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Discriminative stimulus properties of the 5-HT_{1A} receptor agonist BAY x 3702 in the rat

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Abstract

The aminomethylchroman derivative BAY x 3702 (*R*-(−)-2-{4-[(chroman-2-ylmethyl)-amino]-butyl}-1,1-dioxo-benzo[d]isothiazolone HCl) has recently been characterized as a relatively selective, high affinity 5-HT_{1A} receptor agonist with neuroprotective, anxiolytic- and antidepressant-like effects in animal models. It was the aim of the present study to further confirm its receptor binding profile in an in vivo assay. Rats were trained to discriminate BAY x 3702 (0.1 mg/kg, i.p.) from vehicle in a standard two-lever fixed ratio 10 food-reinforced procedure. All rats learned the discrimination, the median number of sessions to reach criterion being 38 (range: 22–58 sessions). Generalization tests with BAY x 3702 showed dose-dependent and complete generalization after different routes of administration; the ED_{50} values being: 0.030, 0.007 and 0.36 mg/kg, after i.p., i.v. and p.o. administration, respectively. Assessment of the duration of action after administration of 0.1 mg/kg BAY x 3702, i.p., resulted in a $T_{1/2}$ of 65 min. Dose-dependent and complete generalization was also obtained with the 5-HT_{1A} receptor agonists 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)-tetralin, ED_{50} in mg/kg, i.p.: 0.086), flesinoxan (0.30), SR 57746A ((4-(3-trifluoromethylphenyl)-*N*-(2-(naphth-2-yl)ethyl)-1,2,3,6-tetrahydropyridine HCl, 1.0), the (+)-enantiomer of BAY x 3702 (1.3) and ipsapirone (1.8); the ED_{50} values being closely correlated with their respective affinities for the 5-HT_{1A} receptor. Pretreatment with the selective 5-HT_{1A} receptor antagonist WAY-100635 ((*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*(2-pyridinyl) cyclohexane carboxamide trihydrochloride) dose-dependently and completely blocked the discriminative effects of 0.1 mg/kg BAY x 3702 (ID_{50}: 0.013 mg/kg, i.p.). WAY-100635, prazosin, idazoxan, raclopride, paroxetine, (−)-BAY k 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methyl-phenyl)-pyridine-5-carboxylate), ethanol, and the putative neuroprotectants MK-801 ((+)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5,10-imine), CNS 1102 (*N*-(1-naphthyl)-*N′*-(3-ethylphenyl)-*N′*-methyl-guanidine), CGS 19755 (*cis*-4-(phosphonomethyl) piperidine-2-carboxylic acid) and nimodipine did not induce more than 20% generalization. It is concluded that the BAY x 3702 cue is mediated by its agonistic activity at 5-HT_{1A} receptors.

Keywords: Animal model; BAY x 3702; Discriminative stimulus; Drug discrimination; (Rat); 5-HT_{1A} receptor

1. Introduction

The novel aminomethylchroman derivative BAY x 3702 (*R*-(−)-2-{4-[(chroman-2-ylmethyl)-amino]-butyl}-1,1-dioxo-benzo[d]isothiazolone HCl) was recently found to possess neuroprotective, as well as anxiolytic- and antidepressant-like effects in animal models (De Vry et al., 1997). Receptor binding studies indicated that BAY x 3702 binds with high affinity to 5-HT_{1A} receptors (K_i values ranging from 0.19 to 0.58 nM, as measured in brain tissue of different species; De Vry et al., 1998). The binding profile of the compound was considered to be relatively selective, as at least one order of magnitude separated its binding to 5-HT_{1A} receptors from its binding to other receptors or binding sites. In biochemical, electrophysiological and behavioral assays, BAY x 3702 was characterized as a 5-HT_{1A} receptor full agonist (De Vry et al., 1998). The effects of BAY x 3702 in these models, as well as its neuroprotective, and anxiolytic- and antidepressant-like effects in preclinical models could be attenuated by cotreatment with the high affinity, selective 5-HT_{1A} receptor antagonist WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*(2-pyridinyl) cyclohexane carboxamide trihydrochloride; Forster et al., 1995), suggesting that the pharmacological effects of the compound are at least partly

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mediated by activation of 5-HT_{1A} receptors (De Vry et al., 1997, 1998; Horvàth et al., 1997).

