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Recent Developments in 5HT-Related Pharmacology of Animal Models of Anxiety

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GARDNER, C. R. *Recent developments in 5HT-related pharmacology of animal models of anxiety.* PHARMACOL BIOCHEM BEHAV 24(5) 1479–1485, 1986.—The proposed anxioselective drug, buspirone, interacts with $5HT_1$ receptors. An analogue, MJ 13805, produces a 5HT behavioural syndrome blocked by central 5HT pathway lesion. Both compounds inhibit 5HT neurone firing. An association of any such action with models of anxiety is not yet possible. Several compounds selective for 5HT receptor sub-types have been tested in models of anxiety. Ritanserin, a selective $5HT_2$ antagonist, shows activity in an emergence test but not conflict models. Preliminary clinical reports indicate qualitatively different anxiolytic activity from that of benzodiazepines. TVXQ 7821 is selective for $5HT_1$ receptors and has shown activity in several models of anxiety. 8OHDPAT and RU 24969 are $5HT_1$ agonists, selective for $5HT_{1A}$ and $5HT_{1B}$ sites respectively. 8OHDPAT released punished drinking but reversed a similar effect of PCPA. Its mode of action remains unclear. RU 24969 has shown no marked anxiolytic-like activity in food or water-motivated conflicts. Further studies are necessary before associating modulation of central 5HT systems with anxiolytic activity, either in animal models or patients.

5HT receptors Anxiety Animal models

EARLY pharmacological studies suggested that reduction of the function of brain 5-hydroxytryptamine (5HT) pathways would lead to an anxiolytic effect, and that the anxiolytic actions of benzodiazepines, the predominant therapy for anxiety, are mediated via a reduction in central 5HT-mediated neurotransmission (for review see Gardner [18]). However, more recent data, particularly with 5HT antagonists have shown less activity in models of anxiety following manipulations of the function of central 5HT pathways [10, 18, 35, 50].

Continued activity of benzodiazepines in conflict models of anxiety after marked suppression of function in 5HT pathways with either agonists for 5HT cell body "autoreceptors" [53] or depletion of 5HT [29,40] argues against depression of 5HT-mediated neurotransmission as a primary mode of the anxiolytic activity. This, however, assumes that the function of the 5HT-containing neurones important in the effects on models of anxiety is completely inhibited by these pharmacological manipulations. Benzodiazepines do decrease dorsal raphe cell activity in freely moving cats, but only at doses which also decrease electromyographic activity and induce ataxia [60]. The anxiolytic triazolopyridazine, CL 218872, is a ligand for benzodiazepine binding sites, but possesses no myorelaxant effect [39]. Contrary to the action of benzodiazepines, CL 218872 does not inhibit 5HT-mediated hyperthermia in rodents; an effect taken to represent anti-5HT properties [39]. Taken together these data question the involvement of 5HT in the anxiolytic effects of benzodiazepines.

Studies of the periaqueductal grey stimulation model of anxiety have suggested that reduction of the activity of 5HT-mediated pathways leads to either an enhancement of fear-like behaviour [8, 36, 45, 52] or no effect [4,62] although benzodiazepines reduce the fear-like behaviour. The relevance of these different models to anxiety in Man is not known and has generally been established empirically. Although there is a report of anxiolytic activity in patients with the 5HT antagonists cyproheptadine and pizotifen [2] this is not a consistent finding with drugs which decrease 5HT neuronal function such as the 5HT depleter parachlorophenylalanine (PCPA) [12,17]. Thus the role of 5HT in anxiety has not been clearly established.

Some recently developed anxiolytic drug candidates and compounds specific for sub-types of 5HT binding sites have opened up new avenues for investigating the role of 5HT in anxiety.

BUSPIRONE AND ANALOGUES

It was originally suggested that the proposed anxiolytic activity of buspirone involved actions on dopamine systems in the brain [56]. However, the activity in some animal models of anxiety of a metabolite, 1-(2-pyrimidinyl)-piperazine (1-PP), [5] and an analogue, MJ 13805 (Fig. 1), [15] without much interaction with dopamine systems [42,64] questioned this hypothesis.

