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The antiaggressive potency of (−)-penbutolol involves both 5-HT_{1A} and 5-HT_{1B} receptors and β-adrenoceptors

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Abstract

The relative importance of 5-HT_{1A} and β-adrenergic activities in the antiaggressive effects of (−)-penbutolol was studied in male mice. (−)-Penbutolol had high affinity for 5-HT_{1A} receptors and β-adrenoceptors, and antagonized the 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT)-induced 5-HT syndrome and the 8-hydroxy-2-(di-*n*-propylamin)tetralin (8-OH-DPAT)-induced discriminatory stimulus in rats. (−)-Penbutolol abolished aggressive behaviour (ED_{50} = 56 μmol/kg), and reversed the antiaggressive effects of 8-OH-DPAT and 1-(3-trifluoromethylphenyl)piperazine (TFMPP) (ED_{50} = 8.1 and 2.1 μmol/kg, respectively). (*N*-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635) reversed the antiaggressive effects of 8-OH-DPAT (ED_{50} = 0.012 μmol/kg), but did not affect the antiaggressive effects of TFMPP. The antiaggressive effect of a submaximal dose of 8-OH-DPAT was markedly potentiated by β-adrenoceptor antagonists without 5-HT_{1A} receptor affinity, whereas (−)-penbutolol was effective at only one dose (4.5 μmol/kg). In conclusion, the 5-HT_{1A} receptor antagonistic potency of (−)-penbutolol in aggressive mice is attenuated by β-adrenoceptor-induced facilitation of serotonergic neurotransmission.

Keywords: Aggression, isolation-induced; 5-HT (5-hydroxytryptamine, serotonin); β-Adrenoceptor, interaction; (Mouse)

1. Introduction

Serotonin (5-HT) receptors play an important role in mediating aggressive behaviour (e.g. reviews by Eichelman, 1990; Miczek et al., 1994; Bell and Hobson, 1994) and the action of serotonergic drugs has been studied extensively in different animal models of aggressive behaviour. Non-selective 5-HT receptor stimulation, e.g. by administration of a selective 5-HT reuptake inhibitor (fluvoxamine (Olivier et al., 1989a); zimeldine (Ögren et al., 1980); sertraline, fluoxetine and femoxetine (Sánchez and Hyttel, 1994)), but also non-selective 5-HT receptor inhibition, e.g. by administration of methiothepin, methysergide and cyproheptadine (Malick and Barnett, 1976; Weinstock and Weiss, 1980) inhibit isolation-induced aggression in male mice. The interpretation of the results depends upon the sites of action involved, i.e. 5-HT receptor subtypes and pre- versus postsynaptic localization of these receptors. It is generally found that 5-HT_1 receptor stimulation inhibits aggressive behaviour in mice and rats (McMillen et al., 1988, 1989; Olivier et al., 1990; Sánchez et al., 1993). 5-HT depletion by treatment with *p*-chlorophenylalanine methyl ester attenuates the antiaggressive effect of the 5-HT releasing agent, fenfluramine, and potentiates the antiaggressive effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamin)tetralin) (Sánchez and Hyttel, 1994). This suggests that postsynaptic 5-HT_{1A} receptors are involved in the mediation of the aggressive behaviour. However, other studies suggest that 5-HT_{1A} autoreceptor stimulation attenuates aggressive behaviour by decreasing social interest (Mos et al., 1992). 5-HT_{1B} receptor stimulation is also found to play a specific role in the control of aggressive behaviour (reviews by Olivier et al., 1989b; Bell and Hobson, 1994). Other 5-HT receptor subtypes may also be involved in mediating aggressive behaviour, e.g. 5-HT_2 or 5-HT_3 receptors (Olivier and Mos, 1992; Bell and Hobson, 1994). Different types of aggressive behaviour (i.e. defensive aggression, maternal aggression, predatory attack, social conflict) and the various behavioural components of the aggressive behaviour may be differently affected

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by compounds with different receptor subtype selectivity (Bell and Hobson, 1994).