Although these findings indicated that BAY x 3702 behaves as a 5-HT_{1A} receptor agonist at the low to moderate doses used in these models, it can not be excluded yet that interactions with other receptors/binding sites may be involved in the pharmacological effects of the compound. Indeed, as it was reported that BAY x 3702 bound also with relatively high to moderate affinity to α_1- and α_2-adrenoceptors (K_i 6 and 7 nM, respectively), 5-HT_7 and 5-HT_{1D} receptors (7 and 36 nM), dopamine D_2 and D_4 receptors (48 and 91 nM), sigma sites (176 nM) and 5-HT_{2C} receptors (310 nM) (De Vry et al., 1998), it can be suspected that interactions with these receptors may contribute to the pharmacological effects of the compound. Functional biochemical studies indicated that BAY x 3702 is an agonist at 5-HT_{1D} receptors, and an antagonist at α_1- and α_2-adrenoceptors and dopamine D_2 receptors with IC_{50} values generally consistent with the reported binding affinities at these receptors (De Vry et al., 1998).

BAY x 3702 was found previously to generalize completely to the 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)-tetralin) cue in rats trained to discriminate the latter compound (0.1 mg/kg, i.p.) from vehicle (De Vry et al., 1998). Although this finding suggested that agonistic properties at 5-HT_{1A} receptors underlie the compound's generalization to the 8-OH-DPAT cue, it was not determined in that study whether the generalization of BAY x 3702 could be antagonized by a selective 5-HT_{1A} receptor antagonist. Moreover, although the 8-OH-DPAT cue is generally considered to be a highly sensitive behavioral assay for 5-HT_{1A} receptor interaction (e.g., Glennon, 1986; Arnt, 1989; De Vry et al., 1991; Sanger and Schoemaker, 1992; Schreiber and De Vry, 1993; Schreiber et al., 1995; Kleven and Koek, 1998), some studies reported generalization with compounds lacking selective affinity for 5-HT_{1A} receptors. Thus, other nonselective serotonergic compounds, such as buspirone, BMY 14802 (4-[4-(5-fluoro-2-pyrimidinyl)-1-piperazinyl]-1-(4-fluorophenyl)butynol), eltoprazine and methysergide, as well as α_2-adrenoceptor antagonists, such as yohimbine, idazoxan and 1-pyrimidinyl piperazine, or β-adrenoceptor antagonists, such as pindolol and metoprolol, have been reported to induce considerable levels of generalization, depending, amongst others, on the training dose of 8-OH-DPAT and species (e.g., Winter, 1988; De Vry et al., 1991; Sanger and Schoemaker, 1992; Rabin and Winter, 1993; Ybema et al., 1993; Sánchez et al., 1996; Wolff and Leander, 1997). In addition, although the 8-OH-DPAT cue was found to be blocked by selective 5-HT_{1A} receptor antagonists, such as WAY-100635 (Sánchez et al., 1996; Wolff and Leander, 1997; Kleven and Koek, 1998), compounds showing no affinity for these receptors, such as mu opioids, have been reported to attenuate the 8-OH-DPAT cue (Morgan and Picker, 1995). Although these findings do not necessarily argue against the concept that the 8-OH-DPAT cue is a highly sensitive in vivo assay for detection of 5-HT_{1A} receptor activity (but see Rabin and Winter, 1993), they should raise caution against premature conclusions about the 5-HT_{1A} selectivity of a compound found to generalize to this cue.

The present study was undertaken to characterize further the discriminative stimulus properties of BAY x 3702. Rats were trained to discriminate a relatively low dose of the compound in a standard two-lever, food-reinforced drug discrimination procedure. The involvement of 5-HT_{1A} receptors in the cue was assessed by means of generalization tests with the selective 5-HT_{1A} receptor ligands 8-OH-DPAT, ipsapirone (Traber et al., 1984), flesinoxan (Schipper et al., 1991), SR 57746A (4-(3-trifluoromethylphenyl)-*N*-(2-(naphth-2-yl)ethyl)-1,2,3,6-tetrahydropyridine HCl; Cervo et al., 1994) and BAY x 3703, the (+)-enantiomer of BAY x 3702, which has a 10-fold lower affinity for 5-HT_{1A} receptors (De Vry et al., 1998). In addition, the role of 5-HT_{1A} receptors was further assessed by antagonism tests with the selective 5-HT_{1A} receptor antagonist WAY-100635. In order to test the possible contribution of α_1- and α_2-adrenoceptors, as well as dopamine D_2 receptors (to which BAY x 3702 has a 20- to 100-fold lower affinity as compared with its affinity to 5-HT_{1A} receptors), additional generalization tests with the α_1-adrenoceptor antagonist prazosin, the α_2-adrenoceptor antagonist idazoxan and the dopamine D_2 receptor antagonist raclopride were performed. Finally, it was investigated whether the discriminative effects of BAY x 3702 could be differentiated from those of other putative neuroprotectants, such as the noncompetitive NMDA receptor antagonists MK-801 (dizocilpine, (+)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5,10-imine; Wong et al., 1986) and CNS 1102 (cerestat, *N*-(1-naphthyl)-*N′*-(3-ethylphenyl)-*N′*-methyl-guanidine; McBurney et al., 1991), the competitive NMDA receptor antagonist CGS 19755 (selfotel, *cis*-4-(phosphonomethyl) piperidine-2-carboxylic acid; Lehmann et al., 1988) and the voltage-dependent calcium channel antagonist nimodipine (Hoffmeister et al., 1982).

