Anpirtoline, a novel, highly potent 5-HT_{1B} receptor agonist with antinociceptive/antidepressant-like actions in rodents

E. Schlicker, *U. Werner, †M. Hamon, †H. Gozlan, *B. Nickel, *I. Szelenyi & ¹M. Göthert

Institute of Pharmacology and Toxicology, University of Bonn, Reuterstr. 2b, D-5300 Bonn 1, Germany; *Department of Pharmacology, ASTA Medica AG, POB 100105, D-6000 Frankfurt 1, Germany and †INSERM U288, Faculté de Médecine Pitié-Salpêtrière, 91 boulevard de l'Hôpital, 75634 Paris cedex 13, France

1 The purpose of the present study was to relate the effects of the novel drug, anpirtoline, on 5-hydroxytryptamine (5-HT) receptor subtypes to its antinociceptive and antidepressant-like actions in rodents.

2 Binding assays with rat brain membranes have shown that anpirtoline bound with a much higher affinity to 5-HT_{1B} receptors ($K_i = 28$ nM) than to 5-HT_{1A} ($K_i = 150$ nM) and 5-HT₂ ($K_i = 1.49$ μ M) receptors.

3 Like 5-HT, anpirtoline concentration-dependently inhibited forskolin-stimulated adenylate cyclase activity in homogenates from the rat substantia nigra. Both effects were not additive, and could be prevented by 5-HT_{1B} receptor antagonists such as propranolol and penbutolol.

4 In superfused rat and pig brain cortex slices preincubated with [³H]-5-HT, the electrically evoked tritium overflow was inhibited by anpirtoline and 5-HT. Whereas 5-HT was equipotent in both tissues ($EC_{50} = 69$ nM), anpirtoline was markedly less potent in pig brain cortex slices ($EC_{50} = 1190$ nM) than in rat brain cortex slices ($EC_{50} = 55$ nM). The concentration-response curve for anpirtoline was shifted to the right by metitepine in both preparations.

5 In the social behaviour deficit test, anpirtoline and trifluoromethylphenyl-piperazine were effective in reversing the isolation-induced impairments in mice, an effect shown only by compounds with agonist properties at the 5-HT_{1B} receptor.

6 In the electrostimulated pain test using mice, anpirtoline dose-dependently increased the pain threshold with an ED_{50} of 0.52 mg kg⁻¹, i.p. The antinociceptive activity of anpirtoline was abolished by pretreatment with cyproheptadine or propranolol.

7 In the forced swimming test in rats, anpirtoline induced a dose-related increase in swimming activity. With an ED_{50} value of 4.6 mg kg⁻¹, i.p., anpirtoline was 4 times more potent than the two standard compounds imipramine and desipramine. The decrease of immobility time or the increase of active periods in this model of behavioural despair is suggested to be characteristic of antidepressant drugs.

8 Anpirtoline exhibits both antinociceptive and antidepressant-like activities in animals. It is probable that anpirtoline elicits these pharmacological effects via its agonist effect on 5-HT_{1B} and 5-HT_{1A} receptors.

Keywords: Anpirtoline; 5-hydroxytryptamine receptors; analgesia; antidepressant activity; 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1A}-receptors

Introduction

Endogenous monoamines, particularly noradrenaline and 5-hydroxytryptamine (5-HT), have been studied in the context of both regulation of nociceptive threshold and the pathophysiology of depressive illness. In recent years, interest in 5-HT has undergone a renaissance. The discovery of new 5-HT receptors and our improved understanding of the functional role of these receptors have contributed to an intensification of research in this field (Whitaker-Azmitia & Peroutka, 1990).

The processing of pain impulses, their perception as painful information, and the organism's response to the perceived pain are highly complex and incompletely understood neurophysiological events. Alterations in brain 5-HT concentration caused by precursor loading or precursor restriction result in analgesia (Lin *et al.*, 1980) and hyperalgesia (Lytle *et al.*, 1975), respectively. It is also known that 5-HT *per se* and the stimulation of raphe nuclei where 5-hydroxytryptaminergic perikarya and dendrites are located increase the nociceptive threshold (Yaksh & Wilson, 1979). Moreover, the dorsal horn of the spinal cord is partly controlled by descending 5-hydroxytryptaminergic pathways (Le Bars, 1988).

Although there are some doubts concerning the altered monoamine metabolism in the pathophysiology of depression, it is likely that 5-HT may play a role in the development of

this mental disorder (Meltzer & Lowy, 1987). Many antidepressants in current use such as imipramine, amitriptyline and fluoxetine block the neuronal uptake of 5-HT (Chouinard, 1985). It has also been demonstrated that administration of amitriptyline was associated with analgesic effects in patients with migraine or postherpetic neuralgia (Taub, 1973; Couch *et al.*, 1976; Watson *et al.*, 1982).

Because anpirtoline (6-chloro-2-[piperidyl-4-thiol]-pyridine hydrochloride (ASTA Medica AG), a new chemical entity (Figure 1), exhibited antinociceptive and antidepressant-like properties in preliminary (unpublished) studies in rats and mice, further investigations have been carried out to examine the possible involvement of 5-hydroxytryptaminergic neurotransmission in the central mechanisms of action of this drug. *In vitro* studies on the possible interaction of anpirtoline with various 5-HT receptor subtypes and extensive behavioural studies strongly suggested that the clearcut antinociceptive and antidepressant-like effects of anpirtoline in mice and rats probably involved its agonist properties toward 5-HT_{1B} and/or 5-HT_{1A} receptors.

Figure 1 Chemical structure of anpirtoline.

¹ Author for correspondence at Bonn address.