The role of β-adrenoceptors in aggressive behaviour is unclear. There are reports of β-adrenoceptor antagonists inhibiting aggressive behaviour. (−)-Propranolol and (±)-propranolol inhibit aggressive behaviour of isolated mice (Yoshimura et al., 1987), whereas (±)-propranolol potentiates target-biting attacks (Matray-Devoti and Wagner, 1993). Other reports suggest that there is no correlation between antiaggressive effects and β-adrenoceptor blockade (DaVanzo et al., 1988). It has been suggested that the antiaggressive effects of β-adrenoceptor antagonists are associated with their 5-HT antagonism rather than their β-adrenoceptor antagonism (Weinstock and Weiss, 1980). However, it cannot be excluded that β-adrenoceptor blockade influences the antiaggressive potency. It has been shown that the 5-HT_{1A} receptor antagonistic potency of (−)-pindolol, measured as reversal of the 8-OH-DPAT-induced hyperlocomotion, is masked by the facilitating effect of β-adrenoceptor blockade (Kalkman and Soar, 1990).

(−)-Penbutolol is a β-adrenoceptor antagonist with high affinity for 5-HT_{1A} and 5-HT_{1B} receptors , also (Langlois et al., 1993). Microdialysis studies and behavioural studies have shown that (−)-penbutolol is an antagonist at 5-HT_{1A} receptors (Hjorth, 1992; Hjorth and Sharp, 1993; Sánchez, 1993) and 5-HT_{1B} receptors (Frances et al., 1994). The aim of the present investigation was to study the effects of (−)-penbutolol in socially isolated aggressive male mice, and to study whether the β-adrenoceptor blockade interacts with the 5-$HT_{1A/1B}$ receptor-mediated effects. The (+)-enantiomer of penbutolol, a non-selective β-adrenoceptor antagonist without 5-$HT_{1A/1B}$ receptor affinity, and the selective β_1/β_2-adrenoceptor antagonists, metoprolol (Rainbow et al., 1984) and *erythro*-DL-1-(7-methylindan-4-yloxy)-3-isopropylamine-butan-2-ol hydrochloride (ICI 118,551; O'Donnell and Wanstall, 1980), respectively, as well as the selective 5-HT_{1A} receptor antagonist, (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100635; Fletcher et al., 1994), were included in the study. The latency to the first attack (i.e. bite or attempt to bite), which has been suggested as a simple and reliable measure of aggressive behaviour (McMillen et al., 1988; Sánchez et al., 1993) was studied. Affinities for 5-HT_{1A} and β-adrenoceptors were assessed by in vitro receptor binding studies. Inhibition of the 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT)-induced 5-HT syndrome and inhibition of the 8-OH-DPAT-induced discriminative stimulus were included as measures of 5-HT_{1A} receptor antagonistic potencies in vivo. Preliminary data have been presented at the European Behavioural Pharmacology Society Meeting in Berlin, 1994 (Sánchez et al., 1994)

2. Materials and methods

2.1. Animals

Male mice (NMRI/BOM, SPF, Møllegård, Denmark) weighing 18–20 g at the beginning of the experiment were used. The mice were housed under a 12-h light/dark cycle (light on at 6 a.m.). The aggressive mice were single-housed in Macrolon type II cages and intruder mice were housed in plastic cages (35 × 30 × 12 cm), ten in each. The room temperature (21 ± 2°C), relative humidity (55 ± 10%), and air exchange (16 times/h) were automatically controlled. The animals had free access to commercial food pellets and tap water between test sessions.

Wistar rats (Mol: Wist, SPF, 170–270 g) were used in receptor binding studies and other in vivo studies. All rats were housed in Macrolon type III cages, four in each. The housing conditions were similar to those described above.

2.2. Procedure

2.2.1. Inhibition of isolation-induced aggressive behaviour in male mice

The test was conducted as described by Sánchez et al. (1993). Briefly, the aggressive mice were kept isolated for about 21 days. After the isolation period the mice were trained to attack a non-aggressive intruder mouse of the same strain. An attack was defined as biting or as an attempt to bite the intruder mouse. The training and the testing sessions took place in the home cage of the isolated mouse. Attack latencies of 10 s or less were reached after daily training sessions for 5–7 days. Only mice with attack latencies of less than 10 s were included in the studies.

In the test sessions the mice were pretested immediately before drug treatment and 30 min later. In the antagonism studies 8-OH-DPAT or TFMPP was administered 15 min after the antagonist, and the post-treatment test was performed after an additional 15 min. The attack latency was measured with a maximum observation time of 180 s. Each group consisted of 8 aggressive (resident) and 16 non-aggressive (intruders for pre- and post-drug testing) mice. A total of two or three separate experiments each including a control group and three or four doses were conducted. The mice were tested immediately before drug administration and only mice with attack latencies of less than 10 s were included in the studies. The mice were randomly allocated to drug or vehicle treatment.

