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Selective agonists and antagonists for 5-hydroxytryptamine receptor subtypes, and interactions with yohimbine and FG 7142 using the elevated plus-maze test in the rat

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The effects of some 5-HT receptor ligands were investigated on measures of anxiety in an elevated plus-maze test in the rat. Quipazine (2 and 4 mg kg^{-1}), a non-specific 5-HT agonist and ritanserin (0·25–10 mg kg^{-1}), a 5-HT_2 receptor antagonist displayed anxiogenic profiles by reducing both of the measures of anxiety used in this test. Two 5-HT_{1A} receptor ligands, buspirone (4 and 8 mg kg^{-1}) and ipsapirone (2·5–10 mg kg^{-1}) and the 5-HT_1 agonist, RU 24969 (0·1875–1·5 mg kg^{-1}) significantly reduced only the percentage of time spent on the open arms. (−)-Propranolol (5 and 10 mg kg^{-1}), a 5-HT_1 receptor antagonist significantly reduced only the percentage of entries made onto the open arms. Metergoline (4 mg kg^{-1}), a non-specific 5-HT antagonist displayed anxiolytic effects in this test by increasing both measures of anxiety. The 5-HT_{1A} receptor agonist, 8-OH-DPAT (0·0625–0·25 mg kg^{-1}) had no effect on either of the measures of anxiety. The results from the non-specific ligands (quipazine and metergoline) are consistent with the theory that a reduction in 5-HT function reduces anxiety. However, in spite of their more selective effects on 5-HT receptors the results in this test from the more specific ligands are not consistent with a strong involvement of any single receptor subtype. The interaction studies with yohimbine and FG 7142 (β-carboline-3-carboxylate methylamide) provided no clear evidence for a major role of 5-HT pathways in the mediation of their anxiogenic effects.

The role of 5-hydroxytryptamine (5-HT) in anxiety has been studied for several years by investigating the effects of lesions of 5-HT pathways and drugs that act at 5-HT receptors or that affect 5-HT synthesis (for review see Johnston & File 1986). Studies have generally shown that small lesions of the dorsal raphe nucleus produce a profile in tests of anxiety that resembles that seen after benzodiazepine treatment: for example, diminished behavioural suppression in conflict procedures (Thiebot et al 1982), and elevated levels of social interaction (File et al 1979). Larger lesions have generally failed to produce such an effect (File & Deakin 1980). Lesions of 5-HT innervation of specific structures have provided differing results: for example, lesioning the innervation of the amygdala led to no specific changes in anxiety (File et al 1981; Thiebot et al 1983), but Thiebot et al (1983) found that lesioning the innervation of the substantia nigra led to an anxiolytic-like profile in a conflict procedure.

However, the generally positive results from lesion studies have not been replicated in studies investigating the effects of drugs that act primarily at 5-HT systems. Although there have been some reports, based on conflict models of anxiety, that 5-HT antagonists such as methysergide and metergoline can have anxiolytic-like effects (Stein et al 1973; Sepinwall & Cook 1978; Leone et al 1983), this conclusion has not been supported by results from other studies (Petersen & Lassen 1981; Commissaris & Rech 1982; Kilts et al 1982), other tests (File 1981), or from clinical investigations (see Soubrie 1986). The converse issue of whether 5-HT agonists can increase anxiety is no clearer; although studies have shown that agonists such as quipazine can decrease punished responding, these drugs also cause a decrease in unpunished responding (Graeff & Schoenfeld 1970; Stein et al 1973; Kilts et al 1982).

One of the reasons why it may have been difficult to obtain clear answers on the role of 5-HT systems in anxiety using those drugs is that as well as having activity at other neurotransmitter systems they are not specific for 5-HT receptor subtypes. A major

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advance in the neuropharmacology of 5-HT systems that has occurred in the last few years, has been the identification of binding sites that may reflect the existence of different subtypes of 5-HT receptors. It is now possible that an examination of the effects of more selective 5-HT receptor ligands may shed some light on the precise role of 5-HT neurons in the expression of anxiety-related behaviours.

The purpose of the first series of experiments was therefore to investigate the possible anxiolytic or anxiogenic effects of these drugs using a non-conflict test of anxiety in the rat. For the second series of experiments we investigated the interaction of these compounds with yohimbine and FG 7142 (β-carboline-3-carboxylate methylamide) to determine whether there was a 5-HT modulation of their anxiogenic effects.

There have been several reports that yohimbine can cause anxiety both in animals (Handley & Mithani 1984; Pellow et al 1985a, b) and in man (Holmberg & Gershon 1961; Charney et al 1983). It is generally assumed that these effects are mediated by an antagonist action at α_2-adrenoceptors (Borowski et al 1976; Goldberg & Robertson 1983), although yohimbine is also reported to possess activity at many other systems including the 5-HT system (Sanghvi & Gershon 1974; Lattimer et al 1984; Pettibone et al 1985). The β-carboline, FG 7142 has also been shown to induce anxiety in animals (Corda et al 1983; File & Pellow 1984; Pellow & File 1986a) and in man (Dorow et al 1983). FG 7142 has been classified as a partial 'inverse' agonist at benzodiazepine receptors, it is therefore believed to produce its effects by altering the effects of endogenous GABA at the GABA/benzodiazepine receptor complex (Braestrup et al 1982). Because of tentative biochemical and behavioural evidence (Wise et al 1972; Rastogi et al 1978; Thiebot et al 1982) implicating a reduction in 5-HT function in the anxiolytic action of benzodiazepines, the inverse agonist FG 7142 might be expected to produce anxiety by increasing 5-HT function.

The 5-HT receptor ligands studied were: quipazine, an agonist that does not distinguish receptor subtypes (Peroutka 1985); 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a putative agonist for the 5-HT_{1A} receptor subtype (Middlemiss & Fozard 1983); ipsapirone (TVX Q 7821) and buspirone, putative ligands for the 5-HT_{1A} subtype (Dompert et al 1985; Peroutka 1985); 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-[1h]-indole (RU 24969), a putative agonist for the 5-HT_1 receptor subtype (Cortes et al 1984; Peroutka 1985); metergoline, a non-selective antagonist at 5-HT receptors (Samanin et al 1977); (−)-propranolol, a putative antagonist at 5-HT_1 receptors (Nahorski & Willcocks 1983) and ritanserin, a putative antagonist at 5-HT_2 receptors (Janssen 1982). Doses of these compounds were selected on the basis of the studies referred to above and those of Goodwin & Green (1985).