Methods

In vitro studies

Receptor binding assays Adult male Sprague-Dawley rats weighing ~250 g were killed by decapitation and membranes from pooled hippocampi, striata or anterior cortices were prepared as described elsewhere (Hall *et al.*, 1985). Three subtypes of 5-HT receptors were investigated by use of binding assays: 5-HT_{1A} with [³H]-8-hydroxy-2-(di-n-propylamino) tetralin ([³H]-8-OH-DPAT) (100 Ci mmol⁻¹, Service des Molécules Marquées, CEA, Saclay, France) as radioligand; 5-HT_{1B} with [³H]-5-HT (12 Ci mmol⁻¹, Amersham, Buckinghamshire, U.K.) plus 0.1 μM 8-OH-DPAT and 0.1 μM mesulergine to prevent the binding of the ³H-amine to 5-HT_{1A} and 5-HT_{1C} sites, respectively; 5-HT₂ with [³H]-ketanserin (66.4 Ci mmol⁻¹, Amersham). Hippocampal, striatal and cortical membranes were used for studies on 5-HT_{1A}, 5-HT_{1B} and 5-HT₂ binding sites, respectively, following the protocols described in detail elsewhere (Adrien *et al.*, 1989).

The inhibition of the specific binding of each radioligand by anpirtoline was examined with at least 10 different concentrations of the drug, between 0.1 nM and 0.1 mM. Calculations of IC₅₀ and n_H values were described by McPherson (1983). The equation of Cheng & Prusoff (1973) was used for converting the IC₅₀s into K_is.

Measurements of adenylate cyclase activity Adenylate cyclase assays were performed on aliquots (10 μl, corresponding to about 5 μg of membrane protein) of substantia nigra homogenates (from male Sprague-Dawley rats) incubated for 20 min at 30°C with 0.5 mM [³²P]-α-ATP (10–20 Ci mmol⁻¹, Amersham) in the presence of 10 μM forskolin, 10 μM GTP and 0.1 M NaCl as described in detail elsewhere (Hamon *et al.*, 1988). The enzymatic activity is expressed as nmol [³²P]-adenosine 3':5'-cyclic monophosphate ([³²P]-cyclic AMP) formed per mg protein and per 20 min. All assays were performed as triplicates, and each experiment was repeated at least twice.

Investigations on brain slices Cerebral cortex slices (0.3 mm thick, 3 mm in diameter) were prepared from male Wistar rats and from pigs of either sex. The slices were incubated for 60 min in physiological salt solution at 37°C (for composition, see Schlicker *et al.*, 1989a) containing 0.1 μM [³H]-5-HT (12.5–30.0 Ci mmol⁻¹, New England Nuclear, Dreieich, Germany). Subsequently, the slices were superfused with physiological salt solution at 1 ml min⁻¹ (37°C; 110 min). Two periods of electrical field stimulation were applied to each slice after 40 and 90 min of superfusion; rectangular pulses of 50 mA and 2 ms were administered at a frequency of 3 Hz. At the end of the superfusion, the radioactivity of the slices (solubilized with Soluene, Packard, Downers Grove, IL, U.S.A.) and superfusate samples was determined by liquid scintillation counting (for details of the method, including calculation of stimulation-evoked tritium overflow, see Schlicker *et al.*, 1989a,b).

In vivo studies

Electrostimulated pain-test The electrostimulation test was performed using the procedure described by Blake *et al.* (1963). The test drug(s) or vehicle were administered to adult male NMRI mice and male Sprague-Dawley rats. After 30 min, each animal was placed in an arena in which square wave electrical stimulation could be applied via the mesh floor. The current was raised until the animal responded vocally. The antinociceptive activity of the compounds was expressed as the ratio (× 100) of the current (in mA) provoking a response in drug- and vehicle-treated animals.

Forced swimming test A situation of behavioural despair was created in the forced swimming test with female Sprague-

Dawley rats weighing 100–150 g. The rats were placed in a container containing about 15 l of water at 25°C. Unlike the method originally described by Porsolt *et al.* (1977) the swimming movements instead of the time of immobility were registered. The swimming activity of the rats was measured by an electronic motor activity monitor (Rhema Labortechnik, Hochheim, F.R.G.). This device records the changes of electrical inductance in an electrical field evoked by swimming movements of the animal. The mean activity over a 5 min test period caused by swimming or diving of the animals is expressed in number of impulses min⁻¹.

Social behaviour deficit test The behavioural deficit test was performed according to Frances (1988) with the male NMRI mice (25–35 g) housed either in groups of 10 per cage or isolated (1 per cage) for 1 week before being tested. One group-housed mouse and one isolated mouse identically treated either with saline or with the test compound were placed together under an inverted glass beaker (diameter = 10 cm). For both mice the number of attempts to escape from this situation were counted for 3 min. The activity of the isolated mice is expressed as a percentage related to their group-housed counterparts. In this experiment a non-significant difference between grouped and isolated mice stands for the reversal of isolation-induced deficit by a compound.

Statistical analysis

In *in vivo* investigations, effective doses (ED₅₀) were calculated by regression analysis. To compare two mean values, Student's *t* test for unpaired samples was used (Cavalli-Sforza, 1969).

In *in vitro* studies, results are given as means ± s.e.mean. For comparison of mean values, Student's *t* test was used; Bonferroni's procedure was employed if two or more experimental groups were compared with the same control series. The apparent pA₂ value for metitepine (against anpirtoline) was calculated according to Furchgott (1972).