Buspirone was found to displace binding to $5HT_1$ sites in hippocampal membranes from the calf [21] but not initially observed to displace binding to either $5HT_1$ or $5HT_2$ sites in rat brain membranes [51]. Displacement of binding to a $5HT_1$ site in rat hippocampus has subsequently been reported [30]. However, caution must be exercised in associating *in vitro*

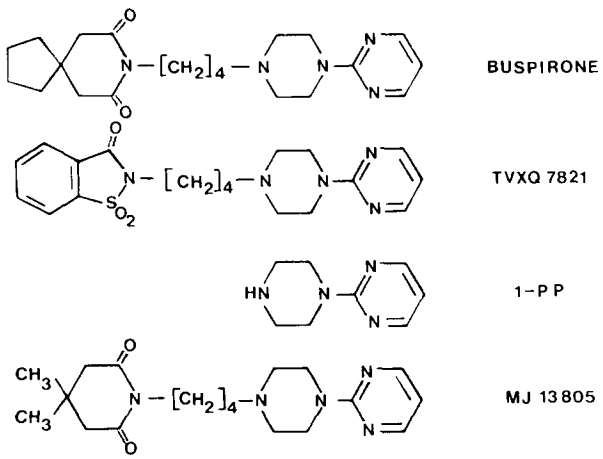

FIG. 1. Structures of buspirone and its analogue MJ 13805 and the proposed active metabolite 1-PP, in comparison with the structure of TVXQ 7821.

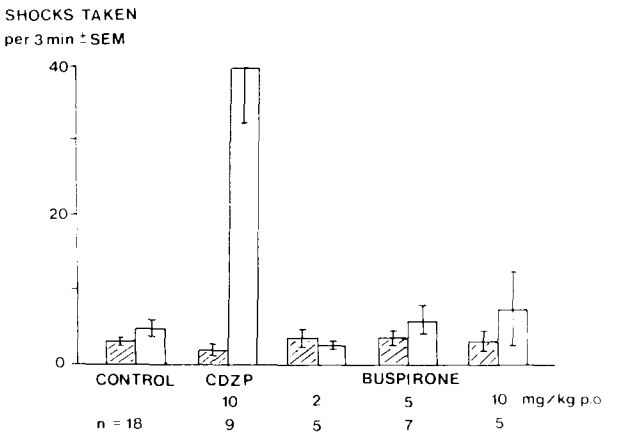

FIG. 2. The effect of buspirone on a licking conflict. The number of footshocks (1 per 20 licks) taken 1 hr after drug administration in a conditioned 3 min punished period (signalled by an on-off 3 kHz tone) is shown (open columns) in comparison to the number taken by the same rats on a previous control day (hatched columns). Chlordiazepoxide (CDZP) was used as a reference anxiolytic agent. Doses in mg/kg PO are shown below the columns with the number of rats tested (n) below that.

binding observations with *in vivo* behavioural effects as buspirone is rapidly absorbed but extensively metabolised, showing a first pass effect [6,57]. Buspirone has been proposed to induce a pro-5HT behavioural syndrome [33] but this was not confirmed by others [37,42] for either buspirone or MJ 13805, regardless of route administration. However, a recent report has claimed a 5HT-like action of MJ 13805 in inducing a myoclonic behavioural syndrome [15]. Furthermore, the activity of MJ 13805 in a licking conflict model of anxiety was prevented by 5,7-dihydroxytryptamine lesions of 5HT-containing brainpathways [15]. This finding suggests that MJ 13805 is not a postsynaptic 5HT receptor agonist but leads to the release of 5HT.

However, the observation of a pro-5HT effect of MJ 13805 being involved in an anxiolytic-like effect in licking conflict appears contrary to previous observations using similar methods. Release of punished licking has been observed with 5HT depletion or lesions and with 5HT antagonists, although such effects were less consistently observed in licking conflicts than in food-motivated conflicts [18]. Interestingly, 5,7-dihydroxytryptamine lesion of 5HT pathways, which prevented the release of punished licking by MJ 13805, did not by itself significantly affect the punished behaviour [15].

