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Monoaminergic involvement in the pharmacological actions of buspirone

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1 Buspirone, MJ-13805 and MJ-13653 did not produce a '5-hydroxytryptamine (5-HT) syndrome' in rats at doses up to $20\,\mathrm{mg\,kg^{-1}}$.

2 These drugs were very weak 5-HT uptake blockers ($\mathrm{IC_{50}} >> 10\,\mu\mathrm{M}$) compared to drugs such as chlorimipramine.

3 These drugs did not inhibit either monoamine oxidase (MAO)-A or MAO-B.

4 The K_i values for these agents as inhibitors of [^{3}H]-5-HT and [^{3}H]-ketanserin binding to rat frontal cortex or hippocampal membranes were in the $\mu\mathrm{M}$ range, well above the brain concentrations achieved after an oral dose of $25\,\mathrm{mg\,kg^{-1}}$.

5 Parenterally administered buspirone blocked apomorphine-induced sterotypy, inhibited the 5-HT syndrome elicited by 5-methoxy-*N*,*N*-dimethyltryptamine, and delayed the onset of *p*-chloroamphetamine induced behaviours.

Introduction

Buspirone, 8-(4-(4-(2-pyrimidinyl)-1-piperazinyl)-butyl)-8-azaspiro [4,5]-decane-7, 9-dione hydrochloride (Figure 1), has demonstrable anticonflict actions in several species (monkey, rat, pigeon) (Riblet *et al.*, 1982; Weissman *et al.*, 1984; Barrett *et al.*, 1984) and anxiolytic actions in man (Goldberg & Finnerty, 1979; Rickels *et al.*, 1982). However, buspirone appears to lack many of the pharmacological properties (e.g. sedative, muscle relaxant, anticonvulsant) associated with other commonly used anxiolytics such as benzodiazepines and barbiturates (Riblet *et al.*, 1982).

The mechanisms by which buspirone exerts these selective anticonflict and anxiolytic actions remain controversial. Although buspirone does not affect the components of a γ-aminobutyric acid (GABA)-benzodiazepine receptor-chloride ionophore complex *in vitro* (see Skolnick *et al.*, 1984 for review), several laboratories have reported an increase in [^{3}H]-benzodiazepine binding *in vivo* in the rat following orally administered buspirone (Garattini *et al.*, 1982; Oakley & Jones, 1983; Taylor *et al.*, 1984; Weissman *et al.*, 1984). However, the failure of either CGS 8216 or Ro 15-1788 to antagonize the anticonflict actions of buspirone (Weissmann *et al.*, 1984), supports the view that the pharmacological actions of buspirone may not involve a direct perturbation of the benzodiazepine-GABA receptor chloride ionophore complex.

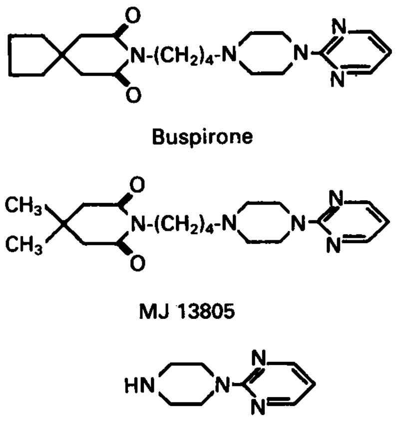

Figure 1 Structure of buspirone and related compounds.

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Monoaminergic pathways have also been proposed to modulate the anticonflict actions of drugs such as the benzodiazepines (see Stein *et al.*, 1977; Koe, 1979 for reviews). Buspirone has been shown to influence monoaminergic (5-HTergic, dopaminergic, noradrenergic) pathways in a complex fashion (see Skolnick *et al.*, 1984 for review). For example, buspirone displays pharmacological actions reminiscent of both a dopamine agonist and antagonist (Riblet *et al.*, 1982) and has been reported to produce a variety of responses resembling the 'serotonin behavioural syndrome' (5-HT syndrome) (Hjorth & Carlsson, 1982). Van der Maelen & Wilderman (1984) observed that buspirone inhibits the firing of dorsal raphé neurones, consistent with a 5-HT-like action. Nevertheless, conflicting reports have appeared regarding the potency of buspirone at 5-HT receptors *in vitro* (Riblet *et al.*, 1982; Glaser & Traber, 1983) and the 5-HTergic qualities of buspirone *in vivo* (McMillen & Mattiace, 1983). Since blockade or disruption of 5-HTergic pathways has been implicated in the anticonflict actions of a number of compounds (Stein *et al.*, 1977; Koe, 1979), and the behavioural and neurochemical actions of buspirone on 5-HTergic pathways are controversial, we have examined the neurochemical and behavioural actions of buspirone, MJ-13805 (a buspirone analogue with anticonflict action), and MJ-13653 (the major metabolite of buspirone) on 5-HTergic systems in an attempt to determine whether these compounds could exert their pharmacological actions through a 5-HTergic mechanism.