Drugs

Anpirtoline HCl was synthesized by the Department of Chemical Research, ASTA Medica AG, Frankfurt, Germany. In addition, the following drugs were used: cyproheptadine HCl, imipramine HCl, desipramine HCl, (±)-propranolol (Sigma, Munich, Germany); tramadol (Tramal, Chemie Grünenthal, Aachen, Germany); buprenorphine (Temgesic, Boehringer Mannheim, Mannheim, Germany); citalopram HCl (Lundbeck, Copenhagen, Denmark); indalpine (Rhône-Poulenc Santé, Gennevilliers, France); metitepine maleate (Hoffmann-La Roche, Basel, Switzerland); phentolamine methane sulphonate (CIBA-Geigy, Wehr, Germany); 5-hydroxytryptamine creatinine sulphate (Merck, Darmstadt, Germany); mesulergine (Sandoz, Basel, Switzerland); trifluoromethylphenyl-piperazine (TFMPP, Interchim, Montluçon, France); (±)-penbutolol (Hoechst, Puteaux, France); MK-212 (6-chloro-2-(1-piperazinyl) pyrazine HCl, Merck, Sharp and Dohme, Munich, Germany); 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, Research Biochem. Inc., Natick, Ma, U.S.A.); cinanserin (Squibb, Princeton, New Jersey, U.S.A.).

Results

Receptor binding studies

Anpirtoline bound differentially to the three 5-HT receptor subtypes 5-HT_{1A}, 5-HT_{1B} and 5-HT₂. The drug exhibited a higher affinity for 5-HT_{1B} sites (K_i = 28 ± 4 nM, n = 3) in striatal membranes than for 5-HT_{1A} sites (K_i = 150 ± 17 nM, n = 3) in hippocampal membranes. As shown in Figure 2, its potency in inhibiting [³H]-ketanserin specific binding to cortical membranes was markedly less than against [³H]-8-OH-

Figure 2 Concentration-dependent inhibition by anpirtoline of the specific binding of [3 H]-5-hydroxytryptamine ([3 H]-5-HT) to 5-HT_{1B} sites, [3 H]-8-hydroxy-2-(di-n-propylamino)tetralin ([3 H]-8-OH-DPAT) to 5-HT_{1A} sites, and [3 H]-ketanserin to 5-HT₂ sites, in rat brain membranes. Binding assays were performed with: 5-HT_{1B} sites—striatal membranes, 2.3 nM [3 H]-5-HT plus 0.1 μ M 8-OH-DPAT and 0.1 μ M mesulergine; non specific binding was determined in the presence of 10 μ M 5-HT; 5-HT_{1A} sites—hippocampal membranes, 0.8 nM [3 H]-8-OH-DPAT; non specific binding was determined in the presence of 10 μ M 5-HT; 5-HT₂ sites—cortical membranes, 0.8 nM [3 H]-ketanserin; non specific binding was determined in the presence of 1 μ M cinanserin. Each point is the mean (vertical bars show s.e.mean) of triplicate determinations in a typical experiment (100%: 5-HT_{1B}: 102.6 fmol [3 H]-5-HT specifically bound mg⁻¹ membrane protein; 5-HT_{1A}: 112.6 fmol [3 H]-8-OH-DPAT mg⁻¹ membrane protein; 5-HT₂: 147.6 fmol [3 H]-ketanserin mg⁻¹ membrane protein). This experiment was repeated 3 times for the calculation of K_i values indicated in the text.

DPAT and [3 H]-5-HT specific binding to 5-HT_{1A} and 5-HT_{1B} sites, respectively, indicating a lower affinity ($K_i = 1.49 \pm 0.22 \mu$ M, $n = 3$) for 5-HT₂ sites. In all cases, concentration-dependent inhibitions of the specific binding of 3 H-ligands occurred with apparent Hill coefficients close to 1.0.

Adenylate cyclase

As shown in Figure 3, the inhibition of forskolin-stimulated adenylate cyclase activity by 5-HT was concentration-dependent, with a maximal effect (-22%) for 1–10 μ M of the indoleamine, and an EC_{50} of about 20 nM. Anpirtoline also exerted a concentration-dependent inhibition of [32 P]-cyclic AMP formation, but to a lesser extent than 5-HT. Thus only a

Figure 3 Concentration-dependent inhibition of forskolin-stimulated adenylate cyclase activity by 5-hydroxytryptamine (5-HT, $\blacksquare$) and anpirtoline ($\square$) in rat substantia nigra homogenates. Assays were performed with 0.5 mM [32 P]-alpha-ATP, 10 μ M forskolin, 10 μ M GTP and 0.1 M NaCl (see Hamon *et al.*, 1988), plus various concentrations of 5-HT, or anpirtoline (abscissa scale). The formation of [32 P]-cyclic AMP is expressed as nmol mg⁻¹ protein 20 min⁻¹ (at 30°C). Each point represents the mean (vertical bars show s.e.mean) of triplicate determinations in three independent experiments. The grey area corresponds to the range of control values (assays without 5-HT or anpirtoline, C on the abscissa scale).

13% reduction of the enzymatic activity was observed in the presence of 10 μ M anpirtoline (Figure 3).

As expected from an effect through the stimulation of 5-HT receptors, the inhibition of forskolin-stimulated adenylate cyclase by anpirtoline was not additive with that due to a saturating concentration (1 μ M) of 5-HT (Table 1). Furthermore, the mixed 5-HT_{1A}, 5-HT_{1B} receptor antagonists ($\pm$)-penbutolol (1 μ M) and ($\pm$)-propranolol (1 μ M) prevented the inhibitory effect of both 5-HT (1 μ M) and anpirtoline (10 μ M) (Table 1). Finally, the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT (10 nM–1 μ M) did not affect the formation of [32 P]-cyclic AMP by substantia nigra homogenates.

In vitro [3 H]-5-hydroxytryptamine release from cortex slices

The effect of anpirtoline on the electrically evoked tritium overflow from rat and pig brain cortex slices was compared to that of 5-HT which had previously been examined under identical experimental conditions (Schlicker *et al.*, 1989a,b). The superfusion medium contained an inhibitor of 5-HT uptake (10 μ M citalopram or 10 μ M indalpine) and, in experiments on pig brain cortex slices, phentolamine (3.2 μ M).

