Effect of acute administration of the 5-HT_{1A} receptor ligand, lesopitron, on rat cortical 5-HT and dopamine turnover

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1 The involvement of presynaptic 5-hydroxytryptamine_{1A} (5-HT_{1A}) autoreceptors in the anxiolytic-like properties of lesopitron (E-4424) (2-[4-[4-(4-chloro-1-pyrazolyl)butyl]-1-piperazinyl]pyrimidine) was studied. Brain microdialysis was used to examine the effect of the drug on the release of 5-hydroxytryptamine (5-HT) and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex of awake, freely moving rats. Moreover, extracellular cortical 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were also studied to assess the possible participation of dopaminergic systems.

2 Lesopitron administered at a dose which induces anxiolytic behaviour in rats (30 µg kg⁻¹, i.p.) markedly reduced 5-HT levels (to 45% of the basal value) in cortical perfusates, having no effect on 5-HIAA, DOPAC and HVA. The effects of lesopitron were compared with those produced by the anxiolytic, and structurally related compound, buspirone.

3 Buspirone administered at a dose inducing anxiolytic-like effects in rats (5 mg kg⁻¹, i.p.) produced a marked decrease in cortical 5-HT levels (to 20% of the basal value), but in contrast to lesopitron, buspirone produced a pronounced increase in cortical DOPAC (to 300% of the basal value) and HVA (to 400% of the basal value) levels. Buspirone administered at a low dose (30 µg kg⁻¹, i.p.) was unable to affect cortical 5-HT levels.

4 To test the hypothesis that the 5-HT decreasing effect of lesopitron could be due to 5-HT_{1A} autoreceptor (somatodendritic)-mediated inhibition of 5-HT neurotransmission, lesopitron was administered locally into the raphe nuclei. Intraraphe administration of 10 µM lesopitron caused a decrease in cortical 5-HT levels (the effect being of the same order as that obtained after systemic injection), with no effect on 5-HIAA, DOPAC and HVA. Raphe 5-HT extracellular levels were not modified after intraraphe administration of lesopitron, indicating the absence of 5-HT reuptake blocking properties.

5 We concluded that lesopitron, at an anxiolytic dose produced a marked inhibition of 5-HT release in the frontal cortex of awake, freely moving rats. This effect was observed after systemic administration as well as after intraraphe administration of the drug, suggesting an agonistic action at raphe 5-HT_{1A} autoreceptors controlling 5-HT release in the projecting areas. In contrast to buspirone, lesopitron treatment had no effect on cortical DOPAC or HVA levels.

Keywords: Lesopitron (E-4424); 5-HT_{1A} receptor; microdialysis; buspirone; anxiolytic

Introduction

Current studies suggest that 5-hydroxytryptaminergic neurotransmission may be involved in the aetiology and treatment of anxiety. Central 5-hydroxytryptamine (5-HT) receptors have been divided into four main classes, named 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ (Peroutka *et al.*, 1990); furthermore several additional 5-HT receptors have been recently cloned, including 5-HT₅, 5-HT₆ and 5-HT₇ receptors (Matthes *et al.*, 1993; Monsma *et al.*, 1993; Shen *et al.*, 1993). The 5-HT₁ class shows extensive heterogeneity (Pedigo *et al.*, 1981) and one of its subtypes, the 5-HT_{1A} receptor, has been particularly implicated in the anxiolytic response (Costall *et al.*, 1992; Amano *et al.*, 1993). 5-HT_{1A} receptors are localized presynaptically on the soma and/or dendrites of 5-hydroxytryptaminergic neurones originating in the raphe nuclei, and postsynaptically on target cells in terminal projection areas (e.g. hippocampus and cortex) (Verge *et al.*, 1985; Pazos & Palacios, 1985).

During the last decade, several nonbenzodiazepine drugs (azaspirones) displaying high affinity and selectivity for the 5-HT_{1A} receptor subtype, including buspirone, gepirone and ipsapirone, have been shown to be clinically effective in the treatment of anxiety (Peroutka, 1985). These drugs, as well as the prototypical 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) have been shown to inhibit the

firing of raphe 5-hydroxytryptaminergic neurones (Van der Maelen *et al.*, 1986; Sprouse & Aghajanian, 1987) and decrease the release of 5-HT in their projecting areas (Sharp *et al.*, 1989; Hutson *et al.*, 1989; Hjorth & Sharp, 1991). These effects have been attributed to activation of presynaptic somatodendritic 5-HT_{1A} autoreceptors. However, an additional involvement of postsynaptic 5-HT_{1A} and/or other 5-HT receptor subtypes (e.g. 5-HT₂) in the anxiolytic response has also been proposed (Schechter *et al.*, 1990; Cowen, 1991; Schreiber & De Vry, 1993).