2. Materials and methods

2.1. Animals

Sixteen male Wistar rats were purchased from Harlan-Winkelmann (Borchen, Germany). Body weight upon arrival at the laboratory was around 160 g, which gradually increased up to about 450 g during the course of the studies. Rats were individually housed in Makrolon® type 3 cages under a normal 12-h light period (light on at 7:00 a.m.), with restricted access to food (approximately 13 g/day, standard pellets; Ssniff Spezialdiäten, Soest, Germany) and water ad libitum. Room temperature was maintained at 22–23°C.

2.2. Apparatus

Sessions were performed in sound- and light-attenuated standard operant chambers (Coulborn Instruments, PA, USA). The chambers were equipped with two levers equidistant from a food tray between the levers. Food reinforcement (45 mg precision pellets; Bio-Serv, NJ, USA) was delivered by an automated food dispenser located outside of the chamber. Data collection and experimental contingencies were programmed using OPN software on an IBM XT microcomputer interfaced with the operant chamber. Ventilation and masking noise were provided by a fan mounted on the wall of the chamber. A white houselight was switched on during the sessions, which were conducted on 9:00 and 12:00 a.m.

2.3. Drug discrimination procedure

In general, the procedure described by De Beun et al. (1996a,b) was followed. After initial shaping to lever press for food reinforcement, the rats ($n = 16$) were trained to discriminate BAY x 3702 (0.1 mg/kg, i.p., *t*-15 min) from vehicle in a standard two-lever, fixed ratio 10 operant procedure. Daily sessions were conducted which were terminated after either 50 reinforcers or after 10 min, whichever came first. For half of the animals, responses on the left lever were reinforced after BAY x 3702; for the other half, responses on this lever were reinforced after the vehicle. The rats were injected with drug or vehicle according to the following sequence: D-D-V-D-V//V-D-V-V-D//D-V-D-V-V//D-D-V-D-V (D = drug, V = vehicle, // = no sessions during the weekends) with repetition. Discrimination criterion consisted of 10 consecutive sessions in which no more than nine responses occurred on the nonreinforced lever before the first reinforcer was obtained. Test sessions were performed when this number of incorrect responding was not more than four on three consecutive training sessions and when at least 20 reinforcers were obtained per session. During test sessions, responding on the selected lever, i.e., the lever on which 10 responses accumulated first, was reinforced for the remainder of the session. Generalization and antagonism tests were separated by at least three training sessions in which vehicle and drug were correctly discriminated, i.e., less than five incorrect responses prior to the first reinforcer. The animals were tested with different doses of the training compound (0.0125–0.4 mg/kg, i.p.) before being submitted to a time-dependency study. BAY x 3702 was tested after i.p., i.v. and p.o. administration, in order to ascertain that the quality of the stimulus was not essentially different after different routes of administration and in order to demonstrate potency equivalence of the doses. Generalization tests were performed 15 min after application of the test compound (except for the time-dependency study, where 0.1 mg/kg BAY x 3702 was tested 30–120 min after application, and the oral dose-dependency study, where BAY x 3702 was tested 30 min after application). In the antagonism study, pretreatment with WAY-100635 (or vehicle, i.p.) occurred 15 min before treatment with BAY x 3702 (0.1 mg/kg or vehicle, i.p.). In general, each dose of a test compound was tested in six to eight rats, randomly allocated to each test condition.