Based on previous studies and theories of 5-HT function, we would anticipate that drugs which increase that function (either through postsynaptic agonist activity or antagonistic activity at the autoreceptor) would be anxiogenic and conversely that drugs which decrease the function would be anxiolytic. However, the picture is complicated by evidence that many of the drugs act both pre- and postsynaptically, the extent to which this occurs possibly varying according to the species used, the test conditions (Goodwin & Green 1985) and even the strain of the animals (Gudelsky et al 1985). The net effect on 5-HT function may therefore be difficult to assess.

The test of anxiety selected was the elevated plus-maze test (Pellow et al 1985b). This test has successfully identified both benzodiazepines, barbiturates and novel anxiolytics as well as several anxiogenic agents (Pellow et al 1985b; Pellow & File 1986a). In the elevated plus-maze the percentage of total entries made onto the two open arms and the percentage of time spent on the open arms is taken as a measure of anxiety. Benzodiazepines and novel putative anxiolytics elevate these measures; anxiogenic agents decrease them (Pellow & File 1986a). The total number of arm entries provides a measure of overall activity.

MATERIALS AND METHODS

Animals

Animals were male hooded Lister rats (Olac Ltd, Bicester), 250–350 g, housed in groups of 6–7 in a room with an 11 h light: 13 h dark cycle, and allowed free access to food and water.

Apparatus

The plus-maze consisted of two open arms (50 × 10 cm) and two enclosed arms of the same size with 40 cm high walls, arranged so that the arms of the same type were opposite each other. There was a central square of 10 cm. The apparatus was wooden and was elevated to a height of 50 cm. The measures indicated were taken by an observer sitting in the same room 1 m from the centre of the maze. The wooden test arena in which the rats were placed before exposure to the maze was 60 × 60 × 35 cm.

Drugs

Metergoline (Farmitalia labs), ritanserin (Janssen) and FG 7142 (Ferrosan) were suspended by ultrasonic dispersion in distilled water with a drop of polysorbate (Tween) 20. Quipazine (Miles Lab Inc.), 8-OH-DPAT (Research Biochemical Inc.), buspirone (Bristol-Myers), ipsapirone (Tropenwerke), RU 24969 (Roussel), (−)-propranolol (ICI) and yohimbine (Sigma) were dissolved in distilled water. Control rats received either distilled water or distilled water to which a drop of polysorbate 20 had been added. All drugs were injected 30 min i.p. before testing (except for 8-OH-DPAT and ritanserin which were given 10 and 60 min, respectively, before testing). The compounds were injected in concentrations to give an injection volume of 2 mL kg^{-1}.

Procedure

As the study took place over several months it was split into several experimental groupings, with control animals in each grouping. The individual test groups and drug doses are shown in Figs 1–4 for the ligands alone (n = 6–8 per drug-treated group and n = 8–19 per control-treated group) and in Table 1 for the ligands in combination with yohimbine and FG 7142. In the experiments with FG 7142 a dose of 10 mg kg^{-1} was used for one of the groupings because those animals were less sensitive to the effects of the β-carboline than animals in other groupings.

For 5 min before the start of the test, each rat was placed individually in a wooden arena (previous studies had found that this procedure resulted in an elevation of the total arm entries on the maze). The rat was then placed in the centre of the maze, facing one of the enclosed arms. During a 5 min test period, the following measures were taken by an observer: the number of entries into, and the time spent on, (a) open and (b) enclosed arms; the total number of arm entries. The maze was cleaned after each trial. Rats were tested between 0800 and 1600h, in an order randomized for drug-treatment.

Statistics

Except as otherwise indicated in the results section, data were analysed by analysis of variance (ANOVA) with drug treatment as the independent factor(s). ANOVA was performed on the percentage of total arm entries made into the open arms and on the percentage of total time spent on the open arms. ANOVA was also performed on the total number of arm entries. Where a drug changed both total arm entries and the percentage of arm entries made into the open arms, analysis of covariance (ANCOVA) was performed to determine to what extent the change in open arm entries was independent of any effect on closed arm entries. Analysis was carried out with open arm entries as the dependent variable and closed arm entries as the covariate. A significant effect is indicated if the reduction in open arm entries is independent of effects on closed arm entries. A non-significant effect indicates that the reduction in open arms entries reflects a non-specific depressant effect of the drug. Posthoc comparisons between drugged animals and controls were made using Dunnett's *t*-tests, and between two drug groups using Duncan's Multiple Range tests. There were control treated animals in each of the test groups and therefore statistical comparisons were only made within the individual groupings.

Table 1. The drug allocations and the numbers of animals per treatment group for experiments with yohimbine and FG 7142.

Group	Treatment (mg kg^{-1})	n
1	Control	19
	Yohimbine 2·5 or FG 7142 7·5	8
	Quipazine 1	8
	Quipazine 1 + yohimbine 2·5 or FG 7142 7·5	8
	Quipazine 2	8
	Quipazine 2 + yohimbine 2·5 or FG 7142 7·5	8
	Metergoline 2	8
	Metergoline 2 + yohimbine 2·5 or FG 7142 7·5	8
	Metergoline 4	8
	Metergoline 4 + yohimbine 2·5 or FG 7142 7·5	8
	8-OH-DPAT 0·125	8
	8-OH-DPAT 0·125 + yohimbine 2·5 or FG 7142 7·5	8
	8-OH-DPAT 0·250	8
	8-OH-DPAT 0·250 + yohimbine 2·5 or FG 7142 7·5	8
2	Control	10
	Yohimbine 2·5 or FG 7142 7·5	8
	Ipsapirone 2·5	8
	Ipsapirone 2·5 + yohimbine 2·5 or FG 7142 7·5	8
	Ipsapirone 5·0	8
	Ipsapirone 5·0 + yohimbine 2·5 or FG 7142 7·5	8
3	Control	13
	Yohimbine 2·5 or FG 7142 10	8
	RU 24969 0·375	8
	RU 24969 0·375 + yohimbine 2·5 or FG 7142 10	8
	RU 24969 1·5	8
	RU 24969 1·5 + yohimbine 2·5 or FG 7142 10	8
4	Control	19
	Yohimbine 2·5 or FG 7142 7·5	8
	(−)-Propranolol 5	8
	(−)-Propranolol 5 + yohimbine 2·5 or FG 7142 7·5	8
	(−)-Propranolol 10	8
	(−)-Propranolol 10 + yohimbine 2·5 or FG 7142 7·5	8
	Ritanserin 0·25	8
	Ritanserin 0·25 + yohimbine 2·5 or FG 7142 7·5	8
	Ritanserin 2·5	8
	Ritanserin 2·5 + yohimbine 2·5 or FG 7142 7·5	8
5	Control	12
	Yohimbine 2·5 or FG 7142 7·5	7
	Buspirone 2	7
	Buspirone 2 + yohimbine 2·5 or FG 7142 7·5	7
	Buspirone 8	7
	Buspirone 8 + yohimbine 2·5 or FG 7142 7·5	7

