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The anxiolytic-like effect of 5-HT$_{1B}$ receptor ligands in rats: a possible mechanism of action

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Abstract

We have examined the effect of lesions of 5-hydroxytryptamine (5-HT) neurons, produced by *p*-chloroamphetamine (*p*-CA; $2 \times 10\,mg\,kg^{-1}$), and the influence of flumazenil (Ro 15-1788, $10\,mg\,kg^{-1}$), a benzodiazepine receptor antagonist, on the anxiolytic-like activity of CP 94253 (5-propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-b]pyridine), a 5-HT$_{1B}$ receptor agonist, SB 216641 (*N*-[3-[3-(dimethylamino)ethoxy]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1′-biphenyl]-4-carboxamide), a 5-HT$_{1B}$ receptor antagonist, and GR 127935 (*N*-[4-methoxy-3-(4-methyl-l-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-l, l′-biphenyl-4-carboxamide), a 5-HT$_{1B/1D}$ receptor antagonist, in the Vogel conflict drinking test in rats. Diazepam was used as a reference compound. CP 94253 ($2.5\,mg\,kg^{-1}$), SB 216641 ($2.5\,mg\,kg^{-1}$), GR 127935 ($10\,mg\,kg^{-1}$) and diazepam ($5\,mg\,kg^{-1}$) significantly increased the number of shocks accepted during experimental sessions in the conflict drinking test in vehicle- and *p*-CA-pretreated rats. Flumazenil did not change the anxiolytic-like effect of CP 94253 ($2.5\,mg\,kg^{-1}$), but wholly blocked the anxiolytic-like effects of SB 216641 ($2.5\,mg\,kg^{-1}$), GR 127935 ($10\,mg\,kg^{-1}$) and diazepam ($5\,mg\,kg^{-1}$). *p*-CA and flumazenil alone were inactive in the conflict drinking test. The results suggested that the anxiolytic-like effect of the 5-HT$_{1B}$ receptor ligands CP 94253, SB 216641 and GR 127935 was possibly linked to the postsynaptic 5-HT$_{1B}$ receptors or/and 5-HT$_{1B}$ heteroreceptors. The results suggested also that benzodiazepine receptors were indirectly involved in the effects of SB 216641 and GR 127935 (but not of CP 94253), which might have been due to a possible interaction between the 5-HT and the GABA/benzodiazepine systems.

Introduction

Pharmacological studies concerning the potential anxiolytic effect of selective 5-HT$_{1B}$ receptor agonists and antagonists are still sparse and the described results are inconsistent. In fact, anxiolytic- and anxiogenic-like effects or no activity at all have been observed after administration of 5-HT$_{1B}$ receptor ligands in different animal models (Mansbach et al 1996; Chopin et al 1998; Schreiber et al 1998; Fish et al 2000; Lin & Parsons 2002; Hudzik et al 2003). Our recent findings have revealed significant anxiolytic-like effects of a 5-HT$_{1B}$ receptor agonist (CP 94253) and antagonists (SB 216641 and GR 127935) in three animal models, including the conflict drinking test in rats (Tatarczyńska et al 2004). These data suggested that stimulation and blockade of 5-HT$_{1B}$ receptors could induce effects characteristic of anxiolytics, hence different hypotheses might have been used to interpret the anxiolytic-like activity of 5-HT$_{1B}$ receptor ligands. The relative contribution of 5-HT$_{1B}$ autoreceptors and postsynaptic 5-HT$_{1B}$ sites, as well as of 5-HT$_{1B}$ heteroreceptors which can remotely modulate the effect of other neurotransmitters involved in anxiety (e.g. γ-aminobutyric acid (GABA)), should be taken into account. A possible interaction between the GABA/benzodiazepine system and 5-HT$_{1B}$ receptors has been indicated by several neuroanatomical (Waeber et al 1990; Bonaventure et al 1998; Peruzzi & Dut 2004), neurophysiological (Johnson et al 1992; Morikawa et al 2000), biochemical (Chadha et al 2000; Yan & Yan 2001) and behavioural (Chopin et al 1998) data.