2.4. Drugs

BAY x 3702 (*R*-(−)-2-{4-[(chroman-2-ylmethyl)-amino]-butyl}-1,1-dioxo-benzo[d]isothiazolone HCl), BAY x 3703 (the (+)-enantiomer of BAY x 3702), 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamino)-tetralin), ipsapirone, flesinoxan, SR 57746A (4-(3-trifluoromethylphenyl)-*N*-(2-(naphth-2-yl)ethyl)-1,2,3,6-tetrahydropyridine HCl), nimodipine, (−)-BAY k 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methyl-phenyl)-pyridine-5-carboxylate), WAY-100635 (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*(2-pyridinyl) cyclohexane carboxamide trihydrochloride), paroxetine, CNS 1102 (cerestat, *N*-(1-naphthyl)-*N′*-(3-ethylphenyl)-*N′*-methylguanidine) and CGS 19755 (selfotel, *cis*-4-(phosphonomethyl) piperidine-2-carboxylic acid) were synthesized by the Chemistry Department of Bayer (Wuppertal, Germany). MK-801 (dizocilpine, (+)-5-methyl-10,11-dihydroxy-5H-dibenzo(a,d)cyclohepten-5,10-imine) (RBI, Cologne, Germany), prazosin (RBI) and idazoxan (Sigma, Deisenhofen, Germany) were purchased, whereas raclopride was kindly donated by Astra (Sodertalje, Sweden). Nimodipine and (−)-BAY k 8644 were suspended in a solvent containing 1% Solutol® HS 15 (12-hydroxystearic acid ethoxilate; BASF, Ludwigshafen, Germany) and 1% ethanol (ethanol absolute, 99.8%; Riedel-de Haen, Sellze, Germany). Prazosin was suspended in distilled water and DMSO (dimethylsulfoxide, 10%; Merck, Darmstadt, Germany). SR 57746A was suspended in distilled water and Cremophor® EL (10%; BASF). Other compounds were dissolved in distilled water or 0.9% NaCl, and a few drops of lactic acid or HCl, if necessary. Application volume was 2 ml/kg (except for ethanol). Ethanol (500–1000 mg/kg) was injected in a fixed concentration of 12.5% w/v and, accordingly, the injection volume was varied among the doses (maximum injection volume being 8.27 ml/kg, at the 1 g/kg dose).

2.5. Data analysis

Test results were expressed as the percentage of rats that selected the drug lever (Percentage Drug Lever Selections). In addition, the percentage of animals that selected a lever (either drug or vehicle lever) was determined as an index of behavioral disruption (i.e., Percentage Lever Selections). Least-square linear regression analysis was used to estimate ED_{50}, ID_{50} and $T_{1/2}$ values and the corresponding 95% confidence limits (CL) after log-probit conversion of the data. ED_{50} or ID_{50} values with nonoverlap-

Table 1
Summary of generalization and antagonism test results with 5-HT_{1A} receptor ligands in rats trained to discriminate BAY x 3702 from vehicle

Test drug	Route of application	Type of test	Percentage drug lever selections (ED_{50}) (95% CL)[a]	Percentage lever selections (ID_{50}) (95% CL)[a]
BAY x 3702	i.p.	General.	0.030 (0.020–0.044)	0.295 (0.233–0.373)
	i.v.	General.	0.007 (0.003–0.016)	> 0.02
	p.o.	General.	0.36 (0.15–0.83)	> 1
8-OH-DPAT	i.p.	General.	0.086 (0.053–0.140)	> 0.3
Flesinoxan	i.p.	General.	0.30 (0.18–0.51)	> 1
SR 57746A	i.p.	General.	1.00 (0.33–3.04)	> 10
BAY x 3703	i.p.	General.	1.28 (NC)	> 3
Ipsapirone	i.p.	General.	1.79 (0.66–4.88)	22.12 (16.65–29.39)
WAY-100635	i.p.	Antagonism	0.013 (0.007–0.025)	> 0.1

[a] Doses in mg/kg.
Abbreviations used: CL (Confidence Limits), General. (Generalization test) and NC (Not Computable).

ping CL limits were considered to be significantly different. Generalization was considered to be complete if at least 80% drug lever selections was obtained, whereas antagonism was considered to be complete if less than 20% drug lever selections was obtained.