2.2.2. Receptor binding studies in vitro

2.2.2.1. 5-HT_{1A} receptor binding. Inhibition of [^{3}H]8-OH-DPAT binding to 5-HT_{1A} receptors in membranes

from rat brain minus cerebellum was determined as described by Hyttel et al. (1988). Briefly, rat brains were homogenized in 10 ml 50 mM Tris buffer (pH 8.0, 25°C) containing 120 mM NaCl, 4 mM $CaCl_2$, and 4 mM $MgCl_2$. The homogenate was centrifuged (20000 $\times g$, 10 min, 4°C). The pellet was homogenized in 10 ml of the same buffer, incubated for 10 min at 37°C, and centrifuged as above. The final pellet was homogenized in 100 volumes (w/v) of the buffer containing 10 μM pargyline. Aliquots (10 mg tissue) were incubated with 1 nM [^{3}H]8-OH-DPAT alone or in the presence of test compound in a total volume of 1200 μl for 15 min at 37°C. After incubation, the samples were filtered under vacuum through Whatman GF/F filters. The filters were washed twice with 5 ml saline and bound radioactivity was estimated by liquid scintillation spectroscopy. Non-specific binding was defined as the binding remaining in the presence of 10 μM 5-HT.

2.2.2.2. β-Adrenoceptor binding. Inhibition of [^{3}H]dihydroalprenolol binding to β-adrenoceptors in membranes from rat cortex was determined as described by Hyttel et al. (1984). Briefly, rat cortical tissue was homogenized in 10 ml 50 mM Tris buffer (pH 8.0, 25°C). The homogenate was centrifuged twice (20000 $\times g$, 10 min, 4°C) with re-homogenization of the pellet in 10 ml ice-cold buffer. The pellet was homogenized in 200 volumes (w/v) ice-cold buffer. Incubation tubes kept on ice were prepared with 100 μl drug solution and 2000 μl tissue suspension. The binding experiment was initiated by adding 100 μl [^{3}H]dihydroalprenolol and placing the tubes in a 25°C water bath. After incubation for 15 min, the samples were filtered under vacuum through Whatman GF/F filters which had been wetted with 0.1% polyethylenimine. The filters were washed twice with 5 ml saline and bound radioactivity was estimated by liquid scintillation spectroscopy. Non-specific binding was defined as the binding remaining in the presence of 0.3 μM (−)-propranolol.

2.2.3. Discriminative stimulus properties induced by 8-OH-DPAT

Rats were trained to discriminate between 8-OH-DPAT (0.4 mg/kg, i.p., 15-min pretreatment time) and physiological saline as described by Arnt (1989). Wire-mesh boxes (29 cm × 24 cm) equipped with a dipper located equidistant between two levers, were used. The boxes were placed in a sound-protected chamber with ventilation fans providing a constant noise level.

A training procedure similar to that described by Nielsen and Jepsen (1985) was followed. Rats were water-deprived by restricting water intake to that received as reinforcers in the training box. The rats were trained to respond under gradually increasing fixed-ratio (FR) schedules for water reward (0.1 ml) up to FR 32. 15 min before the start of the session the rats received an injection of either training drug or saline. The trial began 20 s after the rat was placed in the box, when the house-light was switched on, and lasted for 20 min. Following saline injection only responses on a designated lever (saline lever) were rewarded, and following drug injection, only responses on the opposite lever (drug lever) were rewarded. Incorrect responses had no consequences. Drug and saline levers were randomly allocated to the left and right for different rats. The level of discrimination accuracy was expressed as the percent correct responses on the lever appropriate for the injection of drug or saline before the first reward. Test trials were started when eight out of ten consecutive training sessions showed an accuracy of at least 90% correct discrimination for the group as a mean and at least 75% correct responding for individual animals.

In test trials the experiment finished when a rat had made 32 responses on one of the levers or when 20 min had elapsed. No reinforcement was given. If a rat made fewer than ten responses on one lever during the experiment it was defined as a non-responder. At least half the rats in each dose group comprising at least four rats were required to be responders in order to calculate a drug response. If this criterion was not fulfilled response disruption had occurred.

2.2.4. Inhibition of 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) induced 5-HT syndrome in rats

The test substance was injected 30 min before 5-MeODMT 23 μmol/kg (5 mg/kg, s.c.). 10, 15 and 20 min later the rats were observed for the presence of the 5-HT syndrome: forepaw treading, head weaving, and hindleg abduction. Each part of the syndrome was scored as marked (score 2), weak (score 1) or absent (score 0) and the scores of the three observation times were added. The effect of the test substance was expressed as percent inhibition of the forepaw treading score relative to the control group. Four to eight rats were used for each dose.