Lesopitron, a novel pyrimidinyl-piperazine derivate (Figure 1) potentially reduces behavioural responses to aversive situations in animal models of anxiety (Costall *et al.*, 1991; 1992; Farré, 1992). Binding studies have shown that, like the structurally related compounds buspirone, ipsapirone and gepirone, lesopitron shows affinity for the 5-HT_{1A} receptor in rat

Figure 1 Chemical structure of lesopitron.

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brain. Moreover lesopitron shows high selectivity (having no activity at other 5-HT receptors) and specificity (lacking affinity for adrenoceptors, cholinceptors or benzodiazepine, dopamine, histamine and cholecystokinin receptor sites) (Costall *et al.*, 1992; Farré, 1992).

To assess the participation of somatodendritic 5-HT_{1A} autoreceptors in the anxiolytic properties of lesopitron, we have studied the effects of the drug on the release of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the frontal cortex of awake rats using microdialysis. As it is known that activation of dopamine and noradrenaline turnover is an effect shared by the three azaspiroines, buspirone, ipsapirone and gepirone (Hamon *et al.*, 1988), the effect of lesopitron on the release of the dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the same cerebral area was also investigated.

A preliminary account of some of this work was presented to the British Pharmacological Society (Ballarín *et al.*, 1993).

Methods

Brain microdialysis

Male Wistar rats (Interfauna Ibérica, S.A. Sant Feliu de Codines, Spain) weighing 270–300 g, were anaesthetized with chloral hydrate (440 mg kg⁻¹, i.p.) and placed in a stereotaxic frame.

Concentric dialysis probes were made as described previously (Adell *et al.*, 1989) from 25 G (0.3 mm i.d. × 0.5 mm o.d.) stainless steel tubing (A-M Systems Inc., Everett, U.S.A.). Inlet and outlet cannulae consisted of 0.040 mm i.d. × 0.140 mm o.d. fused silica tubing (Scientific Glass Engineering, Melbourne, Australia). Cuprophane hollow fibres (Gambro GF 80 M) were used for the dialysis membranes (4 mm length).

Probes were implanted into the frontal cortex (3.7 mm anterior to bregma; 3.2 mm lateral to it and 4.4 mm ventral to dura) and/or in the raphe nuclei (7.8 mm posterior to bregma; 0.5 lateral to it and 9.2 mm ventral to dura, according to Paxinos & Watson, 1986). Animals were allowed to recover from surgery, and 18–24 h after implantation, the probes were perfused with artificial cerebrospinal fluid (CSF) (in mM: NaCl 137, KCl 2.4, CaCl₂ 1.2, MgCl₂ 1.2, Na₂HPO₄ 1.4, NaH₂PO₄ 0.9 and glucose 7.2, pH 7.4) at a flow rate of 0.25 µl min⁻¹ (CMA/100 microinjection pump, Carnegie Medicin, Stockholm) and successive 30 min samples (7.5 µl) were collected. At the end of the experiment brains were removed and frozen for histological verification of dialysis probe placement.

Perfusate analysis

Immediately after collection, samples were analysed for 5-HT, 5-HIAA, DOPAC and HVA, by injecting 7 µl/sample (autoinjector CMA/200 Carnegie Medicin, Stockholm) onto a reversed-phase high performance liquid chromatography (h.p.l.c.-e.d.) system.

The column used was an Ultrasphere 3 µm ODS (4.6 mm × 7.5 mm) (Beckman, U.S.A.) and the mobile phase was NaH₂PO₄ 150 mM, EDTA 0.5 mM octane-1-sulphonic acid sodium salt 0.2 mM, 12% methanol, pH 3.1 adjusted with phosphoric acid. The flow rate used was 1 ml min⁻¹. The oxidation potential was set at 700 mV vs an Ag/AgCl reference electrode (Hewlett Packard 1048A). The detection limit of the assay for 5-HT was about 1 fmol/injection using a signal-to-noise ratio of 2.