RESULTS

The effects of the ligands alone

Quipazine. Overall, quipazine had a significant anxiogenic effect as indicated by a reduction in the percentage of total entries made onto the open arms ($F(3,35) = 6{\cdot}48$, $P = 0{\cdot}001$), and a reduction in the percentage of time spent on the open arms ($F(3,35) = 6{\cdot}85$, $P < 0{\cdot}001$), see Fig. 1. Posthoc analysis showed these effects reached significance at both the 2 and the 4 mg kg^{-1} doses (see Fig. 1). Quipazine also reduced the total number of arm entries ($F(3,35) = 4{\cdot}50$, $P < 0{\cdot}01$; see Fig. 1). Because of this reduction in the total arm entries ANCOVA was performed; this revealed that the reduction in open arm entries was independent of the reduction in closed arm entries ($F(3,34) = 3{\cdot}34$, $P < 0{\cdot}05$), and so the observed decrease in exploration of the open arms may reflect anxiogenic activity. In fact, the reduction in total arm entries was not present until the 4 mg kg^{-1} dose, see Fig. 1.

8-OH-DPAT. 8-OH-DPAT (0·0625–0·25 mg kg^{-1}) had no effect on either of the measures of anxiety, see Fig. 1. However, it caused a significant reduction in the total number of arm entries ($F(3,35) = 5{\cdot}37$, $P < 0{\cdot}005$), and as Fig. 1 shows this effect reached significance at the highest dose of the drug.

Buspirone. Buspirone reduced the percentage of time spent on the open arms ($F(5,37) = 2{\cdot}61$, $P < 0{\cdot}05$), this effect reached significance for 4 and 8 mg kg^{-1} on posthoc analysis (see Fig. 2). It had no significant effects on the percentage of entries made onto the open arms or on the total number of arm entries, although in both cases higher doses of buspirone tended to reduce these measures compared with control values (see Fig. 2).

Ipsapirone. Ipsapirone (1·25–5 mg kg^{-1}) reduced the percentage of total time spent in the open arms ($F(3,27) = 3{\cdot}69$, $P < 0{\cdot}05$), this effect reached significance for 2·5 and 5 mg kg^{-1} (see Fig. 2). This compound also reduced the total number of arm entries ($F(3,27) = 3{\cdot}39$, $P < 0{\cdot}05$ see Fig. 2). Ipsapirone (1·25–5 mg kg^{-1}) was without significant effect on the percentage of total entries made onto the open arms, although there was a trend towards a reduction in this measure (see Fig. 2). The data obtained from rats treated with 10 mg kg^{-1} of ipsapirone was not normally distributed and was therefore compared with the control-treated group using Mann-Whitney U-tests. At this dose the percentage of entries made onto the open arms, the percentage of time spent on the open arms and the total arm entries were significantly reduced compared with control values (see Fig. 2).

RU 24969. RU 24969 reduced the percentage of total time spent on the open arms ($F(3,31) = 7{\cdot}51$, $P = 0{\cdot}01$), this effect reached significance for all doses on posthoc analysis (see Fig. 3). Although there was a significant overall drug factor for the percentage of total arm entries made onto the open arms ($F(3,31) = 3{\cdot}19$, $P < 0{\cdot}05$), the dose–response curve for this measure as illustrated in Fig. 3 is complicated and shows a non-significant decrease for the lower doses of RU 24969 and a non-significant increase for the highest dose of RU 24969 (1·5 mg kg^{-1}) compared

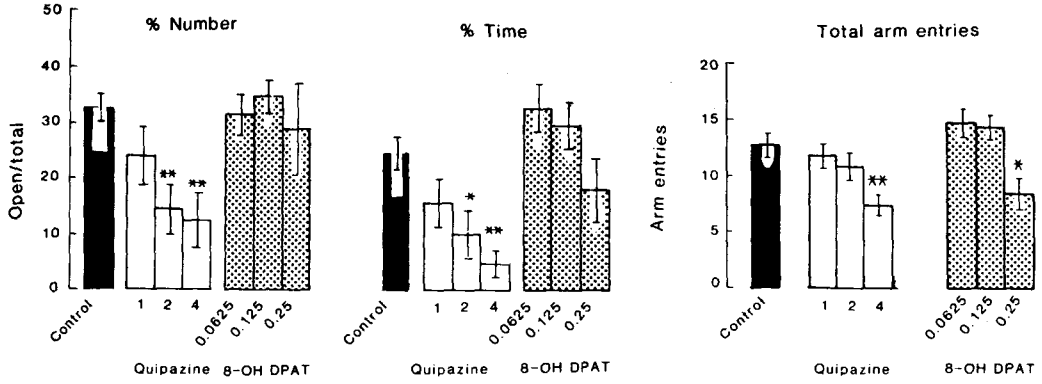

FIG. 1 Mean (± s.e.m.) percentage of arm entries made onto the open arms (% Number), percentage of time spent on the open arms (% Time) and total number of arm entries in rats given a 5 min test in the elevated plus-maze, 30 and 10 min after i.p. injection with quipazine (1–4 mg kg^{-1}) or 8-OH-DPAT (0·0625–0·25 mg kg^{-1}), respectively. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, significantly different from controls, Dunnett's *t*-test after analysis of variance.