2.3. Statistics

The in vivo results were expressed as ED_{50} values with 95% confidence limits, calculated by means of log-probit analysis. Attack latencies were also expressed as means (±S.E.M.); one-way analysis of variance (ANOVA) and post-hoc comparisons of means (Dunnett's *t*-test) were used for dose-response comparisons.

Receptor binding affinities were expressed as IC_{50} values in nM (logarithmic means). Two full concentration-response curves were measured using five concentrations of test drug in triplicate (covering three decades). In a series of 100 determinations the variance of the log ratio (log R) between the double determinations ($\Sigma(\log R^2)/2n \times 100$)) was deter-

mined. When the log ratio was greater than corresponding to 3 × sd (99% confidence interval) extra determinations were performed and outliers were discarded. Antilogs (sd) of 1.4 and 2.1 were obtained for 5-HT_{1A} and β-adrenoceptor binding, respectively.

2.4. Drugs

(−)-Penbutolol and (+)-penbutolol molecular weight (mw) 292; 8-hydroxy-2-(di-*n*-propylamin)tetralin HBr (8-OH-DPAT), mw 328; 1-(3-trifluoromethylphenyl)piperazine dihydrochloride (TFMPP) mw 303; (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide oxalate (WAY 100635), mw 513 (all synthesized at the Department of Medicinal Chemistry, H. Lundbeck A/S); metoprolol tartrate mw 685 and 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT) mw 218 (Sigma, USA); *erythro*-DL-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol hydrochloride (ICI 118,551) mw 314 (ICI, UK). All drugs were dissolved in saline. Injection volumes were 10 ml/kg for mice, 5 ml/kg for rats in studies of 5-MeODMT inhibition and 1 ml/kg in drug discrimination studies.

[^{3}H]OH-DPAT and [^{3}H]dihydroalprenolol with specific activities of approximately 235 Ci/mmol and 108 Ci/mmol, respectively, were obtained from Amersham International, UK.

3. Results

3.1. In vitro and in vivo effects of (−)-penbutolol on β-adrenoceptors and 5-HT_{1A} receptors

(−)-Penbutolol had high affinity for both β-adrenoceptors and 5-HT_{1A} receptors in vitro (Table 1). The (+)-enantiomer of penbutolol had an about 30 times lower affinity for 5-HT_{1A} receptors than the (−)-enantiomer, and the β-adrenoceptor antagonists, metoprolol and ICI 118,551, were devoid of 5-HT_{1A} receptor affinity. WAY 100635 had high affinity for 5-HT_{1A} receptors and no affinity for β-adrenoceptors.

(−)-Penbutolol antagonized the 5-MeODMT-induced 5-HT syndrome and the 8-OH-DPAT-induced discriminatory stimulus in rats, whereas (+)-penbutolol, metoprolol, and ICI 118, 551 were inactive (Table 1). WAY 100635 was a potent antagonist in both in vivo tests. Neither (−)-penbutolol nor WAY 100635 substituted for 8-OH-DPAT in the drug discrimination test (data not shown).

3.2. The effect of (−)-penbutolol on inhibitory potency of 8-OH-DPAT or TFMPP on isolation-induced aggression in mice

(−)-Penbutolol itself antagonized the aggressive behaviour at a relatively high dose (ED_{50} = 56 μmol/kg; Table 2 and Fig. 1). (−)-Penbutolol reversed the antiaggressive effect induced by submaximal doses of 8-OH-DPAT (0.95 μmol/kg) and TFMPP (8.3 μmol/kg), respectively (Fig. 1 and Table 2). The ED_{50} values of (−)-penbutolol were ED_{50} = 8.1 μmol/kg in 8-OH-DPAT-treated mice and 2.1 μmol/kg in TFMPP-treated mice, respectively. Furthermore, the antiaggressive effect of 8-OH-DPAT (0.95 μmol/kg) was potentiated significantly by a low dose of (−)-penbutolol, i.e. 4.5 μmol/kg (Fig. 1). However, this effect was only observed at one dose.

