Behavioral Effects of 5-HT Receptor Ligands in the Aversive Brain Stimulation, Elevated Plus-Maze and Learned Helplessness Tests

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GRAEFF, F. G., E. A. AUDI, S. S. ALMEIDA, E. O. GRAEFF AND M. H. L. HUNZIKER. Behavioral effects of 5-HT receptor ligands in the aversive brain stimulation, elevated plus-maze and learned helplessness tests. NEUROSCI BIOBEHAV REV 14(4) 501-506, 1990.—In order to illustrate the use of animal models in the study of the anxiolytic and antidepressant properties of drugs acting on 5-HT receptors, a series of experiments is described. With electrical stimulation of the midbrain central gray (CG), an aversive area of the brain, the 5-HT-1 receptor antagonist propranolol raised the aversive threshold in a dose-dependent way, following its microinjection into the CG. This antiaversive effect of propranolol, which is similar to that of benzodiazepine anxiolytics, was prevented by microinjection into the same brain site of the 5-HT-2 receptor blocker ritanserin. Ritanserin itself and the 5-HT-1A receptor ligand ipsapirone caused either little or no effect. In another animal model of anxiety, the elevated plus-maze, intra-CG propranolol also caused an anxiolytic-like effect, antagonized by ritanserin, indicating a 5-HT mediation. However, systemically injected isamoltane, a congener of propranolol, was ineffective in the elevated plus-maze, whereas ipsapirone caused an anxiolytic effect. Ritanserin was again inactive. Finally, both ipsapirone as well as another 5-HT-1A receptor ligand BAY R 1531, given IP, reversed the learning deficit resulting from exposure to uncontrollable foot-shocks, an effect characteristic of antidepressant drugs.

5-HT receptor ligands

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THE identification of subtypes of 5-HT receptors in binding assays (32, 33, 37) and the verification that specific ligands of such receptors caused anxiolytic and/or antidepressant effects in preclinical as well as in clinical tests (3, 9, 11, 12, 14, 23, 42, 43, 47) revived interest in the role played by 5-HT in emotional and mood disorders.

One approach that may prove useful in clarifying the function of 5-HT in brain processes underlying anxiety and depression is to measure the behavioral effects of drugs acting selectively on different kinds of 5-HT receptors in several animal models of anxiety and depression. The following series of experiments is an illustration of this research strategy. Two laboratory tests related to anxiety, namely aversive brain electrical stimulation (17) and the elevated plus-maze (19), and one to depression, the learned helplessness test (27), were used.

AVERSIVE BRAIN STIMULATION

Electrical stimulation of brain aversive areas, in particular the

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midbrain central gray (CG), induces defensive reactions and/or flight behavior in several species and, therefore, may be viewed as an animal model of anxiety. Indeed, either systemic or intracerebral injection of benzodiazepine anxiolytics have been shown to attenuate the behavioral effects of aversive brain stimulation [for reviews see (16–18)].

As regards 5-HT, it has been reported that intraperitoneal injection of the nonselective 5-HT receptor antagonists cyproheptadine and methysergide, as well as of the synthesis inhibitor p-chlorophenylalanine (PCPA), enhance the aversive effect of electrical stimulation applied to the CG of rats trained to press a lever in order to either switch off or decrease the electrical current. Conversely, administration of drugs supposed to facilitate 5-HT neurotransmission, such as the 5-HT uptake inhibitor chlorimipramine or the synthesis precursor 5-hydroxytryptophan (5-HTP), caused antiaversive effects, like the benzodiazepine anxiolytics (24, 25, 39). Therefore, 5-HT seems to decrease the aversive consequences of CG electrical stimulation.

This conclusion is further supported by studies performed in rats having an electrode glued to a guide cannula (chemitrode) chronically implanted into their brains, allowing microinjection of drugs and electrical stimulation to be applied to the same area. Using this technique, it was found that 5-HT increased tolerance to aversive electrical stimulation of the CG or of the medial hypothalamus (26,41). The last study has additionally shown that the nonselective 5-HT receptor agonist 5-methoxydimethyltryptamine (5-MeODMT) and the 5-HT uptake inhibitor zimelidine increased the aversive threshold following intra-CG administration. Moreover, the antiaversive effect of 5-HT was antagonized by local pretreatment with either metergoline or ketanserin. Since the latter drug is a selective 5-HT-2 receptor blocker, Schütz et al. (41) suggested a mediation by this type of 5-HT receptor of the antiaversive action of the neurotransmitter in the CG. Also, because zimelidine is likely to act by way of endogenous 5-HT, a functional role of 5-HT in the modulation of aversion in the CG was further suggested.