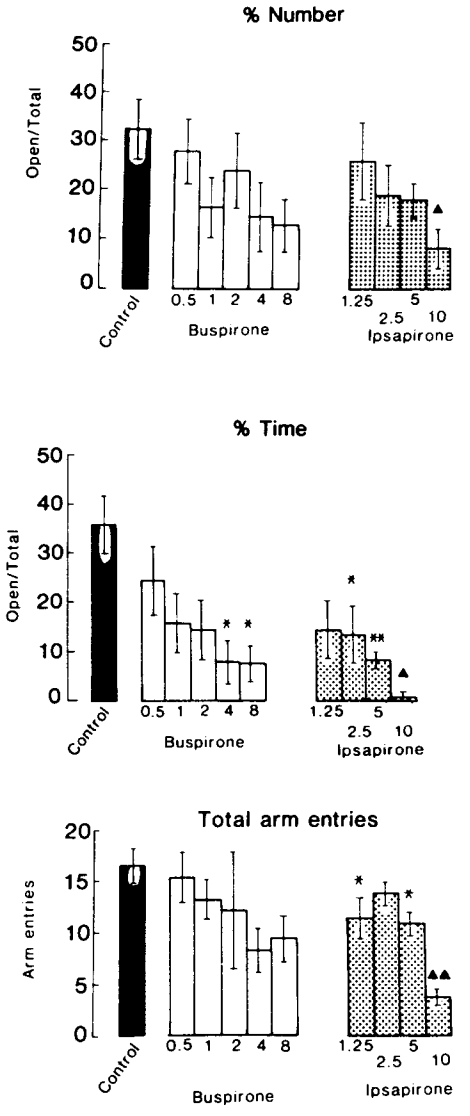

FIG. 2. Mean (± s.e.m.) percentage of arm entries made onto the open arms (% Number), percentage of time spent on the open arms (% Time) and total number of arm entries in rats given a 5 min test in the elevated plus-maze, 30 min after i.p. injection with buspirone (0·5–8 mg kg^{-1}) or ipsapirone (2·5–10 mg kg^{-1}). *P < 0·05, **P < 0·01, significantly different from controls, Dunnett's t-test after analysis of variance. ▲ P < 0·05, ▲▲ P < 0·01, significantly different from controls, Mann-Whitney U-test.

with control values. RU 24969 also reduced the total number of arm entries ($F(3,31) = 7·51$, $P < 0·005$; see Fig. 3) and posthoc analysis showed that the reduction in arm entries reached significance only at the highest dose of RU 24969.

Metergoline. Metergoline increased the percentage of total arm entries made onto the open arms ($F(3,24) = 2·83$, $P = 0·06$) and percentage of total time spent on the open arms ($F(3,24) = 4·87$, $P < 0·01$) indicating anxiolytic activity. Posthoc analysis revealed that this effect was significant only for 4 mg kg^{-1} of metergoline (see Fig. 3). The drug had no effect on the total arm entries.

(−)-Propranolol. (−)-Propranolol reduced the percentage of total arm entries made into the open arms ($F(3,39) = 5·36$, $P < 0·005$; posthoc tests showed that this was significant at 5 and 10 mg kg^{-1}, see Fig. 4) but it was without effect on the percentage of time spent on the open arms, see Fig. 4. There was a significant overall effect on total number of arm entries ($F(3,39) = 4·54$, $P < 0·01$) and Fig. 4 shows that at the 5 mg kg^{-1} dose only, there was a significant elevation in arm entries above the control scores ($P < 0·01$).

Ritanserin. Ritanserin had a significant anxiogenic effect overall, as indicated by a reduction in the percentage of total entries made onto the open arms, ($F(5,53) = 4·14$, $P < 0·005$) and a reduction in the percentage of total time spent on the open arms, ($F(5,53) = 3·25$, $P < 0·05$). Posthoc analysis showed that these effects reached significance for the 0·25, 1·25 and 10 mg kg^{-1} doses (see Fig. 4). Ritanserin also reduced total arm entries ($F(5,53) = 3·91$, $P < 0·0005$), see Fig. 4, however, this effect was significant on posthoc analysis only for 0·25 and 2·5 mg kg^{-1} doses. Because of this reduction in total arm entries, ANCOVA was performed and this showed that the decrease in the entries into the open arms was independent of the decrease in enclosed arm entries ($F(5,52) = 3·82$, $P < 0·01$). The reduction in exploration of the open arms is therefore likely to reflect anxiogenic activity.

The effects of the ligands in combination with yohimbine

Yohimbine produced an anxiogenic effect in all the groupings, this was indicated by a significant reduction in the percentage of entries made onto the open arms and a significant reduction in the percentage of time spent on the open arms compared with control-treated rats. The dose of yohimbine used (2·5 mg kg^{-1}) produced a submaximal effect on both measures of anxiety, except in the studies with ipsapirone, buspirone, (−)-propranolol and ritanserin, when it would not have been possible to

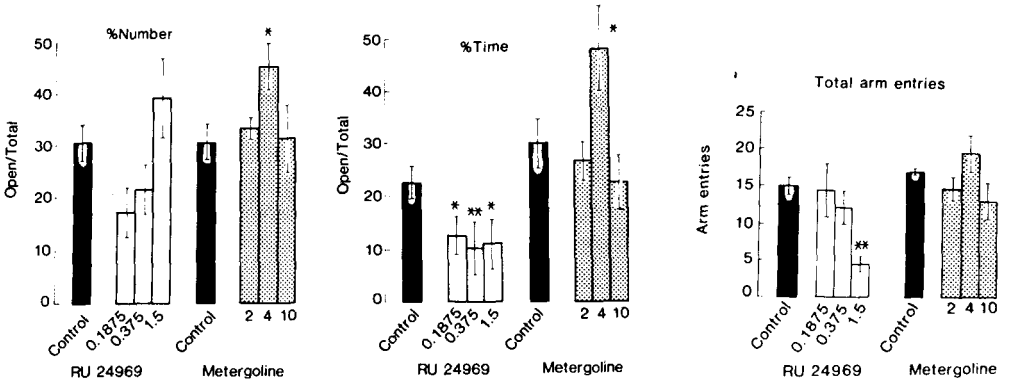

FIG. 3. Mean (± s.e.m.) percentage of arm entries made onto the open arms (% Number), percentage of time spent on the open arms (% Time) and total number of arm entries in rats given a 5 min test in the elevated plus-maze, 30 min after i.p. injection with RU 24969 (0·1875–1·5 mg kg^{-1}) or metergoline (2–10 mg kg^{-1}). *$P < 0·05$, **$P < 0·01$, significantly different from controls, Dunnett's t-test after analysis of variance.

observe a significant potentiation of its effect on the percentage of time spent on the open arms, and also in the study with buspirone when it would not have been possible to observe a significant potentiation of yohimbine's effect on the percentage of entries made onto the open arms.

Only the combination experiments that produced significant yohimbine × 5-HT ligand interaction terms on the measures of anxiety are discussed.