3.3. The effect of WAY 100635 on inhibitory potency of 8-OH-DPAT or TFMPP on isolation-induced aggression in mice

The selective 5-HT_{1A} receptor antagonist, WAY 100635, had no antiaggressive effect at very high doses (Table 1 and Fig. 2). WAY 100635 potently reversed the antiaggressive effect induced by a submaximal dose

Table 1
In vitro affinity of (−)-penbutolol, (+)-penbutolol, metoprolol and ICI 118,551 for β-adrenoceptors and 5-HT_{1A} receptor; antagonistic potency of (−)-penbutolol, (+)-penbutolol, WAY 100635, metoprolol, and ICI 118,551 on 5-MeODMT-induced 5-HT syndrome and 8-OH-DPAT-induced discriminatory stimulus in rats

Drug	In vivo 5-HT_1 receptor antagonism (ED_{50} (μmol/kg) and 95% conf. int.)		In vitro receptor affinity (IC_{50} (nM))	
	Antagonism of 5-MeODMT	Antagonism of 8-OH-DPAT cue	β-Adrenoceptors	5-HT_{1A} receptors
8-OH-DPAT	–	–	> 100 000	3.5
TFMPP	–	–	1100	460
(−)-Penbutolol	6.0 (2.5–14)	15 (7.5–30)	2.8	9.9
(+)-Penbutolol	> 34	> 69	5.3	300
WAY 100635	0.62 (0.24–1.6)	0.024 (0.0096–0.060)	> 1000	2.2
Metoprolol	> 29	> 29	48	> 10 000
ICI 118,551	> 32	> 32	36	20 000

Table 2
Antiaggressive potency of 8-OH-DPAT, TFMPP, (−)-penbutolol and WAY 100635, and antagonistic potency of (−)-penbutolol and WAY 100635 on 8-OH-DPAT (0.95 μmol/kg = 0.31 mg/kg)- or TFMPP (8.3 μmol/kg = 2.5 mg/kg)-induced inhibition of aggressive behaviour in socially isolated mice

Drug	ED_{50} (μmol/kg) (95% confidence interval)		
	Antagonism of aggression	Reversal of 8-OH-DPAT-induced inhibition of aggression	Reversal of TFMPP-induced inhibition of aggression
8-OH-DPAT	0.78 (0.43–1.4)	–	–
TFMPP	7.0 (4.7–11)	–	–
(−)-Penbutolol	56 (51–62)	8.1 (5.4–12)	2.1 (1.6–2.7)
WAY 100635	> 39	0.012 (0.0063–0.023)	> 1.2

of 8-OH-DPAT (0.95 μmol/kg; ED_{50} = 0.012 μmol/kg), but did not affect the antiaggressive effect of TFMPP (8.3 μmol/kg; Fig. 2 and Table 1).

3.4. The effect of (+)-penbutolol, metoprolol and ICI 118,551 on inhibitory potency of 8-OH-DPAT on isolation-induced aggression in mice

The β-adrenoceptor antagonist, (+)-penbutolol, and the β_2-adrenoceptor antagonist, ICI 118,551, antagonized the aggressive behaviour, but at relatively high doses, whereas the β_1-adrenoceptor antagonist, metoprolol, was inactive at doses up to 92 μmol/kg (40 mg/kg; Fig. 3). At lower doses (+)-penbutolol, metoprolol and ICI 118,551 all potentiated the antiaggressive effect of 8-OH-DPAT (Fig. 3).

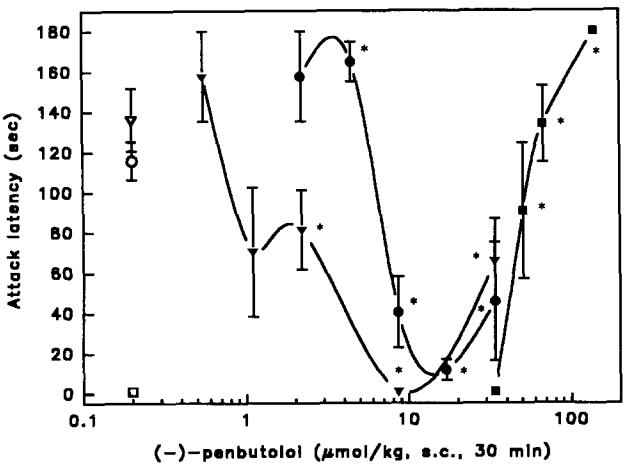

Fig. 1. Antiaggressive potency of (−)-penbutolol (■) alone or combined with 8-OH-DPAT (0.95 μmol/kg = 0.31 mg/kg) (●) or TFMPP (8.3 μmol/kg = 2.5 mg/kg) (▼) in socially isolated male mice. Saline (□), 8-OH-DPAT (0.95 μmol/kg) (○) and TFMPP (8.3 μmol/kg) (▽). Mean (±S.E.M.) increase in attack latency versus dose is shown. (−)-Penbutolol was administered 15 min before 8-OH-DPAT or TFMPP and the post-treatment test was performed 30 min after (−)-penbutolol administration. * $P < 0.05$; one-way ANOVA followed by post-hoc comparison to a control group. n = 8–16.