Buspirone and MJ 13805 also modulate central 5HT systems in another way. They both inhibit the firing of dorsal raphe neurones after intraperitoneal, intravenous or microiontophoretic application [15,61]. Perhaps this decrease in 5HT cell activity has greater functional significance than any pro-5HT effects with these agents, although the prevention of release of punished licking with MJ 13805 by 5HT lesion would argue against this view. Clearly, the activity of these compounds has not helped to clarify the role of 5HT in anxiety and may be confused by other pharmacological actions of these agents.

It is additionally worth noting that the activity of buspirone in models of anxiety is not consistently observed. Activity in licking conflict has been observed by several groups [49, 51, 55, 63] although in differing dose ranges, but not by others [25] or by ourselves (Fig. 2). Reports of release of punished food-motivated behaviour in rats [20, 32, 34] have also not been confirmed by our group with buspirone or 1-PP, although the method employed detects benzodiazepine receptor ligands of weak intrinsic activity which have been proposed as non-sedative anxiolytic drugs (Fig. 3).

Buspirone has also been reported to have strong [20,32], weak [3,63] or no [25] anticonflict activity in primates. An anxiolytic-like effect has, however, been observed in a rat social interaction model of anxiety [19]. The emergence of such new drugs with different modes of action leads to questioning either of the consistent clinical activity of the molecule on the one hand or of the predictivity of animal models on the other. Further clinical and pharmacological research is necessary before this basic question can be resolved and before the specific mode of action of these agents can be clarified.

RITANSERIN

Ritanserin is claimed to be a selective $5HT_2$ antagonist [47] with no partial agonist properties; primarily based on ligand binding techniques and the ability of ritanserin to antagonise but not generalise with the LSD discriminative stimulus [9]. Preliminary clinical trials suggest that ritanserin may have anxiolytic properties but that they may be qualitatively different from those of benzodiazepines [1,7]. Ritanserin was more effective than chlordiazepoxide in an emergence model of anxiety at 1–20 mg/kg IP [9] but not in another non-operant model, social interaction, at 10 mg/kg IP (Fig. 4). Furthermore, little activity has been observed from ritanserin in conflict models, either licking conflict [9] or food-motivated conflict (Fig. 5).

It is true to say, however, that a range of classical $5HT_{1/2}$ antagonists so far tested do not show consistent effects in animal models of anxiety [18]. For example, metergoline is ineffective in the social interaction model referred to above [18] and both metergoline and cyproheptadine were without marked activity in the food-motivated conflict cited [18]. Ritanserin has weak but potentially functional effects on dopamine receptors [47] and the pharmacological specificity as well as the clinical efficacy of this compound require es-

