacol. 10, 647–656.

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Ducobu, J., 2005. The endocannabinoid system and the regulation of the metabolism. J. Pharm. Belg. 60, 84–88.

Fegley, D., Kathuria, S., Mercier, R., Li, C., Goutopoulos, A., Makriyannis, A., Piomelli, D., 2004. Anandamide transport is independent of fatty-acid amide hydrolase activity and is blocked by the hydrolysis-resistant inhibitor AM1172. Proc. Natl. Acad. Sci. U. S. A. 101, 8756–8761.

Fegley, D., Gaetani, S., Duranti, A., Tontini, A., Mor, M., Tarzia, G., Piomelli, D., 2005. Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3′-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleoylethanolamide deactivation. J. Pharmacol. Exp. Ther. 313, 352–358.

Filip, M., Bubar, M.J., Cunningham, K.A., 2006. Contribution of serotonin (5-HT) 5-HT2 receptor subtypes to the discriminative stimulus effects of cocaine in rats. Psychopharmacology 183, 482–489.

Forget, B., Hamon, M., Thiebot, M.H., 2005. Cannabinoid CB1 receptors are involved in motivational effects of nicotine in rats. Psychopharmacology 181, 722–734.

Frankowska, M., Filip, M., Przegaliński, E., 2005. Effects of direct and indirect dopamine receptor agonists on the cannabinoid CB1 receptor agonist CP 55,940-induced discrimination. Acta Neurobiol. Exp. 65, 311.

Gold, L.H., Balster, R.L., Barrett, R.L., Britt, D.T., Martin, B.R., 1992. A comparison of the discriminative stimulus properties of delta 9-tetrahydrocannabinol and CP 55,940 in rats and rhesus monkeys. J. Pharmacol. Exp. Ther. 262, 479–486.

Goldberg, S.R., Henningfield, J.E., 1988. Reinforcing effects of nicotine in humans and experimental animals responding under intermittent schedules of i.v. drug injection. Pharmacol. Biochem. Behav. 30, 227–234.

Goldberg, S.R., Risner, M.E., Stolerman, I.P., Reavill, C., Garcha, H.S., 1989. Nicotine and some related compounds: effects on schedule-controlled behaviour and discriminative properties in rats. Psychopharmacology 97, 295–302.

Gommans, J., Stolerman, I.P., Shoaib, M., 2000. Antagonism of the discriminative and aversive stimulus properties of nicotine in C57BL/6J mice. Neuropharmacology 39, 2840–2847.

Gong, J.P., Onaivi, E.S., Ishiguro, H., Liu, Q.R., Tagliaferro, P.A., Brusco, A., Uhl, G.R., 2006. Cannabinoid CB2 receptors: Immunohistochemical localization in rat brain. Brain Res. 107, 10–23.

Gonzalez, S., Cascio, M.G., Fernandez-Ruiz, J., Fezza, F., Di Marzo, V., Ramos, J.A., 2002. Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. Brain Res. 954, 73–81.

Gotti, C., Riganti, L., Vailati, S., Clementi, F., 2006. Brain neuronal nicotinic receptors as new targets for drug discovery. Curr. Pharm. Des. 12, 407–428.

Grottick, A.J., Trube, G., Corrigall, W.A., Huwyler, J., Malherbe, P., Wyler, R., Higgins, G.A., 2000. Evidence that nicotinic alpha(7) receptors are not involved in the hyperlocomotor and rewarding effects of nicotine. J. Pharmacol. Exp. Ther. 294, 1112–1119.

Herkenham, M., Lynn, A.B., Little, M.D., Johnson, M.R., Melvin, L.S., de Costa, B.R., Rice, K.C., 1990. Cannabinoid receptor localization in brain. Proc. Natl. Acad. Sci. U. S. A. 87, 1932–1936.

Herzberg, U., Eliav, E., Bennett, G.J., Kopin, I.J., 1997. The analgesic effects of *R*(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. Neurosci. Lett. 221, 157–160.

James, J.R., Villanueva, H.F., Johnson, J.H., Arezo, S., Rosecrans, J.A., 1994. Evidence that nicotine can acutely desensitize central nicotinic acetylcholinergic receptors. Psychopharmacology 114, 456–462.

