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Assesment of relative efficacies of 5-HT_{1A} receptor ligands by means of in vivo animal models

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Abstract

We have evaluated the effects of ligands with varying efficacies at β-adrenoceptors and 5-HT_{1A} receptors in three in vivo models reflecting pre- and/or postsynaptic 5-HT_{1A} receptor activation. Forepaw treading in rats is mediated by postsynaptic 5-HT_{1A} receptors, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propylamin)tetralin)-induced discriminative stimulus is predominantly mediated by postsynaptic, but presynaptic 5-HT_{1A} receptors might also be involved, and footshock-induced ultrasonic vocalization involves predominantly presynaptic 5-HT_{1A} receptors. In vitro receptor binding studies demonstrated high β-adrenoceptor and 5-HT_{1A} receptor affinity of (−)-penbutolol, high β-adrenoceptor and 60 times lower 5-HT_{1A} receptor affinity of (+)-penbutolol, high β-adrenoceptor affinity and about 100 times lower 5-HT_{1A} receptor affinity of pindolol and (−)-tertatolol, only affinity for β-adrenoceptors of metoprolol and ICI 118,551 (*erythro*-D,L-1-(7-methylindan-4-yloxy)-3-isopropylamine-butan-2-ol, and only affinity for 5-HT_{1A} receptors of WAY 100.635 ((*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclo-hexane-carboxamide). (−)-Penbutolol, (−)-tertatolol, pindolol and WAY 100.635 antagonized 5-MeODMT-induced (5-methoxy-*N*,*N*-dimethyltryptamine) forepaw treading in rats, and (+)-penbutolol, ICI 118,551 and metoprolol were inactive. (−)-Penbutolol, WAY 100.635 and (−)-tertatolol antagonized 8-OH-DPAT-induced discriminative stimulus in rats, pindolol and metoprolol showed a mixed antagonistic and agonistic profile. Pindolol antagonized footshock-induced ultrasonic vocalization in rats, tertatolol inhibited maximum 36% and WAY 100.635, (−)-penbutolol, (+)-penbutolol, metoprolol and ICI 118,551 were inactive. (−)-Penbutolol and WAY 100.635 reversed 8-OH-DPAT-induced inhibition of ultrasonic vocalization completely, (−)-tertatolol reversed maximum 52% and (+)-penbutolol and pindolol were inactive. It is concluded, that efficacies at 5-HT_{1A} receptors can be estimated by applying a battery of in vivo test models that involve post- and presynaptic receptors to a variable degree. The in vivo ranking order of efficacy at 5-HT_{1A} receptors was: WAY 100.635 = (−)-penbutolol < (−)-tertatolol < pindolol.

Keywords: 5-HT_{1A} receptor; Efficacy; Ultrasonic vocalization; Drug discrimination; (Rat)

1. Introduction

A number of β-adrenoceptor antagonists have high affinity for subtypes of serotonin (5-HT) receptors, particularly of the 5-HT_{1A} and 5-HT_{1B} receptor subtypes (Langlois et al., 1993). Until recently these compounds were the only available compounds with 5-HT_1 receptor antagonistic properties and they have been widely used in pharmacological experiments. In the present study we have evaluated the effects of these drugs and β-adrenoceptor antagonists in three in vivo models reflecting 5-HT_{1A} receptor activation.

The in vivo studies in rats included induction of forepaw treading or inhibition of 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT)-induced forepaw treading, generalization to or inhibition of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT)-induced discriminative stimulus and inhibition of footshock-induced ultrasonic vocalization or reversal of 8-OH-DPAT-induced inhibition of ultrasonic vocalization. These test models involve presynaptic and postsynaptic 5-HT_{1A} receptors to variable degrees and are therefore appropriate for in vivo studies of differences in functional responses at pre- and postsynaptic receptors of 5-HT_{1A} receptor ligands. Serotonergically induced forepaw treading in rats is mediated by postsynaptic 5-HT_{1A} receptors (Nisbet and Marsden, 1984). 8-OH-DPAT-induced discriminative stimulus is predominantly

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mediated by postsynaptic 5-HT_{1A} receptors, as the response to 8-OH-DPAT was unchanged after *p*-chlorophenylalanine-induced 5-HT depletion (Kalkman, 1990). However, a study of local administration of 8-OH-DPAT into dorsal raphe nucleus to rats trained to discriminate 8-OH-DPAT suggested that presynaptic 5-HT_{1A} receptors also might be involved (Schreiber and De Vry, 1993a). Footshock-induced ultrasonic vocalization emitted by adult rats was potently inhibited by anxiolytics with selectivity for 5-HT_{1A} receptors, i.e., buspirone, ipsapirone and gepirone (Miczek et al., 1991; Sánchez, 1993; De Vry and Schreiber, 1993; Molewijk et al., 1995). Even low efficacy 5-HT_{1A} receptor active compounds, which exert antagonistic activity in other models, e.g., (−)-pindolol and (−)-alprenolol, inhibit ultrasonic vocalization potently (Sánchez, 1993). Studies involving 5-HT depletion by means of 5,7-dihydroxytryptamine or *p*-chlorphenylalanine and local application of 5-HT_{1A} receptor agonists suggest that footshock-induced ultrasonic vocalization involves predominantly presynaptic 5-HT_{1A} receptors (Schreiber and De Vry, 1993b).