Table 1 Blockade by penbutolol and propranolol of the inhibitory effect of anpirtoline and/or 5-hydroxytryptamine (5-HT) on forskolin-stimulated adenylate cyclase in substantia nigra homogenates and lack of effect of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) on the enzymatic activity

Addition		[32 P]-cyclic AMP (nmol mg ⁻¹ protein)		
		None	Penbutolol (1 μ M)	Propranolol (1 μ M)
None		4.36 $\pm$ 0.11	4.52 $\pm$ 0.12	4.55 $\pm$ 0.13
Anpirtoline	10 μ M	3.81* $\pm$ 0.11	4.31 $\pm$ 0.10	4.36 $\pm$ 0.09
5-HT	1 μ M	3.54* $\pm$ 0.06	4.26 $\pm$ 0.12	4.30 $\pm$ 0.14
{ Anpirtoline + 5-HT	10 μ M	3.62* $\pm$ 0.10	4.38 $\pm$ 0.11	4.32 $\pm$ 0.12
	1 μ M			
8-OH-DPAT	1 μ M	4.30 $\pm$ 0.08	4.56 $\pm$ 0.14	4.58 $\pm$ 0.11

Assays were performed as described in the legend to Figure 3. Each value (in nmol [32 P]-cyclic AMP synthesized mg⁻¹ protein 20 min⁻¹ at 30°C) is the mean $\pm$ s.e.mean of triplicate determinations in at least three separate experiments.

* $P < 0.05$ when compared to [32 P]-cyclic AMP formation in the absence of agonists (no addition).

Table 2 Antinociceptive activity of anpirtoline in comparison with buprenorphine and tramadol in electrostimulated pain test in mice and rats

Compound	n Species	Doses (mg kg ⁻¹)	Route	Nociceptive threshold (% of controls ¹)	ED ₅₀ ² (mg kg ⁻¹)
Anpirtoline	10 mice	1.5	p.o.	121 ± 4*	2.8
		3.0	p.o.	152 ± 7**	(2.3–3.4)
		6.0	p.o.	184 ± 6***	
Anpirtoline	10 rats	1.0	p.o.	127 ± 7*	1.86
		2.0	p.o.	148 ± 6**	(1.4–2.4)
		4.0	p.o.	184 ± 6***	
Tramadol	10 mice	10.0	p.o.	114 ± 3 NS	25.7
		20.0	p.o.	139 ± 5**	(22.3–28.6)
		40.0	p.o.	168 ± 8**	
Anpirtoline	10 mice	0.25	i.p.	125 ± 4**	0.52
		0.5	i.p.	150 ± 3**	(0.41–0.63)
		1.0	i.p.	171 ± 5***	
Buprenorphine	10 mice	0.05	i.p.	122 ± 4**	0.1
		0.1	i.p.	150 ± 7**	(0.08–0.12)
		0.2	i.p.	170 ± 6***	
Anpirtoline + cyproheptadine	10 mice	6.0	p.o.	117 ± 3 #	—
		12.0	i.p.		
Anpirtoline + propranolol	10 mice	6.0	p.o.	121 ± 5 #	—
		5.0	i.p.		

¹ Control animals received 1% methocel p.o. or 0.9% NaCl i.p. Values represent means ± s.e.mean of *n* animals. * *P* < 0.005; ** *P* < 0.01; *** *P* < 0.001, NS, not significantly different from nociceptive threshold of methocel- (p.o.) or saline- (i.p.) treated animals (= 100%).

P < 0.01 significantly different from anpirtoline 6 mg kg⁻¹, p.o., alone.

Significance determined by Student's *t* test.

² ED₅₀ is given with 95% confidence limits.

Anpirtoline did not affect basal tritium efflux (results not shown), but concentration-dependently inhibited the electrically evoked tritium overflow from rat (Figure 4a) and pig (Figure 4b) brain cortex slices. The maximum inhibitory effects were approximately 55 and 50%, respectively (Figure 4). The maximum degree of inhibition obtainable with 5-HT tended to be higher (by 65–70%) but the difference between the maximal effects due to both compounds was not significant (*P* > 0.05). The respective pEC₅₀ values for anpirtoline and 5-HT were 7.25 and 7.16 with rat brain cortex slices and 5.93 and 7.16 with pig brain cortex slices. The concentration-response curve for anpirtoline was shifted to the right by metitepine in either tissue (Figure 4). The apparent pA₂ values of metitepine were 7.03 and 6.71 for rat and pig brain cortex slices, respectively.

Effect of anpirtoline on nociception

As shown in Table 2, both oral and intraperitoneal administration of anpirtoline resulted in a dose-dependent increase in the nociceptive threshold of electrical stimulation in mice and/or rats. After intraperitoneal administration, the antinociceptive-like activity of 0.25–1.0 mg kg⁻¹ anpirtoline was similar to that induced by 0.05–0.2 mg kg⁻¹ buprenorphine, a strong centrally acting antinociceptive opiate compound (Nickel & Aledter, 1987). Via the oral route, anpirtoline was more potent than tramadol (also a strong analgesic of the opiate type) as doses about 10 times higher of the latter drug were required to produce the same increase in the nociceptive threshold as anpirtoline (Table 2; see also Nickel & Aledter, 1987). The antinociceptive activity of anpirtoline was not influenced by naloxone or idazoxan (not shown). However, it was abolished after pretreatment of the animals with cyproheptadine or propranolol (Table 2).

