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Stimulation of postsynaptic 5-HT_{1A} receptors is responsible for the anticonflict effect of ipsapirone in rats

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Abstract—Ipsapirone (1·25–10 mg kg^{-1}), a non-benzodiazepine anxiolytic drug with high affinity for 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors, increased dose-dependently the number of punished licks in the drinking conflict test (Vogel test) in rats. The anticonflict effect of the drug administered at a dose of 5 mg kg^{-1} was not modified in animals with lesions of 5-HT neurones, produced by *p*-chloroamphetamine (PCA, 2 × 10 mg kg^{-1}). The anticonflict effect of ipsapirone in PCA-pretreated rats was antagonized by the 5-HT_{1A} receptor and α_1-adrenoceptor antagonist NAN-190 (1-(2-methoxyphenyl)-4-[4-(2-phthalimmido)]butylpiperazine hydrobromide; 0·5–1 mg kg^{-1}), but not by the selective α_1-adrenoceptor blocker prazosin (0·5 mg kg^{-1}). Neither NAN-190 nor prazosin affected the punished response in PCA-pretreated rats. The present results indicate that the anticonflict effect of ipsapirone depends on stimulation of postsynaptic 5-HT_{1A} receptors.

We have recently shown in rats that the anticonflict effect of ipsapirone, a non-benzodiazepine anxiolytic drug with high affinity for 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}) receptors (Traber et al 1984; Peroutka 1985), results from its interaction with postsynaptic 5-HT_{1A} receptors (Chojnacka-Wójcik & Przegaliński 1991). The effect of ipsapirone was antagonized by 5-HT_{1A} receptor antagonists (e.g. NAN-190 and SDZ 21009), but was not modified in animals with lesions of 5-HT neurones, produced by *p*-chloroamphetamine (PCA). However, those results did not allow us to conclude whether an agonist or an antagonist action of ipsapirone on postsynaptic 5-HT_{1A} receptors was responsible for its anticonflict effect. Ipsapirone is a partial agonist of these receptors (Smith & Peroutka 1986; Martin & Mason 1987; Maj et al 1987), and the results of electrophysiological studies strongly suggest that ipsapirone acts as an antagonist of postsynaptic 5-HT_{1A} receptors in the hippocampus (Andrade & Nicoll 1987; Colino & Halliwell 1987); on the other hand, results showing an agonist activity of ipsapirone at these receptors have also been reported (Rowan & Anwyl 1986).

Materials and methods

Animals. The experiments were performed on male Wistar rats, 180–220 g. The animals were kept at a room temperature (19–21°C) on a 12 h light/12 h dark cycle; they were housed in standard laboratory conditions and had free access to food and water before the experiment. Each experimental and control group consisted of 7–9 animals.

Apparatus and anticonflict procedure. A modification of the method of Vogel et al (1971) was used. On the first day of the experiment the rats were adapted to the test chamber for 5 min. The chamber was a plexiglass box (27 × 27 × 50 cm), equipped with a grid floor of stainless steel bars and a drinking bottle containing tap water. An electric shock (0·5 mA, 1 s) could be applied between the spout of the drinking bottle and the grid floor. After an initial adaptation period the animals were deprived of water for 24 h, and were then placed in the test chamber for another 5 min adaptation period during which they had free access to the drinking bottle. Afterwards they were allowed free access to drinking water in their home cage for 30 min. After another 24 h water deprivation period, the rats were placed again in the test chamber and were allowed to drink water for 30 s. Immediately afterwards drinking attempts were punished with an electric shock (every 2 s). The number of shocks accepted throughout a 5 min experimental session was recorded. All animals were used only once in the experiment.

Biochemical determinations. 5-Hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) in the hippocampus were determined by HPLC (Przegaliński et al 1990b).

Drugs. Ipsapirone hydrochloride (Troponwerke, Germany) and 1-(2-methoxyphenyl)-4-[4-(2-phthalimmido)]butylpiperazine hydrobromide (NAN-190; synthesized by Dr J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków) were suspended in a 1% aqueous solution of Tween 80. Prazosin hydrochloride (Pfizer, USA) and *p*-chloroamphetamine hydrochloride (PCA; Regis, USA) were dissolved in saline. All compounds were injected intraperitoneally in a volume of 4 mL kg^{-1}. Ipsapirone was given at 30 min, NAN-190 and prazosin, at 60 min, and PCA at 9 and 8 days before the test.