Drug treatment

All reagents used were analytical or h.p.l.c. grade: 5-HT hydrochloride, 5-HIAA, DOPAC and HVA (Sigma Chemical Co., St Louis, MO, U.S.A.); NaCl, KCl, CaCl₂, MgCl₂,

NaH₂PO₄, EDTA (Merck, Germany), octane-1-sulphonic acid sodium salt (Romil Chemicals, GB). 2-[4-(4-Chloro-1-pyrazolyl)butyl]-1-piperazinylpyrimidine (lesopitron) (Lab. Dr Esteve, Barcelona) and buspirone (Lab. Dr Esteve, Barcelona) were administered either through the dialysis probe (dissolved in artificial CSF) or i.p. (dissolved in 0.9% saline, 2 ml kg⁻¹ body weight).

Statistical analysis

Dialysate levels of 5-HT, 5-HIAA, DOPAC and HVA were expressed as percentages (mean ± s.e.mean) of the average amount of four samples collected before the administration of the drug (time = 0). Statistical significance (**P* < 0.05) between the mean values and saline controls at each time point, was calculated using a two-factor (one between groups, the drug administered, and the other within each group, the time points after administration) repeated measurement analysis of variance. We have used a GLM (General Linear Models) procedure (Hardy, 1989) of the SAS statistical package (SAS Institute Inc., 1989).

Results

Effect of systemic administration of lesopitron and buspirone on 5-HT and 5-HIAA in cortical dialysates

Basal levels of 5-HT and 5-HIAA, measured in frontal cortex dialysates (not corrected for fibre recovery) in awake rats, ranged from 3 to 15 fmol 7 µl⁻¹ dialysate and from 3 to 8 pmol 7 µl⁻¹ dialysate, respectively. These results were obtained by using artificial CSF as a perfusion medium without addition of a 5-HT uptake blocker.

Peripheral administration of lesopitron (30 µg kg⁻¹, i.p.) (dose with anxiolytic properties) (Costall *et al.*, 1992), caused an immediate reduction in cortical 5-HT levels when compared to saline-treated controls. This effect was significant 90 to 180 min after drug administration, the level decreasing to 45% of the basal value (Figure 2a and Table 1). However, 5-HIAA levels in cortical dialysates were not affected by the drug (Table 1). A high dose of lesopitron (5 mg kg⁻¹, i.p.) decreased cortical 5-HT release to the same extent (to 42% of the basal value; Figure 2a) without affecting 5-HIAA levels (Table 1).

Buspirone (5 mg kg⁻¹, i.p., a dose that produces potential anxiolytic effects in adult rats, Costall *et al.*, 1992) produced a marked reduction of cortical 5-HT levels when compared with saline-treated controls. The effect was significant 60 to 120 min after drug administration, the level decreasing to 24% of the basal value (Figure 2b and Table 1). 5-HIAA levels were unaffected by the drug (Table 1). Moreover, buspirone administered at a low dose (30 µg kg⁻¹, i.p.), had no significant effect on cortical 5-HT levels or 5-HIAA levels (Figure 2b and Table 1).

Effect of systemic administration of lesopitron and buspirone on DOPAC and HVA levels in cortical dialysates

Basal levels of DOPAC and HVA, measured in frontal cortex dialysates (not corrected for fibre recovery) in awake rats, ranged from 100 to 600 fmol 7 µl⁻¹ and from 200 to 1000 fmol 7 µl⁻¹ respectively. In contrast to lesopitron, which at an anxiolytic-like dose (30 µg kg⁻¹, i.p.) caused no change in DOPAC and HVA cortical levels (Figure 3a,c and Table 1), buspirone (5 mg kg⁻¹, i.p.), caused a pronounced increase in cortical DOPAC (up to 324% of the basal value) (Figure 3b and Table 1) and HVA (up to 388% of the basal value) (Figure 3d and Table 1). These results are in agreement with the reported decreasing 5-HT turnover and increasing dopamine turnover effect of buspirone at anxiolytic doses (Hamon *et al.*, 1988).

Lesopitron, even at doses 100 fold greater than the minimally effective behavioural dose (5 mg kg^{-1} , i.p.), only slightly increased cortical DOPAC and HVA (to 120–130% of basal values) (Figure 3a,c and Table 1).