Antidepressant-like activity of anpirtoline

Anpirtoline significantly increased the swimming activity in female rats with an ED₅₀ of 4.6 mg kg⁻¹, i.p. The two refer-

Figure 4 Effects of anpirtoline (○) and 5-hydroxytryptamine (□) (5-HT) on the electrically evoked tritium overflow from superfused rat (a) and pig (b) brain cortex slices, and interaction of anpirtoline with metitepine (●). Anpirtoline or 5-HT was added to the medium from the 62nd min of superfusion onward whereas the following drugs were present throughout the superfusion: rat brain cortex slices (a)—10 μM citalopram (5-HT uptake inhibitor) and, if relevant, 3.2 μM metitepine; pig brain cortex slices (b)—10 μM indalpine (5-HT uptake inhibitor), 3.2 μM phentolamine, and, if relevant, 1 μM metitepine. Tritium overflow was evoked twice, after 40 and 90 min of superfusion (S₁, S₂), and the ratio of the overflow evoked by S₂ over that evoked by S₁ was determined (S₂/S₁). The evoked tritium overflow is given as a percentage of the ratio S₂/S₁ in the corresponding controls. Tritium overflow evoked by S₂ in the control series related to anpirtoline was (in % of tissue tritium): 6.78 ± 0.69 and 11.17 ± 1.83 (means ± s.e.mean, *n* = 7 each, *P* < 0.05) in rat brain cortex slices superfused in the absence and presence of metitepine, respectively. The respective values for pig brain cortex slices were 3.54 ± 0.49 and 5.67 ± 0.89 (means ± s.e.mean, *n* = 7 each, *P* < 0.05). Means of 4–9 experiments (s.e.mean shown by vertical lines); * *P* < 0.05; ** *P* < 0.005.

Table 3 Antidepressant-like activity of anpirtoline in the forced swimming test in rats

Compound	n	Doses (mg kg ⁻¹)	Route	Animal activity (% of controls)	ED ₅₀ ¹ (mg kg ⁻¹)
Anpirtoline	10	2	i.p.	111.5 NS	4.6
	10	4	i.p.	144.2**	(4.1–5.2)
	10	8	i.p.	177.5**	
Imipramine	10	20	i.p.	150**	—
Desipramine	10	20	i.p.	156***	—

** $P < 0.01$; *** $P < 0.001$, NS, not significantly different from saline-treated rats (100%); *n*, number of rats. Significance determined by Student's *t* test

¹ ED₅₀ is given with 95% confidence limits.

ence compounds imipramine (20 mg kg⁻¹, i.p.) and desipramine (20 mg kg⁻¹, i.p.), as expected, also increased the swimming activity by 50% and 56% over the controls, respectively (Table 3).

Social behaviour

In the social behaviour deficit test, active compounds show a bell-shaped dose-response curve (Frances, 1988). Saline-treated isolated mice make about half as many attempts to find a way out of a confined situation as do saline-treated grouped mice. Active compounds are able to reverse this behavioural deficit when the treated isolated mice act similarly to their group-housed counterparts within the same trial (Frances, 1988). In this study, imipramine (8–32 mg kg⁻¹, i.p.) and desipramine (5–20 mg kg⁻¹, i.p.) were not able to reverse the isolation-induced behavioural deficit or even increased it (Table 4). In contrast, anpirtoline (2.5 mg and 5 mg kg⁻¹, i.p.) and the 5-HT_{1B} receptor agonist, TFMPP (Titeler *et al.*, 1987; Schechter, 1988), at 1–4 mg kg⁻¹, i.p., increased the number of escape attempts in isolated mice to a level not significantly different from that of grouped mice (Table 4). As shown in Table 4, pretreatment with the mixed 5-HT_{1A}, 5-HT_{1B} receptor antagonist (±)-propranolol (5 mg kg⁻¹, i.p.) prevented the effects of anpirtoline and TFMPP in this test.

Discussion

Anpirtoline, a new chemical entity which is not related to known analgesic and/or antidepressant drugs, has been

demonstrated to have a relatively high affinity for 5-HT_{1B} binding sites and to influence forskolin-stimulated adenylate cyclase activity in the rat substantia nigra. In agreement with previous data (Bouhelal *et al.*, 1988; Hamon *et al.*, 1990b), the 5-HT_{1A} receptor agonist, 8-OH-DPAT, did not mimic 5-HT, but mixed 5-HT_{1A}, 5-HT_{1B} receptor antagonists such as (±)-penbutolol and (±)-propranolol prevented the inhibitory effect of both the indoleamine and anpirtoline on this enzymatic activity. These results further supported the view that the 5-HT receptor negatively coupled with adenylate cyclase in the rat substantia nigra is the 5-HT_{1B} receptor subtype. Indeed, Bouhelal *et al.* (1988) and Hamon *et al.* (1990b) have already shown that in addition to 5-HT, 5-HT_{1B} receptor agonists such as RU-24969, TFMPP and CGS-12066B inhibited forskolin-stimulated adenylate cyclase activity in the rat substantia nigra. Accordingly, anpirtoline could be considered as a 5-HT_{1B} receptor agonist in the rat brain. Although the inhibition of forskolin-activated adenylate cyclase was less with anpirtoline than with 5-HT at the highest concentration tested (10 μM), it cannot be concluded that anpirtoline is a partial agonist because, in contrast to what is expected for such a drug, anpirtoline (10 μM) did not reduce the inhibitory effect of 5-HT on adenylate cyclase activity (Table 1).