Methods

Male Sprague-Dawley rats (200–250 g) from Taconic Farms, Germantown, NY, were used in all experiments.

Neurochemical procedures

[^{3}H]-5-HT uptake Rats were killed by decapitation and the brains rapidly removed and placed on ice. Frontal cortices and hippocampi were dissected and homogenized in 20 volumes of ice-cold 0.32 M sucrose with a glass-teflon homogenizer (10 strokes). The homogenate was then centrifuged for 10 min at 1000 *g*. The supernatant (S_1) was then centrifuged for 20 min at 20,000 *g* and the resulting pellet (P_2) resuspended in 50 volumes of modified Krebs-Ringer-phosphate buffer, pH 7.4 (Hyttel, 1978): 50 µl of this crude synaptosomal suspension (P_2) was added to assay tubes containing test substances in a volume of 850 µl. [^{3}H]-5-HT (100 µl, final concentration 10 nM) was added to control tubes and incubated at 4°C for 10 min. A parallel incubation was performed at 37°C for 5 min, [^{3}H]-5-HT added and incubated for an additional 10 min at 37°C. The reaction was terminated by rapid filtration under vacuum over GF/C filters using a Brandel M-24R Cell Harvester, with 2 × 5 ml washes with the same buffer. Filters were transferred to scintillation vials containing 8 ml of Ready-Solv MP; radioactivity was determined in a Beckman LS-5800 liquid scintillation counter. Specific uptake was estimated by substracting the d.p.m. obtained at 4°C from that obtained at 37°C. The uptake at 4°C usually represented ~7% of total uptake (37°C).

[^{3}H]-5-HT binding Binding assays were performed essentially as described by Mallat & Hamon (1982) for membrane preparations of frontal cortex and hippocampus. In brief, tissue was homogenized in 30 volumes of 50 mM Tris-HCl buffer, pH 7.4, with a Brinkman Polytron (setting 6–7, 15 s) and centrifuged for 20 min at 40,000 *g*. The resulting pellet was resuspended in an identical volume of buffer and recentrifuged. Following resuspension in the original volume of buffer, the pellet was incubated for 10 min at 37°C, and recentrifuged. The final pellet was resuspended in 60 volumes of 50 mM Tris-HCl (pH 7.4) containing 4 mM $CaCl_2$, 10 µM pargyline, and 0.1% ascorbic acid. Incubations consisted of: 0.8 ml (0.3–0.8 mg protein) of tissue, [^{3}H]-5-HT (2 nM final concentration), and test substances in a total volume of 2 ml. Nonspecific binding was determined with 10 µM unlabelled 5-HT. Incubations were carried out at 37°C for 10 min and terminated by rapid filtration through GF/B filters and 2 × 5 ml washes with ice-cold buffer. Radioactivity was estimated as described above.

[^{3}H]-ketanserin binding Binding assays for [^{3}H]-ketanserin were performed according to the procedure of Leysen *et al.* (1982) with the following modifications: prefrontal cortex was homogenized in 10 volumes of 0.32 M sucrose with glass-teflon homogenizer (10 strokes) and centrifuged for 10 min at 1000 *g*. The supernatant was diluted (1:4) with a mixture of 1 volume sucrose (0.32 M):3 volumes 50 mM Tris-HCl (pH 7.4) buffer and centrifuged for 10 min at 35,000 *g*. the pellet was washed once by resuspension with a pipette in the same buffer and centrifuged under the same conditions. The final pellet was resuspended in 60 volumes of 50 mM Tris-HCl pH 7.4 buffer and 0.5 ml added to each tube containing 0.1 ml [^{3}H]-ketanserin (final concentration: 2 nM), 0.2 ml drugs and the appropriate amount of buffer (total volume of 2 ml). Nonspecific binding was determined in the presence of 10 µM lysergic acid diethylamide (LSD). Incubations were performed at 37°C for 15 min and were terminated by rapid filtration over GF/B filters using a Brandel Cell Harvester. Radioactivity was estimated as described above.

