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5-HT and Anxiety: Promises and Pitfalls

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JOHNSTON, A. L. and S. E. FILE. *5-HT and anxiety: Promises and pitfalls.* PHARMACOL BIOCHEM BEHAV **24**(5) 1467–1470, 1986.—This review examines the evidence implicating serotonergic systems in the control of anxiety. The effects of manipulations of 5-HT, including 5-HT lesions, pharmacological reduction and enhancement of serotonergic function in animal tests of anxiety are reviewed. Biochemical and behavioral evidence implicating serotonergic pathways in the anxiolytic action of benzodiazepines is presented. In each section the promises of a sertonergic involvement, as indicated by positive findings, and the pitfalls, evidenced by inconsistent and conflicting results, are discussed. Finally the dangers of a superficial interpretation of the behavioral and biochemical findings is stressed.

Benzodiazepines 5-HT Anxiety Sedation

ALTHOUGH serotonergic pathways have been implicated in the control of anxiety for more than a decade, the evidence for this is both conflicting and controversial. With the introduction of several new and more specific drugs that act on serotonin receptors there has been a resurgence of interest in the possible role of these pathways in the control of anxiety. The purpose of this article is to review the evidence from earlier work in order to show the promise that was offered by some of the results and to warn against too superficial and optimistic conclusions from these studies. In each area that we discuss, it will be seen that alongside the positive findings, it is necessary also to consider the negative results and the failure to replicate. The evidence comes from two main areas: the effects in animal tests of anxiety of manipulating serotonergic systems; and the effects of anxiolytic drugs, in particular the benzodiazepines, on the turnover of serotonin in the brain.

EVIDENCE FROM BEHAVIOURAL TESTS OF ANXIETY

Effects of 5-HT Lesions

Lesions of serotonergic pathways, resulting from the injection of the neurotoxins 5,6 or 5,7-dihydroxytryptamine have been reported to produce anxiolytic profiles in conflict paradigms [25,40]. The dorsal raphe ascending pathways seem to be of particular importance. Small 5,7-dihydroxytryptamine (5,7-DHT) lesions of this nucleus produced anxiolytic effects in the social interaction test [14] and a similar effect was produced by injecting the toxin into the lateral septum [3]. Thiebot *et al.* [38] also found that 5,7-DHT lesions of the dorsal raphe resulted in an anti-conflict effect. However, this result is in contrast with the results of an earlier study where it was found that 5-HT lesioned rats did not differ from the controls in a conflict task [37]; Commissaris *et al.* [6] also failed to find any effect on shock-suppressed responding after intracerebroventricular injection of 5,7-DHT. Attempts to investigate this effect further by making larger lesions of the dorsal raphe nucleus or by lesioning the input from the dorsal raphe into the amygdala were unsuccessful because the lesions resulted in a marked general hypoactivity [12,15].

Effects of Pharmacological Reduction of Serotonergic Function

The 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) has produced anxiolytic effects in several animal tests, including conflict procedures and social interaction [13, 17, 29, 35, 36], and Stein *et al.* [34] were able to reverse the anxiolytic effect of PCPA by administering the 5-HT precursor 5-hydroxytryptophan. However, other studies have found the effects of PCPA on punished responding to be weak, transient and inconsistent [1,8]; in one, there was a small anti-conflict effect, but the effect was poorly correlated with the depletion of 5-HT [22]. In a modified water-lick conflict procedure in which trained rats were used, PCPA was found to be ineffective [25].

The effects of 5-HT antagonists in tests of anxiety are even more conflicting. An anxiolytic action for some antagonists has been demonstrated in rat conflict procedures, for example methysergide [34] and cinanserin [8,18] and methysergide and D-2-bromolysergic acid diethylamide have been reported to increase punished response rates in the pigeon [19]. However, others have failed to find anti-conflict effects with these antagonists [7, 22, 26]. No anxiolytic effects were found for metergoline or methysergide in the social interaction test [10] and in a task in which rats were lever-pressing to avoid stimulation of the peri-aqueductal grey 5-HT antagonists were found to have an anxiogenic profile [30].

Microiontophoretic injections of 5-HT into the dorsal raphe were found by Thiebot *et al.* [37] to release punished

TABLE 1

THE EFFECT OF QUIPAZINE (1–4 mg/kg) ON SOCIAL INTERACTION AND MOTOR ACTIVITY

	Mean social interaction scores (sec) ± SEM	Mean motor scores ± SEM
Control	211.5 ± 10.3	670.2 ± 40.1
Quipazine 1 mg/kg	209.7 ± 25.7	760.4 ± 56.8
Quipazine 2 mg/kg	193.6 ± 27.0	623.0 ± 58.4
Quipazine 4 mg/kg	159.8 ± 16.4	457.8 ± 32.5

Social interaction is measured as time spent in active social interaction by pairs of rats given a 7.5 min test, 30 min after intraperitoneal injection with quipazine or control. The motor scores reflect the mean number of breaks of infra-red photobeams per test session. The rats were tested in a low light, familiar arena.

responding. Because of evidence that these 5-HT injections may depress the firing rate of dorsal raphe neurons, probably through an action on autoreceptors, the results were interpreted as support for the hypothesis that decreased 5-HT activity results in an attenuation of behavioral suppression.