Järbe, T.U., Andrzejewski, M.E., DiPatrizio, N.V., 2002. Interactions between the CB1 receptor agonist Delta 9-THC and the CB1 receptor antagonist SR-141716 in rats: open-field revisited. Pharmacol. Biochem. Behav. 73, 911–919.

Järbe, T.U., Harris, M.Y., Li, C., Liu, Q., Makriyannis, A., 2004. Discriminative stimulus effects in rats of SR-141716 (rimonabant), a cannabinoid CB1 receptor antagonist. Psychopharmacology 177, 35–45.

Järbe, T.U., Liu, Q., Makriyannis, A., 2006. Antagonism of discriminative stimulus effects of delta(9)-THC and (*R*)-methanandamide in rats. Psychopharmacology 184, 36–45.

Kathuria, S., Gaetani, S., Fegley, D., Valino, F., Duranti, A., Tontini, A., Mor, M., Tarzia, G., La Rana, G., Calignano, A., Giustino, A., Tattoli, M., Palmery, M., Cuomo, V., Piomelli, D., 2003. Modulation of anxiety through blockade of anandamide hydrolysis. Nat. Med. 9, 76–81.

Khan, I.M., Yaksh, T.L., Taylor, P., 1994. Ligand specificity of nicotinic acetylcholine receptors in rat spinal cord: studies with nicotine and cytisine. J. Pharmacol. Exp. Ther. 270, 159–166.

Kleefstra, A., Glennon, J., McCreary, A.C., 2004. Nicotinic receptor-induced hypothermia: A pharmacological evaluation. Eur. Neuropsychopharmacol. 14 (Suppl. 3), S357.

Koren, A.O., Horti, A.G., Mukhin, A.G., Gundisch, D., Kimes, A.S., Dannals, R.F., London, E.D., 1998. 2-, 5-, and 6-Halo-3-(2(*S*)-azetidinylmethoxy) pyridines: synthesis, affinity for nicotinic acetylcholine receptors, and molecular modeling. J. Med. Chem. 41, 3690–3698.

Lambert, D.M., Fowler, C.J., 2005. The endocannabinoid system: drug targets, lead compounds, and potential therapeutic applications. J. Med. Chem. 48, 5059–5087.

Le Foll, B., Goldberg, S.R., 2004. Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. NeuroReport 15, 2139–2143.

Little, P.J., Compton, D.R., Johnson, M.R., Melvin, L.S., Martin, B.R., 1988. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. J. Pharmacol. Exp. Ther. 247, 1046–1051.

Liu, X., Koren, A.O., Yee, S.K., Pechnick, R.N., Poland, R.E., London, E.D., 2003. Self-administration of 5-iodo-A-85380, a beta2-selective nicotinic receptor ligand, by operantly trained rats. NeuroReport 14, 1503–1505.

Mackie, K., 2006. Cannabinoid receptors as therapeutic targets. Annu. Rev. Pharmacol. Toxicol. 46, 101–122.

Mansbach, R.S., Chambers, L.K., Rovetti, C.C., 2000. Effects of the competitive nicotinic antagonist erysodine on behavior occasioned or maintained by nicotine: comparison with mecamylamine. Psychopharmacology 148, 234–242.

Maskos, U., Molles, B.E., Pons, S., Besson, M., Guiard, B.P., Guilloux, J.P., Evrard, A., Cazala, P., Cormier, A., Mameli-Engvall, M., Dufour, N., Cloez-Tayarani, I., Bemelmans, A.P., Mallet, J., Gardier, A.M., David, V., Faure, P., Granon, S., Changeux, J.P., 2005. Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. Nature 436, 103–107.

Millar, N.S., 2003. Assembly and subunit diversity of nicotinic acetylcholine receptors. Biochem. Soc. Trans. 31, 869–874.

Miller, D.K., Wilkins, L.H., Bardo, M.T., Crooks, P.A., Dwoskin, L.P., 2001. Once weekly administration of nicotine produces long-lasting locomotor sensitization in rats via a nicotinic receptor-mediated mechanism. Psychopharmacology 156, 469–476.

Miyata, H., Ando, K., Yanagita, T., 2002. Brain regions mediating the discriminative stimulus effects of nicotine in rats. Ann. N.Y. Acad. Sci. 965, 354–363.