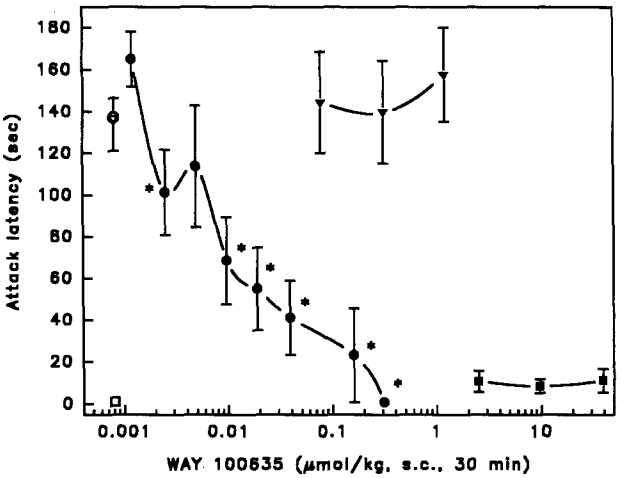

Fig. 2. Antiaggressive potency of WAY 100635 (■) alone or combined with 8-OH-DPAT (0.95 μmol/kg = 0.31 mg/kg) (●) or TFMPP (8.3 μmol/kg = 2.5 mg/kg) (▼) in socially isolated male mice. Saline (□), 8-OH-DPAT (0.95 μmol/kg) (○) and TFMPP (8.3 μmol/kg) (▽). Mean increase in attack latency (±S.E.M.) versus dose is shown. WAY 100635 was administered 15 min before 8-OH-DPAT or TFMPP and post-treatment test was performed 30 min after WAY 100635 administration. * $P < 0.05$; one-way ANOVA followed by post-hoc comparison to a control group. n = 8–16.

4. Discussion

The relative importance of 5-HT_{1A} and β-adrenergic activities in the antiaggressive effects of (−)-penbutolol was studied.

In vitro, (−)-penbutolol had high affinity in the nanomolar range for both 5-HT_{1A} receptors and β-adrenoceptors. The IC_{50} value was only 3–4 times lower for 5-HT_{1A} receptors than for β-adrenoceptors. Furthermore, the in vitro 5-HT_{1A} IC_{50} value for (−)-penbutolol was only 4–5 times lower than the IC_{50} value for the selective 5-HT_{1A} receptor antagonist WAY 100635. The (+)-enantiomer of penbutolol had a much lower IC_{50} value for 5-HT_{1A} receptors than for β-adrenoceptors, and metoprolol and ICI 118,551 were devoid of 5-HT_{1A} receptor affinity. In agreement with the 5-HT_{1A} receptor affinities, the in vivo potency of (−)-penbutolol measured as antagonism of the 5-MeODMT-induced 5-HT syndrome was about 10 times lower than that of WAY 100635. In contrast, the potency of (−)-penbutolol was more than 600 times lower than that of WAY 100635 measured as antagonism of the 8-OH-DPAT-induced discriminative stimu-