Recent work has resulted in the development of several drugs that act at sub-types of 5-HT receptors. Ritanserin, claimed to act at 5-HT_2 receptors, has a possibly anxiolytic effect in a mouse test of light:dark crossing [5]. Another new compound, buspirone, with proposed clinical efficacy as an anxiolytic, inhibits firing of 5-HT dorsal raphe neurons after iontophoretic and systemic administration [42]. Buspirone has little efficacy in animal tests of anxiety [11], but if it does work clinically it raises the possibility of a mechanism of anxiolytic action to which existing animal tests are relatively insensitive.

Effects of Pharmacological Enhancement of 5-HT Function

The effects of intraventricular injection of 5-HT, whilst complex, were in general in the direction of a proconflict effect in the rat [34]. The 5-HT agonist, α-methyltryptamine suppressed punished responding in both the pigeon and the rat, but since unpunished responding was also decreased the results may simply reflect a non-specific depressant effect of the drug [19,34]. A similar interpretation can be given to the effects of the 5-HT agonist m-chlorophenylpiperazine (mCPP) which reduced both punished and unpunished responding [22]. Another 5-HT agonist, 5-methoxy,N,N-dimethyltryptamine (5-MeODMT) had no effect on punished response rates in a conflict procedure and reduced unpunished rates [31]. However, in this study 5-MeODMT was able to reverse the anti-conflict effects of chronic administration of PCPA.

The effects of quipazine, a non-selective 5-HT agonist have been studied in the social interaction test. Table 1 shows that quipazine (1–2 mg/kg) was without effect on time spent in social interaction, and at 4 mg/kg it reduced both social interaction and motor activity, indicating a general depressant effect. Thus it did not appear to be anxiogenic in this test, since an anxiogenic action is shown by a specific decrease in social interaction, without a concomitant drop in motor activity [11].

Fenfluramine, which releases 5-HT, had no effect on punished responding, whereas the 5-HT precursor, 5-hydroxytryptophan (5HTP) did have a proconflict effect [22]. However, this effect was blocked by the addition of carbidopa, which blocks peripheral decarboxylation, indicating that a peripheral action of 5-HTP was responsible for the behavioral effect.

The effects of several 5-HT uptake inhibitors have been examined in the social interaction test. An anxiogenic profile might have been expected, but this was displayed only be WY 25093; others, including S3344, clomipramine, CGP 15210 and CGP 4718 were generally without effect [11]. Amitriptyline, which also inhibits 5-HT uptake, had the non-specific effect of depressing both punished and unpunished responding in a conflict task [22]. This non-specific depressant effect of 5-HT uptake inhibitors can also be seen in the social interaction test [11].

5-HT agonists have also been developed which preferentially bind to 5-HT_1 or 5-HT_2 receptors. One of these, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) a putative agonist at 5-HT_{1A} sites had an antianxiety effect in that it released punished licking, but also reversed a similar effect that followed PCPA treatment [9]. This apparent contradiction and the unexpected anxiolytic profile of 8-OH-DPAT has been explained by suggesting that the compound is a partial agonist that acts either as an antagonist or as an agonist, or that the two effects reflect actions at pre- and post-synaptic receptors, respectively.

BIOCHEMICAL AND BEHAVIORAL EVIDENCE IMPLICATING 5-HT SYSTEMS IN THE ANXIOLYTIC ACTION OF BENZODIAZEPINES

It has been suggested that since the inhibition of serotonergic systems can mimic the profile of benzodiazepines in animal tests of anxiety the anxiolytic action of these drugs is mediated through their actions on serotonergic pathways [21,33]. We have already seen that the evidence to support the first part of this contention is controversial. We now discuss the effects of benzodiazepines on the serotonin system and lastly, consider the evidence to link their effect on 5-HT with their anxiolytic action.

Increased brain concentrations of l-tryptophan, the precursor of 5-HT, have been reported following benzodiazepine administration (e.g., [27,41]) and from *in vitro* studies Hockel *et al.* [20] have suggested that this is due to an increased uptake of l-tryptophan. Several studies have found that benzodiazepines increase brain 5-HT concentrations, probably by decreasing 5-HT turnover [2,23]. Pratt *et al.* [27] found that clonazepam increased 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and tryptophan levels in mice.