Mogg, A.J., Whiteaker, P., McIntosh, J.M., Marks, M., Collins, A.C., Wonnacott, S., 2002. Methyllycaconitine is a potent antagonist of alpha-conotoxin-MII-sensitive presynaptic nicotinic acetylcholine receptors in rat striatum. J. Pharmacol. Exp. Ther. 302, 197–204.

Mogg, A.J., Jones, F.A., Pullar, I.A., Sharples, C.G., Wonnacott, S., 2004. Functional responses and subunit composition of presynaptic nicotinic receptor subtypes explored using the novel agonist 5-iodo-A-85380. Neuropharmacology 47, 848–859.

Mukhin, A.G., Gundisch, D., Horti, A.G., Koren, A.O., Tamagnan, G., Kimes, A.S., Chambers, J., Vaupel, D.B., King, S.L., Picciotto, M.R., Innis, R.B., London, E.D., 2000. 5-Iodo-A-85380, an alpha4beta2 subtype-selective ligand for nicotinic acetylcholine receptors. Mol. Pharmacol. 57, 642–649.

Oz, M., Ravindran, A., Diaz-Ruiz, O., Zhang, L., Morales, M., 2003. The endogenous cannabinoid anandamide inhibits alpha7 nicotinic acetylcholine receptor-mediated responses in Xenopus oocytes. J. Pharmacol. Exp. Ther. 306, 1003–1010.

Oz, M., Jackson, S.N., Woods, A.S., Morales, M., Zhang, L., 2005. Additive effects of endogenous cannabinoid anandamide and ethanol on alpha7-nicotinic acetylcholine receptor-mediated responses in Xenopus Oocytes. J. Pharmacol. Exp. Ther. 313, 1272–1280.

Pertwee, R.G., 2005. The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J. 7, E625–E654.

Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L.M., Pich, E.M., Fuxe, K., Changeux, J.P., 1998. Acetylcholine receptors containing the beta2 subunit are involved in the reinforcing properties of nicotine. Nature 391, 173–177.

Picciotto, M.R., Caldarone, B.J., King, S.L., Zachariou, V., 2000. Nicotinic receptors in the brain: links between molecular biology and behavior. Neuropsychopharmacology 22, 451–465.

Pratt, J.A., Stolerman, I.P., Garcha, H.S., Giardini, V., Feyerabend, C., 1983. Discriminative stimulus properties of nicotine: further evidence for mediation at a cholinergic receptor. Psychopharmacology 81, 54–60.

Przegaliński, E., Göthert, M., Frankowska, M., Filip, M., 2005. WIN 55,212-2-induced reduction of cocaine hyperlocomotion: possible inhibition of 5-HT (3) receptor function. Eur. J. Pharmacol. 517, 68–73.

Reavill, C., Jenner, P., Kumar, R., Stolerman, I.P., 1988. High affinity binding of [3H] (−)-nicotine to rat brain membranes and its inhibition by analogues of nicotine. Neuropharmacology 27, 235–241.

Rinaldi-Carmona, M., Barth, F., Heaulme, M., Alonso, R., Shire, D., Congy, C., Soubrie, P., Breliere, J.C., Le Fur, G., 1995. Biochemical and pharmacological characterisation of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. Life Sci. 56, 1941–1947.

Rinaldi-Carmona, M., Barth, F., Millan, J., Derocq, J.M., Casellas, P., Congy, C., Oustric, D., Sarran, M., Bouaboula, M., Calandra, B., Portier, M., Shire, D., Breliere, J.C., Le Fur, G.L., 1998. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. J. Pharmacol. Exp. Ther. 284, 644–650.

Rosecrans, J.A., Villanueva, H.F., 1991. Discriminative stimulus properties of nicotine: mechanisms of transduction. NIDA Res. Monogr. 116, 101–116.

Schechter, M.D., 1995. Scopolamine–physostigmine combination does not substitute for nicotine. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 19, 499–508.

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

Shoaib, M., Stolerman, I.P., Kumar, R.C., 1994. Nicotine-induced place preferences following prior nicotine exposure in rats. Psychopharmacology 113, 445–452.

Shoaib, M., Zubaran, C., Stolerman, I.P., 2000. Antagonism of stimulus properties of nicotine by dihydro-beta-erythroidine (DHbetaE) in rats. Psychopharmacology 149, 140–146.