Affinities for 5-HT_{1A} receptors and β-adrenoceptors were assessed by in vitro receptor binding techniques. The following compounds were included in the study: (−)-penbutolol, (+)-penbutolol, (−)-pindolol, (−)-tertatolol, *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide (WAY 100.635), metoprolol, *erythro*-D,L-1-(7-methylindan-4-yloxy)-3-isopropylamine-butan-2-ol (ICI 118,551).

Preliminary data were presented at the European Behavioral Pharmacology Society meeting in Berlin, 1994 (Sánchez et al., 1994).

2. Materials and methods

2.1. Animals

Male Wistar WU rats (Charles River, Germany) weighing 150–175 g at the beginning of the study were used for studies of footshock-induced ultrasonic vocalization. Wistar rats (Mol: Wist, Møllegaard, Denmark) weighing 170–270 g were used in receptor binding studies and other in vivo studies. All rats were housed in groups of 4 in Macrolon type III cages. The room temperature (21 ± 2°C), relative humidity (55 ± 5%) and air exchange (16 times per h) were automatically controlled. The animals had free access to commercial food pellets and tap water between test sessions.

2.2. Procedure

2.2.1. Receptor binding studies in vitro

2.2.1.1. 5-HT_{1A} receptors. 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 (20 000 × *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. The non-specific binding was defined as binding remaining in the presence of 10 μM 5-HT.

2.2.1.2. β-Adrenoceptors. 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 (20 000 × *g*, 10 min, 4°C) with rehomogenization of the pellet in 10 ml icecold buffer. The pellet was homogenized in 200 volumes (w/v) icecold 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 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% polyethylenimin. The filters were washed twice with 5 ml saline and bound radioactivity was estimated by liquid scintillation spectroscopy. The non-specific binding was defined as binding remaining in the presence of 0.3 μM (−)-propranolol.

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

Rats were trained to discriminate between 8-OH-DPAT (1.2 μmol/kg = 0.40 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. Rats were trained to respond under gradually increasing fixed ratio (FR) schedules for water reward (0.1 ml) up to FR 32. Fifteen minutes before 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 or until 32 responses were

recorded. Following saline injection only responses on a designated lever (saline lever) were rewarded and following drug 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 percentage 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 the 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 less than ten responses on one lever during the experiment it was defined as a non-responder. At least half of 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. The responses were calculated as percentage of drug lever responses relative to total number of responses. The latency to complete the test trial was recorded as reaction time. If less than 32 but more than 10 responses were made on one lever, reaction time was assigned the total length of trial. Generalization to 8-OH-DPAT-induced discriminative stimulus was tested 30 min after drug administration. Antagonism of 8-OH-DPAT-induced discriminative stimulus was tested 30 min after test drug and 15 min after 8-OH-DPAT administration.

2.2.3. 5-Methoxy-N,N-dimethyltryptamine (5-MeODMT)-induced forepaw treading in rats

The rats were observed 30 min after injection of test substance for presence of forepaw treading which was scored as marked (score 2), weak (score 1) or absent (score 0). 5-MeODMT (23 μmol/kg = 5 mg/kg, s.c.) was administered immediately after the scoring, and the presence of forepaw treading was scored again 10 min later. The effect of the test substance was expressed as percentage inhibition relative to the control group. Four to eight rats were used for each dose.

2.2.4. Inhibition of footshock-induced ultrasonic vocalization in adult rats

The test was conducted as described by Sánchez (1993). Briefly, the test cages (22 cm × 22 cm × 22 cm) were made of gray perspex and equipped with a metal grid floor. Footshocks were delivered from a two pole shocker. A microphone sensitive to ultrasounds in the range of 20–30 kHz was placed in the center of the lid of the test cage. The ultrasounds were sent from the microphone to a preamplifier and converted from AC signals to DC signals in a signal rectifier. The accumulated time in which the voltage of the rectified signal was larger than the voltage of a previously determined threshold level was recorded. Furthermore, the number of calls and the mean duration of the calls were determined. Animals were shocked 24 h before the first test session. The rat was placed in the test cage and immediately received four 1.0 mA inescapable footshocks each of 10 s duration and with an intershock interval of 5 s. The animals were left in the test cage for a total of 6 min after the last shock. The same shock regimen was followed on test days. The rats were tested on a weekly basis for up to 7–8 weeks. The animals were housed in groups of four and the groups were randomly allocated to drug treatment or vehicle treatment. Inhibitory potency against footshock-induced ultrasonic vocalization was tested 30 min after drug administration. Reversal of 8-OH-DPAT-induced inhibition of ultrasonic vocalization was tested 30 min after drug and 15 min after 8-OH-DPAT (0.24 μmol/kg, s.c.) administration. The effects were expressed as means (± S.E.M.) of total duration of vocalization, number of calls and mean duration of calls, and as percentage relative to controls. Eight to 24 rats were used for each dose.