Statistical analysis. Data are expressed as the mean ± s.e.m. When only one drug was given (e.g. dose-response effect), the data were subjected to an analysis of variance, followed by Dunnett's test. When two drugs were given, the data were analysed by analysis of variance (2 × 2) followed by the Newman-Keuls test.

Results

As shown in Table 1, ipsapirone (1·25–10 mg kg^{-1}) produced an anticonflict effect, increasing dose-dependently the number of punished responses in the licking conflict paradigm. The effect of the drug administered at a dose of 5 mg kg^{-1} was not modified in PCA-pretreated animals. The anticonflict effect of ipsapirone in PCA-pretreated rats was significantly reduced by NAN-190, (0·5 and 1 mg kg^{-1}) but was not affected by prazosin (0·5 mg kg^{-1}). Neither NAN-190 nor prazosin affected the punished response in PCA-pretreated rats.

PCA reduced the hippocampal concentrations of 5-HT and 5-HIAA by about 84 and 87%, respectively (absolute values: controls, 450 ± 36 and 310 ± 14 ng g^{-1}, respectively, n = 8; PCA-treated, 72 ± 7 and 40 ± 4 ng g^{-1}, respectively, n = 7, $P < 0.01$).

Discussion

The present paper confirms the results of our previous study (Chojnacka-Wójcik & Przegaliński 1991), as it indicates that ipsapirone has an anticonflict effect in the Vogel test in rats, this effect being still observed in animals with lesions of 5-HT neurones produced by PCA. These results, together with our earlier findings showing that the anticonflict effect of ipsapirone

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Table 1. Effects of NAN-190 and prazosin on the anticonflict action of ipsapirone in *p*-chloroamphetamine (PCA)-pretreated rats.

Treatment and doses (mg kg^{-1})		Number of shocks accepted/5 min Mean ± s.e.m.
Control		9·3 ± 1·1
Ipsapirone (1·25)		16·4 ± 4·0
Ipsapirone (2·5)		24·0 ± 1·7*
Ipsapirone (5·0)		32·7 ± 3·7*
Ipsapirone (10·0)		34·8 ± 4·1*
Control		12·6 ± 3·0
PCA		13·1 ± 3·1
PCA + ipsapirone (5·0)		35·2 ± 3·2*
PCA + NAN-190 (0·5)	+ ipsapirone (5·0)	19·1 ± 3·4**
PCA + NAN-190 (1·0)	+ ipsapirone (5·0)	17·0 ± 3·2**
PCA + prazosin (0·5)	+ ipsapirone (5·0)	35·6 ± 5·5*
Control		9·6 ± 2·1
PCA		9·1 ± 1·6
PCA + NAN-190 (0·5)		12·3 ± 2·9
PCA + NAN-190 (1·0)		8·0 ± 1·7
PCA + prazosin (0·5)		11·1 ± 2·1

PCA (2 × 10 mg kg^{-1}) was given 9 and 8 days before the test. Ipsapirone was injected 30 min before the test. NAN-190 and prazosin were administered 30 min before ipsapirone. n = 7–9 rats per group. *$P < 0.01$ vs control or PCA (Dunnett's test) **$P < 0.01$ vs PCA + ipsapirone (Newman-Keuls test).

is antagonized by 5-HT$_{1A}$ receptor blockers (Chojnacka-Wójcik & Przegaliński 1991), lead to the conclusion that the effect of this drug depends on its interaction with postsynaptic rather than presynaptic 5-HT$_{1A}$ receptors. Such a conclusion is consistent with the results of Davis et al (1988) and Kostowski et al (1989) who suggested a role of postsynaptic 5-HT$_{1A}$ receptors in the anxiolytic effects of buspirone (an analogue of ipsapirone) in other experimental models. However, involvement of presynaptic 5-HT$_{1A}$ receptors in the anxiolytic activity of ipsapirone) in different animal models has also been suggested (Eison et al 1986; Higgins et al 1988; Carli et al 1989).