3. Results

All 16 rats learned to discriminate BAY x 3702 (0.1 mg/kg, i.p.) from vehicle; the median number of sessions to reach criterion being 38 (range: 22–58 sessions). The generalization obtained with BAY x 3702 was dose-dependent (ED_{50} value in Table 1; complete generalization obtained at 0.1 mg/kg; Fig. 1) and time-dependent ($T_{1/2}$ and 95% CL in min: 65; 47–90; Fig. 2). Testing of 0.2 and 0.4 mg/kg of the training drug resulted in 100% drug lever selections and coincided with a dose-dependent decrease in percentage lever selections (87.5% and 20% lever selections, respectively; ID_{50} value in Table 1). Dose-dependent and complete generalization was also obtained after i.v. and p.o. application of the compound (i.v.: 83% drug lever selections at 0.02 mg/kg, 100% lever selections at each dose; p.o.: 89% drug lever selections at 1 mg/kg, 100% lever selections at each dose; Fig. 1).

As shown in Fig. 3, dose-dependent and complete generalization was also obtained with the 5-HT_{1A} receptor agonists 8-OH-DPAT, flesinoxan, SR 57746A, BAY x 3703 and ipsapirone (ED_{50} values in Table 1). In

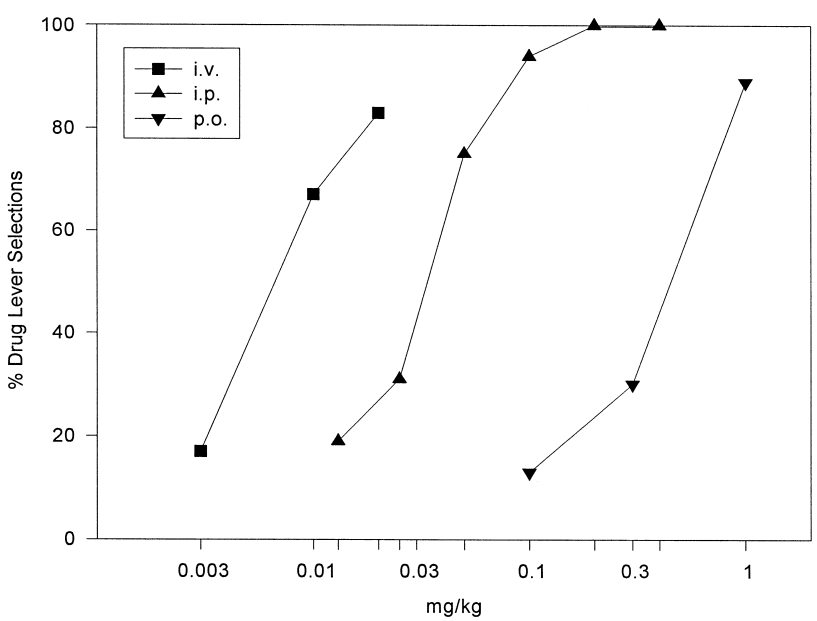

Fig. 1. Dose-dependent generalization obtained with BAY x 3702 after different routes of administration in rats ($n = 16$) trained to discriminate BAY x 3702 (0.1 mg/kg, i.p.) from vehicle in a two-lever food-reinforced procedure. Vehicle control values were: 0% and 13% drug lever selections, after i.v. and i.p. administration, respectively (p.o. vehicle control was not performed). Injection-test time intervals (number of animals tested) were: 15 min ($n = 6$, i.v.), 15 min ($n = 16$, i.p.) and 30 min ($n = 8$–10, p.o.).