To determine whether the integrity of 5-HT neurons was necessary to reveal the anti-anxiety activity of 5-HT$_{1B}$ receptor ligands, we have examined the anxiolytic-like

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effect of the 5-HT_{1B} receptor agonist CP 94253 (Koe et al 1992), the 5-HT_{1B} receptor antagonist SB 216641 (Hagan et al 1997; Schlicker et al 1997) and the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 (Starkey & Skingle 1994; Skingle et al 1995) in the conflict drinking test (Vogel test) in rats, whose 5-HT neurons were destroyed by prior administration of *p*-chloroamphetamine. The effect of the benzodiazepine receptor antagonist flumazenil on the anxiolytic-like activity of these compounds was studied also. The dosage and time schedules of the tested 5-HT_{1B} receptor ligands were based on the results of earlier studies; we chose doses in which those ligands induced a maximal anxiolytic-like effect in rats (Tatarczyńska et al 2004).

Materials and Methods

Chemicals

Diazepam (Polfa-Poznań, Poland), flumazenil (Ro 15-1788, Hoffman-La Roche & Co, Ltd, Basel, Switzerland) and *N*-[4-methoxy-3-(4-methyl-l-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-l, 1′-biphenyl-4-carboxamide (hydrochloride, GR 127935; GlaxoSmithKline, Stevenage, UK) were suspended in a 1% aqueous solution of Tween 80. 5-Propoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-pyrrolo[3,2-b]pyridine (hydrochloride, CP 94253; Tocris, Cookson Ltd, UK), *N*-[3-[3-(dimethylamino) ethoxy]-4-methoxyphenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-[1,1′-biphenyl]-4-carboxamide (hydrochloride, SB 216641; Tocris, Cookson Ltd, UK) and *p*-chloroamphetamine (hydrochloride, *p*-CA, Regis, USA) were dissolved in distilled water. All these compounds were administered intraperitoneally (i.p.).

Animals

The experiments were carried out on male Wistar rats (240–260 g). The animals, in groups of eight, were caged ($60 \times 38 \times 20$ cm) at an ambient temperature of 20 ± 1°C. They had free access to food (standard laboratory pellets) and water before the experiment. All the experiments were conducted in the light phase on a natural light/dark cycle (February to March), between 0900 and 01600 h. The animals were used only once in each test. All injections were made at a volume of 2 mL kg^{-1}. The experiments were performed by an observer unaware of the applied treatment. All the experimental procedures were approved by the Local Bioethics Commission at the Institute of Pharmacology, Polish Academy of Sciences in Kraków.

Conflict drinking test (Vogel test)

A modification of the method of Vogel et al (1971), described below, was used. On the first day of the experiment, the rats were adapted to the test chamber for 10 min. It was a plexiglas box ($27 \times 27 \times 50$ cm), equipped with a grid floor of stainless steel bars and a drinking bottle containing tap water. After the adaptation period, the animals were deprived of water for 24 h and were then placed in the test chamber for another 10-min adaptation period during which they had free access to the drinking bottle. Afterwards, they were allowed a 30-min free-drinking session in their home cage. After another 24-h period of water deprivation, the rats were placed again in the test chamber and were allowed to drink for 30 s. Immediately afterwards, some of their drinking attempts were punished with an electric shock (0.5 mA). The impulses were released every 2 s (timed from the moment when a preceding shock was delivered), via the spout of the drinking bottle. Each shock lasted 1 s, and if a rat was drinking when an impulse was released, it received a shock. The number of shocks accepted throughout a 5-min experimental session was recorded. *p*-CA was administered on two consecutive days, on the ninth and eighth days before the test, SB 216641 and GR 127935 60 min before, and CP 94253, diazepam and flumazenil 30 min before.

Statistical analysis

All the data are presented as the mean ± s.e.m. A two-way analysis of variance was applied, and statistical comparisons between individual groups were carried out by the Newman–Keuls post-hoc test.

Results

As shown in Tables 1 and 2, CP 94253 (2.5 mg kg^{-1}), SB 216641 (2.5 mg kg^{-1}) and GR 127935 (10 mg kg^{-1}) given alone exerted an anxiolytic-like effect, having significantly increased the number of shocks accepted in the conflict drinking test in rats. The depletion of 5-HT with *p*-CA (2×10 mg kg^{-1}) did not modify the anti-anxiety-like effect of CP 94253 (2.5 mg kg^{-1}), SB 216641 (2.5 mg kg^{-1}) and

Table 1 Effect of *p*-CA on the anxiolytic-like action of CP 94253, SB 216641 and GR 127935 in the conflict drinking test in rats