Propranolol and similar β-adrenoceptor blockers are useful anxiolytics, seemingly because they attenuate many of the autonomic changes that occur in anxiety states, by acting at the periphery of the organism. Nevertheless, there is a group of patients which respond to high doses (160 mg daily) given for 2 to 3 weeks, with improvement of both somatic and psychological manifestations of anxiety, and in this case, a central mechanism of action is a likely possibility (48). In this regard, there is also experimental evidence showing that propranolol was more effective than atenolol, another β-blocker which crosses the blood-brain barrier less easily than propranolol, in releasing key-pecking behavior suppressed by punishment in pigeons (8).

This presumed central action of β-adrenoceptor blockers may be exerted through serotonergic rather than adrenergic mechanisms. Thus, in vitro experiments have shown that β-blockers prevent the inhibition caused by 5-HT of the release of radioactively labeled serotonin from slices of brain tissue stimulated either electrically or by adding potassium ions to the bathing fluid. Such action of β-blockers has been ascribed to the competitive blockade of autoreceptors localized in serotonergic nerve endings, which in the rat belong to the 5-HT-1B subtype (10, 28, 31). Nevertheless, 5-HT-1A receptors, which are localized either postsynaptically or in the soma-dendrites of 5-HT containing neurons, are also blocked by propranolol-like drugs (10,30).

Supporting the view that the anxiolytic effect of β-adrenoceptor antagonists is, at least in part, mediated by 5-HT, the relatively more selective 5-HT-1B ligand isamolate was found to be more effective than propranolol in attenuating experimental anxiety in human volunteers at doses causing comparable blockade of peripheral β-adrenoceptors (38,49). Notice, however, that presynaptic autoreceptors in serotonergic nerve endings of the human frontal cortex do not have the binding properties of 5-HT-1B receptors (20), although serotonin release is similarly modulated by 5-HT autoreceptors (40).

Because a high density of 5-HT-1B receptors in the CG has been reported by Pazos et al. (31), the possibility of a direct action of β-blockers in this brain region has been explored by two of us (E.A.A. and F.G.G.). For this purpose, indwelling chemitrodes were implanted in rats and, following recovery, increasing doses of propranolol were microinjected into the dorsal CG. The threshold of aversive electrical stimulation was measured before and after the injection, according to the method described by Audi and Graeff (1).

As illustrated in Fig. 1, propranolol induced dose-dependent increases in aversive threshold, similar to the effect of midazolam, being considerably more potent than the benzodiazepine. Nevertheless, the mechanisms of action of the two drugs appear to be different. Thus, reported results show that the log dose-effect functions of midazolam and chloridiazepoxide are represented by parallel straight lines (1), as expected from drugs acting upon the same type of receptor. In contrast, the straight lines of Fig. 1, corresponding to propranolol and midazolam, are not parallel to each other.

In addition, further results evidenced that the antiaversive effect of propranolol was antagonized by pretreatment with the selective blocker of 5-HT-2 receptors, ritanserin, whereas that of midazolam remained unchanged (Fig. 2). Analysis of variance indicated that the overall effect of drug-treatments was significant, F(5,48) = 8.453, p<0.001, while post hoc analysis with the Newman-Keul's test revealed that the groups treated with vehicle plus propranolol, vehicle plus midazolam and ritanserin plus midazolam differed significantly (p<0.01) from the group treated with vehicle plus saline. Regarding midazolam, Audi and Graeff

![FIG. 1. Antiaversive effect caused by microinjection (0.5 μl in 30 sec) of propranolol (left line) and midazolam (right line) into the dorsal midbrain central gray (CG) of the rat. The aversive threshold was the lowest current intensity inducing at least nine midline crossings (switch-off response) in ten successive trials with electrical stimulation applied to the CG of rats placed inside a shuttle-box. The change in threshold was the difference between the threshold determined 10 min after the injection and the basal threshold, determined before the injection in the same animal. Points in the figure represent the mean and vertical bars ± SEM of 9 rats. The straight lines are the calculated linear regressions. [Redrawn from data reported by Audi and Graeff (1) and by Audi et al. (2).]](image-url)
p = 0.05. In this test, the percentage of open arm entries is p = 0.015, the percentage of total entries made onto the open arms drug-induced blockade of presynaptic autoreceptors which inhibit evidence obtained in vitro (10, 28, 31), this may result from the chlordiazepoxide act directly upon benzodiazepine receptors, whereas CG.