Quipazine and yohimbine. There was a significant quipazine × yohimbine interaction term only for the percentage of time spent on the open arms ($F(2,49) = 3·21$, $P < 0·05$) (see Fig. 5).

RU 24969 and yohimbine. There were significant RU 24969 × yohimbine interaction terms for the percentage of entries made onto the open arms ($F(2,47) = 5·62$, $P < 0·01$) and the percentage of time spent on the open arms $F(2,47)$, $P < 0·05$). Fig. 5 shows a significant reversal of the effects of yohimbine by the lower dose (0·375 mg kg^{-1}) of RU 24969 for the percentage of open arm entries. This reversal of the effects of yohimbine was no longer evident with the higher dose of RU 24969 (1·5 mg kg^{-1}), and neither did we see simple addition; instead, there was a slight, but non-significant potentiation by RU 24969 of the effects of yohimbine (see Fig. 5). Similarly, on the percentage of time spent in the open arms, Fig. 5 shows that although there was some antagonism by the lower dose of RU 24969 and enhancement by the higher dose RU 24969 of the reduction caused by yohimbine, these combination groups were not significantly different from those treated with yohimbine alone.

8-OH-DPAT and yohimbine. There was a significant 8-OH-DPAT × yohimbine interaction term for the percentage of open arm entries ($F(2,49) = 3·56$, $P < 0·05$), because 8-OH-DPAT potentiated the reduction caused by yohimbine alone (see Fig. 6). There was no significant interaction between the two drugs on the percentage of time spent in the open arms, although the scores of the group treated with yohimbine alone are already very low so it would be difficult to see a significant potentiation of this effect.

Ipsapirone and yohimbine. There were significant ipsapirone × yohimbine interactions on the measures of percentage of arm entries made into the open arms ($F(2,44) = 5·07$, $P = 0·01$) and percentage of time spent in the open arms ($F(2,44) = 7·02$, $P < 0·005$). For the latter measure the interaction term was significant because the effects of these drugs were less than additive when given in combination (see Fig. 6). However, this interaction may be complicated by a 'floor' effect, since the scores of the yohimbine group alone are already very low. The significant interaction between the two drugs on the percentage of arm entries made into the open arms arose because a combination of the lower dose (2·5 mg kg^{-1}) of ipsapirone and yohimbine produced a mutual antagonism of the reduction caused by the two drugs alone (see Fig. 6). However, ipsapirone (2·5 mg kg^{-1}) did not significantly reverse the effects of yohimbine and this combination group was still significantly different from controls. This mutual antagonism was not seen with the higher dose of ipsapirone and yohimbine; instead the combination was additive (see Fig. 6).

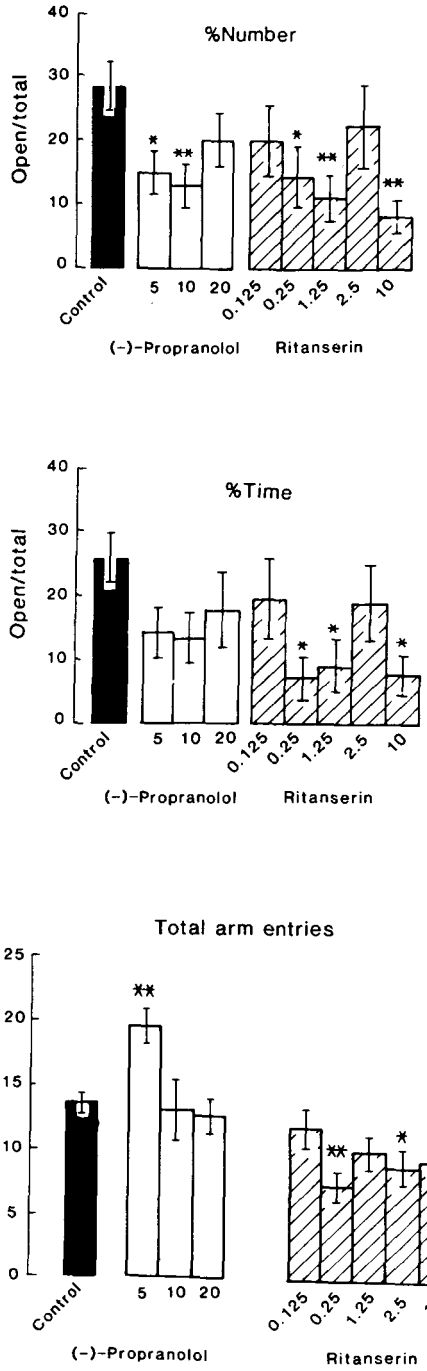

FIG. 4. Mean (± s.e.m.) percentage of arm entries made onto the open arms (% Number), percentage of time spent on the open arms (% Time) and total number of arm entries in rats given a 5 min test in the elevated plus-maze, 30 min and 60 min after i.p. injection with (−)-propranolol (5–20 mg kg^{-1}) and ritanserin (0·125–10 mg kg^{-1}) respectively. *$P < 0.05$, **$P < 0.01$, significantly different from controls, Dunnett's t-test after analysis of variance.

Ritanserin and yohimbine. There was a significant ritanserin × yohimbine interaction on the percentage of time spent in the open arms ($F(2,53) = 3.12$, $P = 0.05$). Fig. 6 shows that the effects of ritanserin (0·25 mg kg^{-1}) and yohimbine are less than additive; however, because the scores of the yohimbine group alone are low it is possible that the interaction reflects a 'floor' effect, rather than a mutual antagonism.

The effects of the ligands in combination with FG 7142

FG 7142 produced an anxiogenic effect in all the groupings, this was indicated by a significant reduction in the percentage of entries made onto the open arms and a significant reduction in the percentage of time spent on the open arms compared with control-treated rats. The dose of FG 7142 (either 7·5 or 10 mg kg^{-1}) produced a submaximal effect on both measures of anxiety in all the studies.