Monoamine oxidase activity Monoamine oxidase (MAO) activity was measured in a rat brain mitochondrial preparation as described by Tipton & Youdim (1983), using [^{14}C]-5-HT binoxalate and [^{14}C]-β-phenylethylamine hydrochloride as substrates. In brief, enzyme (100 μl) was incubated in 50 mM potassium phosphate buffer, pH 7.4 (300 μl), with either 200 or 20 μM 5-HT or β-phenylethylamine (100 μl) in the presence or absence of buspirone, MJ-13653 or MJ-13805 for 20 min. The reaction was stopped with 100 μl or 1 mM pargyline and the deaminated metabolites separated on Amberlite CG-200 and counted.

Behavioural procedures

A 5-HT behavioural syndrome was induced by either the 5-HT releasing agent *p*-chloroamphetamine (PCA) (Trulson & Jacobs, 1976; Kuhn *et al.*, 1985) or the 5-HT agonist 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) (Grahame-Smith, 1971) and measured in groups of 3 animals housed in clear Plexiglas cages. After receiving the test injection, animals were observed for 5 min at 15 min intervals for up to 2 h for the appearance of a 5-HT behavioural syndrome (Kuhn *et al.*, 1985). Symptoms of this syndrome included head weaving, reciprocal forepaw treading, hindlimb abduction, salivation, wet dog shakes, and hyperactivity. An 'all-or-none' method of scoring the presence or absence of the 5-HT behavioural syndrome was used (Sloviter *et al.*, 1978; Kuhn *et al.*, 1985). Individual rats were rated by observers 'blind' to the treatment. The concurrent appearance of three or more of the responses characteristic of the 5-HT syndrome, i.e. head weaving, forepaw treading, hindlimb abduction, or salivation were scored as a positive expression of the syndrome (Sloviter *et al.*, 1978; Jacobs, 1976; Green & Grahame-Smith, 1976; Kuhn *et al.*, 1985). A 'dopamine dependent behavioural syndrome' was produced by apomorphine (2 mg kg^{-1}, i.p.). This syndrome included sniffing, head weaving and circling. An 'all or none' method of scoring was used to define the presence or absence of this syndrome.

Materials

DL-*p*-Chloroamphetamine, apomorphine and 5-methoxy-*N*,*N*-dimethyltryptamine were purchased from Sigma, St. Louis, MO. Pargyline was a gift of Abbott Laboratories, Chicago, IL. [^{3}H]-ketanserin hydrochloride (64.6 Ci mmol^{-1}), [^{3}H]-5-hydroxytryptamine binoxalate (24.1 Ci mmol^{-1}), 1-[^{14}C]β-phenethylamine hydrochloride (50 mCi mmol^{-1}) and 1-[^{14}C]5-hydroxytryptamine creatinine bisulphate (50 mCi mmol^{-1}) were purchased from New England Nuclear, Boston, MA. Buspirone, MJ-13805 (4-4-dimethyl-1-[4-[4-(2-pyrimidinyl)-1-piperazinyl]butyl]-2,6-piperidinedione hydrochloride, and MJ-13653 (1-pyrimidinylpiperazine hydrochloride) were supplied by Dr K. Wheeler, Mead-Johnson Co., Evansville, IN.

Table 1 Inhibition of [^{3}H]-5-hydroxytryptamine ([^{3}H]-5-HT) uptake in to rat brain synaptosomes by pharmacological agents.