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genic drugs decrease the percentage of open arm entries (19, 34, 36). The results obtained in rats provided with guide cannula aimed at the dorsal CG are illustrated in Fig. 3. It may be seen that propranolol significantly increased, F(2,12)=6.016, other specifications are in Fig. 1. (1) showed that its antiaversive effect as well as that of chlordiazepoxide were blocked by the selective benzodiazepine receptor antagonist Ro 15-1788 (flumazenil), previously injected into the CG.

From the above results it may be concluded that midazolam and chlordiazepoxide act directly upon benzodiazepine receptors, whereas propranolol raises the aversive threshold by enhancing the action of 5-HT upon type 2 receptors. In view of the aforementioned evidence obtained in vitro (10, 28, 31), this may result from the drug-induced blockade of presynaptic autoreceptors which inhibit 5-HT release.

It is interesting to point out that ritanserin, per se, did not increase the aversive threshold when injected into the CG. Although this drug has been claimed to be effective in some animal models of anxiety, as well as in clinical assays (3-5), negative results have also been reported (35). Similarly, the buspirone analog ipsapirone caused only minor increases in the aversive threshold (up to 10 μA) following its microinjection into the CG, at the doses of 10, 20 and 40 nmol.

THE ELEVATED PLUS-MAZE

An anxiolytic-like effect of intra-CG propranolol also occurred in the elevated plus-maze, a widely used animal model of anxiety (19, 34, 36). The results obtained in rats provided with guide cannula aimed at the dorsal CG are illustrated in Fig. 3. It may be seen that propranolol significantly increased, F(2,12)=6.016, p = 0.015, the percentage of total entries made onto the open arms of the maze [100 × open/(open + closed)], while not affecting the total (open + closed) number of arm entries. F(2,12)=0.566, p=0.05. In this test, the percentage of open arm entries is supposed to reflect the level of fear/anxiety, while the total number of arm entries is viewed as a measure of overall activity; accordingly, anxiolytic drugs generally increase, whereas anxiogenic drugs decrease the percentage of open arm entries (19, 34, 36). Therefore propranolol caused a selective anxiolytic effect in the elevated plus-maze, following its microinjection into the dorsal CG, since it increased the percentage of open arm entries without affecting the total number of arm entries. In the same way as the antiaversive effect discussed before, this anxiolytic effect of intra-CG propranolol seems to be mediated by endogenous 5-HT acting upon 5-HT-2 receptors, since it was also antagonized by ritanserin (Fig. 4).

Nevertheless, whether the CG is involved in the anxiolytic action of systemically administered β-blockers remains an open question. In order to test this hypothesis, two of us (S.S.A. and F.G.G.) studied the effect of intraperitoneal injection of isamoltane on the exploratory behavior of rats placed in the elevated plus-maze. However, doses ranging from 2.5 to 20 mg/kg of isamoltane, given 30 min before the test, did not significantly change the percentage of open arm entries, F(4,36)=1.026, p = 0.407. Doses above 20 mg/kg were not used, because with this dose there was a clear, though statistically nonsignificant, tendency to decrease the total number of arm entries. Negative results with IP injection of several β-blockers—alprenolol, timolol, metoprolol and ICI 118551—were similarly reported by Critchley et al. (6), though only one dose of each drug was tested. In contrast, pindolol significantly increased the percentage of open arm entries, while not affecting general activity. Nevertheless, this anxiolytic effect of pindolol was limited to a narrow range of doses, and a higher dose of the drug even caused an anxiogenic effect (5,6). Finally, a significant decrease in the percentage of open arm entries together with an increase in total arm entries caused by propranolol was reported by Pellow et al. (35). Therefore, β-blockers seemingly do not cause consistent anxiolytic or anxiogenic effects in the elevated plus-maze when administered systemically.

On the other hand, ipsapirone, which was little effective when given intracerebrally, caused a selective anxiolytic effect in the elevated plus-maze, as shown in Fig. 5. Analyses of variance
on the action of either 5-HT-1A agonists or 5-HT-2 antagonists can yet be drawn from behavioral measures taken in the elevated plus-maze, because both increases and decreases of the percentage of open arm entries and/or of time spent in the open arms have been reported following administration of these drugs (5, 6, 29, 34, 35).

LEARNED HELPLESSNESS

The boundaries between anxiety and depression, in special reactive depression or dysthymia, are sometimes difficult to draw. Not only do the two conditions often occur together in the same patient, but also drug treatments presently tend to overlap. Thus, tricyclic antidepressants have been reported to be more effective than benzodiazepine anxiolytics in the long-term management of generalized anxiety disorder (21), besides being the treatment of choice in panic and obsessive-compulsive disorders (22). Conversely, an antidepressant action of certain benzodiazepine anxiolytics, such as alprazolam, has been claimed (44). Concerning drugs acting on 5-HT-1A receptors, it has been found that buspirone and ipsapirone alleviate symptoms of depression as well as of anxiety (14, 43, 47) and that 5-HT-1A receptor agonists are effective in animal models of depression (12, 23, 42).