Figure 2 Time-course change in 5-hydroxytryptamine (5-HT) levels in cortical dialysates following (a) lesopitron at different doses: (○) $30 \mu\text{g kg}^{-1}$, i.p.; (●) 5 mg kg^{-1} , i.p.; (▽) 0.9% saline 2 ml kg^{-1} and (b) buspirone at different doses: (○) $30 \mu\text{g kg}^{-1}$, i.p.; (●) 5 mg kg^{-1} , i.p.; (▽) 0.9% saline 2 ml kg^{-1} . Values are expressed as mean percentages $\pm$ s.e.mean, ($n = 6$) of the average amount of four samples collected before injection of the drug ($t = 0$). The dashed line represents 100% baseline level. * $P < 0.05$ compared to saline controls (two-factor repeated-measurement design using GLM procedures of SAS SYSTEM).

Effect of intraraphe perfusion with lesopitron and buspirone on cortical and raphe dialysates

To test the hypothesis that lesopitron decreases 5-HT levels by 5-HT_{1A} autoreceptor (somatodendritic)-mediated inhibition of 5-hydroxytryptaminergic neurotransmission, some rats were implanted with two probes: one close to the dorsal and medial raphe nuclei and the other one in the frontal cortex. Once four basal values were obtained, the artificial CSF perfusing the probe placed in the raphe nuclei, was changed to one containing the drug dissolved in artificial CSF. Perfusion continued for 180 min. During this time, perfusates were collected simultaneously from the raphe nuclei and the frontal cortex.

Cortical dialysates Intraraphe administration of lesopitron $10 \mu\text{M}$ (without correction for dialysis probe permeability) caused a significant decrease in cortical 5-HT levels (Figure 4a and Table 2) with no effect in 5-HIAA, DOPAC and HVA (Table 2). 5-HT reduction was maximal (to 45% of the basal value) 120 min after starting the administration of the drug (Figure 4a). These results strongly suggest an agonistic action of lesopitron on raphe 5-HT_{1A} autoreceptors modulating 5-HT release in the frontal cortex.

Intraraphe administration of buspirone $100 \mu\text{M}$ (without correction for dialysis probe recovery) caused a marked decrease in cortical 5-HT release, this was significant from 90 to 180 min after the administration of the drug (Figure 4b and Table 2). 5-HIAA levels were not affected (Table 2) and DOPAC and HVA were only slightly increased (to 120–130% of the basal value) after drug infusion (Table 2).

Raphe dialysates In the raphe nuclei, dialysate basal levels ranged from 10 to $30 \text{ fmol } 7 \mu\text{l}^{-1}$ for 5-HT, from 30 to $50 \text{ pmol } 7 \mu\text{l}^{-1}$ for 5-HIAA, from 400 to $1500 \text{ fmol } 7 \mu\text{l}^{-1}$ for DOPAC and from 600 to $1800 \text{ fmol } 7 \mu\text{l}^{-1}$ for HVA. In contrast with the effect on cortical 5-HT release, intraraphe administration of lesopitron ($10 \mu\text{M}$) failed to affect 5-HT extracellular levels in the raphe nuclei (Figure 4a and Table 2). Raphe 5-HIAA, DOPAC and HVA extracellular levels were also unaffected (Table 2).

Intraraphe buspirone ($100 \mu\text{M}$) also had no effect on 5-HT raphe levels (Figure 4b and Table 2). Similarly, raphe levels of 5-HIAA, DOPAC and HVA were unaffected (Table 2).

Discussion

The present study demonstrates that systemic administration of lesopitron markedly decreases 5-HT extracellular levels in the frontal cortex of awake freely moving rats. This effect is

Table 1 Comparison of the effects of lesopitron and buspirone administered i.p. at different doses, on the extracellular levels of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the rat frontal cortex

Treatment (mg kg^{-1} i.p.)	5-HT (1) (% of basal release)	5-HIAA (2) (% of basal release)	DOPAC (3) (% of basal release)	HVA (4) (% of basal release)
Vehicle	87.9 ± 11.0	81.4 ± 3.2	85.8 ± 4.7	92.8 ± 9.8
Control				
Lesopitron				
0.03	$58.7 \pm 7.0^*$	100.0 ± 7.4	96.9 ± 7.1	95.7 ± 7.5
Buspirone				
0.03	77.0 ± 10.4	106.5 ± 11.7	104.6 ± 17.1	122.6 ± 17.7
Lesopitron				
5.0	$42.4 \pm 7.0^*$	79.1 ± 3.0	119.9 ± 17.8	127.5 ± 10.6
Buspirone				
5.0	$24.0 \pm 5.8^*$	90.7 ± 6.3	$324.0 \pm 45.8^*$	$388.3 \pm 105.6^*$