Compound	*Concentration* (μM)	*Frontal cortex*	*Hippocampus*
		% inhibition	
Buspirone	1	3.3 ± 1.0	− 1.7 ± 4.5[a]
	10	27.6 ± 2.6	29.5 ± 3.0
MJ-13805	10	19.2 ± 3.0	19.0 ± 3.8
MJ-13653	10	16.9 ± 1.2	15.3 ± 2.7
Chlorimipramine	1	97.0 ± 1.0	95.9 ± 1.0

[^{3}H]-5-HT uptake assays were performed as described in Methods. Values represent $\bar{x}$ ± s.e.mean from 3 independent experiments.

[a] The mean inhibition at this concentration is not different from 0.

Results

Effects of buspirone, MJ-13805 and MJ-13653 on monoamine oxidase activity

The effects of buspirone, MJ-13805 and MJ-13653 on monoamine oxidase (MAO) activity were measured with 5-HT and β-phenylethylamine as substrates to measure MAO type A and B, respectively. Buspirone, MJ-13805 and MJ-13653 did not affect either MAO-A or MAO-B at concentrations from 10^{-8} to 10^{-4} M (data not shown).

Inhibition of [^{3}H]-5-hydroxytryptamine uptake

The effects of buspirone, MJ-13805, MJ-13653, and chlorimipramine (CIMP) on [^{3}H]-5-HT uptake in crude synaptosomal preparations of frontal cortex and hippocampus are shown in Table 1. In agreement with previous observations (Hyttel, 1978), CIMP at a concentration of 10^{-6} M completely inhibited [^{3}H]-5-HT uptake in both preparations. In contrast, none of the other three drugs examined was a potent inhibitor of 5-HT uptake; buspirone (10^{-5} M) (the most potent of the three compounds examined) inhibited [^{3}H]-5-HT uptake by 27.6 ± 2.6 and 29.5 ± 3.0% in synaptosomes from frontal cortex and hippocampus, respectively.

Table 2 [^{3}H]-5-hydroxytryptamine ([^{3}H]5–HT) binding to rat frontal cortex and hippocampus: effects of pharmacological agents

	Frontal cortex			*Hippocampus*		
Agent	n	*IC$_{50}$* (M)	*Hill coefficient*	n	*IC$_{50}$* (M)	*Hill coefficient*
5-HT	6	2.72×10^{-9}	1.00 ± 0.05	5	2.28×10^{-9}	0.95 ± 0.03
5-MeODMT	3	5.90×10^{-8}	0.64 ± 0.03	3	3.21×10^{-8}	0.70 ± 0.02
Buspirone	6	2.65×10^{-6}	0.41 ± 0.03	3	4.11×10^{-7}	0.38 ± 0.05
MJ-13805	5	9.60×10^{-6}	0.53 ± 0.02	4	2.94×10^{-6}	0.48 ± 0.05
MJ-13653	3	9.57×10^{-6}	1.03 ± 0.03	3	1.16×10^{-6}	0.79 ± 0.08

Membranes were incubated for 10 min at 37°C with six concentrations (10^{-10}–10^{-4} M) of inhibitors; IC$_{50}$ values were calculated from a Hill plot.

Table 3 The effects of buspirone, MJ-13805 and MJ-13653 on 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT) induced behaviour

Drug	*Dose* (mg kg^{-1})	*Route of administration*	n	*Behavioural score (5-HT syndrome)*	*Time of appearance of 5-HT syndrome* (min)
Saline	—	i.p.	18	18/18	2
Buspirone	1.0	i.p.	6	6/6	2
	2.5	i.p.	6	6/6	2
	5.0	i.p.	6	3/6	5
	10.0	i.p.	6	1/6*	—**
	20.0	i.p.	12	0/12*	—**
	20.0	p.o.	6	6/6	2
MJ-13805	20.0	i.p.	6	0/6*	—**
	20.0	p.o.	6	6/6	2
MJ-13653	20.0	i.p.	6	6/6	2
	20.0	p.o.	6	6/6	2
Metergoline	5.0	i.p.	6	0/6*	—**

Drugs were administered either orally or parenterally 30 min before the injection of 5 mg kg^{-1}, i.p. of 5-MeODMT. Control animals received saline. The rats were observed for 30 min following the injection of 5-MeODMT. The behavioural score represents the number of animals displaying the syndrome/number of treated animals.

*significantly different ($P < 0.05$) from controls according to a χ^2-analysis.