Although ipsapirone is a partial agonist of 5-HT$_{1A}$ receptors (Smith & Peroutka 1986; Martin & Mason et al 1987; Maj et al 1987), and although the results of electrophysiological studies indicate that it acts as an antagonist of postsynaptic 5-HT$_{1A}$ receptors in the hippocampus (Andrade & Nicoll 1987; Colino & Halliwell 1987); a brain structure whose 5-HT system has been postulated to control anxiety (Gray 1982), the results of the present study indicate that an agonist rather than an antagonist action of ipsapirone on postsynaptic 5-HT$_{1A}$ receptors is responsible for the anticonflict effect of the drug. We have demonstrated that the anticonflict effect of ipsapirone in PCA-pretreated rats is antagonized by NAN-190, a drug which shows high affinity for 5-HT$_{1A}$ but not for other subtypes of 5-HT receptors (Glennon et al 1988), and which acts like a 5-HT$_{1A}$ receptor antagonist in functional in-vivo models (Glennon et al 1988; Hjorth & Sharp 1990; Przegaliński et al 1990a). It should be noted, however, that in addition to 5-HT$_{1A}$ receptors, NAN-190 shows high affinity for α_1-adrenoceptors and may thus be regarded as their antagonist (Chojnacka-Wójcik & Przegaliński 1991). However, the α_1-adrenoceptor blocking activity of NAN-190 does not seem to be important for its antagonism of the anticonflict effect of ipsapirone in PCA-pretreated rats, since the effect of ipsapirone was not affected by the selective α_1-adrenoceptor antagonist prazosin. Therefore, our conclusion that the anticonflict activity of ipsapirone results from stimulation of postsynaptic 5-HT$_{1A}$ receptors by the drug, differs from the above cited results of electrophysiological studies of Andrade & Nicoll (1987) and Colino & Halliwell (1987). It is noteworthy, however, that the agonist activity of ipsapirone at hippocampal postsynaptic 5-HT$_{1A}$ receptors could also be demonstrated electrophysiologically (Rowan & Anwyl 1986).

Further evidence showing that the antagonism of postsynaptic 5-HT$_{1A}$ receptors does not seem to be involved in the anticonflict activity comes from the experiment in which no effect of NAN-190 on the punished response in the licking conflict paradigm was shown in either normal (Chojnacka-Wójcik & Przegaliński 1991) or PCA-pretreated (present study) rats.

In conclusion, our results indicate that the anticonflict effect of ipsapirone results from its agonist action on postsynaptic 5-HT$_{1A}$ receptors.

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BQ-153, a novel endothelin (ET)$_A$ antagonist, attenuates the renal vascular effects of endothelin-1

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Abstract—Endothelin (ET)-1, leukotriene D_4 and the thromboxane analogue, U-44069, were all shown to produce dose-dependent reductions in renal blood flow after direct injection into the renal artery of anaesthetized pigs. The effects of ET-1 differed from the other two mediators in that ET-1 caused a transient vasodilator followed by a prolonged vasoconstrictor response. The pressor response was not mediated by the secondary release of either leukotriene D_4 or thromboxane A_2 as evidenced by the lack of effect of appropriate receptor antagonist MK571 (3-{-2(7-chloro-2 quinolinyl) ethenyl}phenyl{3-(dimethylamino-3-oxopropyl)thio}methyl thio propionic acid) and L-670,596 respectively. This response, however, could be inhibited in a dose-dependent fashion by the selective ET_A antagonist, BQ-153 (cyclo-D-sulphalanine-L-Pro-D-Val-L-Leu-D-Trp-). Following blockade by BQ-153 the vasodilator response was unaffected and a residual pressor response remained, suggesting that either or both of these effects were mediated either through an ET_B or a novel, as yet undefined, endothelin receptor.