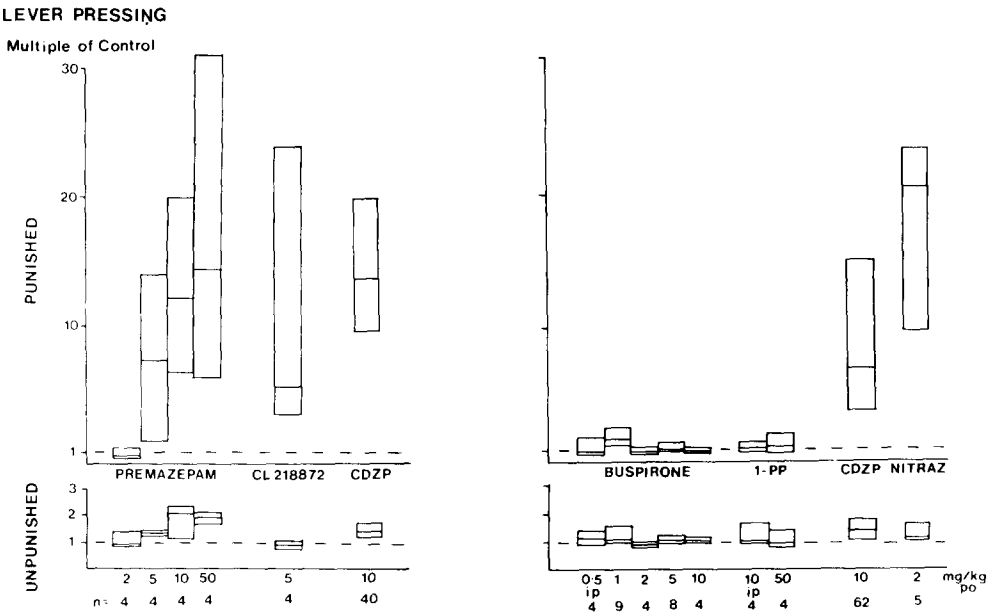

FIG. 3. Effects of proposed non-sedative benzodiazepine ligands premazepam and CL 218872 in comparison with the effects of buspirone and its metabolite 1-PP in food motivated conflict. The schedule consisted of five cycles of a light signalled 4 min FI 30 sec unpunished session followed by 3 min FR 5 punished lever pressing for food pellets. As this data does not follow a Gaussian distribution the total punished or unpunished lever pressing after drug administration (30 min prior to beginning testing) is expressed as a multiple of the activity on two previous vehicle injection control days, and the results are shown as bars representing interquartile ranges, bisected by the median value. Doses (mg/kg) are shown below the columns and are after oral administration except where indicated as intraperitoneal (IP). The effects of reference anxiolytics chlordiazepoxide (CDZP) and nitrazepam (NITRAZ) were obtained (for each section of the figure) from the same rats, within four administrations before and/or after the test substance. The number of rats tested at each dose (n) is indicated below the doses.

tablishing before a link between selective $5HT_2$ receptor blockade and anxiolytic effects can be made.

TVXQ 7821

TVXQ 7821 is selective for $5HT_1$ binding sites in calf hippocampus *in vitro* [14, 58, 59]. This compound has some structural similarity to buspirone (Fig. 1) which the same authors also found to be active on $5HT_1$ sites in the calf hippocampus [21]. It is not yet clear whether TVXQ 7821 is functionally an agonist or an antagonist on $5HT_1$ sites or whether it shows any selectivity for $5HT_{1A}$ or $5HT_{1B}$ sites.

This compound is active in a range of tests claimed to be models of anxiety, being equi-active with diazepam in inhibiting passive avoidance in rat and more active in reducing fear-related behaviour in defeated rats and in a social interaction test. In all these tests and in blocking conditioned place aversion in rats TVXQ 7821 was active at 2.5 mg/kg IP [14,28]. Other effects displayed by benzodiazepines, such as motor incoordination and anticonvulsant effects were not induced by TVXQ 7821.

The nature and specificity of the interaction of TVXQ 7821 with $5HT_1$ receptors requires further investigation as well as initiation of clinical studies before any firm association can be made.

$5HT_1$ RECEPTOR AGONISTS

There is growing evidence that $5HT_1$ and $5HT_2$ receptors are separable on the basis of function [23, 26, 27, 48]. For example, the hallucinogens, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM) and lysergic acid diethylamide (LSD) produce interoceptive stimuli with $5HT_2$ characteristics. Compounds which preferentially bind to $5HT_1$ sites, such as 8-hydroxy-2-(di-n-propylamino) tetralin (8OHDPAT) and 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-indole (RU 24969), do not show any generalisation of these interoceptive stimuli and selective $5HT_2$ antagonists block these stimuli [22,24]. In contrast, 5-hydroxytryptophan or 1-(3-trifluoromethylphenyl) piperazine (TMPP) induce interoceptive stimuli with some $5HT_1$ characteristics. The TMPP stimulus generalises to RU 24969 and selective $5HT_2$ antagonists do not block these interoceptive stimuli [22, 23, 46]. Recent evidence has suggested that there are two sub-sites involved in the binding of 5HT to $5HT_1$ sites. Spiperone displaces ^{3}H-5HT with a distinctly biphasic curve with, in the order of 3000-fold difference between the dissociation constants for high and low affinity sites [44]. These sites have been designated $5HT_{1A}$ and $5HT_{1B}$. In addition, there is some behavioural evidence that $5HT_{1A}$ and $5HT_{1B}$ sites may be separated functionally [26,28].