Shoaib, M., Gommans, J., Morley, A., Stolerman, I.P., Grailhe, R., Changeux, J.P., 2002. The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. Neuropharmacology 42, 530–539.

Stolerman, I.P., Garcha, H.S., Pratt, J.A., Kumar, R., 1984. Role of training dose in discrimination of nicotine and related compounds by rats. Psychopharmacology 84, 413–419.

Stolerman, I.P., Chandler, C.J., Garcha, H.S., Newton, J.M., 1997. Selective antagonism of behavioural effects of nicotine by dihydro-beta-erythroidine in rats. Psychopharmacology 129, 390–397.

Stolerman, I.P., Chamberlain, S., Bizarro, L., Fernandes, C., Schalkwyk, L., 2004. The role of nicotinic receptor alpha 7 subunits in nicotine discrimination. Neuropharmacology 46, 363–371.

Takada, K., Swedberg, M.D., Goldberg, S.R., Katz, J.L., 1989. Discriminative stimulus effects of intravenous L-nicotine and nicotine analogs or metabolites in squirrel monkeys. Psychopharmacology 99, 208–212.

Tallarida, R.J., Murray, R.B., 1987. Manual of Pharmacologic Calculations with Computer Programs, 2nd ed. Springer-Verlag, New York. pp. 16–18 and 26–31.

Tapper, A.R., McKinney, S.L., Nashmi, R., Schwarz, J., Deshpande, P., Labarca, C., Whiteaker, P., Marks, M.J., Collins, A.C., Lester, H.A., 2004. Nicotine activation of alpha4* receptors: sufficient for reward, tolerance, and sensitization. Science 306, 1029–1032.

Thomas, B.F., Gilliam, A.F., Burch, D.F., Roche, M.J., Seltzman, H.H., 1998. Comparative receptor binding analyses of cannabinoid agonists and antagonists. J. Pharmacol. Exp. Ther. 285, 285–292.

Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K., Walker, J.M., 1998. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83, 393–411.

Turek, J.W., Kang, C.H., Campbell, J.E., Arneric, S.P., Sullivan, J.P., 1995. A sensitive technique for the detection of the alpha 7 neuronal nicotinic acetylcholine receptor antagonist, methyllycaconitine, in rat plasma and brain. J. Neurosci. Methods 61, 113–118.

Vailati, S., Moretti, M., Longhi, R., Rovati, G.E., Clementi, F., Gotti, C., 2003. Developmental expression of heteromeric nicotinic receptor subtypes in chick retina. Mol. Pharmacol. 63, 1329–1337.

van Haaren, F., Anderson, K.G., Haworth, S.C., Kem, W.R., 1999. GTS-21, a mixed nicotinic receptor agonist/antagonist, does not affect the nicotine cue. Pharmacol. Biochem. Behav. 64, 439–444.

Varanda, W.A., Aracava, Y., Sherby, S.M., VanMeter, W.G., Eldefrawi, M.E., Albuquerque, E.X., 1985. The acetylcholine receptor of the neuromuscular junction recognizes mecamylamine as a noncompetitive antagonist. Mol. Pharmacol. 28, 128–137.

Watkins, S.S., Epping-Jordan, M.P., Koob, G.F., Markou, A., 1999. Blockade of nicotine self-administration with nicotinic antagonists in rats. Pharmacol. Biochem. Behav. 62, 743–751.

Wei, Z.L., Xiao, Y., George, C., Kellar, K.J., Kozikowski, A.P., 2003. Functionalization of the alicyclic skeleton of epibatidine: synthesis and nicotinic acetylcholine receptor binding affinities of epibatidine analogues. Org. Biomol. Chem. 1, 3878–3881.

Wiley, J.L., Barrett, R.L., Lowe, J., Balster, R.L., Martin, B.R., 1995. Discriminative stimulus effects of CP 55,940 and structurally dissimilar cannabinoids in rats. Neuropharmacology 34, 669–676.

Zakharova, E.S., Danysz, W., Bespalov, A.Y., 2005. Drug discrimination analysis of NMDA receptor channel blockers as nicotinic receptor antagonists in rats. Psychopharmacology 179, 128–135.