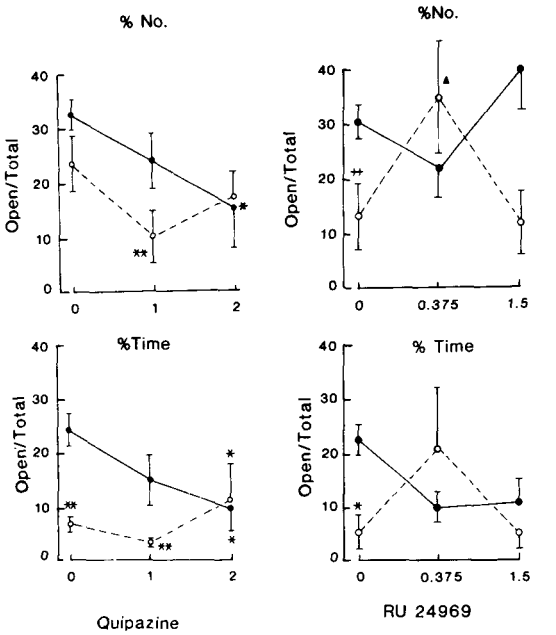

FIG. 5. Mean (± s.e.m.) percentage of arm entries made onto the open arms (% Number) and percentage of time spent on the open arms (% Time) and in rats given a 5 min test in the elevated plus-maze, 30 min after i.p. injection with quipazine (1 and 2 mg kg^{-1}) or RU 24969 (0·375 and 1·5 mg kg^{-1}) alone ● and in combination with yohimbine (2·5 mg kg^{-1}) ○. *$P < 0.05$, **$P < 0.01$, significantly different from controls, Dunnett's t-test after analysis of variance. ▲ $P < 0.05$ significantly different from groups treated with yohimbine alone, Duncan's test after analysis of variance. ++ $P < 0.01$ significantly different from controls, Student's t-test.

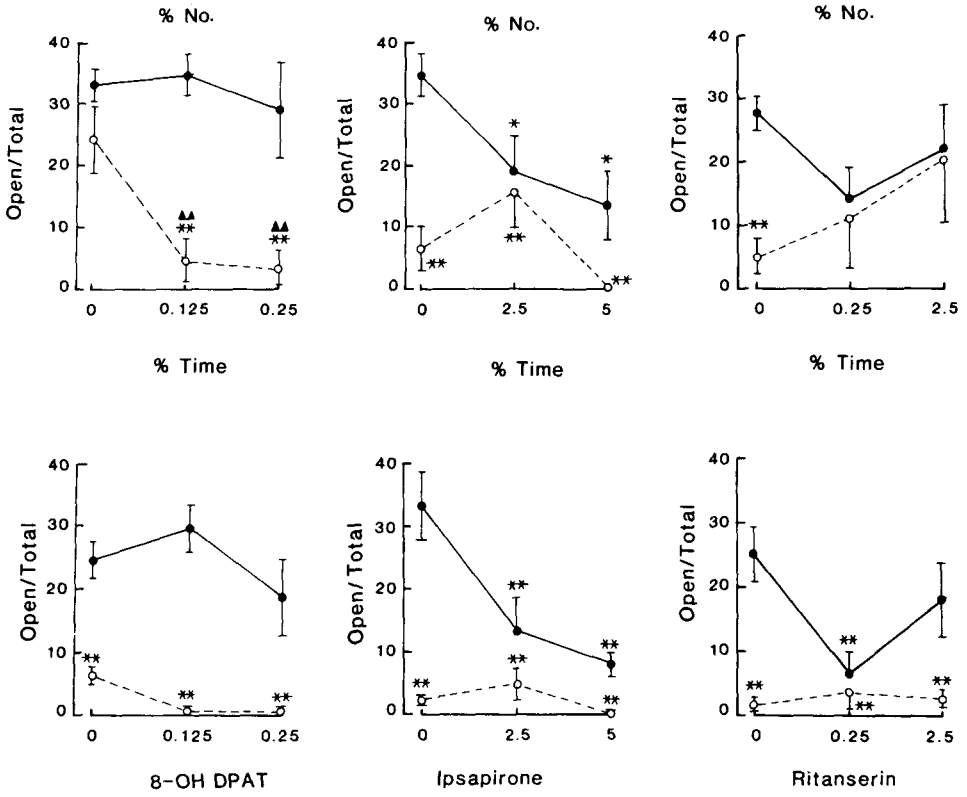

FIG. 6. Mean (± s.e.m.) percentage of arm entries made onto the open arms (% Number) and percentage of time spent on the open arms (% Time) in rats given a 5 min test in the elevated plus-maze, 10 min after i.p. injection with 8-OH-DPAT (0·125 and 0·25 mg kg^{-1}) or 30 min after i.p. injection with ipsapirone (2·5 and 10 mg kg^{-1}) or 60 min after i.p. injection with ritanserin (0·25 and 2·5 mg kg^{-1}) alone ● and in combination with yohimbine (2·5 mg kg^{-1}) ○. *$P < 0·05$, **$P < 0·01$, significantly different from controls, Dunnett's t-test after analysis of variance. ▲▲ $P < 0·01$ significantly different from groups treated with yohimbine alone, Duncan's test after analysis of variance.

Only the combination experiments which produced significant FG 7142 × 5-HT ligand interaction terms on the measures of anxiety are discussed.

RU 24969 and FG 7142. There was a significant RU 24969 × FG 7142 interaction term for the percentage of time spent in the open arms ($F(2,47) = 4·30$, $P < 0·05$). Fig. 7 shows that the addition of RU 24969 at 1·5 mg kg^{-1} significantly reversed the reduction in the percentage of total time spent on the open arms that occurred with FG 7142 alone.

Ritanserin and FG 7142. The ritanserin × FG 7142 interaction term was significant for the percentage of arm entries made into the open arms ($F(2,53) = 3·37$, $P < 0·05$) and this term just missed significance ($F(2,53) = 2·42$, $P = 0·10$) for the percentage of total time spent on the open arms. The lower dose of ritanserin (0·25 mg kg^{-1}) partially reversed the reduction in the percentage of entries made into the open arms caused by FG 7142 alone: although this reversal was not significant on posthoc analysis, the combination group was no longer significantly different from the controls (see Fig. 7). Also, on the percentage of total time spent on the open arms, the combination of ritanserin 0·25 mg kg^{-1} and FG 7142 were less than additive (see Fig. 7), indicating some interaction between the two drugs.