Treatment and dose (mg kg^{-1})	Number of shocks accepted
Vehicle + vehicle	9.6 ± 1.5
p-CA + vehicle	11.1 ± 2.5
Vehicle + CP 94253 (2.5)	40.4 ± 6.2*
p-CA + CP 94253 (2.5)	45.6 ± 5.3*
	F (1,25) = 0.279, ns
Vehicle + vehicle	8.7 ± 1.6
Vehicle + SB 216641 (2.5)	25.6 ± 4.3*
p-CA + SB 216641 (2.5)	27.6 ± 4.2*
	F (1,25) = 0.233, ns
Vehicle + vehicle	9.3 ± 1.9
Vehicle + GR 127935 (10)	35.4 ± 6.6*
p-CA + GR 127935 (10)	34.5 ± 5.3*
	F (1,25) = 0.027, ns

p-CA (10 mg kg^{-1}) was administered on two consecutive days, on the ninth and eighth days before the test, SB 216641 and GR 127935 60 min before, and CP 94253 30 min before. *$P < 0.01$ compared with the respective vehicle + vehicle group, n = 7–8. ns, not significant.

Table 2 Effect of flumazenil on the anxiolytic-like action of CP 94253, SB 216641, GR 127935 and diazepam in the conflict drinking test in rats

Treatment and dose (mg kg^{-1})	Number of shocks accepted
Vehicle + vehicle	8.1 ± 1.2
Vehicle + flumazenil	7.9 ± 1.2
CP 94253 (2.5) + vehicle	38.4 ± 4.4*
CP 94253 (2.5) + flumazenil	42.3 ± 4.4*
	F (1,24) = 0.405, ns
Vehicle + vehicle	8.2 ± 1.4
SB 216641 (2.5) + vehicle	26.4 ± 4.5*
SB 216641 (2.5) + flumazenil	9.1 ± 1.8^{+}
	F (1,25) = 11.933, $P < 0.01$
Vehicle + vehicle	10.6 ± 2.3
GR 127935 (10) + vehicle	30.9 ± 4.1*
GR 127935 (10) + flumazenil	8.4 ± 1.1^{+}
	F (1,24) = 15.211, $P < 0.001$
Vehicle + vehicle	7.4 ± 1.0
Diazepam (5) + vehicle	41.1 ± 6.7*
Diazepam (5) + flumazenil	9.4 ± 1.4^{+}
	F (1,26) = 18.631, $P < 0.001$

SB 216641 and GR 127935 were administered 60 min before the test, while CP 94253, diazepam and flumazenil (10 mg kg^{-1}) 30 min before. *$P < 0.01$, compared with the respective vehicle + vehicle group; $^{+}P < 0.01$, compared with the respective compound + vehicle group; n = 7–8. ns, not significant.

GR 127935 (10 mg kg^{-1}) (Table 1). *p*-CA (2 × 10 mg kg^{-1}) given alone did not affect the punished response of rats (Table 1).

The benzodiazepine receptor antagonist flumazenil (10 mg kg^{-1}) did not change the anxiolytic-like activity of CP 94253 (2.5 mg kg^{-1}), while the effect of SB 216641 (2.5 mg kg^{-1}) and GR 127935 (10 mg kg^{-1}) – as in the case of diazepam (5 mg kg^{-1}) – was completely abolished by flumazenil (10 mg kg^{-1}) (Table 2). Flumazenil (10 mg kg^{-1}) given alone did not affect the punished response of rats (Table 2).

Discussion

In agreement with our recent findings (Tatarczyńska et al 2004), the 5-HT$_{1B}$ receptor agonist CP 94253, as well as the 5-HT$_{1B}$ receptor antagonist SB 216641 and the 5-HT$_{1B/1D}$ receptor antagonist GR 127935, induced a visible anti-anxiety-like effect in the conflict drinking test in rats. CP 94253 was the most effective in that test, whereas of the antagonists used GR 127935 seemed to be slightly more efficient in relieving anxiety than was SB 216641. The anxiolytic-like effect of those 5-HT$_{1B}$ ligands was observed in other animal models (Mansbach et al 1996; Chopin et al 1998; Fish et al 2000; Hudzik et al 2003; Tatarczyńska et al 2004). It seemed that the potential anti-anxiety activity of the 5-HT$_{1B}$ receptor agonist and antagonists might have been linked to their interaction with diversely localized 5-HT$_{1B}$ receptors.