2.3. Statistics

The in vivo results (inhibition of 5-MeODMT-induced effects, inhibition of 8-OH-DPAT-induced discriminative stimulus and inhibition of the time spent emitting ultrasonic sounds) were expressed as ED_{50} values with 95% confidence limits, calculated by means of log-probit analysis. Furthermore ultrasonic vocalizations (total vocalization time, number of calls and mean duration of calls) were also expressed as means (± S.E.M.) and one-way analysis of variance (ANOVA) and post hoc comparisons of means were used for dose-response comparisons.

The receptor binding affinities were expressed as IC_{50} values in nM (logarithmic means). Two full concentration-response curves were measured using 5 concentrations of test drug in triplicate (covering 3 decades). In a series of 100 determinations the variance of the log ratio (log R) between the double determinations ((log $R)^2/2 \times 100$) was determined. In case the log ratio was greater than corresponding to 3 × sd (99% confidence interval) extra determinations were performed and outliers were discarded. An antilog (sd) of 1.4 and 2.1 were obtained for 5-HT_{1A} receptor and β-adrenoreceptor bindings, respectively.

2.4. Drugs

(−)-Penbutolol and (+)-penbutolol, molecular weight (mw) 292; 8-hydroxy-2-(di-*n*-propylamino)tetralin HBr (8-OH-DPAT), mw 328; (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide oxalate (WAY-100635), mw 513; 1-(trifluoromethylphenyl)piperazine dihydrochloride (TFMPP) mw 303; (−)-tertatolol mw 332 (all synthesized at Department of

Medicinal Chemistry, H. Lundbeck A/S, Denmark); 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) mw 218 and metoprolol tartrate mw 685 (both Sigma, St. Louis, MO, USA); *erythro*-D,L-1-(7-methylindan-4-yloxy)-3-isopropylamine-butan-2-ol hydrochloride (ICI 118,551) mw 314 (ICI, UK) were all dissolved in saline. Pindolol mw 248 (Dumex A/S, Denmark) was dissolved in minimum amounts of 0.1 M citric acid. *p*-Chlorophenylalanine methyl ester HCl, mw 249 (synthesized at Department of Medicinal Chemistry, H. Lundbeck A/S, Denmark) was dissolved in H_2O.

Injection volumes were 5 ml/kg for rats in studies of ultrasonic vocalization and 5-MeODMT inhibition and 1 ml/kg in drug discrimination studies. The route of administration was s.c.

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

3. Results

3.1. In vitro β-adrenoceptor and 5-HT$_{1A}$ receptor affinities

(−)-Penbutolol had high affinity in the nanomolar range for β-adrenoceptors and 5-HT$_{1A}$ receptors, whereas its (+)-isomer had about 60 times lower affinity for 5-HT$_{1A}$ receptors without change in β-adrenoceptor affinity (Table 1). Pindolol and (−)-tertatolol had high β-adrenoceptor affinity and about 10 times lower 5-HT$_{1A}$ receptor affinity. Metoprolol and ICI 118,551 had only affinity for β-adrenoceptors, and WAY 100.635 had only affinity for 5-HT$_{1A}$ receptors.

Table 1
In vitro β-adrenoceptor and 5-HT$_{1A}$ receptor affinities of 8-OH-DPAT, pindolol, (−)-tertatolol, (−)-penbutolol, (+)-penbutolol, metoprolol, ICI 118,551 and WAY 100.635

Drug	In vitro receptor affinity (IC_{50}, nM)	
	β-Adrenoceptors	5-HT$_{1A}$ receptors
8-OH-DPAT	> 100000	3.5
Pindolol	0.89	85
(−)-Tertatolol	0.66	94
(−)-Penbutolol	2.9	9.9
(+)-Penbutolol	5.3	300
Metoprolol	48	> 10000
ICI 118,551	36	20000
WAY 100.635	> 1000	2.2

For further details, see Materials and methods.

3.2. Antagonism of 5-MeODMT-induced 5-HT syndrome and 8-OH-DPAT-induced discriminative stimulus

(−)-Penbutolol and (−)-tertatolol antagonized 5-MeODMT-induced forepaw treading and 8-OH-DPAT-induced discriminative stimulus at very similar potencies (Tables 2 and 3). The potency of pindolol was weaker than those of (−)-penbutolol and (−)-tertatolol against 5-MeODMT-induced forepaw treading, and pindolol showed a mixed antagonistic and agonistic profile in the drug discrimination studies. The mixed $\beta_{1/2}$-adrenoceptor antagonist (+)-penbutolol and the β_2-adrenoceptor antagonist ICI 118,551 were inactive against 5-MeODMT-induced forepaw treading and 8-OH-DPAT-induced discriminative stimulus. The β_1-adrenoceptor antagonist metoprolol did not antagonize 5-MeODMT-induced forepaw treading, but generalized to and antagonized 8-OH-DPAT-induced discriminative stimulus partially at the same dose levels. WAY 100.635 was a very potent antagonist in both test models.