Further characterization of anpirtoline was carried out in two functional models, i.e. in superfused rat and pig brain cortex slices preincubated with [³H]-5-HT. The electrically evoked tritium overflow in either tissue is tetrodotoxin-sensitive and Ca²⁺-dependent and, thus, represents quasi-physiological 5-HT release (Fink *et al.*, 1988; Göthert, 1980). 5-HT release in either tissue is subject to modulation via inhibitory presynaptic autoreceptors (Moret, 1985; Timmer-

Table 4 Effects of anpirtoline and other drugs on isolation-induced social behaviour deficit in mice

Compound	n	Dose (mg kg ⁻¹ , i.p.)	Number of escape attempts (mean ± s.e.mean)		%
			Grouped mice	Isolated mice	
NaCl 0.9%	99	—	32.6 ± 1.5	17.5 ± 0.9***	54
Imipramine	8	8	45.6 ± 7.2	19.0 ± 2.9**	42
	8	16	38.4 ± 4.1	22.9 ± 5.0*	60
	8	32	51.4 ± 4.0	7.9 ± 3.0***	15
	8	5	34.1 ± 6.6	11.6 ± 2.7**	34
Desipramine	8	10	27.9 ± 3.0	16.0 ± 2.7*	57
	8	20	29.9 ± 4.3	8.4 ± 1.1***	28
	21	1.25	31.2 ± 1.9	21.9 ± 2.4**	70
Anpirtoline	21	2.5	31.3 ± 2.4	27.7 ± 3.5 NS	89
	21	5	31.7 ± 3.6	24.8 ± 3.2 NS	78
	8	1	45.9 ± 4.5	46.8 ± 10.5 NS	102
TFMPP	8	2	32.6 ± 3.7	44.5 ± 5.4 NS	136
	8	4	34.8 ± 4.7	29.0 ± 3.6 NS	83
Propranolol		5			
+ anpirtoline	8	2.5	24.9 ± 4.2	10.6 ± 2.7*	43
Propranolol		5			
+ TFMPP	8	2	33.0 ± 4.4	22.7 ± 3.4*	69

Duration of isolation: 7 days; significance determined by Student's *t* test: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significantly different from grouped controls; for each condition, the percentage of escape attempts in isolated vs. grouped mice is indicated in the right hand column. *n*, number of mice.

TFMPP = trifuoromethylphenyl-piperazine.

mans & Thoolen, 1987; Middlemiss, 1988; Starke *et al.*, 1989). The autoreceptor could be classified as a 5-HT_{1B} receptor in the rat brain and as a 5-HT_{1D} receptor in the pig brain (Engel *et al.*, 1986; Schlicker *et al.*, 1989b; Hoyer & Middlemiss, 1989; Starke *et al.*, 1989). Like 5-HT_{1B} and 5-HT_{1D} receptor agonists, anpirtoline inhibited the electrically evoked tritium overflow from rat and pig brain cortex slices. Furthermore, the rightward shift of its concentration-response curve by metitepine in either tissue supported the view that this effect was mediated via the stimulation of 5-HT_{1B} and 5-HT_{1D} receptors, respectively. The potency of anpirtoline was similar to that of 5-HT in rat brain cortex slices, but markedly lower (by more than one order of magnitude) than that of the indoleamine in pig brain cortex slices.

Of the three behavioural tests which have been investigated in the present study, two are especially responsive to 5-HT_{1B} receptor agonists, i.e. the isolation-induced social behaviour deficit in mice (Frances, 1988; Frances *et al.*, 1990) and the vocalization elicited by nociceptive electrical stimulation (see Hamon *et al.*, 1990a). In agreement with the inferences from the *in vitro* studies, anpirtoline also behaved as a 5-HT_{1B} receptor agonist in these two tests since it reversed the isolation-induced social behaviour deficit in mice, as did the 5-HT_{1B} receptor agonist TFMPP (Titeler *et al.*, 1987; Schechter, 1988; see Table 4), and increased the nociceptive threshold in the electrostimulated pain-test, as previously found with the potent 5-HT_{1B} receptor agonists, TFMPP and *m*-chlorophenyl-piperazine (Zemlan *et al.*, 1988). In addition to 5-HT_{1B} receptor agonists, opiates and α_2 -adrenoceptor agonists also exhibit antinociceptive properties in relevant animal tests (Sullivan *et al.*, 1987; Yaksh & Stevens, 1988). However, opiate receptors and α_2 -adrenoceptors were very probably not involved in the antinociceptive effect of anpirtoline since neither the opiate receptor antagonist, naloxone, nor the α_2 -adrenoceptor antagonist, idazoxan, reversed this effect. Conversely, 5-HT receptor antagonists such as cyproheptadine and ($\pm$)-propranolol abolished the anpirtoline effect. Similarly ($\pm$)-propranolol prevented the reversal by anpirtoline of isolation-induced social behaviour deficit in mice. Although further investigations have to be performed with selective 5-HT_{1B} receptor antagonists (which, unfortunately, are not yet available) for a definitive demonstration, the data obtained so far strongly support the view that anpirtoline acts *in vitro* and *in vivo* as 5-HT_{1B} receptor agonist in both rats and mice.

Another interesting property of anpirtoline is its antidepressant-like action in the forced swimming test (Porsolt *et al.*, 1977) in rats. Indeed, anpirtoline was at least as potent as classical antidepressants such as imipramine and desipramine in reducing the immobility time. However the efficient doses of anpirtoline in this test (2–8 mg kg⁻¹, i.p.) were generally higher than those required to elicit antinociceptive activity (0.25–1.0 mg kg⁻¹, i.p.; Table 2) and reversing the isolation-induced social behaviour deficit (maximal reversion was obtained with 2.5 mg kg⁻¹, i.p. of anpirtoline, see Table 4). These data are compatible with the possibility that different receptors might be responsible for the antidepressant-like action of anpirtoline on the one hand and its antinociceptive effect and the ability to reverse isolation-induced social behaviour deficit on the other hand. Interestingly, the binding studies have shown that anpirtoline bound not only to 5-HT_{1B} receptors but also to 5-HT_{1A} receptors, and the ratio of its respective K_i values for these two receptors: $K_i(5-HT_{1A})/K_i(5-HT_{1B}) = 5.4$, was approximately in the same range as that of the efficient doses in the forced swimming test over those in the other two behavioural tests. As 5-HT_{1A} receptor agonists exhibit potent antidepressant-like activity in relevant tests in rodents (Wieland & Lucki, 1990; Martin *et al.*, 1990; Martin & Puech, 1991), it might be tentatively proposed that the stimulation of central 5-HT_{1A} receptors accounted for the antidepressant-like properties of anpirtoline. However, further studies are necessary to prove or reject this hypothesis.