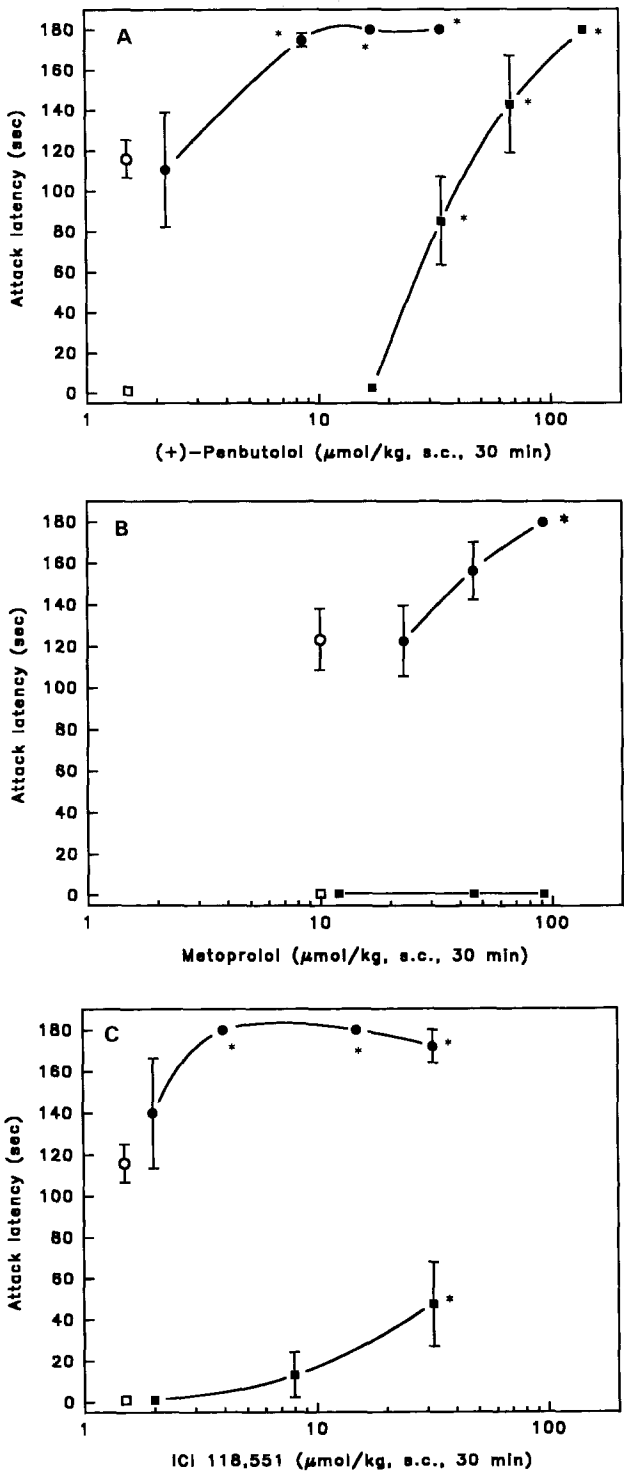

Fig. 3. Effect of (+)-penbutolol (A), metoprolol (B), and ICI 118,551 (C) alone (■) or combined with 8-OH-DPAT (0.95 μmol/kg = 0.31 mg/kg) (●), saline (□) or 8-OH-DPAT (0.95 μmol/kg = 0.31 mg/kg) alone (○) on aggressive behaviour in socially isolated male mice. Mean increase in attack latency (±S.E.M.) versus dose is shown. Drug was administered 30 min before post-treatment test was performed. * $P < 0.05$; one-way ANOVA followed by post-hoc comparison to a control group. $n = 8$–16.

lus and reversal of 8-OH-DPAT-induced inhibition of aggressive behaviour. The potent effects of WAY 100635 in the drug discrimination procedure and to reverse the 8-OH-DPAT-induced inhibition of aggression agree with potencies reported in the literature (Fletcher et al., 1994; Critchley et al., 1994). This suggests that the antagonistic potency of WAY 100635 against the 5-MeODMT-induced 5-HT syndrome was atypical. As 5-MeODMT is a non-selective 5-HT_1 receptor agonist (Glennon, 1987), the non-selective 5-$HT_{1A/1B}$ receptor antagonist, (−)-penbutolol, may prove to be a relatively more efficient antagonist than the selective 5-HT_{1A} receptor antagonist, WAY 100635.

(−)-Penbutolol, (+)-penbutolol and the β_2-adrenoceptor-selective antagonist, ICI 118,551, all increased the attack latency of aggressive male mice significantly. The minimal effective doses were rather high, i.e. from 30 to 60 μmol/kg, but the mice showed no sedation or any other behavioural changes at the doses tested. The β_1-adrenoceptor antagonist, metoprolol, was inactive at doses up to 100 μmol/kg. This might suggest that β_2-adrenoceptors rather than β_1-adrenoceptors are involved in mediation of aggressive behaviour. This agrees with the findings of Matsumoto et al. (1994), that ICI 118,551 blocked the desipramine-induced enhancement of aggressive behaviour in long-term isolated mice at doses from 1.3 mg/kg (1.9 μmol/kg), whereas metoprolol was inactive at doses up to 20 mg/kg (29 μmol/kg). However, results of other studies suggest that metoprolol has a low potency for reducing aggressive behaviour in mice, i.e. $ED_{50} = 100$ mg/kg (150 μmol/kg) (DaVanzo et al., 1988).