The above evidence prompted us (E.O.G., M.H.L. and F.G.G.) to investigate the effect of a partial 5-HT-1A agonist, ipsapirone, and of a full agonist, BAY R 1531 (13), in the learned helplessness model of depression (27,45). In this model, anxiety is likely to play an important role in the development of learning deficits caused by uncontrollable (inescapable and unpredictable) shocks, since benzodiazepine anxiolytics exert a preventive action when given before the shock session; they are nevertheless ineffective in reversing the deficit, as antidepressants typically do, once this is established (7, 45, 46).

The test of learned helplessness used consisted in the delivery through the grid floor of a square box of 60 unescapable shocks of 1 mA, 10-sec duration, at variable intervals averaging 1 min. Five days later, the test session was conducted in a shuttle-box. Then, a similar series of 30 shocks was delivered, but rats were allowed to escape from the shocks by jumping through a window to the opposite side of the shuttle-box. Maximum shock duration was 10 sec. Drugs were administered IP twice daily in unequal doses for five days, starting with the higher dose, 6 hr after the session of uncontrollable shocks. On the next three days, the lower dose was administered in the morning and the higher dose 6 hr later, in the afternoon. On the fifth day, the lower dose was given 30 min before the test session.

As shown in Fig. 6, subchronic treatment with ipsapirone (3 mg/kg in the morning and 10 mg/kg in the afternoon) and with BAY R 1531 (0.25 mg/kg in the morning and 0.5 mg/kg in the afternoon) reversed the learning deficit produced by the uncontrollable shocks. Analyses of variance indicated that the difference between shocked and nonshocked rats was significant in animals treated with saline, F(1,14)=15.82, p<0.005, becoming nonsignificant following administration of either ipsapirone, F(1,14)= 0.80, p>0.250, or BAY R 1531, F(1,14)=0.57, p>0.250.

The above effect is characteristic of antidepressant drugs (45,46), thus indicating that ipsapirone and BAY R 1531 may be clinically useful in depressive disorders. Similar results with several ligands of 5-HT-1A receptors, including 8-OH-DPAT and ipsapirone, but not BAY R 1531 have recently been reported by Giral et al. (12).

DISCUSSION

The results presented in this review, concur with several reported studies [see e.g., (11, 18, 35)] to show that animal
models of anxiety developed for detecting benzodiazepines are not always sensitive to new anxiolytics acting outside the GABA-receptor complex. Thus, in the presently described results isamol-tane and ritanserin failed to show an anxiolytic profile in the elevated plus-maze while ritanserin and ipsapirone were either little or not effective in the test of brain stimulation-induced aversion. Even the anxiolytic-like effect of ipsapirone in the maze, shown in Fig. 5, does not correlate well with the clinical response, which appears only after one to two weeks of repeated administration (47). In especial, the lack of anxiolytic effect of β-blockers in the elevated plus-maze found in the present as well as in previously reported studies (5, 6, 35) is puzzling, since this experimental condition looks very much like the acute stressful situations where these drugs are therapeutically useful (48).

Such limitations of the animal models of anxiety, presently available, indicate that further knowledge about the brain mechanisms operating in each test and in the behavioral effects of anxiolytic drugs is badly needed. In this respect, the fact that intra-CG injection of propranolol both attenuated brain stimulation-induced aversion and increased exploration of open arms suggests that CG neurons elaborating aversion inhibit exploratory avoidance in the elevated plus-maze. Nevertheless, the CG is unlikely to be the only nervous structure involved, since ipsapirone caused a clear anxiolytic-like effect in the maze, in spite of being only marginally effective when injected into the CG.

On the other hand, the fact that ipsapirone and other 5-HT-1A receptor ligands are active in animal models of depression, as shown by the present as well as previously reported results (12, 23, 42), alleviate depressive symptoms in clinical trials (14, 43, 47) and need a lag time of more than one week for their therapeutic action to take place, makes these drugs more similar to antidepressants than to benzodiazepine anxiolytics. Yet, unlike the former, though less effectively than the latter drugs, they show anxiolytic-like effects after single administration in certain animal tests (11, 47), as ipsapirone presently did in the elevated plus-maze.

It is hoped that additional studies on the effect of 5-HT receptor ligands on behavior of laboratory animals submitted to tests seemingly related to anxiety and depression will shed more light on the mechanisms of their anxiolytic and antidepressant action.

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REFERENCES