8OHDPAT is a selective agonist for $5HT_{1A}$ binding sites [31, 38, 44] whilst RU 24969 shows selectivity for $5HT_{1B}$ sites [11, 43, 54]. 8OHDPAT released punished licking but reversed a similar effect of PCPA [16]. Although agonist or antagonist effects at different sites or under different circumstances have been suggested, the mode of action of 8OHDPAT in this model of anxiety remains to be elucidated. RU 24969 in contrast, depressed responding in a licking con-

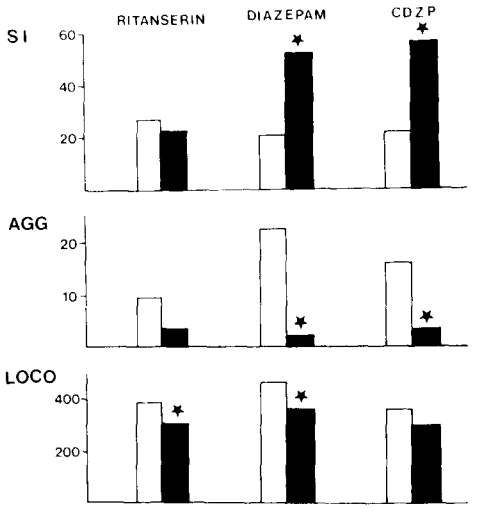

FIG. 4. Effect of ritanserin on social interaction in groups of 10 pairs of male rats according to the method of Gardner and Guy [19]. Acute administration of benzodiazepines such as diazepam (6 mg/kg PO) and chlordiazepoxide (CDZP, 10 mg/kg PO) increase novelty-suppressed active social interaction (SI in sec), decrease aggressive behaviours (AGG in sec) and have only a small depressant effect on locomotor activity (LOCO as counts from electromagnetic floor sensors). Open columns are mean activity of control groups and closed columns the mean activity of drug-treated groups. A star indicates $p<0.05$ using the Mann-Whitney U test. Ritanserin at a dose which was active in an emergence test (10 mg/kg IP [9]) decreased all behaviours, the decrease in locomotor activity being statistically significant. Thus ritanserin does not show an anxiolytic-like profile.

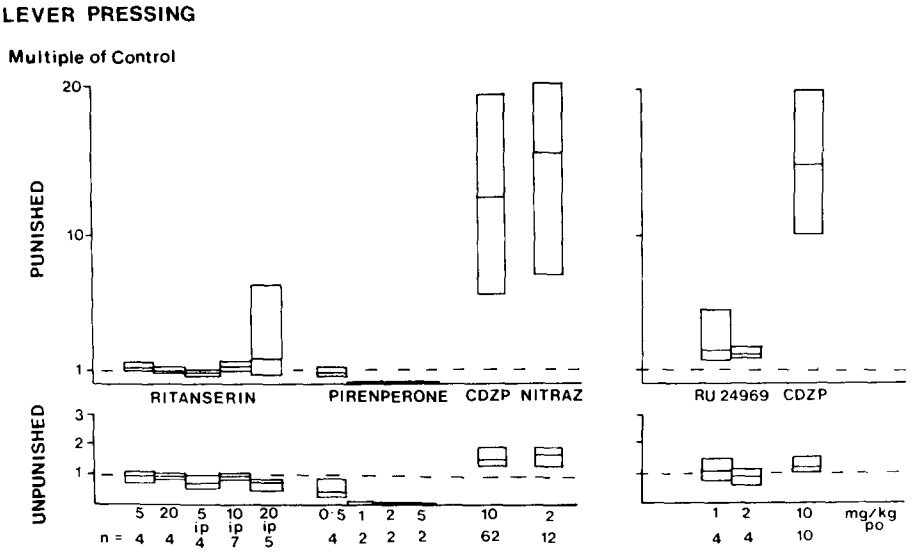

FIG. 5. Effects of ritanserin, pirenperone and RU 24969 on food-motivated conflict in male Lister rats. The method and data presentation are as described in Fig. 3.

flict [18] but this effect may be due to disruption of responding by drug-induced hyperactivity. Low doses of RU 24969 evoked only a slight increase in punished responding in a food-motivated conflict (Fig. 5).