A similar anxiolytic-like effect of the tested 5-HT$_{1B}$ receptor ligands in the conflict drinking test was observed in rats whose 5-HT neurons were destroyed by prior administration of *p*-CA. It should be stressed that *p*-CA given alone did not affect the anxiety behaviour of animals in that model. As was demonstrated in a previous study, *p*-CA reduced the hippocampal concentrations of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) by approximately 85% (Wesołowska et al 2003). The above results indicated that the anxiolytic-like activities of CP 94253, SB 216641 and GR 127935 did not require any integrity of 5-HT neurons; moreover, it might be speculated that the 5-HT$_{1B}$ receptors involved in the anxiolytic-like effect of 5-HT$_{1B}$ receptor ligands were located postsynaptically or presynaptically as heteroreceptors. Such a concept of the role of these receptors in anxiety is in line with the results showing that the anxiolytic-like effect of 1-(3-chlorophenyl)piperazine (m-CPP), a nonselective 5-HT receptor agonist, which stemmed from stimulation of 5-HT$_{1B}$ receptors, was not modified in rats with 5-HT lesions produced by *p*-CA (Chojnacka-Wójcik & Kłodzińska 1992). Moreover, the anti-aggressive effects of CP 94253 and zolmitriptan (a 5-HT$_{1B/1D}$ receptor agonist) were unaltered by 5,7-dihydroxytryptamine lesions (de Almeida et al 2001).

An intriguing question arose as to whether the anxiolytic-like effect of 5-HT$_{1B}$ receptor ligands depended exclusively on serotonergic mechanisms. Only the results obtained by Chopin et al (1998) indicated that the anxiolytic-like effect of GR 127935 in mice was reduced by the benzodiazepine receptor antagonist flumazenil. This study showed that the anxiolytic-like effect of SB 216641 and GR 127935, but not of CP 94253, was blocked by flumazenil at a dose reported to antagonize various effects of diazepam and/or other benzodiazepines (Boast et al 1983; File et al 1985; Löscher & Hönack 1994), including anxiolytic effects (Liliequist & Engel 1984; this study). Therefore it seemed that benzodiazepine receptors were not involved in the anxiolytic-like activity of the 5-HT$_{1B}$ receptor agonist CP 94253, while the effect of the 5-HT$_{1B}$ receptor antagonists SB 216641 and GR 127935 resulted from some functional interaction between the 5-HT and the GABA/benzodiazepine systems. Indeed, such a conclusion seemed to be supported by several neuroanatomical and functional studies. For example, the majority of neurons in the inferior colliculus, which had been immunostained for GABA, were also positive towards the 5-HT$_{1B}$ receptors (probably located postsynaptically) (Peruzzi & Dut 2004). Some convincing evidence indicated that 5-HT$_{1B}$ receptors mediated presynaptic inhibition in many areas of the central nervous system, including GABAergic transmission in the ventral midbrain (Johnson et al 1992; Morikawa et al 2000). Moreover, Bagdy et al (2000) concluded that there was reciprocal influence between serotonergic projection neurons and GABAergic interneurons or afferents in the raphe nuclei, and that that interaction might be mediated by 5-HT$_{1A/1B}$ and GABA$_{A/B}$ receptors. It has been reported that 5-HT$_{1B}$ receptors within the ventral tegmental area and globus pallidus can function as heteroreceptors inhibiting the release of GABA (Chadha et al 2000; Yan & Yan 2001).

However, the above-quoted data did not show how the anxiolytic-like effects of 5-HT_{1B} receptor ligands developed as a result of an interaction between the 5-HT and GABA/benzodiazepine systems. Further studies are necessary to explain how such an interaction may occur. Importantly, benzodiazepine receptors seem to be involved in the anxiolytic-like activity of not only 5-HT_{1B} receptor antagonists (Chopin et al 1998; this study), but also other potential non-benzodiazepine anxiolytics including 5-HT_{1A}- (López-Rubalcava et al 1992; Wesołowska et al 2003), *N*-methyl-D-aspartate- and $glycine_B$ (Kłodzińska & Chojnacka-Wójcik 2000; Przegaliński et al 2000) receptor ligands.

Conclusions

The results suggested that the anxiolytic-like activities of the 5-HT_{1B} receptor agonist CP 94253, the 5-HT_{1B} receptor antagonist SB 216641 and the 5-$HT_{1B/1D}$ receptor antagonist GR 127935 were probably linked to 5-HT_{1B} receptors located postsynaptically or/and acting as heteroreceptors. The effects of SB 216641 and GR 127935 (but not of CP 94253) might have arisen from the indirect activation of the $GABA_A$/benzodiazepine receptor complex.

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