Table 2
Forepaw treading-inducing potencies and inhibitory potencies against 5-MeODMT-induced forepaw treading of ligands with varying efficacies at 5-HT$_{1A}$ receptors and β-adrenoceptors. Potencies are shown as ED_{50} values (μmol/kg and mg/kg) with 95% confidence intervals in parentheses and as maximum effects (Max, %)

Drug	5-HT syndrome			Antagonism of 5-MeODMT		
	ED_{50}		Max	ED_{50}		Max
	μmol/kg	mg/kg	%	μmol/kg	mg/kg	%
8-OH-DPAT	4.4 (2.8–7.0)	1.4	100	> 30	> 10	0
Pindolol	> 81	> 20	0	36 (19–68)	8.9	0
(−)-Tertatolol	> 30	> 10	0	11 (4.4–28)	3.7	100
(−)-Penbutolol	> 34	> 10	0	6.0 (2.5–14)	1.8	92
(+)-Penbutolol	> 34	> 10	0	> 34	> 10	19
Metoprolol	> 29	> 20	0	> 29	> 20	4
ICI 118,551	> 32	> 10	0	> 32	> 10	0
WAY 100.635	> 20	> 10	0	0.62 (0.24–1.6)	0.31	100

For further details, see Materials and methods.

Table 3
Generalization to and antagonism of 8-OH-DPAT-induced discriminative stimulus of ligands with varying efficacies at 5-HT_{1A} receptors and β-adrenoceptors. Potencies are shown as ED_{50} values (μmol/kg and mg/kg) with 95% confidence intervals in parentheses and as maximum effects (Max, %)

Drug	8-OH-DPAT-induced discriminative stimulus					
	Agonism, ED_{50}			Antagonism, ED_{50}		
	μmol/kg	mg/kg	Max %	μmol/kg	mg/kg	Max %
8-OH-DPAT	0.10 (0.063–0.16)	0.31	100	Not tested		–
Pindolol	67 (9.7–460)	17	59	14 (3.1–26)	3.5	81
(−)-Tertatolol	> 60	> 20	27	13 (3.4–49)	4.3	66
(−)-Penbutolol	> 69	> 20	24	15 (7.5–30)	4.4	81
(+)-Penbutolol	Not tested			> 69	> 20	5
Metoprolol	Approx. 29[1]	20	51	> 29[a]	> 20	49
ICI 118,551	Not tested			> 32	> 10	17
WAY 100.635	> 1.2	> 0.63	20	0.024 (0.0096–0.060)	0.012	95

For further details, see Materials and methods. [a]Significantly increased response latency.

3.3. Inhibition of footshock-induced ultrasonic vocalization in adult male rats

Pindolol antagonized footshock-induced ultrasonic vocalization by itself (Table 4). (−)-Tertatolol partially inhibited the vocalization 36% at 30 μmol/kg (10 mg/kg). (−)-Penbutolol, (+)-penbutolol, metoprolol and ICI 118,551 were all inactive against footshock-induced ultrasonic vocalization (Table 4). WAY 100.635 partially inhibited the vocalization (maximum effect 34% at 1.2 μmol/kg = 0.63 mg/kg, s.c.).

(−)-Penbutolol and WAY 100.635 reversed 8-OH-

Table 4
Inhibition of footshock-induced ultrasonic vocalization in adult rats and reversal of 8-OH-DPAT (0.24 μmol/kg)-induced inhibition of ultrasonic vocalization. Inhibitory potencies are assessed as inhibition of total vocalization time and shown as ED_{50} values (μmol/kg and mg/kg) with 95% confidence intervals in parentheses and as maximum effects (Max, %)