**5-MeODMT induced behaviours absent.

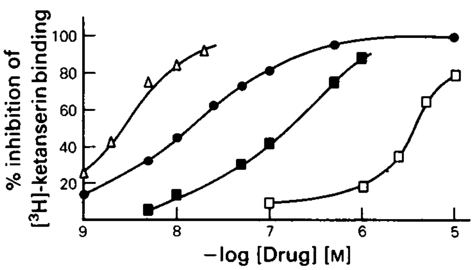

Figure 2 Concentration-response inhibition of [^{3}H]-ketanserin by pharmacological agents. The ability of various compounds to displace [^{3}H]-ketanserin (2.01 nM) from rat prefrontal cortex membranes was examined as described in Methods. Values represent data from a single experiment repeated 3–5 times with similar results. MJ-13805 and MJ-13653 were found to have IC$_{50}$'s-$> 1 \times 10^{-5}$ and $> 5 \times 10^{-5}$ M, respectively (results not shown). Symbols: spiroperidol, (△); LSD, (●); chlorimipramine, (■); buspirone, (□).

Inhibition of [^{3}H]-5-hydroxytryptamine binding

Buspirone was found to inhibit [^{3}H]-5-HT binding to cortical and hippocampal membranes with IC$_{50}$ values of 2.7×10^{-6} and 4.1×10^{-7} M, respectively (Table 2). Both MJ-13805 and MJ-13653 were slightly less potent inhibitors of [^{3}H]-5-HT binding with IC$_{50}$ values ranging from 2.9 to 11.7×10^{-6} M. 5-HT and the 5-HT agonist 5-MeODMT were included in this study for purposes of comparison. The IC$_{50}$ values obtained for these compounds (~2.5×10^{-9} for 5-HT and 5.9×10^{-8} and 3.2×10^{-8} M for 5-MeODMT, in frontal cortex and hippocampus, respectively), are consonant with values previously reported by Mallat & Hamon (1982) and Nelson *et al.* (1983). The Hill coefficients of MJ-13805 and buspirone were significantly less than 1.0, while the coefficients of 5-HT, MJ-13653 and 5-MeODMT were ~1 (Table 2).

Table 4 The effect of buspirone, MJ-13805 and MJ-13653 on the behavioural syndrome induced by *p*-chloroamphetamine (PCA)

Drug	*Dose* (mg kg^{-1})	*Route of administration*	n	*Behavioural score (5-HT syndrome)*	*Time of appearance of 5-HT syndrome* (min)
Saline	—	i.p.	24	24/24	8
Buspirone	2.5	i.p.	6	6/6	15
	5.0	i.p.	6	6/6	30–40*
	10.0	i.p.	8	8/8	60–75*
	20.0	i.p.	6	6/6	55–75*
	5.0	p.o.	6	6/6	8
	10.0	p.o.	12	12/12	8
	20.0	p.o.	6	6/6	15–20*
MJ-13653	2.5	i.p.	6	6/6	8
	5.0	i.p.	6	6/6	8
	10.0	i.p.	6	6/6	8
MJ-13805	10.0	i.p.	6	6/6	20–30*
	20.0	i.p.	6	6/6	40–55*
	5.0	p.o.	6	6/6	10
	10.0	p.o.	6	6/6	10
	20.0	p.o.	6	6/6	10
Metergoline	5.0	i.p.	6	0/6	—

Control rats received saline; all other animals were treated with the drugs indicated 30 min before PCA (10 mg kg^{-1}, i.p.). The behaviour was recorded for the next 2 h and scored as described in Table 3. The time of appearance of the 5-HT syndrome is indicated as either a mean or a range. *$P < 0.01$ compared with controls.

Inhibition of [^{3}H]-ketanserin binding

The effects of buspirone, MJ 13805, MJ 13653, and three reference substances (LSD, spiroperidol, and chlorimipramine) were examined on [^{3}H]-ketanserin binding to rat prefrontal cortex (Figure 2). Buspirone inhibited [^{3}H]-ketanserin binding with an IC$_{50}$ value of 4.28×10^{-6} M, while MJ 13805 and MJ 13653 had IC$_{50}$ values > than 10^{-5} and $> 5 \times 10^{-5}$ M, respectively. LSD, spiroperidol and chlorimipramine, which have been previously shown to be potent inhibitors of [^{3}H]-ketanserin binding in a similar preparation (Leysen *et al.*, 1982) inhibited [^{3}H]-ketanserin binding with IC$_{50}$ values of 1.31×10^{-8}, 3.31×10^{-9} and 1.37×10^{-7} M, respectively (Figure 2).

