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Buspirone and 1-(2-pyrimidinyl)-piperazine attenuate xylazine-induced antinociception in the mouse

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Abstract—The effects of subcutaneous pretreatment with buspirone and its major metabolite 1-(2-pyrimidinyl)-piperazine (1-PP) on the antinociceptive effect of xylazine were examined using the mouse acetic acid assay. Both buspirone and 1-PP dose-dependently attenuated the antinociceptive action of subcutaneously administered xylazine (0·8 mg kg^{-1}), with ED50 values of 7·3 mg kg^{-1} for buspirone and 3·4 mg kg^{-1} for 1-PP. Pretreatment with either buspirone (8 mg kg^{-1}) or 1-PP (4 mg kg^{-1}) increased the antinociceptive ED50 of xylazine 3–4-fold. These data support the involvement of α_2-adrenoceptor and 1-PP in the pharmacological activity of buspirone.

1-(2-Pyrimidinyl)-piperazine (1-PP) is the major metabolite of buspirone, a non-benzodiazepine anxiolytic. Following acute systemic administration of buspirone, 1-PP appears in plasma and accumulates in the central nervous system of rats in higher concentrations than the parent compound (Caccia et al 1983, 1986). It has been proposed that 1-PP may at least, in part, contribute to the pharmacological activity of buspirone, since the metabolite exhibited anti-conflict effects comparable with that of the parent drug in the Vogel drinking test in rats, a paradigm commonly used to evaluate anti-anxiety agents (Gower & Tricklebank 1988). On the other hand, the opposite conclusion has been drawn from the results of some other experiments. For example, unlike buspirone, 1-PP lacked antidepressant-like activity (Wieland & Lucki 1990). Furthermore, it has been reported that 1-PP antagonized or reversed the antidepressant properties of buspirone in the forced-swimming test and the learned-helplessness paradigm in rats (Cervo et al 1988; Martin 1991). In addition, 1-PP abolished the inhibitory effect of buspirone on 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head shakes in mice (Dursun & Handley 1993). These conflicting results suggest that the involvement of 1-PP in the pharmacological activity of buspirone remains to be clarified.

A number of studies have demonstrated that buspirone is a 5-HT$_{1A}$-receptor partial agonist and has very low affinity for α_2-adrenoceptors (Taylor 1988). In contrast, 1-PP is a potent α_2-adrenoceptor antagonist with negligible binding to the 5-HT$_{1A}$ receptor, as demonstrated by biochemical, electrophysiological and pharmacological studies (Giral et al 1987; Bianchi & Garattini 1988; Engberg 1989; Gobbi et al 1990). α_2-Adrenoceptor agonists, such as clonidine and xylazine, produce antinociception that can be antagonized by yohimbine and other α_2-adrenoceptor antagonists (Luttinger et al 1985). The analgesic effect of α_2-adrenoceptor agonists is thought to be mediated by descending noradrenergic pathways, which may also be activated by opiates (Yaksh 1985). Recently, it has been found that buspirone but not 1-PP attenuates antinociception induced by morphine and sufentanil (Millan & Colpaert 1990 a,b). It was also found that buspirone prevented the analgesic consequences of social defeat in male mice via 5-HT$_{1A}$ receptor mechanisms (Rodgers & Shepherd 1989). To provide further data for interpreting the influence of buspirone and its metabolite on opioid and non-opioid analgesia, we considered it worthwhile to determine whether buspirone and 1-PP can modify the antinociceptive effect of xylazine in the mouse.

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Materials and methods

Male Swiss albino mice, 18–22 g, were allowed free access to food and water until 1 h before the experiment. All experiments were conducted in a temperature (20 ± 2°C)-controlled room. Each mouse was used only once.

The antinociceptive effect of xylazine was assessed using the acetic acid assay essentially as described by Hayashi & Takemori (1971). Briefly, acetic acid (0·6%; 10 mL kg^{-1}) was given intraperitoneally. Beginning 5 min after the injection of acetic acid, the mice were observed for abdominal constrictions for a period of 5 min. A single constriction was defined as a wave of constriction of the abdominal musculature followed by a stretching of hind limbs. Antinociception was quantified as the percent reduction in abdominal constrictions compared with the control group.

Inhibition was quantified as the percentage change in nociception caused by the test material. Comparisons of multiple treatment groups with a control group were carried out using analysis of variance followed by Dunnett's test. Drug potency was expressed as the ED50 (dose producing 50% antinociception or 50% inhibition) together with 95% confidence limit obtained by regression analysis of the log dose-response relationship.

All drugs were dissolved in 0·9% NaCl (saline) on the day of use and administered subcutaneously in a volume of 10 mL kg^{-1}. Xylazine was given 20 min before acetic acid challenge. Buspirone and 1-PP were administered 10 min before xylazine. These pretreatment times were found in preliminary experiments to be near the time of onset of maximum effects. Doses of xylazine and buspirone are expressed as the hydrochloride salt.