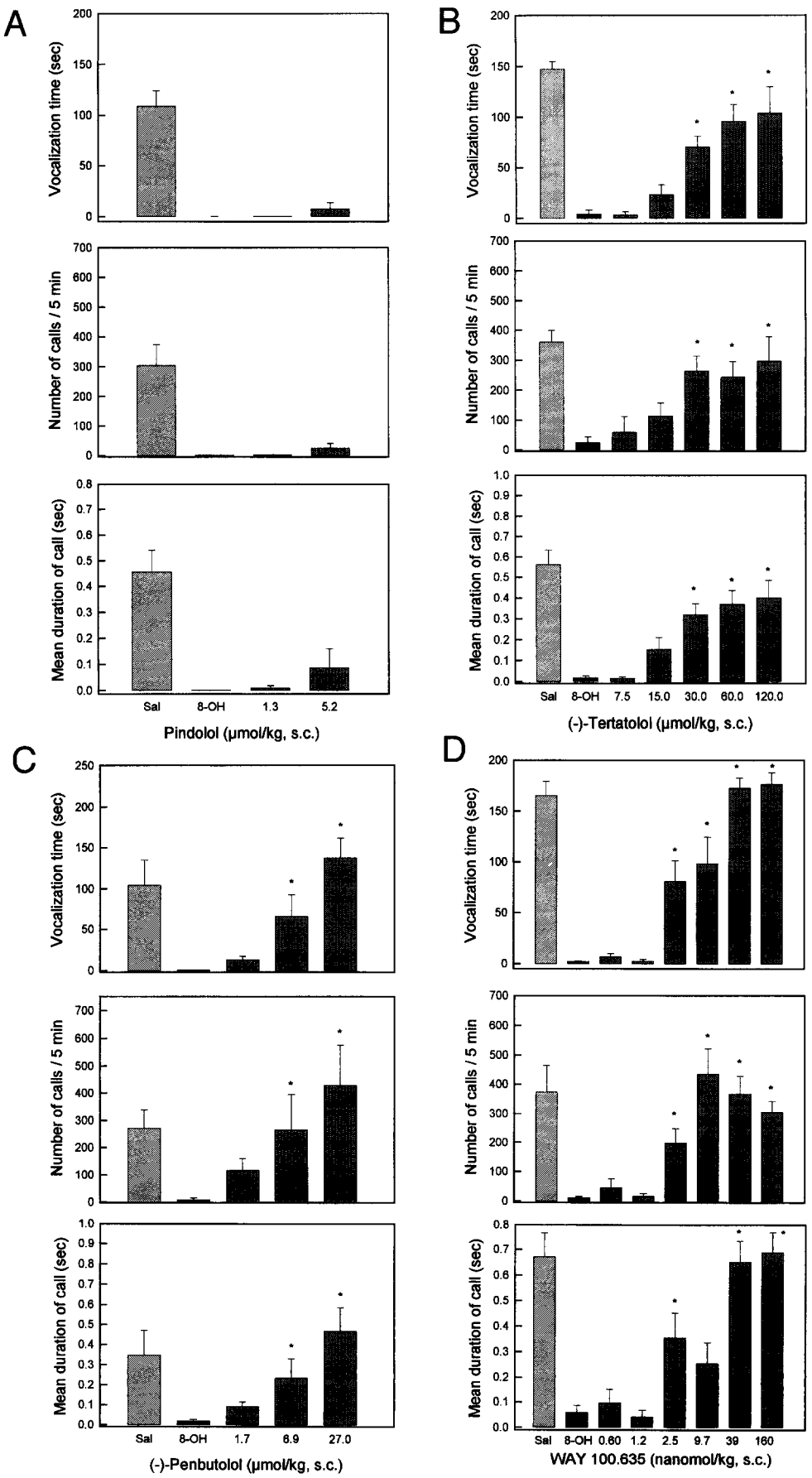
Drug	Inhibition of ultrasonic vocalization			Reversal of 8-OH-DPAT-induced inhibition of ultrasonic vocalization		
	ED_{50}		Max	ED_{50}		Max
	μmol/kg	mg/kg	%	μmol/kg	mg/kg	%
8-OH-DPAT	0.053 (0.038–0.074)[a]	0.017	100	–		–
Pindolol	5.9 (3.1–11)[a]	1.5	80	> 5.2	> 1.3	6
(−)-Tertatolol	> 60	> 20	36	42 (23–76)	14	52
(−)-Penbutolol	> 27	> 8.0	0	4.7 (3.1–7.1)	1.4	100
(+)-Penbutolol	> 27	> 8.0	0	> 27	> 8.0	10
Metoprolol	> 58[a]	> 40	0	> 58	> 40	0
ICI 118,551	> 32[a]	> 10	6	Not tested	–	–
WAY 100.635	> 39	> 20	34	0.0037 (0.0022–0.0063)	0.0019	100

For further details, see Materials and methods. [a]Sánchez, 1993.

Table 5
Inhibition of footshock-induced ultrasonic vocalization (total time) in adult rats and reversal of TFMPP (4.1 μmol/kg = 1.2 mg/kg) or pindolol (10 μmol/kg = 2.5 mg/kg)-induced inhibition of ultrasonic vocalization. Results are shown as ED_{50} values (μmol/kg and mg/kg) with 95% confidence interval in parentheses and as maximum effects (Max, %)

Drug	Inhibition of ultrasonic vocalization			Reversal of TFMPP-induced inhibition			Reversal of pindolol-induced inhibition		
	ED_{50}		Max	ED_{50}		Max	ED_{50}		Max
	μmol/kg	mg/kg	%	μmol/kg	mg/kg	%	μmol/kg	mg/kg	%
TFMPP	1.7 (1.2–2.4)	0.52	100	–	–	–	–	–	–
Pindolol	5.9 (3.1–11)	15	80	–	–	–	–	–	–
(−)-Penbutolol	> 27	> 8.0	0	2.5 (1.4–4.5)	0.73	88	3.4 (1.5–8)	1.0	93
WAY 100.635	> 39	> 20	34	> 1.2	> 0.63	0	0.029 (0.019–0.044)	0.057	100

For further details, see Materials and methods.


A
B
C
D
Vocalization time (sec)
Number of calls / 5 min
Mean duration of call (sec)
Sal
8-OH
1.3
5.2
Pindolol (μmol/kg, s.c.)
7.5
15.0
30.0
60.0
120.0
(-)-Tertatolol (μmol/kg, s.c.)
1.7
6.9
27.0
(-)-Penbutolol (μmol/kg, s.c.)
0.60
1.2
2.5
9.7
39
160
WAY 100.635 (nanomol/kg, s.c.)