Table 5 The effect of buspirone and MJ-13805 on apomorphine-induced behaviour

Drug	*Dose* (mg kg^{-1})	n	*Apomorphine-induced behaviour*
Saline	—	9	9/9
Buspirone	5.0	6	4/6
	10.0	6	1/6*
	20.0	6	0/6*
MJ-13805	10.0	6	6/6
	20.0	12	11/12

Buspirone and MJ-13805 were injected i.p. at the doses indicated and 30 min later apomorphine (2 mg kg^{-1}, i.p.) was administered. Control rats received saline followed by apomorphine. The behavioural score was derived as described in Table 3.

*Significantly different ($P < 0.05$) from control animals according to χ^2 analysis.

The effects of buspirone, MJ-13805 and MJ-13653 on 5-hydroxytryptamine and dopamine behavioural syndromes

Buspirone (5–20 mg kg^{-1}, i.p.) reduced motor activity in rats within 5 min of administration. Animals assumed a flat posture and were tame and unresponsive to handling. These effects were absent following administration of either MJ-13653 or MJ-13805.

Intraperitoneal, but not oral administration of busipirone blocked the behavioural syndrome induced by the 5-HT agonist 5-MeODMT (Table 3). A similar blockade of 5-MeODMT-induced effects was observed with MJ-13805 but not MJ-13653. PCA-induced behavioural changes in control rats are generally observed 8-10 min after injection. The behavioural syndrome caused by PCA was delayed in a dose-dependent fashion after i.p. administration of buspirone, while MJ-13805 was less potent than buspirone in delaying this syndrome (Table 4). In contrast, orally administered buspirone, MJ-13805 and MJ-13653 did not prevent or delay the effects of PCA. The 5-HT antagonist, metergoline

(5 mg kg^{-1}, i.p.) potently inhibited the behaviour induced by either 5-MeODMT or PCA (Tables 3 and 4)

Intraperitoneally administered buspirone but not MJ-13805 antagonized the apomorphine induced behaviours (Table 5). This effect of buspirone was dose-dependent and reached its peak at about 10 mg kg^{-1}.

Discussion

Neurochemical, electrophysiological and pharmacological studies have shown that buspirone can affect neurotransmitter systems which have been linked to the anticonflict actions of compounds such as the benzodiazepines. Nonetheless, a causal relationship between perturbation of one or more of these systems and the anticonflict actions of buspirone has not been established. Since disruption or blockade of 5-HTergic pathways has been associated with the anticonflict actions of a number of compounds (Stein *et al.*, 1977; Koe, 1979) and the influence of buspirone on 5-HTergic systems is controversial (Hjorth & Carlsson, 1982; McMillen & Mattiace, 1983; Eison *et al.*, 1983b; Glaser & Traber, 1983 Van der Maelen & Wilderman, 1984), we examined the effects of buspirone, MJ-13805 (a buspirone analogue), and MJ-13653 (the major metabolite of buspirone) on 5-HTergic pathways to determine whether an action on these systems is consistent with the pharmacological actions of these compounds.

Buspirone, MJ-13805 and MJ-13653 appear to have a marginal influence on the uptake and metabolism of 5-HT. In comparison to chlorimipramine, these compounds are virtually inactive as inhibitors of [^{3}H]-5-HT uptake into cortical and hippocampal synaptosomes (Table 1). The concentrations of buspirone needed to inhibit [^{3}H]-5-HT uptake *in vitro* would not be achieved in brain following the minimally effective anticonflict doses of either buspirone and MJ-13805 in rat (1 mg kg^{-1}, orally) (Garattini *et al.*, 1982; Riblet *et al.*, 1982; Eison *et al.*, 1983a; Weissman *et al.*, 1984).