The results are expressed as percentages, mean $\pm$ s.e.mean ($n = 6$), of the average amount of four samples collected before injection of the drug. The average basal values for each group are: (1) $8.4 \pm 1.8 \text{ fmol/sample}$; (2) $4.7 \pm 0.6 \text{ pmol/sample}$; (3) $236.4 \pm 63.7 \text{ fmol/sample}$ and (4) $554.3 \pm 88.3 \text{ fmol/sample}$. Values are taken 90 min after injection of the drug (time point of maximum effect for buspirone on 5-HT output, and at which both drugs produce significant changes, see Figure 2a). * $P < 0.05$ compared to vehicle controls (two-factor repeated-measurement design using GLM procedures of SAS system).

also shared by the anxiolytic, structurally related compounds buspirone, ipsapirone and gepirone (Sharp *et al.*, 1989; Routledge *et al.*, 1993). The fact that a 60% reduction of 5-HT release is achieved by a $30 \mu\text{g kg}^{-1}$, i.p. dose of lesopitron, while higher doses are needed for buspirone to obtain that effect, is in accordance with the more potent anxiolytic activity showed by lesopitron in animal behavioural tests (Costall *et al.*, 1992).

Our results support electrophysiological data demonstrating that lesopitron inhibits the firing rate of 5-hydroxy-

trptaminergic neurones in the dorsal raphe nucleus at doses as low as $10 \mu\text{g kg}^{-1}$ i.v. through a (-)-tertatolol-sensitive mechanism (Haj-Dahmane *et al.*, 1994). It is then quite plausible that lesopitron can effect cortical 5-HT upon acute administration, at least in part, through activation of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei, which mediate inhibition of 5-hydroxytryptaminergic neurotransmission.

Since systemically injected drugs can affect transmitter release by acting on somatodendritic autoreceptors located in

Figure 3 Time-course change in 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) levels in cortical dialysates following (a) (c): lesopitron at different doses (○) $30 \mu\text{g kg}^{-1}$, i.p.; (●) 5 mg kg^{-1} , i.p.; (▽) 0.9% saline 2 ml kg^{-1} and (b), (d): buspirone (○) $30 \mu\text{g kg}^{-1}$, i.p.; (●) 5 mg kg^{-1} , i.p.; (▽) 0.9% saline 2 ml kg^{-1} . Values are mean percentages $\pm$ s.e.mean ($n = 6$). See legend to Figure 2 for further details.

Table 2 Comparison of the effects of lesopitron and buspirone administered intraraphe on cortical and raphe perfusate levels of 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA)

Treatment (μM i.r)	Cerebral area	5-HT (1) (% of basal release)	5-HIAA (2) (% of basal release)	DOPAC (3) (% of basal release)	HVA (4) (% of basal release)
Lesopitron	Cortex	$45.5 \pm 7.5^*$	85.2 ± 8.6	86.3 ± 8.4	96.1 ± 12.4
	Raphe	95.0 ± 6.5	108.3 ± 4.0	107.8 ± 2.8	111.9 ± 4.2
Buspirone	Cortex	$57.1 \pm 11.7^*$	99.9 ± 7.3	112.9 ± 7.0	120.0 ± 2.7
	Raphe	105.2 ± 20.0	116.3 ± 8.7	98.9 ± 5.8	102.6 ± 6.0

Results are expressed as percentages mean $\pm$ s.e.mean ($n = 5$) of the average values of four samples taken before perfusion with the drug (basal values). The average basal values for each group are: (1) cortex 5.1 ± 1.4 fmol/sample and raphe 15.2 ± 4.6 fmol/sample; (2) cortex 4.0 ± 0.4 pmol/sample and raphe 34.5 ± 2.3 pmol/sample; (3) cortex 190.0 ± 30.1 fmol/sample and raphe 1100.0 ± 150.0 fmol/sample; (4) cortex 600.2 ± 110.1 fmol/sample and raphe 1230.3 ± 160.0 fmol/sample. Values are taken 120 min after starting drug infusion (time point of which lesopitron produces the maximum effect on 5-HT release and buspirone is close to its maximum effect) and are a mean of five rats. $*P < 0.05$ compared to basal values (two-factor repeated-measurement design using GLM procedures of SAS system).