Results

Xylazine, producing about 90% antinociception (0·8 mg kg^{-1})

Table 1. Dose-response relationships of buspirone and 1-(2-pyrimidinyl)-piperazine (1-PP) for antagonizing xylazine-induced antinociception in the mouse acetic acid test. Xylazine (0·8 mg kg^{-1}) was administered subcutaneously 20 min before acetic acid challenge. Buspirone and 1-PP were injected subcutaneously 10 min before xylazine.

Pretreatment (mg kg^{-1})		Antinociception (%)	Inhibition (%)
Saline		89·8 ± 6·1	—
Buspirone	(2)	88·1 ± 8·8	1·9
	(4)	75·3 ± 6·6*	16·1
	(8)	44·6 ± 7·0*	50·3
	(16)	11·4 ± 4·8*	87·3
1-PP	(1)	85·0 ± 8·6	5·3
	(2)	64·9 ± 5·8*	27·7
	(4)	44·0 ± 7·2*	51·0
	(8)	12·6 ± 5·7*	86·0

Data are means ± s.e.m. (n = 10). *$P < 0·01$ compared with saline pretreatment group.

Table 2. Summary of ED50 values and their 95% confidence limits (CL) for antagonizing xylazine-induced antinociception by buspirone and 1-(2-pyrimidinyl)-piperazine (1-PP) pretreatment in the mouse acetic acid test.

Pretreatment ($mg\,kg^{-1}$)	Xylazine ($mg\,kg^{-1}$)	ED50 (95% CL) ($mg\,kg^{-1}$)
Saline	0·2–0·8	0·39 (0·27–0·58)[a]
Buspirone (8)	0·8–3·2	1·39 (1·29–1·49)[a]
1-PP (4)	0·8–3·2	1·42 (1·24–1·62)[a]
Buspirone (2–16)	0·8	7·3 (5·8–9·3)[b]
1-PP (1–8)	0·8	3·4 (3·0–4·0)[b]

[a]Antagonism of xylazine (three doses) by a fixed dose of buspirone or 1-PP, [b]antagonism of a fixed dose ($0·8\,mg\,kg^{-1}$) of xylazine by four doses of buspirone or 1-PP. Ten mice were used for each dose.

in the mouse acetic acid assay, was chosen to study the effects of pretreatment with buspirone and 1-PP on xylazine-induced antinociception. The effects of pretreating mice with various doses of buspirone and 1-PP on the antinociceptive effects of xylazine are shown in Table 1. Both buspirone and its major metabolite 1-PP inhibited the antinociceptive action of xylazine in a dose-dependent fashion. In terms of dosage, 1-PP was twice as active as buspirone in antagonizing xylazine (Table 2).

The influence of pretreatment with a fixed dose of buspirone or 1-PP on the antinociceptive ED50 of xylazine was compared with saline pretreatment. As shown in Table 2, after subcutaneous administration of buspirone ($8\,mg\,kg^{-1}$) and 1-PP ($4\,mg\,kg^{-1}$), the ED50 (95% confidence limit in parentheses) of xylazine was increased from 0·39 (0·27–0·58) to 1·39 (1·29–1·49) and 1·42 (1·24–1·62) $mg\,kg^{-1}$, respectively. Both buspirone and 1-PP significantly decreased the potency of xylazine.

Discussion

Other investigators have reported that buspirone and its major metabolite 1-PP suppressed the central and peripheral effects of clonidine, another α_2-adrenoceptor agonist, including hypothermia and hypomotility (Giral et al 1987), mydriasis (Gower & Tricklebank 1988) and the slowing of gastrointestinal transit in rats (Bianchi & Garattini 1988). In this study, we have demonstrated that buspirone and 1-PP could attenuate xylazine-induced antinociception measured with the mouse acetic acid assay. The effects of buspirone and 1-PP appear to be mediated by their α_2-adrenoceptor blocking activity, since xylazine produces its antinociception via stimulation of the α_2 adrenoceptor (Luttinger et al 1985).

There seems no possibility of involvement of the 5-HT-ergic system in the inhibition of xylazine antinociception by 1-PP (Fuller & Perry 1989). In addition, this inhibitory effect does not appear to be due to a decrease in the distribution of xylazine to the central nervous system, as we found that the antinociceptive effect of intrathecally administered xylazine in mice was also attenuated by subcutaneous administration of buspirone and 1-PP (unpublished observation).

Buspirone has a very low affinity for the α_2 adrenoceptor in-vitro (Taylor 1988) and is less active in-vivo as an α_2-adrenoceptor antagonist, as demonstrated by this and previous studies (Giral et al 1987; Bianchi & Garattini 1988; Bianchi et al 1988). These facts suggest that the antagonistic action of systemically administered buspirone on antinociception induced by xylazine may be due to the formation of the metabolite 1-PP, which exerts α_2-adrenoceptor antagonist activity. This conclusion is supported by the findings that pretreatment with proadifen, an inhibitor of liver microsomal enzymes, prevented the antagonistic action of buspirone on clonidine-induced hypothermia in mice (Giral et al 1987). 1-PP was 10-fold more potent than buspirone in antagonizing clonidine-induced inhibition of gastrointestinal transit in rats (Bianchi et al 1988).