In conclusion, anpirtoline is a new 5-HT receptor agonist acting preferentially on central 5-HT_{1B} receptors in rodents. Its potent antinociceptive and antidepressant-like properties and ability to reverse isolation-induced social behaviour deficit further support the theory that central 5-HT neurotransmission plays a major role in the control of pain and mood (see Meltzer & Lowy, 1987; Le Bars, 1988).

We thank the Service des Molécules Marquées (CEA), CIBA-Geigy, Hoffmann-La Roche, Lundbeck, Rhône-Poulenc Santé and Sandoz for kindly providing [³H]-8-OH-DPAT, phentolamine, metitepine, citalopram, indalpine and mesulergine, respectively.

We also thank Mrs H. Kotowsky, Mrs C. Müller, Mrs D. Petri and Mr A. Aledter for their skilled technical assistance and Mrs U. Kolb for typing the manuscript.

References

- ADRIEN, J., LANFUMEY, L., GOZLAN, H., FATTACCINI, C.M. & HAMON, M. (1989). Biochemical and electrophysiological evidence for an agonist action of CM 57493 at pre- and postsynaptic 5-hydroxytryptamine_{1A} receptors in brain. *J. Pharmacol. Exp. Ther.*, **248**, 1222–1230.
- BLAKE, L., GRAEME, M.L. & SIGG, E.B. (1963). Grid shock test for analgesic assay in mice. *Med. Exper.*, **9**, 146–150.
- BOUHELAL, R., SMOUNYA, L. & BOCKAERT, J. (1988). 5-HT_{1B} receptors are negatively coupled with adenylate cyclase in rat substantia nigra. *Eur. J. Pharmacol.*, **151**, 189–196.
- CAVALLI-SFORCA, L. (1969). *Biometrie, Grundzüge biologisch-medizinischer Statistik*. Stuttgart: G. Fischer Verlag.
- CHENG, Y.C. & PRUSOFF, W.H. (1973). Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.*, **22**, 3099–3108.
- CHOUINARD, G. (1985). A double-blind controlled clinical trial of fluoxetine and amitriptyline in the treatment of outpatients with major depressive disorder. *J. Clin. Psychiat.*, **46**, 32–37.
- COUCH, J.R., ZIERGLER, D.R.T. & HASSANEIN, R. (1976). Amitriptyline in the prophylaxis of migraine. *Neurology*, **26**, 121–127.
- ENGEL, G., GÖTHERT, M., HOYER, D., SCHLICKER, E. & HILLENBRAND, K. (1986). Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **332**, 1–7.
- FINK, K., SCHLICKER, E., BETZ, R. & GÖTHERT, M. (1988). Identification of presynaptic 5-HT₁ autoreceptors in pig brain cortex synaptosomes and slices. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **338**, 14–18.
- FRANCES, H. (1988). New animal model of social behaviour deficit: reversal by drugs. *Pharmacol. Biochem. Behav.*, **29**, 467–470.
- FRANCES, H., LIÉNARD, C. & FERMANIAN, J. (1990). Improvement of the isolation-induced social behavioural deficit involves activation of the 5-HT_{1B} receptors. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.*, **14**, 91–102.
- FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Handbook of Experimental Pharmacology*, Vol. XXXIII. ed. Blaschko, H. & Muscholl, E. pp. 283–335. Berlin: Springer Verlag.
- GÖTHERT, M. (1980). Serotonin-receptor-mediated modulation of Ca²⁺-dependent 5-hydroxytryptamine release from neurones of the rat brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **314**, 223–230.
- HALL, M., EL MESTIKAWY, S., EMERIT, M.B., PICHAT, L., HAMON, M. & GOZLAN, H. (1985). [³H]8-hydroxy-2-(di-n-propylamino)tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J. Neurochem.*, **44**, 1685–1696.
- HAMON, M., FATTACCINI, C.M., ADRIEN, J., GALLISSOT, M.C., MARTIN, P. & GOZLAN, H. (1988). Alterations of central serotonin and dopamine turnover in rats treated with ipsapirone and other