Figure 4 Effect of intraraphe perfusion with CSF containing (a) 10 μ M lesopitron and (b) 100 μ M buspirone on cortical (●) and raphe (○) 5-hydroxytryptamine (5-HT) dialysate levels. After four samples had been collected to obtain basal values, the artificial CSF was changed (at time 0) to one containing the drug and perfusion continued for 180 min. Results are expressed as percentages (mean $\pm$ s.e. mean, $n = 5$) of the average values of the four samples taken before perfusion with the drug (basal values). * $P < 0.05$ compared to basal values (two-factor repeated-measurement design using GLM procedures of SAS SYSTEM).

the cell body and/or on receptors located on the nerve terminal region, in another group of experiments drugs were administered by local perfusion into the raphe nuclei. Both dorsal and ventral regions were perfused, as the 4 mm dialysis membrane was located in close vicinity to both areas. Comparison between the drug effects following systemic and/or local perfusion, can reveal the contribution of each receptor population. Both routes of administration (lesopitron 30 μ g kg⁻¹, i.p. or 10 μ M intraraphe) caused a maximal decrease in cortical 5-HT to about 40–50% of controls, indicating that the raphe 5-HT_{1A} population is mainly implicated in lesopitron's activity.

In contrast to its effect on cortical 5-HT levels, intraraphe

administration of lesopitron failed to affect 5-HT extracellular levels in the raphe nuclei. It has been shown that extracellular 5-HT in raphe nuclei originates from a cytoplasmic pool and is not dependent on neuronal activity in 5-hydroxytryptaminergic neurones or local activation of 5-HT_{1A} receptors (Adell *et al.*, 1993). It seems plausible that 5-HT output in the raphe nuclei may be regulated by the reuptake process, as *in vivo* blockade with 5-HT uptake inhibitors markedly increases the extracellular 5-HT in this area (Adell & Artigas, 1991). Considering these facts, our results obtained from raphe dialysates suggest that lesopitron, at the doses tested, does not behave as a 5-HT uptake blocker, since direct intraraphe administration (10 μ M) failed to affect 5-HT extracellular levels. Moreover our results also suggest that the 5-HT decreasing effect of lesopitron in the frontal cortex is not due to an increase in raphe extracellular 5-HT levels acting on the 5-HT_{1A} autoreceptors, as would be the case in a reuptake blocking situation, and as has been suggested for other compounds (Adell & Artigas, 1991).

Buspirone (100 μ M) also failed to affect 5-HT output in raphe nuclei when administered locally into this area. The low influence of buspirone, gepirone and 1-(2-pyrimidinyl)-piperazine (1-PP) on 5-HT uptake into cortical and hippocampal synaptosomes has been described by others (Skolnick *et al.*, 1985). The work of Skolnick *et al.* (1985) suggests that the concentration of buspirone needed to inhibit [³H]-5-HT uptake sites *in vitro*, would not be achieved in rat brain following administration of the minimally effective anti-conflict doses of either buspirone or gepirone.

Lesopitron, as well as buspirone, failed to affect 5-HIAA output. The same effect has been reported with the related compounds, ipsapirone and gepirone (Sharp *et al.*, 1989). However it should be remembered that much of the extracellular 5-HIAA is due to catabolism of unreleased 5-HT and that release and metabolism can be affected differently (Crespi *et al.*, 1990). Thus, changes in the extracellular levels of 5-HIAA do not necessarily indicate corresponding changes in 5-HT release.

Activation of dopamine and noradrenaline turnover constitutes an effect shared by the azaspiroones, ipsapirone, gepirone and buspirone (Hamon *et al.*, 1988) that probably takes place through their common metabolite 1-PP (Giral *et al.*, 1987). However, the fact that 1-PP, which has a marked stimulatory effect on catecholaminergic systems, but is without influence on 5-hydroxytryptaminergic neurones (Hamon *et al.*, 1988; Fuller & Perry, 1989; Sharp *et al.*, 1989) has no anxiolytic activity (Young *et al.*, 1987; Amano *et al.*, 1993), and that lesopitron, while having a potent anxiolytic activity in animal tests, has no influence on dopamine metabolites, supports the suggestion that anxiolytic activity does involve mainly 5-hydroxytryptaminergic neurones.

In summary, this study showed that acute administration of lesopitron, at a dose that reduces anxiety-like behaviour, significantly inhibits 5-HT release in the frontal cortex of rats *in vivo*. This effect was obtained after systemic as well as intraraphe administration of lesopitron, suggesting the involvement of raphe 5-HT_{1A} autoreceptors controlling 5-HT release at projecting areas. In contrast with buspirone, lesopitron did not influence cortical dopamine metabolites, indicating that the anxiolytic properties of lesopitron do not appear to be dependent upon an action on dopamine systems.

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