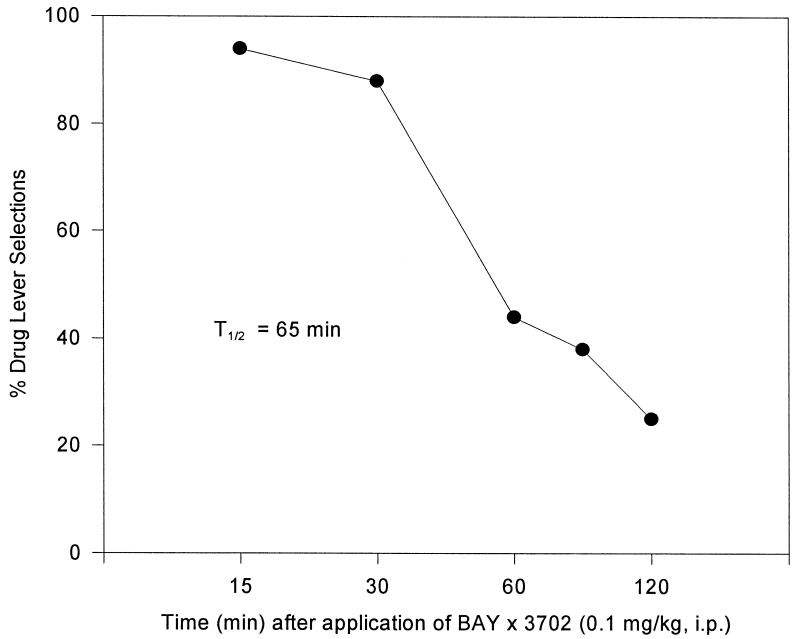

Fig. 2. Time-dependency of the discriminative stimulus induced by the training dose of BAY x 3702 in rats trained to discriminate this compound from vehicle (0.1 mg/kg, i.p., *t*-15 min). For each time point, $n = 16$, except 120 min ($n = 8$).

contrast to the other compounds, complete generalization to ipsapirone coincided with severe response suppression (ID_{50} value in Table 1) and the slope of the dose-response curve tended to be less steep.

Pretreatment with the 5-HT_{1A} receptor antagonist WAY-100635 dose-dependently and completely blocked the BAY x 3702 cue (Fig. 4). Testing of the antagonist alone (in combination with vehicle) did not induce more than 17% drug lever selections (0.01 mg/kg). In the experiments with WAY-100635 (either alone or in combination with BAY x 3702), all rats selected a lever at each dose.

No generalization was obtained with the α_1-adrenoceptor antagonist prazosin (dose range tested in mg/kg, i.p.: 1–5; maximal percentage drug lever selections: 13% at 3 mg/kg), nor with the α_2-adrenoceptor antagonist idazoxan (dose range tested in mg/kg, i.p.: 3–10; maximal percentage drug lever selections: 20% at 10 mg/kg) or the dopamine D_2 receptor antagonist raclopride (0% drug lever responses at 0.3 mg/kg, i.p.). None of the putative

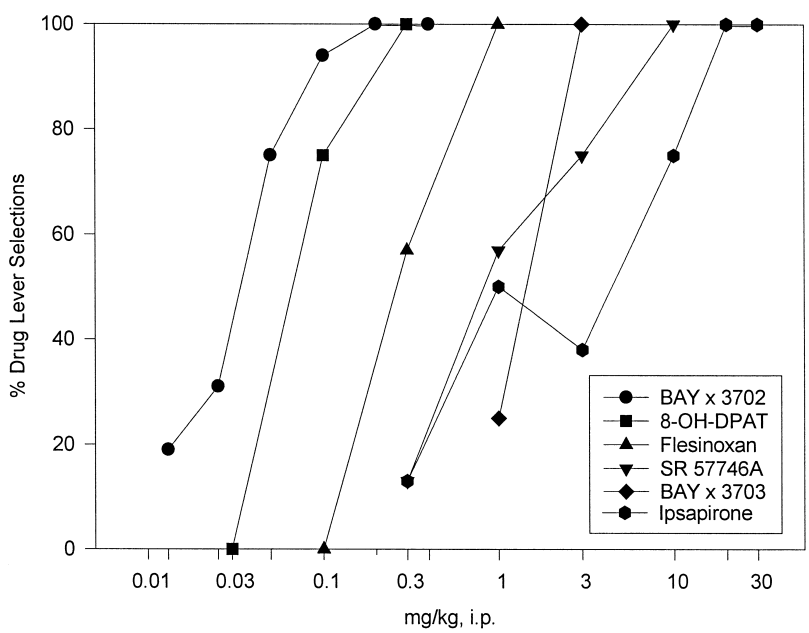

Fig. 3. Dose-dependent generalization induced by 5-HT_{1A} receptor agonists in rats trained to discriminate BAY x 3702 (0.1 mg/kg) from vehicle.