Although the raised tryptophan levels indicated an effect on 5-HT synthesis, this was not found as judged by the accumulation of 5-HTP following inhibition of central decarboxylase activity. However, a decrease in 5-HT utilisation and an inhibition of the egress of 5-HIAA from the brain was confirmed. Increased brain concentrations of the metabolite 5-HIAA have been found in several studies in which acute treatment with high doses, or chronic treatment with low doses of benzodiazepines have been used [2, 8, 43].

Rastogi *et al.* [28] found that both acute and chronic diazepam elevated synaptosomal 5-HT, suggesting that the release of 5-HT was reduced; this effect was much more

marked with chronic treatment. Synaptosomal uptake and synthesis of 5-HT was enhanced by chronic diazepam treatment, but was unaffected by acute treatment. However other studies on 5-HT release in different brain regions have not been in agreement. Soubrie *et al.* [32] studied the effects on 5-HT release of both intra-raphe and systemic application of chlordiazepoxide. Systemic injection enhanced *in vivo* 5-HT release in the dorsal raphe of encephale isole cats, an effect that was blocked by the intra-raphe administration of the benzodiazepine antagonist Ro 15-1788. These results are consistent with *in vitro* studies in which benzodiazepines facilitated the potassium-evoked release of 5-HT from midbrain raphe slices [4,37].

The most important question is whether any changes in serotonergic function can be related to the anxiolytic action of the benzodiazepines. Wise *et al.* [45] attempted to relate the behavioral actions of the benzodiazepines to their effects on monoamine turnover in the brain. They reported a reduction in 5-HT turnover after both acute and chronic oxazepam treatment, but a reduction in noradrenaline turnover only after acute treatment. These results led to the theory that the anxiolytic effect of the benzodiazepines was due to their effect of reducing 5-HT turnover, whereas their depressant effects (to which tolerance rapidly develops with chronic treatment) were due to reduction in noradrenaline turnover. The study also found an antagonism of the anxiolytic effect of oxazepam by the intraventricular administration of 5-HT.

However, other studies have failed to replicate these results. Cook and Sepinwall [8] found a reduction in 5-HT turnover only after chronic treatment with chlordiazepoxide. Although the lack of effect on 5-HT turnover of acutely administered chlordiazepoxide corresponded with a weak anti-conflict effect, the maximum anti-conflict effect was not accompanied by the maximum reduction in 5-HT turnover. File and Vellucci [16] found no changes in 5-HT turnover after a single dose of chlordiazepoxide (which was sedative), but did find reduced 5-HT turnover and an anxiolytic effect in the social interaction test after five days of drug administration. These results are not consistent with another study [24] in which an increased concentration of 5-HT was found after acute administration of a higher dose of chlordiazepoxide, but no change was found after 5–10 days of treatment. These differences suggest that the dose-response effects of benzodiazepines on 5-HT synthesis and metabolism may differ. The results of Lister and File [24] suggest that tolerance may have developed to the reduction in 5-HT turnover and Cook and Sepinwall [8] also found tolerance to the decrease in 5-HT turnover, occurring between the second and third days of treatment. This raises the possibility that the link between reduced 5-HT turnover and the sedative effect of benzodiazepines has been prematurely dismissed. Lister and File [24] found that tolerance to the sedative effects of chlordiazepoxide developed at the same time as tolerance to the effects on 5-HT. However, they interpret the increase in 5-HT levels, observed acutely, as a result of a decrease in 5-HT turnover rather than an increase in 5-HT activity; this may be incorrect since Wambebe [44] reports a link between serotonergic function and the sedative-hypnotic effect of benzodiazepines. Thus neither the particular behavioral effect (anxiolysis or sedation) nor the direction of the link with 5-HT function can be unequivocally established, and these findings highlight the difficulty of interpreting behavioral changes in the light of biochemical results.

CONCLUSIONS

The studies designed to investigate the role of central 5-HT systems in the anxiolytic effects of the benzodiazepines have failed to produce consistent results. At every stage the promise of a serotonergic involvement was held out by some positive results. However, the conflicting results point out the pitfalls which arise from a superficial survey of the field. Evidence against a serotonergic involvement comes from the inability of PCPA pretreatment or the administration of a 5-HT agonist to modify the anti-conflict effect of chlordiazepoxide [31] and by the continued anxiolytic effect of systemic diazepam after destruction of the ascending 5-HT pathways with 5,7-dihydroxytryptamine [39]. However, the interpretation of even this study is not simple since the anxiolytic effect of chlordiazepoxide applied directly to the dorsal raphe is prevented by the prior administration of the neurotoxin [37]. This could suggest that more than one pathway can mediate the anxiolytic effects of benzodiazepines. Certainly, some of the manipulations of the 5-HT systems have produced anxiolytic effects. It is hoped that some of the newer and more specific drugs will help to unravel some of these paradoxes.

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