INTERPRETATION PROBLEMS WITH 5HT AGONISTS AND ANTAGONISTS

The net effects of these pharmacological agents will depend on the nature and degree of their effect on 5HT receptors at different sites in the neurotransmission. This, in turn will depend upon the pharmacological characteristics of the receptors at these different sites. Besides the different $5HT_1/5HT_2$ postsynaptic 5HT receptors there are receptors on the nerve terminals of 5HT releasing neurones which modulate release and receptors on the 5HT-containing cell bodies. In addition to these receptors there are 5HT receptors on the nerve terminals of neurones releasing other transmitters, presumably modulating their release (see Gardner [18]).

8OHDPAT is thought to stimulate postsynaptic $5HT_{1A}$ sites but also can act as an agonist at presynaptic sites, decreasing transmission by reducing 5HT release [30,31]. Further to this, after microiontophoretic or systemic administration, 8OHDPAT powerfully inhibits the firing activity of 5HT-containing neurones in the dorsal raphe nucleus [13], suggesting that it possesses agonist activity at this site that would lead to a decrease in net 5HT-mediated neurotransmission. Thus, for example, it is difficult to interpret the net effect of 8OHDPAT on 5HT-mediated transmission during its effects on licking conflict in the presence or absence of PCPA pretreatment [16]. RU 24969, in addition to its effects on postsynaptic $5HT_1$ receptors, has agonist properties at presynaptic receptors [41,43] but not at the cell body sites, at least for the raphe-suprachiasmatic nucleus pathway [41]. Whether such differences alone can explain the functionally different effects of these compounds is not clear.

A further major problem in interpretation of behavioural effects is that activation of different synaptic 5HT receptors may lead to different, even opposing, functional effects [18, 26, 27]. The relative contributions of different pathways with degrees of involvement of different postsynaptic receptors, to any given behaviour is poorly understood.

SUMMARY

The activities in animal models of anxiety of a range of newer compounds which interact with 5HT mechanisms in the brain have been reviewed. In several cases, few models of anxiety have been investigated (MJ 13805, 8OHDPAT, RU 24969 and ritanserin) and comparison of activities in a wider range of models would help to classify both the compounds and the models. Buspirone has been tested by several groups in a range of tests although activity has not been consistently observed. TVXQ 7821 has been tested by one group but in a range of models and appeared to be consistently active.

Several basic sets of information are required before association of actions on 5HT mechanisms in the brain and anxiolytic activity can be associated: (A) confirmed clinical anxiolytic activity, (B) specificity for 5HT systems as opposed to other neurotransmitter systems or other pharmacological effects, and (C) the net effect of association with various sites in the 5HT pathway on the 5HT transmissions involved in modulation of anxiety. This latter information is clearly the most difficult to achieve and could be complicated by the presence of functionally distinct and possibly opposed 5HT pathways involved in models of anxiety [18]. Although it is possible to determine the net effect of a compound on some specific 5HT pathways, it is not known whether the effects of this compound would be the same on all 5HT pathways or whether the pathways studied are involved in anxiety.

Of the compounds reviewed here, the specificities of buspirone and to some extent ritanserin are known to be compromised at least by activities on dopamine pathways [47,56]. There may be as yet unidentified pharmacological actions of the other compounds reviewed. However, there is growing knowledge of the net functional effects of $5HT_1$ agonists on 5HT pathways. This, together with extension of the range of receptor-specific compounds studied, should lead to a clarification of the role of 5HT both in models of anxiety and in clinical anxiety.

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