- 5-HT_{1A} agonists with potential anxiolytic properties. *J. Pharmacol. Exp. Ther.*, **246**, 745–752.
- HAMON, M., COLLIN, E., CHANTREL, D., DAVAL, G., VERGÉ, D., BOURGOIN, S. & CESSÉLIN, F. (1990a). Serotonin receptors and the modulation of pain. In *Serotonin and Pain*. ed. Besson, J.M. pp. 53–72. Amsterdam: Elsevier Sci. Publ.
- HAMON, M., EMERIT, M.B., EL MESTIKAWY, S., GALLISSOT, M.C. & GOZLAN, H. (1990b). Regional differences in the transduction mechanisms of 5-hydroxytryptamine receptors in the mammalian brain. In *Cardiovascular Pharmacology of 5-Hydroxytryptamine*. ed. Saxena, P.R., Wallis, D.I., Wouters, W. & Bevan, P. pp. 41–49. Dordrecht: Kluwer Acad. Publ.
- HOYER, D. & MIDDLEMISS, D.N. (1989). Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends Pharmacol. Sci.*, **10**, 130–132.
- LE BARS, D. (1988). Serotonin and pain. In *Neuronal Serotonin*. ed. Osborne, N.N. & Hamon, M. pp. 171–229. Chichester: John Wiley & Sons Ltd.
- LIN, M.-T., CHANDRA, A., CHI, M.-L. & KAU, C.L. (1980). Effects of increasing serotonergic activity in brain on analgesic activity in rats. *Exp. Neurol.*, **68**, 548–554.
- LYTLE, L.D., MESSING, R.A., FISHER, L. & PHEBUS, L. (1975). Effects of long-term corn consumption on brain serotonin and the response to electric shock. *Science*, **190**, 692–694.
- MARTIN, P., BENINGER, R.J., HAMON, M. & PUECH, A.J. (1990). Antidepressant-like action of 8-OH-DPAT, a 5-HT_{1A} agonist, in the learned helplessness paradigm: evidence for a postsynaptic mechanism. *Behav. Brain Res.*, **38**, 135–144.
- MARTIN, P. & PUECH, A.J. (1991). Is there a relationship between 5-HT_{1B} receptors and the mechanisms of action of antidepressant drugs in the learned helplessness paradigm in rats? *Eur. J. Pharmacol.*, **192**, 193–196.
- McPHERSON, G.A. (1983). Kinetics, EBDA, Ligand, Lowry. A collection of radioligand binding analysis programs. Cambridge, U.K.: Elsevier, Biosoft.
- MELTZER, H.Y. & LOWY, M.T. (1987). The serotonin hypothesis of depression. In *Psychopharmacology: the Third Generation of Progress*. ed. Meltzer, H.Y. pp. 513–526. New York: Raven Press.
- MIDDLEMISS, D.N. (1988). Autoreceptors regulating serotonin release. In *The Serotonin Receptors*. ed. Sanders-Bush, E. pp. 201–224. Clifton, New Jersey: Humana Press.
- MORET, C. (1985). Pharmacology of the serotonin autoreceptor. In *Neuropharmacology of Serotonin*. ed. Green, A.R. pp. 21–49. Oxford: Oxford University Press.
- NICKEL, B. & ALEDTER, A. (1987). Comparative physical dependence studies in rats with flupirtine and opiate receptor stimulating analgesics. *Postgrad. Med. J.*, **63**, 41–43.
- PORSOLT, R.D., LE PICHON, M. & JALFRE, M. (1977). Depression: a new animal model sensitive to antidepressant treatments. *Nature*, **266**, 730–732.
- SCHECHTER, M.D. (1988). Use of TFMPP stimulus properties as a model of 5-HT_{1B} receptor activation. *Pharmacol. Biochem. Behav.*, **31**, 53–57.
- SCHLICKER, E., BETZ, R. & GÖTHERT, M. (1989a). Investigation into the age-dependence of release of serotonin and noradrenaline in the rat brain cortex and of autoreceptor-mediated modulation of release. *Neuropharmacology*, **28**, 811–815.
- SCHLICKER, E., FINK, K., GÖTHERT, M., HOYER, D., MOLDERINGS, G., ROSCHKE, I. & SCHOEFFTER, P. (1989b). The pharmacological properties of the presynaptic serotonin autoreceptor in the pig brain cortex conform to the 5-HT_{1D} receptor subtype. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**, 45–51.
- STARKE, K., GÖTHERT, M. & KILBINGER, H. (1989). Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol. Rev.*, **69**, 864–989.
- SULLIVAN, A.F., DASHWOOD, M.R. & DICKENSON, A.H. (1987). Alpha₂-Adrenoceptor modulation of nociception in rat spinal cord: location, effects and interactions with morphine. *Eur. J. Pharmacol.*, **138**, 169–177.
- TAUB, A. (1973). Relief of post-herpetic neuralgia with psychotropic drugs. *J. Neurosurg.*, **39**, 235–241.
- TIMMERMANS, P.B.M.W.M. & THOOLEN, M.J.M.C. (1987). Autoreceptors in the central nervous system. *Med. Res. Rev.*, **7**, 307–332.
- TITELER, M., LYON, R.A., DAVIS, K.H. & GLENNON, R.A. (1987). Selectivity of serotonergic drugs for multiple brain serotonin receptors: role of [³H]4-bromo-2,5-dimethoxyphenylisopropylamine ([³H]DOB), a 5-HT₂ agonist radioligand. *Biochem. Pharmacol.*, **36**, 3265–3271.
- WATSON, C.P., EVANS, R.J., REID, K., MERSKEY, H., GOLDSMITH, L. & WARSH, J. (1982). Amitriptyline versus placebo in postherpetic neuralgia. *Neurology*, **32**, 671–673.
- WHITAKER-AZMITIA, P.M. & PEROUTKA, S.J. ed. (1990). The neuropharmacology of serotonin. *Ann. N.Y. Acad. Sci.*, **600**, 715p.
- WIELAND, S. & LUCKI, I. (1990). Antidepressant-like activity of 5-HT_{1A} agonists measured with the forced swim test. *Psychopharmacology*, **101**, 497–504.
- YAKSH, T.L. & STEVENS, C.W. (1988). Properties of the modulation of spinal nociceptive transmission by receptor-selective agents. In *Proceedings Vth World Congress on Pain*. ed. Dubner, R., Gebhart, G.F. & Bond, M.R. pp. 417–435. Amsterdam: Elsevier.
- YAKSH, T.L. & WILSON, P.R. (1979). Spinal serotonin terminal system mediates antinociception. *J. Pharmacol. Exp. Ther.*, **208**, 446–453.
- ZEMLAN, F.B., BEHBEHANI, M.M. & MURPHY, R.M. (1988). Serotonin receptor subtypes and the modulation of pain transmission. *Progr. Brain Res.*, **77**, 349–355.

(Received September 2, 1991)

Revised November 7, 1991

Accepted November 11, 1991)