Endothelin (ET)-1 is a potent, 21 amino acid, vasoconstrictor peptide first isolated from medium conditioned by cultured endothelial cells (Yanagisawa et al 1988). Subsequent studies have revealed the presence of two additional, closely related peptides (ET-2 and ET-3), all three peptides being coded for by three separate genes (Inoue et al 1989). Further research has shown the presence of two distinct endothelin receptors, which have been termed ET_A (selective for ET-1) and ET_B (nonselective for ET isopeptides) (Arai et al 1990; Sakurai et al 1990). These peptides and receptors are widely distributed in a number of tissues and mediate a number of biological responses (Lerman et al 1990).

There has been a considerable interest in the role of endothelin peptides in renal pathology (Simonson & Dunn 1991). Evidence for a role for ET-1 in post-ischaemic acute renal failure and cyclosporine-induced glomerular dysfunction has been obtained through the use of anti-endothelin antibodies (Kon et al 1989, 1990; Shibouta et al 1990). The ET-1 gene is expressed not only in vascular endothelial cells, but also in mesangial cells where its expression can be upregulated by various inflammatory mediators (Sakamoto et al 1990; Zoja et al 1991). ET-1-induced reductions in renal blood flow and function can be mediated through both efferent and afferent arteriolar constriction (Kon et al 1989), as well as through effects on ET_A receptors on mesangial cells (Simonson & Dunn 1990). The vasoconstriction mediated by ET-1 may be counteracted in the kidney by the secondary release of vasodilators, such as prostaglandin I_2 (Chou et al 1990). Because ET-1 can induce eicosanoid synthesis, it is possible that the renal vasoconstrictor effects could be mediated, in part, by the release of pressor arachidonic acid metabolites, such as thromboxane A_2 and leukotriene D_4. This has been investigated in the present work through the use of specific receptor antagonists. Recently a highly selective, but relatively weak, ET_A antagonist, BE-18257B, a novel cyclic pentapeptide, has been isolated from *Streptomyces misakiensis* (Ihara et al 1991). Analogues of this have been synthesized and potent, selective ET_A antagonists, such as BQ-153 (cyclo-D-sulphalanine-L-Pro-D-Val-L-Leu-D-Trp-), have been described (Ihara et al 1992). The effects of BQ-153 on renal vasoconstriction in the pig have been investigated in the present work.

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Materials and methods

Materials. Leukotriene D_4, L-670,596 and MK-571 (3-{-2(7-chloro-2 quinolinyl) ethenyl}phenyl{3-(dimethylamino-3-oxo-propyl)thio}methyl thio propionic acid), synthesized at Merck Frosst; BQ-153 (cyclo-D-sulphalanine-L-Pro-D-Val-L-Leu-D-Trp-) synthesized at Banyu Central Research Laboratories; U-44069, Upjohn; ET-1, Peninsula labs; Azaperone, Pitman-Moore Ltd; sodium pentobarbitone, MTC Pharmaceuticals.

In-vivo procedures. Yorkshire domestic pigs, 2 months old, with a mean weight of $15{\cdot}0 \pm 2$ kg were sedated with azaperone (2–4 mg kg^{-1}, i.m.) and anaesthetized with sodium pentobarbitone (20 mg kg^{-1}, i.v.) Surgical anaesthesia was maintained with a continuous infusion of the sodium pentobarbitone (5–10 mg kg^{-1} h^{-1}, i.v.) from which the animals were not permitted to recover. Body temperature was maintained at 37·5°C with a homeothermic blanket (Harvard Instruments).

An endotracheal tube was inserted through a tracheostomy and the animals were ventilated with room air (Harvard Instruments respirator). Systemic arterial pressure was monitored with a catheter-tip pressure transducer (Millar Instruments) inserted into the right femoral artery. A port located 5 mm from the tip of the catheter permitted the withdrawal of arterial blood (0·3 mL) for blood gas (pO_2, pCO_2) and pH determinations (Instrumentation Laboratories blood gas analyser, model 1312). The respiratory volume (150–200 mL/stroke) and frequency (10–20 breaths min^{-1}) were adjusted to maintain pO_2 at 80–100 mmHg, pCO_2 at 35–45 mmHg, and pH at 7·35–