(−)-Penbutolol reversed the 8-OH-DPAT-induced inhibition of aggressive behaviour at doses ranging from about 7–30 μmol/kg. This agrees with the 5-HT_{1A} receptor antagonistic potencies against the 5-MeODMT-induced 5-HT syndrome and antagonism of the 8-OH-DPAT-induced discriminative stimulus. According to the literature, (−)-penbutolol has similar in vitro affinity for 5-HT_{1A} and 5-HT_{1B} receptors, i.e. $K_i = 2.4$ nM and $K_i = 3.6$ nM, respectively, measured by means of [^{3}H]8-OH-DPAT binding in rat hippocampus and [^{3}H]5-HT binding in striatum (Langlois et al., 1993). The antagonistic potency of (−)-penbutolol to reverse the antiaggressive effect of TFMPP was about 4 times higher than that of 8-OH-DPAT (Fig. 1). The 8-OH-DPAT and TFMPP doses were comparable as regards antiaggressive potential, showing an about 70% inhibition of aggressive behaviour. The antiaggressive potency of TFMPP may be mediated by 5-HT_{1B} receptors, as the selective 5-HT_{1A} receptor antagonist, WAY 100635, did not alter the effect of TFMPP, whereas the mixed 5-HT_{1A} and 5-HT_{1B} receptor antagonist, (−)-penbutolol, reversed the effect of TFMPP. TFMPP has

affinity for 5-HT_{2C} receptors in vitro and the selectivity ratios for other 5-HT receptor subtypes are not high (Glennon, 1990; Schoeffter and Hoyer, 1989; Mos et al., 1992). Drug discrimination studies suggest that both 5-HT_{1B} and 5-HT_{2C} receptors are important in the mediation of the TFMPP discriminative stimulus (Arnt, 1989; Herndon et al., 1992). Results of studies of the hypophagic, hypolocomotor and anxiogenic effects of TFMPP also suggest that 5-HT_{2C} receptors are involved in its effects in vivo (Kennett and Curzon, 1988a, b; Kennett et al., 1989). However, (−)-penbutolol does not have affinity for 5-HT_{2C} receptors (Frederiksen, H. Lundbeck A/S, personal communication). A recent study of mutant mice lacking the 5-HT_{1B} receptor suggested an important role of 5-HT_{1B} receptors in aggressive behaviour in the resident intruder test (Saudou et al., 1994).

A low dose of (−)-penbutolol (4.5 μmol/kg) facilitated the antiaggressive effect of 8-OH-DPAT (0.95 μmol/kg = 0.31 mg/kg) significantly. Similarly, (+)-penbutolol and ICI 118,551 potentiated the effects of 8-OH-DPAT at doses below 10 μmol/kg. Metoprolol also potentiated 8-OH-DPAT but at much higher doses. Thus, all β-adrenoceptor antagonists potentiated the antiaggressive effect of 8-OH-DPAT irrespective of their affinities for the 5-HT_{1A} receptor subtype. This suggests a modulatory role of β-adrenoceptors in 5-HT_{1A} receptor-mediated effects in aggressive mice. There is extensive anatomical and biochemical evidence for a close connection between the noradrenergic and serotonergic systems (reviewed by Caldecott-Hazard et al., 1991), and results of a number of behavioural studies also suggest interactions between the two systems. β-Adrenoceptor agonists like clenbuterol and salbutamol potentiated and antagonists like oxprenolol attenuated 5-HTP-induced head-twitches and tremors (Ortman et al., 1981; Martin et al., 1986; Hallberg, 1986; Weinstock et al., 1977), whereas β-adrenoceptor antagonists like ICI 118,551 and betaxolol enhanced 8-OH-DPAT-induced motor responses (Kalkman and Soar, 1990). Thus, the net effect of interaction between the serotonergic and adrenergic system can, depending on the animal model, be expressed as either potentiation or attenuation.

In conclusion, the mixed β-adrenoceptor and 5-HT_{1A} and 5-HT_{1B} receptor antagonist (−)-penbutolol shows a complex response pattern in the resident-intruder model in male mice. The 5-HT_{1A} receptor antagonistic potency of (−)-penbutolol in aggressive mice appears to be attenuated by β-adrenoceptor-induced facilitation of serotonergic neurotransmission. This shows that caution is warranted in the interpretation of results obtained with compounds with a mixed β-adrenoceptor and 5-HT_{1A} receptor antagonist profile.

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