Buspirone and FG 7142. Buspirone (2 and 8 mg kg^{-1}) significantly enhanced the effects of FG 7142 (10 mg kg^{-1}), bringing the scores for % time spent on the open arms to 2·4 ± 1·3 and 1·1 ± 1·1, respectively. However, the reason for these low scores was not simply an enhancement of anxiety. One rat in the FG 7142 + buspirone (2 mg kg^{-1})

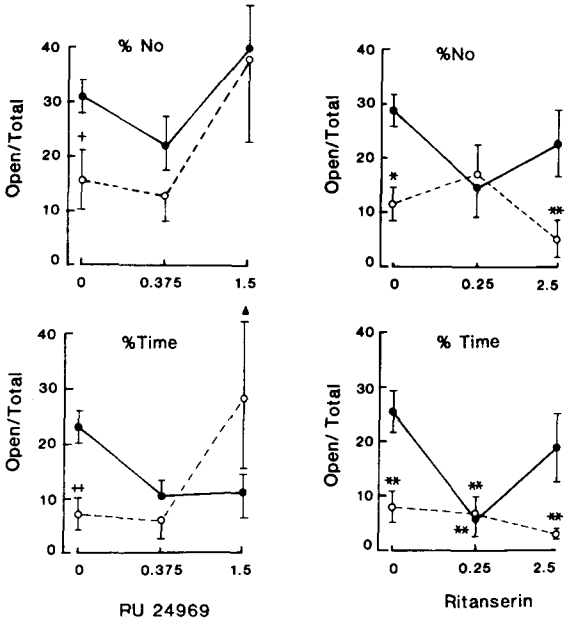

FIG. 7. Mean (± s.e.m.) percentage of arm entries made onto the open arms (% Number) and percentage of time spent on the open arms (% Time) in rats given a 5 min test in the elevated plus-maze, 30 min after i.p. injection with RU 24969 (0·375 and 1·5 mg kg^{-1}) or 60 min after i.p. injection with ritanserin (0·25 and 2·5 mg kg^{-1}) alone ● and in combination with FG 7142 (10 mg kg^{-1}) ○. *P < 0·05, **P < 0·01, significantly different from controls, Dunnett's t-test after analysis of variance. ▲ P < 0·05 significantly different from the group treated with FG 7142 alone, Duncan's test after ANOVA. +P < 0·05, ++P < 0·01, significantly different from controls, Student's t-test.

group had full and prolonged tonic-clonic convulsions and 5/6 of the others had repeated myoclonic jerks; 5/7 of the rats in the FG 7142 and buspirone (8 mg kg^{-1}) group also showed repeated myoclonus.

In these interaction studies three of the 5-HT ligands produced effects which were not the same as those previously observed in the first series of experiments with the ligands alone (see above). In the interaction studies, metergoline no longer produced a significant anxiolytic effect, ipsapirone decreased both measures of anxiety thus indicating a stronger anxiogenic effect than had previously been observed, and with RU 24969 only a non-significant reduction (possibly due to a wide variability in the data) was observed on the percentage of time spent on the open arms.

DISCUSSION

Quipazine, a non-selective agonist (Peroutka 1985) displayed an anxiogenic profile in this test of anxiety. This result could have been predicted since quipazine is thought to increase 5-HT function, either by acting as a postsynaptic agonist (Goodwin & Green 1985) and/or by displaying antagonistic activity at the autoreceptor (Martin & Sanders-Bush 1982). Despite demonstrating anxiogenic activity in these studies, we have found the effects of quipazine to be inconsistent (unpublished data, this laboratory).

Metergoline, a non-selective antagonist (Samanin et al 1977) appeared anxiolytic in the plus-maze at one of the doses studied and so also lent support to the theory. However, as with quipazine this effect appeared to be weak and inconsistent and further studies with metergoline (in the interaction experiments reported here) revealed little evidence for anxiolytic activity. In conflict studies both anti-conflict (Leone et al 1983; Sullivan et al 1985) and no effect (Commissaris & Rech 1982; Deacon & Gardener 1986) of metergoline has been reported. Metergoline's effect on 5-HT function may be complicated by the possibility of antagonistic activity at the autoreceptor which would increase 5-HT function (Martin & Sanders-Bush 1982). Indeed, one clinical study has reported the surprising finding that metergoline increased anxiety in a study on healthy volunteers, subjected to an anxiogenic situation (Graeff et al 1985).

The actions of certain of the more selective ligands on overall 5-HT function in the CNS are often unclear because it is not known whether they behave as agonists or antagonists at the various 5-HT subtypes. There is also controversy over which 5-HT subtype corresponds to the 5-HT autoreceptor, although evidence now seems to favour the 5-HT$_{1A}$ subtype for the dorsal raphe somatodendritic autoreceptor and the 5-HT$_{1B}$ subtype for the nerve terminal autoreceptor (see Middlemiss 1985; Dourish et al 1986; Sprouse & Aghajanian 1987).

8-OH-DPAT, a putative 5-HT$_{1A}$ agonist (Middlemiss & Fozard 1983) was without effect in this test of anxiety. However, in another study using the elevated plus-maze 8-OH-DPAT produced an anxiogenic-like response (Critchley & Handley 1986a); the reason for this discrepancy is unclear. Conflict studies have reported both inactivity (Boast et al 1983; Deacon & Gardener 1986) and anti-conflict activity (Engel et al 1984; Amrick & Bennett 1986). Quantitative differences in behavioural responses to this drug have previously been reported between rat strains (Gudelsky et al 1985). 8-OH-DPAT produces the 5-HT syndrome that is characteristic of an increase in 5-HT activity (Goodwin & Green 1985); however, other behaviours induced by 8-OH-DPAT are more indicative of a reduction in 5-HT function,

e.g. sexual (Ahlenius et al 1981) and feeding (Dourish et al 1985) behaviour and hypothermia (Goodwin et al 1986). 8-OH-DPAT could reduce 5-HT function either by agonist activity at 5-HT autoreceptors or by antagonist activity at postsynaptic receptors. There is electrophysiological evidence to suggest that this drug behaves as an antagonist at postsynaptic receptors (Colino & Halliwell 1986). There are also studies demonstrating both activity (Gozlan et al 1983) and inactivity at the 5-HT autoreceptor (Middlemiss 1984). These studies are not necessarily conflicting as evidence now suggests that 8-OH-DPAT is active on the autoreceptor on the cell bodies but not on the autoreceptors on presynaptic terminals (see Dourish et al 1986).