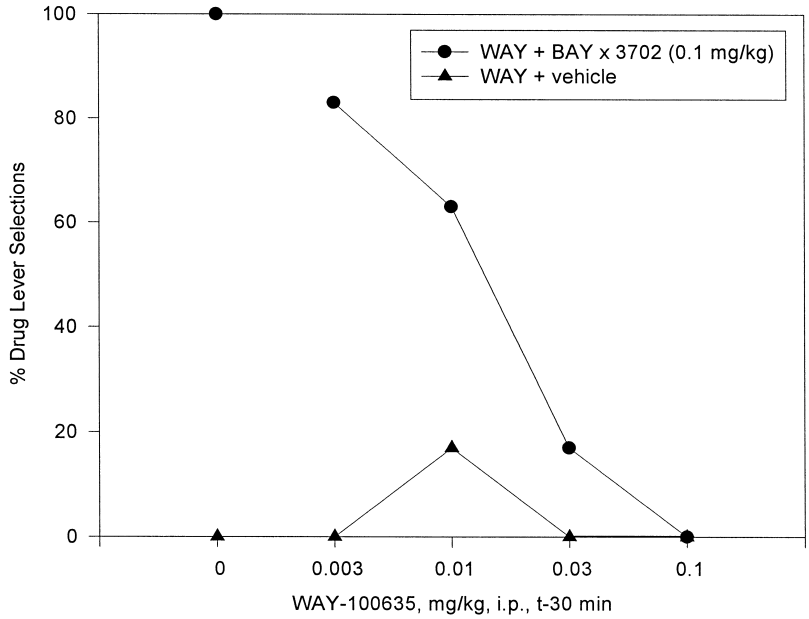

Fig. 4. Dose-dependent antagonism of the BAY x 3702 cue by pretreatment with the selective 5-HT_{1A} receptor antagonist WAY-100635 (WAY). WAY-100635 was administered 15 min before BAY x 3702 or vehicle.

neuroprotectants tested induced more than 20% drug lever selections (compound, doses tested in mg/kg: MK-801, 0.1–0.3; CGS 19755, 10; nimodipine, 3–10; CNS 1102, 3). Finally, no generalization (0% drug lever selections) was obtained with ethanol (500–1000 mg/kg, i.p.), the selective 5-HT re-uptake inhibitor paroxetine (1–3 mg/kg, i.p.), or the calcium channel agonist (−)-BAY k 8644 (0.3 mg/kg, i.p.). Testing of higher doses of these compounds was precluded due to severe response rate suppression.

4. Discussion

In the present study, the novel aminomethylchroman derivative BAY x 3702 was found to induce a discriminative stimulus in rats, and by means of generalization and antagonism tests, it was confirmed that this compound is a relatively potent 5-HT_{1A} receptor agonist in vivo (De Vry et al., 1998). BAY x 3702 induced dose-dependent generalization after different routes of administration. Thus, as compared with i.p. administration, a four-fold parallel shift of the generalization gradient to the left and a 12-fold shift to the right was obtained after i.v. and p.o. administration, respectively. These findings indicate that the ED_{50} values after the different routes of administration were well within the dose range which was found to be effective in animal models of cerebral ischemia/trauma, anxiety and depression (De Vry et al., 1997; Horvàth and Augstein, 1997; Horvàth et al., 1997). The time-dependency study showed that the duration of the cue was relatively short ($T_{1/2}$ = approximately 1 h), and therefore appeared to be in close accordance with pharmacokinetic data reported elsewhere (De Vry et al., 1997; Schwarz et al., 1997). After each route of administration, complete generalization occurred in a dose range which was devoid of any effects on percentage lever selections. After i.p. administration, doses higher than the training dose still induced complete generalization, but this coincided with a dose-dependent reduction in percentage lever selection. Nevertheless, the separation between ED_{50} value for generalization and ID_{50} for behavioral disruption was about factor 10, and this difference was considered to be statistically significant. In general, almost identical findings were reported in rats trained to discriminate 8-OH-DPAT in a similar drug discrimination procedure, where it was found that BAY x 3702 induced dose-dependent generalization (De Vry et al., 1998). The occurrence of symmetrical generalization between BAY x 3702 and 8-OH-DPAT, therefore, suggests that their discriminative stimulus properties are qualitatively very similar.

Generalization studies with a number of relatively selective 5-HT_{1A} receptor agonists indicated that these compounds generalized dose-dependently and completely to the BAY x 3702 cue. The order of potency after i.p. administration was: BAY x 3702 > 8-OH-DPAT > flesinoxan > SR 57746A = BAY x 3703 > ipsapirone, and correlated with the previously reported affinity of these compounds to 5-HT_{1A} receptors (Schipper et al., 1991; Cervo et al., 1994; De Vry et al., 1998). Interestingly, the slope of the dose-response curve of ipsapirone tended to be less steep as compared with the other curves. This finding was also observed in some studies using the 8-OH-DPAT cue (e.g., Sanger and Schoemaker, 1992; Schreiber and De Vry, 1993) and may be a reflection of the fact that