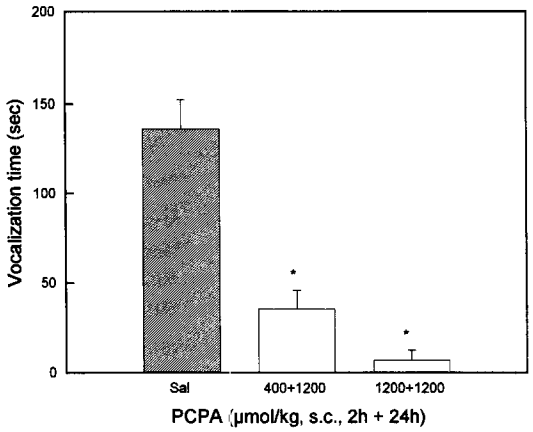


Fig. 2. Inhibition of footshock-induced ultrasonic vocalization in male rats by inhibition of 5-HT synthesis with PCPA. Data are presented as mean time spent vocalizing (±S.E.M.). PCPA was given 24 h (1200 μmol/kg = 300 mg/ kg, s.c.) and 2 h (400 or 1200 μmol/kg = 100 or 300 mg/kg, s.c.). Hatched bar, saline + saline; open bars, PCPA + PCPA. One minute after the last footshock ultrasonic vocalization was recorded for 5 min. $n = 16$ For further details see Methods. * $P < 0.05$; one-way ANOVA followed by post hoc comparison to a saline-treated control group (Dunnett's t-test).

DPAT-induced inhibition of ultrasonic vocalization completely (Table 4 and Fig. 1), WAY 100.635 showing high potency. (−)-Tertatolol reversed 8-OH-DPAT-induced inhibition of ultrasonic vocalization partially with a maximum effect of about 52%, whereas (+)-penbutolol and pindolol were inactive (Table 4 and Fig. 1). In addition to total duration of vocalization, Fig. 1 shows the number of ultrasonic calls and the mean duration of the calls. The dose-response relationships of these test parameters were very similar to those of the total duration of vocalization.

The 5-HT_1 receptor agonist TFMPP inhibited footshock-induced ultrasonic vocalization and the inhibitory effect of TFMPP (4.1 μmol/kg) was reversed by (−)-penbutolol (Table 5). In contrast, WAY 100.635 did not reverse the effects of TFMPP (4.1 μmol/kg). WAY 100.635 and (−)-penbutolol did also reverse the inhibitory effect of pindolol (10 μmol/kg = 2.5 mg/kg) (Table 5).

Treatment with the 5-HT depleting compound p-chlorophenylalanine methyl ester (1200 + 1200 μmol/kg = 300 + 300 mg/kg) 2 and 24 h before test abolished the ultrasonic response after footshock stimulation (Fig. 2).

4. Discussion

We have evaluated the 5-HT_{1A} receptor antagonistic potency of ligands with varying activity at 5-HT_{1A} receptors and β-adrenoceptors in three animal models reflecting 5-HT_{1A} receptor activity.

(−)-Penbutolol binds non-selectively to β_1- and β_2-adrenoceptors and is a partial agonist at peripheral β-adrenoceptors (Martindale, 1993). In the present study (−)-penbutolol had high affinity for β-adrenoceptors and 5-HT_{1A} receptors (Table 1) and acted as a 5-HT_{1A} receptor antagonist at both pre- and postsynaptic 5-HT_{1A} receptors. (−)-Penbutolol reversed agonist-induced effects completely in all three models and was inactive in the tests reflecting 5-HT_{1A} receptor agonistic activity (Tables 2–4). Microdialysis and behavioral studies (e.g., inhibition of 8-OH-DPAT-induced 5-HT syndrome or hypothermia) reported in the literature have also shown that (−)-penbutolol is an antagonist at both pre- and postsynaptic 5-HT_{1A} receptors (Hjorth, 1992; Hjorth and Sharp, 1993).

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 rat striatum (Langlois et al., 1993). In the present study the antagonistic potency of (−)-penbutolol was similar in reversing inhibition of ultrasonic vocalization induced by the non-selective 5-HT receptor agonist TFMPP and the 5-HT_{1A} receptor selective agonist 8-OH-DPAT, respectively (Tables 4 and 5). The in vitro selectivity of TFMPP is not very high (Glennon, 1992; 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 stimulus (Herndon et al., 1992). Studies on the hypophagic, hypolocomotor and anxiogenic effects of TFMPP suggest that 5-HT_{2C} receptors are involved in these in vivo effects. However, (−)-penbutolol has very low affinity for 5-HT_{2C} receptors ($IC_{50} = 2400$ nM; Frederiksen, personal communication). Thus, the inhibitory potency of TFMPP against footshock-induced ultrasonic vocalization is most likely to be mediated by 5-HT_{1B} receptors, as the selective 5-HT_{1A} receptor antagonist WAY-100635 was inactive. Furthermore, (−)-penbutolol has been shown to antagonize TFMPP-induced increase in escape attempts of isolated mice, a behavior

Fig. 1. Reversal of 8-OH-DPAT-induced inhibition of footshock-induced ultrasonic vocalization in adult male rats by pindolol (A), (−)-tertatolol (B), (−)-penbutolol (C) and WAY 100.635 (D). Data are presented as mean time spent vocalizing, number of calls and mean duration of calls (±S.E.M.) as a function of dose. Test compound or saline was given 30 min before test and 15 min before 8-OH-DPAT (0.24 μmol/kg, s.c.). Hatched bars, saline + saline; solid bars, saline + 8-OH-DPAT; double hatched bars, test drug + 8-OH-DPAT. One minute after the last footshock ultrasonic vocalization was recorded for 5 min. $n = 8$–24. For further details see Methods. * $P < 0.05$; one-way ANOVA followed by post hoc comparison to a saline-treated control group (Dunnett's t-test).

that has been shown to be mediated by 5-HT_{1B} receptors (Frances et al., 1994).