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References

Bianchi, G., Garattini, S. (1988) Blockade of α_2-adrenoceptors by 1-(2-pyrimidinyl)-piperazine (PmP) in vivo and its relation to the activity of buspirone. Eur. J. Pharmacol. 147: 343–350

Bianchi, G., Caccia, S., Vedova, F. D., Garattini, S. (1988) The α_2-adrenoceptor antagonist activity of ipsapirone and gepione is mediated by their common metabolite 1-(2-pyrimidinyl)-piperazine (PmP). Eur. J. Pharmacol. 151: 365–371

Caccia, S., Muglia, M., Mancinelli, A., Garattini, S. (1983) Disposition and metabolism of buspirone and its metabolite 1-(2-pyrimidinyl)-piperazine in the rat. Xenobiotica 13: 147–153

Caccia, S., Conti, I., Vigano, G., Garattini, S. (1986) 1-(2-Pyrimidinyl)-piperazine as active metabolite of buspirone in man and rat. Pharmacology 33: 46–51

Cervo, L., Grignaschi, G., Samanin, R. (1988) Different effects of intracerebral and systemic administration of buspirone in the forced swimming test: involvement of a metabolite. Life Sci. 43: 2095–2102

Dursun, S. M., Handley, S. E. (1993) The effects of α_2-adrenoceptor antagonists on the inhibition of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head shakes by 5-HT_{1A} receptor agonists in the mouse. Br. J. Pharmacol. 109: 1046–1052

Engberg, G. (1989) A metabolite of buspirone increases locus coeruleus activity via alpha$_2$-receptor blockade. J. Neural Transm. 76: 91–98

Fuller, R. W., Perry, K. W. (1989) Effects of buspirone and its metabolite, 1-(2-pyrimidinyl)-piperazine, on brain monoamines and their metabolites in rats. J. Pharmacol. Exp. Ther. 248: 50–56

Giral, P., Soubrie, P., Puech, A. J. (1987) Pharmacological evidence for the involvement of 1-(2-pyrimidinyl)-piperazine (PmP) in the interaction of buspirone or gepirone with noradrenergic systems. Eur. J. Pharmacol. 134: 113–116

Gobbi, M., Frittoli, E., Mennini, T. (1990) Antagonist properties of 1-(2-pyrimidinyl)-piperazine at presynaptic α_2-adrenoceptors in the rat brain. Eur. J. Pharmacol. 180: 183–186

Gower, A. J., Tricklebank, M. D. (1988) α_2-Adrenoceptor antagonist activity may account for the effects of buspirone in an anticonflict test in the rat. Eur. J. Pharmacol. 155: 129–137

Hayashi, G., Takemori, A. E. (1971) The type of analgesic-receptor interaction involved in certain analgesic assays. Eur. J. Pharmacol. 16: 63–66

Luttinger, R., Ferrari, R., Perrone, M. H., Haubrich, D. R. (1985) Pharmacological analysis of alpha-2 adrenergic mechanisms in nociception and ataxia. J. Pharmacol. Exp. Ther. 232: 883–889

Martin, P. (1991) 1-(2-Pyrimidinyl)-piperazine may alter the effects of 5-HT_{1A} agonists in the learned helplessness paradigm in rats. Psychopharmacology 104: 275–278

Millan, M. J., Colpaert, F. C. (1990a) 5-Hydroxytryptamine(HT)$_{1A}$ receptors and tail-flick responses. III. Structurally diverse 5-HT_{1A} partial agonists attenuate mu- but not kappa-opioid antinociception in mice and rats. J. Pharmacol. Exp. Ther. 256: 993–1001

Millan, M. J., Colpaert, F. C. (1990b) Attenuation of opioid-induced antinociception by 5-HT_{1A} partial agonist in the rat. Neuropharmacology 29: 315–318

Rodgers, R. J., Shepherd, J. K. (1989) Prevention of the analgesic consequences of social defeat in male mice by 5-HT_{1A} anxiolytics, buspirone, gepirone and ipsapirone. Psychopharmacology 99: 374–380

Taylor, D. P. (1988) Buspirone, a new approach to the treatment of anxiety. FASEB J. 2: 2445–2452

Wieland, S., Lucki, I. (1990) Antidepressant-like activity of the 5-HT_{1A} agonists measured with the forced swim test. Psychopharmacology 101: 497–504

Yaksh, T. L. (1985) Pharmacology of spinal adrenergic systems which modulate spinal nociceptive processing. Pharmacol. Biochem. Behav. 22: 845–858