Buspirone has been reported to bind to 5-HT_{1A} receptors (Peroutka 1985), although interactions with many other neurotransmitter systems, in particular the dopaminergic system, have been recorded (see Taylor et al 1985). This compound is a putative anxiolytic with reported efficacy in clinical studies of anxiety (Taylor et al 1985). In general, animal tests have failed to identify buspirone as an anxiolytic (see File 1986) and in the elevated plus-maze we were unable to detect anxiolytic activity; in fact, the action of this drug on one of the measures of anxiety was towards an anxiogenic-like effect. There is conflicting evidence on whether buspirone induces the 5-HT behavioural syndrome (see Eison et al 1986; Gardener 1986; Smith & Peroutka 1986). It has been suggested that, in a similar manner to 8-OH-DPAT, buspirone behaves as an agonist at autoreceptors of the 5-HT_{1A} subtype (Dourish et al 1986). However, buspirone has been reported to block the effects of 8-OH-DPAT on guinea-pig ileum preparations (Fozard & Kilbinger 1985) and some components of the 5-HT syndrome induced by 8-OH-DPAT (Smith & Peroutka 1986) possibly indicating some 5-HT_{1A} antagonist activity.

Ipsapirone is another compound which is also thought to bind to 5-HT_{1A} sites (Dompert et al 1985); again, it is not yet clear whether it functions as an agonist or as an antagonist at these sites. Ipsapirone bears some structural similarity to buspirone (Glaser & Traber 1985) and so may possess similar activity to the latter. Along with 8-OH-DPAT and buspirone, ipsapirone produces hyperphagia in rats and can depress firing of 5-HT neurons in the raphe nuclei, leading to the suggestion that ipsapirone behaves as an agonist on 5-HT_{1A} autoreceptors (Dourish et al 1986). However, ipsapirone has also been reported to antagonize 8-OH-DPAT-induced hypothermia in rodents (Goodwin et al 1986) and some of the components of the 5-HT syndrome induced by 8-OH-DPAT (Smith & Peroutka 1986). Conflict studies have reported both inactivity (Deacon & Gardener 1986; Meert & Colpaert 1986) and a weak anti-conflict effect at one dose (Traber et al 1986) of ipsapirone; in a test measuring social interaction between pairs of rats ipsapirone was reported to increase the time spent in interaction (Schuurman et al 1986). We were unable to detect anxiolytic activity in the elevated plus-maze. The results we obtained were similar to those for buspirone in that a reduction rather than the expected increase in one of the measures of anxiety was observed and this effect was even stronger in the yohimbine and FG 7142 interaction studies reported here. Ipsapirone decreased overall activity, particularly at the highest dose tested, this is in contrast to the reported lack of effect of ipsapirone in a balance rod test in mice (Traber et al 1984), which the authors interpreted as evidence for a low incidence of benzodiazepine-like side effects, such as sedation.

RU 24969 is a putative 5-HT receptor agonist which, until recently, was claimed to have a selectivity for the 5-HT_{1B} site. Again there is doubt as to the net effect of this compound on 5-HT function because of its reported effects on both 5-HT_{1A} and 5-HT_{1B} receptor subtypes (Peroutka 1985) and because of possible activity at the autoreceptor (Middlemiss 1985). Although RU 24969 displayed weak anti-conflict activity in a food-motivated conflict test (Gardener 1986) it was reported to show anxiogenic-like effects in an investigation using the elevated plus-maze (Critchley & Handley 1986a). In this study RU 24969 reduced the percentage of total time spent in the open arms (this is in same direction as anxiogenic compounds) but was without effect on the percentage of entries into the open arms. Unfortunately we were unable to attempt meaningful studies with doses of RU 24969 above 1·5 mg kg^{-1}, because at these doses we encountered circling behaviour which confounded measurement in the plus-maze. If RU 24969 can be shown to increase overall 5-HT function possibly through its agonist activity on postsynaptic 5-HT_1 sites, the partial anxiogenic profile observed would support the theory that an increase in 5-HT function increases anxiety.

(−)-Propranolol, a non-selective 5-HT_1 antagonist, reduced the percentage of entries made onto the open arms but was without significant effect on the percentage of time spent on the open arms. This drug would be expected to reduce 5-HT function and hence an anxiolytic profile rather than the observed

anxiogenic-like effect on one of the measures might have been anticipated. However, there is evidence for antagonistic activity at the autoreceptor (Middlemiss 1985) and its effects on anxiety may be complicated by its β-adrenoceptor antagonist properties.

Finally, ritanserin, a putative 5-HT_2 antagonist displayed an anxiogenic profile in the elevated plus-maze. There has been evidence for anxiolytic activity in a clinical trial (Ceulemans et al 1985), in a light : dark crossing test (Colpaert et al 1985) in rats and in another study using the elevated plus-maze (Critchley & Handley 1986b); however, other investigations using conflict tests have reported no activity (Meert & Colpaert 1986) or activity at only one dose (Colpaert et al 1985; Amrick & Bennett 1986) and ritanserin gave no evidence for an anxiolytic profile in a social interaction test (Gardener 1986). Consistent with one of the previous behavioural studies, we found that ritanserin was active over a wide dose range (Colpaert et al 1985).

In the interaction studies with yohimbine, there was evidence for a significant reversal of the anxiogenic effects of yohimbine by only one of the ligands: RU 24969, a 5-HT_1 receptor agonist. Quipazine, ipsapirone and ritanserin had a non-significant tendency to reverse the anxiogenic effects of yohimbine. There was some evidence for a potentiation of the anxiogenic effects of yohimbine with 8-OH-DPAT. Based on evidence that yohimbine increases 5-HT function (Papeschi et al 1971; Sanghvi & Gershon 1974; Maura et al 1982) we conclude that the results from the 5-HT ligands were generally not in the direction predicted, and when they were, these effects were weak.

There was evidence for an interaction with FG 7142 on the measures of anxiety only in the combinations with RU 24969 and ritanserin. These ligands partially reversed the anxiogenic actions of FG 7142, but the effects were weak and only evident on one of the two measures used in this test. The seizure-like activity observed in combinations of FG 7142 and buspirone is consistent with a previous report of pro-convulsant activity following buspirone treatment (Eison & Eison 1984). In summary, the studies investigating interactions with the two anxiogenic agents suggest that 5-HT pathways are not primarily involved in mediating the effects of yohimbine and FG 7142.

With the introduction of several new and more specific drugs that act on 5-HT receptors it was hoped that more light might be shed on the link between 5-HT and anxiety. However, our results with these compounds have not helped to clarify the role of 5-HT in anxiety. The net effect of these compounds on 5-HT function is still not clear and we found no strong and consistent pattern of results in the elevated plus-maze. As we have discussed, it appears that evidence is now emerging that many of these specific 5-HT ligands produce weak, inconsistent and non-dose related effects in other animal tests of anxiety. It is therefore possible that there is a mechanism of anxiety reduction to which current animal tests are insensitive (see also Pellow & File 1986b).

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