ipsapirone is rather a *partial* agonist than a *full* agonist at 5-HT_{1A} receptors (for review, see De Vry, 1995). Generalization with BAY x 3702 and its (+)-enantiomer, BAY x 3703, showed the same stereoselectivity (about 10- to 30-fold potency difference) as obtained in other models of 5-HT_{1A} receptor function, including the 8-OH-DPAT cue (De Vry et al., 1998). Therefore, these data support the suggestion that the discriminative stimulus effects of BAY x 3702 are mediated by 5-HT_{1A} receptors (De Vry et al., 1998). This suggestion was further underscored by the present finding that the BAY x 3702 cue was completely and potently blocked by pretreatment with the selective 5-HT_{1A} receptor antagonist WAY-100635. The ID_{50} value of WAY-100635 was very close to the ED_{50} value obtained with BAY x 3702, and this relationship is in accordance with the affinity of these compounds to 5-HT_{1A} receptors (Forster et al., 1995; De Vry et al., 1998). When tested alone, none of the doses of WAY-100635 induced more than 20% generalization, even at a high dose of 3 mg/kg. These findings support the suggestion that, in contrast with other previously claimed 5-HT_{1A} receptor antagonists, such as NAN-190 and BMY-7378, which were found to partially generalize to, and partially blocked the 8-OH-DPAT cue (De Vry et al., 1992; Schreiber and De Vry, 1993; Ybema et al., 1993; Kleven and Koek, 1998), WAY-100635 appears to be a 5-HT_{1A} receptor antagonist virtually devoid of any intrinsic activity (Forster et al., 1995). Also in rats or pigeons trained to discriminate either 8-OH-DPAT or flesinoxan from vehicle, WAY-100635 was found to be a powerful antagonist with virtually no intrinsic activity (Gommans et al., 1995; Sánchez et al., 1996; Mos et al., 1997; Wolff and Leander, 1997; Kleven and Koek, 1998).

Because it was reported that BAY x 3702 shows, in addition to its high affinity to 5-HT_{1A} receptors, a relatively high to moderate affinity to α_1- and α_2-adrenoceptors (about 20-fold lower affinity), as well as dopamine D_2 receptors (about 100-fold lower affinity), and because BAY x 3702 was characterized in vitro as an antagonist at these receptors (De Vry et al., 1998), appropriate reference compounds for these receptors were also tested in subsequent generalization tests. These studies showed that neither the α_1-adrenoceptor antagonist prazosin, nor the α_2-adrenoceptor antagonist idazoxan, or the dopamine D_2 receptor antagonist raclopride were found to induce more than 20% generalization at behaviorally active doses. Therefore, it appears unlikely that interactions with these receptors are involved in the discriminative stimulus induced by a moderately low training dose of BAY x 3702. It remains to be determined to what extent other receptors or binding sites, such as serotonergic 5-HT_7, 5-HT_{1D} and 5-HT_{2C} receptors, dopamine D_4 receptors, or sigma sites, to which BAY x 3702 shows also some affinity in binding studies, are involved in the BAY x 3702 cue. However, the fact that the highly selective 5-HT_{1A} receptor antagonist WAY-100635 was found to completely block the BAY x 3702 cue, suggests that their possible contribution is, at best, very modest. Further selectivity of the BAY x 3702 cue was suggested by the absence of generalization in tests with ethanol, the selective 5-HT re-uptake inhibitor paroxetine and the calcium channel agonist (−)-BAY k 8644, when tested at doses which were shown to be behaviorally active or discriminable in a similar procedure (e.g., De Beun et al., 1996a,b).

In order to investigate whether BAY x 3702 shares discriminative stimulus effects with putative neuroprotectants selected from diverse pharmacological classes, generalization tests were performed with the noncompetitive NMDA receptor antagonists MK-801 and CNS 1102, the competitive NMDA receptor antagonist CGS 19775 and the voltage-dependent calcium channel antagonist nimodipine. It was found, however, that none of these compounds induced more than 20% generalization when tested in a dose range which was behaviorally active (as indicated by a reduction of percentage lever selections, or by the fact that these compounds were previously shown to induce a cue at the tested dose(s) in a similar drug discrimination procedure; e.g., De Vry and Traber, 1989; De Vry et al., 1996). Therefore, it is suggested that the putative neuroprotectant BAY x 3702 may have a mechanism of action which is at least partially related to its high affinity and intrinsic activity at 5-HT_{1A} receptors (De Vry et al., 1997, 1998; Horvàth and Augstein, 1997).

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