Since MAO inhibitors have been reported to be effective in treating some forms of anxiety (Youdim & Finberg, 1982), we examined the effects of buspirone, MJ-13805 and MJ-13653 on brain MAO activity. These studies revealed that none of the compounds inhibited either MAO-A or MAO-B at concentrations up to 10^{-4}M (see Results).

Buspirone, MJ-13805 and MJ-13653 were examined for their abilities to displace the 5-HT$_2$ receptor ligand [^{3}H]-ketanserin (a 5-HT antagonist) from binding sites in rat prefrontal cortex. The potencies of these compounds in displacing [^{3}H]-ketanserin are in the μM range, at least three orders of magnitude higher than reference compounds (spiroperidol, LSD and CIMP) included in this study for comparison. Further, the potencies of these compounds at 5-HT$_1$-receptors are too low to be of consequence to the anticonflict actions of these compounds since doses of buspirone approximately 100 fold higher than those needed for an anticonflict action would be needed to achieve brain concentrations in the μM range.

In contrast to findings suggesting a 5-HT like action of buspirone (Hjorth & Carlsson, 1983), a 5-HT behavioural syndrome was not observed with either buspirone or its derivatives at doses up to 20 mg kg^{-1} (Tables 3, 4). In agreement with the results of McMillen & Mattiace (1983), we observed that doses of 10 and 20 mg kg^{-1} of buspirone (i.p.) produce a 'taming' effect in the animals such that they are unresponsive to handling, but are not cataleptic. A reduction in motor activity was also observed. Parenterally, but not orally, administered buspirone (5–20 mg kg^{-1}) or MJ-13805 (20 mg kg^{-1}) blocked the 5-HT syndrome produced by 5-MeODMT and delayed the appearance of the 5-HT syndrome produced by PCA. These data suggest that some of the pharmacological effects of buspirone could be attributed to 5-HT receptor antagonism or inhibition of 5-HT release.

It could be argued that parenteral administration of relatively high doses of buspirone or MJ-13805 lead to sufficient brain levels to block 5-HT receptors and antagonize the actions of 5-MeODMT and delay the actions of PCA. Nevertheless, 5-HT receptor blockade does not appear to be the sole mechanism for the anticonflict actions of these drugs since the doses needed to block the 5-MeODMT syndrome and delay the PCA syndrome are much higher than minimally effective anticonflict doses. An alternative explanation for the inhibition of the 5-HT behavioural syndrome by buspirone could be related to the dopamine blocking properties of these compounds (Riblet *et al.*, 1982). Green & Grahame-Smith (1976) have reported that the 5-HT behavioural syndrome may have a dopaminergic component, since both chlorpromazine and haloperidol can block this syndrome. The observation that buspirone (10–20 mg kg^{-1}, i.p.) can inhibit apomorphine-induced sterotypy would support this view (Riblet *et al.*, 1982; Table 5). Nonetheless, MJ-13805 appears to be devoid of dopamine blocking properties (Eison *et al.*, 1983a) but can also antagonize the 5-MeODMT syndrome. Thus, these findings appear to be inconsistent with buspirone and MJ-13805 acting solely via dopaminergic pathways.

In summary, the present findings do not permit a firm conclusion regarding the mechanism of anticonflict action of buspirone, MJ-13805, and MJ-13653. However, these findings argue against a 5-HT antagonist action, since the doses of buspirone, MJ-13805 or MJ-13653 needed to block 5-HT receptors are many fold higher than would be found after a minimally effective anticonflict dose (Garrattini *et al.*, 1982; Gammans *et al.*, 1983). Furthermore, electrophysiological studies demonstrating a 5-HT-like action of buspirone in the dorsal raphé are inconsistent with evidence suggesting that anticonflict actions are achieved or augmented by blockade or disruption of 5-HT pathways (Stein *et al.*, 1977; Koe, 1979). Eison & Eison (1984) have proposed that the anticonflict action of buspirone and related compounds could be related to a complex action on both dopaminergic and 5-HTergic pathways. The observations that under certain circumstances dopamine antagonists have been reported to possess anticonflict activity in experimental animals and antianxiety activity in man (Lippa *et al.*, 1979 and references cited therein) would support this contention. Certainly, the question of the mechanism by which these compounds achieve their therapeutic effects merits further investigation.

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