The (+)-enantiomer of penbutolol, which had similar affinity for β-adrenoceptors as the (−)-isomer, but about 60 times lower affinity for 5-HT_{1A} receptors, neither induced nor reversed 5-MeODMT-induced forepaw treading and was inactive in the ultrasonic vocalization models. This suggests that β-adrenoceptors are not involved in mediating these effects. This is further supported by the facts that the selective β_2-adrenoceptor antagonist ICI 118,551 (O'Donnell and Wanstall, 1980; Beer et al., 1988) and the β_1-adrenoceptor antagonist metoprolol (Rainbow et al., 1984) were inactive. In contrast, metoprolol both generalized to the 8-OH-DPAT-induced discriminatory stimulus and antagonized the 8-OH-DPAT-induced stimulus. The maximum effects of about 50% were achieved at the same dose levels, but only at doses that prolonged the latency time significantly. This lack of specific effects of β-adrenoceptor antagonists in the 8-OH-DPAT-induced discriminative stimulus paradigm agrees with the findings of Tricklebank et al. (1987).

Pindolol binds non-selectively to central β_1- and β_2-adrenoceptors and is a partial agonist at peripheral β-adrenoceptors (Rainbow et al., 1984; Martindale, 1993). In the present study pindolol had very high affinity for β-adrenoceptors and about 100 times lower affinity for 5-HT_{1A} receptors. It is intriguing that pindolol showed activity at 5-HT_{1A} receptors in vivo, whereas (+)-penbutolol, which had a slightly more favorable 5-HT_{1A} receptor/β-adrenoceptor selectivity ratio, was inactive. It might be that the in vivo selectivity ratios are different from the in vitro selectivities. Furthermore, different hydrophilicity and consequently differences in regional distribution of pindolol and penbutolol might play a role.

In the present study pindolol antagonized 5-MeODMT-induced forepaw treading completely suggesting that pindolol is an antagonist at the postsynaptic 5-HT_{1A} receptors involved in forepaw treading. This agrees with former findings in which *l*-5-HTP-induced 5-HT syndrome was antagonized by pindolol (Weinstock et al., 1977). Pindolol showed a mixed agonistic/antagonistic profile in the 8-OH-DPAT drug discrimination test. Pindolol antagonized 8-OH-DPAT completely, but at higher doses it generalized to 8-OH-DPAT discriminative stimulus with a maximum response of 59%. This might suggest that pindolol is acting as a partial 5-HT_{1A} receptor agonist at presynaptic 5-HT_{1A} receptors. However, the effect might also be interpreted as a non-specific effect due to the β-adrenoceptor antagonistic properties of pindolol as the response latency was significantly prolonged at the highest dose tested. Pindolol abolished footshock-induced ultrasonic vocalization by itself and did not reverse 8-OH-DPAT-induced inhibition of footshock-induced ultrasonic vocalization. Furthermore, pindolol-induced inhibition of footshock-induced ultrasonic vocalization was abolished by WAY 100635. Thus, pindolol acted like a 5-HT_{1A} receptor agonist in this model. Microdialysis studies also suggest that pindolol is a partial agonist with low efficacy at presynaptic 5-HT_1 receptors, as pindolol reversed 8-OH-DPAT-induced inhibition of 5-HT release but, unlike (−)-penbutolol, was unable to increase the 5-HT level by itself (Hjorth and Sharp, 1993).

Pindolol has high affinity for 5-HT_{1B} receptors (Langlois et al., 1993) and acts like a 5-HT_{1B} receptor agonist as it increased escape attempts of isolated mice and failed to antagonize TFMPP-induced increases (Frances et al., 1994). In the present study pindolol also failed to attenuate TFMPP-induced inhibition of ultrasonic vocalization (unpublished observation).

(−)-Tertatolol is a non-selective β-adrenoceptor antagonist at peripheral receptors (Martindale, 1993). The in vitro affinity for 5-HT_{1A} receptor was more than 100 times lower than for β-adrenoceptors. However, in the present study (−)-tertatolol antagonized 5-MeODMT-induced 5-HT syndrome completely suggesting that (−)-tertatolol is an antagonist at postsynaptic 5-HT_{1A} receptors. (−)-Tertatolol showed only a weak agonistic effect in the drug discrimination test and antagonized 8-OH-DPAT-induced discriminative stimulus with a maximum effect of 66% at doses that did not affect response latency. Furthermore, (−)-tertatolol inhibited the ultrasonic vocalization weakly and reversed 8-OH-DPAT-induced inhibition of ultrasonic vocalization partially, i.e., about 50%. Thus, (−)-tertatolol acted like a low efficacy 5-HT_{1A} receptor agonist in the two latter models. (−)-Tertatolol has high affinity for 5-HT_{1A}, but much lower affinity for 5-HT_{1B} receptors (Millan et al., 1994). Thus it is unlikely that the inhibition of footshock-induced ultrasonic vocalization is mediated by 5-HT_{1B} receptors. In the literature (−)-tertatolol is suggested to be an antagonist at both pre- and postsynaptic 5-HT_{1A} receptors in vivo. (−)-Tertatolol reduced the inhibitory influence of 8-OH-DPAT on the accumulation of 5-hydroxytryptophan after treatment with the *l*-amino acid decarboxylase inhibitor NSD-1015 and (−)-tertatolol prevented the inhibitory effects of 5-HT_{1A} receptor agonists on the firing rate of 5-HT neurons in the dorsal raphe nucleus (Prisco et al., 1993; Jolas et al., 1993).

WAY 100.635 is a selective and potent 5-HT_{1A} receptor antagonist at both pre- and postsynaptic situated receptors (Fletcher et al., 1994; Critchley et al., 1994). Accordingly WAY 100.635 reversed 5-MeODMT-induced 5-HT syndrome, 8-OH-DPAT-induced discriminative stimulus and 8-OH-DPAT-induced inhibition of footshock-induced ultrasonic vocalization completely and with high potency (Tables 2–4). WAY 100.635 by itself partially antagonized footshock-induced ultrasonic vocalization, but the effective doses were about 1000-fold higher than those necessary for reversal of the 5-HT_{1A} receptor agonist-induced effects (Table 4). The inhibitory effect might as well be ascribed to an α_1-adrenergic mechanism, as WAY 100.635 shows a selectivity ratio of about 50 between the affinities for 5-HT_{1A} receptors and α_1-adrenoceptors in in vitro binding

studies (unpublished observation). α_1-Adrenoceptor antagonists, e.g., prazosin, inhibit footshock-induced ultrasonic vocalization (Sánchez, 1993).

Both (−)-penbutolol and pindolol are partial agonists at peripheral β-adrenoceptors. It could be hypothesized that β-adrenoceptor stimulation interferes with effects mediated by 5-HT receptors, although the efficacy of the present compounds at the central β-adrenoceptors involved remains to be clarified. There is extensive anatomical and biochemical evidence for a close connection between noradrenergic and serotonergic systems (reviewed by Caldecott-Hazard et al., 1991) and a number of behavioral studies also suggest interactions between the two systems: β-adrenoceptor agonists like clenbuterol and salbutamol potentiate, and antagonists like oxprenolol attenuate 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 enhance 8-OH-DPAT-induced motor response (Kalkman and Soar, 1990). The β-adrenoceptor agonist clenbuterol partially generalized to 8-OH-DPAT drug discrimination with a maximum effect of 52% at 0.99 μmol/kg, but the response latency was increased significantly at the same dose (Arnt, unpublished). Clenbuterol was inactive against footshock-induced ultrasonic vocalization and did not affect 8-OH-DPAT-induced inhibition (Sánchez, 1993; unpublished observations). Clenbuterol is a β_2-selective agonist in peripheral tissues, but binding studies of rat brain tissue suggest that the selectivity is lost centrally (Beer et al., 1988). Thus, there is no evidence that β_1- or β_2-adrenergic stimulation influences serotonergically mediated effects of (−)-penbutolol and pindolol in these models.

The fact that low efficacy 5-HT_{1A} receptor active compounds, which inhibited 5-MeODMT-induced 5-HT syndrome, still showed agonistic activity in the ultrasonic vocalization model supports the assumption that presynaptic 5-HT_{1A} receptors play a key role in this model. It is characteristic for partial 5-HT_{1A} receptor agonists that they can antagonize the effect of full agonists at postsynaptic receptors while they activate the somatodendritic autoreceptors (reviewed by Fletcher et al., 1993). Recent studies suggest that the inhibitory effects of 5-HT_{1A} receptor agonists are mediated by presynaptic receptors in dorsal raphe nucleus (Schreiber and De Vry, 1993b; Jolas et al., 1995). In the present study pretreatment with *p*-chlorophenylalanine methyl ester (1200 μmol/kg 2 and 24 h before test), which profoundly depletes cerebral pools of 5-HT, also abolished the ultrasonic response after footshock stimulation. This supports that presynaptic mechanisms are involved in the serotonergically mediated effects. On the other hand, a previous study demonstrated that increased serotonergic activity achieved by treatment with the 5-HT releasing agent fenfluramine, the selective 5-HT reuptake inhibitor, citalopram, or the 5-HT precursor, *l*-5-HTP, decreased footshock-induced ultrasonic vocalization, too (Sánchez, 1993 and unpublished observation). These results may suggest that the effects are mediated by postsynaptic receptors. As both pre- and postsynaptically situated 5-HT receptor subtypes (e.g., 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptors) are involved in mediation of ultrasonic vocalization (Sánchez, 1993 and present study) this might account for the apparently contradictory results.

In conclusion, it is stated that efficacies at 5-HT_{1A} receptors can be estimated by applying a battery of in vivo test models that involve post- and presynaptic receptors to a variable degree. The in vivo ranking order of efficacy at 5-HT_{1A} receptors was: WAY 100.635 and (−)-penbutolol were antagonists at both pre- and postsynaptic receptors, (−)-tertatolol was a postsynaptic antagonist and a partial agonist at presynaptic receptors, pindolol was a postsynaptic antagonist and an agonist at presynaptic receptors.

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