Pharmacology Biochemistry & Behavior, Vol. 24, pp. 1471–1477, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Are Serotonergic Neurons Involved in the Control of Anxiety and in the Anxiolytic Activity of Benzodiazepines?

M. H. THIEBOT

Département de Pharmacologie, Faculté de Médecine Pitié-Salpêtrière
91, boulevard de l'Hôpital, 75634 Paris Cedex 13, France

THIEBOT, M. H. *Are serotonergic neurons involved in the control of anxiety and in the anxiolytic activity of benzodiazepines?* PHARMACOL BIOCHEM BEHAV **24**(5) 1471–1477, 1986.—Several studies have shown that, like benzodiazepines (BZP), treatments able to reduce or block the activity of CNS serotonergic (5-HT) neurons released punished behavior. Therefore, 5-HT mechanisms have been tentatively implicated in the anti-punishment (anxiolytic?) activity of BZP. Numerous data, however, are not in keeping with this hypothesis. Since not responding enables the animals to avoid punishment but also delays the receipt of food-reward, one of these factors could be an alteration of waiting capacities. Indeed, we have shown that diazepam released behavioral suppression in conflict schedules only when the duration of the punished periods exceeded 1 minute. Moreover, in rats allowed to choose in a T-maze between immediate-but-small vs. delayed-but-large reward, BZP significantly decreased the frequency with which the delayed reward was chosen, with 5-HT uptake blockers producing opposite effects. Therefore, one can hypothesize that BZP render the animals less prone than controls to tolerate delay of reward and that 5-HT mechanisms may be involved in this phenomenon. An altered tolerance to delay of reward should be taken into account when interpreting the BZP-induced release of behavioral inhibition in classical conflict procedures.

Benzodiazepines Serotonin Anxiety Behavior Waiting capacities

SEVERAL lines of pharmacological, behavioral and biochemical evidence reviewed in a recent paper by Iversen [23] suggest that serotonergic (5-HT) neurons may be involved in the control of anxiety, and more precisely of behavioral inhibition. However, experiments designed to demonstrate a role of central 5-HT neurons in the anxiolytic effects of benzodiazepines (BZP) have not yielded consistent results. The aim of this review is to examine whether or not common behavioral processes may account for the observed similarities between the effects of reduced 5-HT transmission and those of BZP.

Before engaging in such a study, it must be mentioned that the research strategy which consists in isolating one system of neurotransmission from the others and in analysing its implications in one specific function, or hypothesizing that it preferentially controls one neurobiological process, may be viewed as an oversimplification. However, investigating whether or not a given neuronal system is more directly involved in the control of one type of behavior, or in the action of one class of psychotropic drugs thereon, does not imply that further neuronal systems do not participate (directly or indirectly) in the same processes.

EVIDENCE FOR THE INVOLVEMENT OF 5-HT NEURONS IN ANXIETY

The proposition that 5-HT neurons may be involved in the control of anxiety arises from the following experimental findings:

(a) Numerous studies indicate that a reduction or a blockade of 5-HT transmission specifically releases, as do BZP, response suppression in punishment paradigms [12, 14, 36, 43, 48, 49, 51, 57].

(b) Electric stimulation of the median raphe causes a behavioral inhibition resembling fear responses to threatening events in situations where escape is impossible [15]. Treatments which increase 5-HT availability in the synaptic clefts have been shown to reinforce response suppression in conflict procedures. This effect, however, seems unspecific in that non-punished behavior is usually also reduced [14,47].

(c) BZP have been reported to decrease 5-HT turnover [8, 18, 34, 39], to slow down the firing rate of 5-HT neurons [26, 33, 55] and to reduce 5-HT released from nerve endings [46].

(d) BZP applied to the raphe nuclei (the main origin of ascending 5-HT neurons) or microinjections of either GABA mimetics or 5-HT itself into the raphe dorsalis, elicit various effects which mimic those of peripherally-administered BZP. These treatments enhance locomotor activity [38], they facilitate feeding behavior [35] and they release suppressed behavior in a conditioned conflict paradigm, an effect which is prevented by the destruction of 5-HT raphe neurons [50].

Some clinical data are in agreement with these findings since antiserotonergic drugs have been reported to have beneficial effects on target symptoms of anxiety in humans [1,60].

Even if the data which support the 5-HT hypothesis in

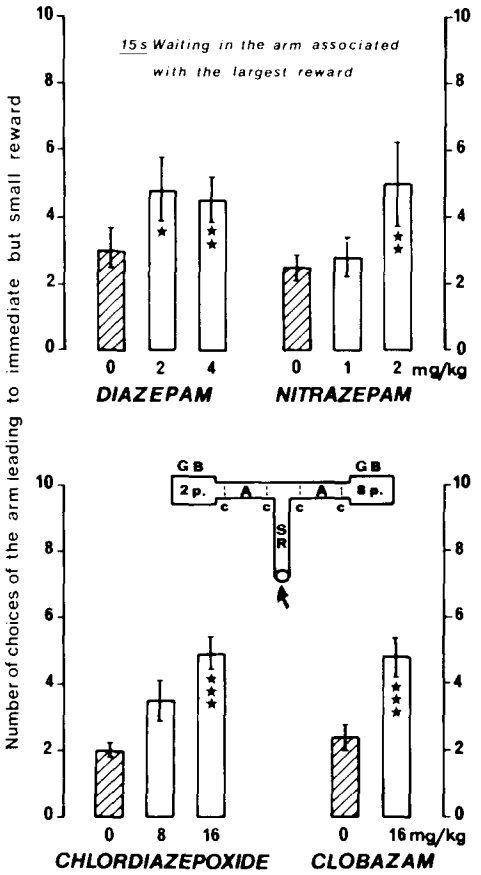

FIG. 1. Effects of four benzodiazepines (BZP) on the frequency with which the arm leading to immediate-but-small reward was chosen, during sessions for which a 15-sec waiting period was imposed in the large-reward arm. Separate groups of rats (n=6–10) were injected IP with either diazepam (2–4 mg/kg), nitrazepam (1–2 mg/kg), chlordiazepoxide (8–16 mg/kg) or clobazam (16 mg/kg) 30 min before each of two consecutive test-sessions (5 trials each) performed 24 hr apart. Columns represent the number of choices (mean±SEM) of the small-reward arm over ten trials. Open columns represent performances of treated rats during the two sessions. Hatched columns represent performances of the rats of the same groups during the last two training sessions (for clarity of the figure, when two doses of a BZP were tested, the previous performances of the two groups are combined, but statistics were performed on individual variations across sessions). $\star p<0.05$; $\star\star p<0.02$; $\star\star\star p<0.01$ compared with rats' choice during the last two training sessions (ANOVA). Inset: schematic representation of the T-maze. A=arm; c=clefts for guillotine doors; GB=goal-box; SR=starting runway; p=pellets.

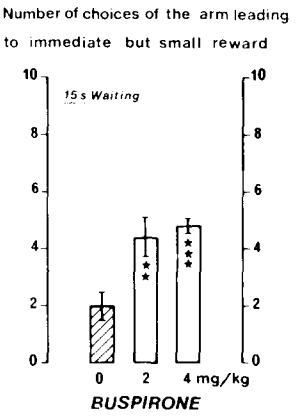

FIG. 2. Effects of buspirone on the frequency with which the arm leading to immediate-but-small reward was chosen during sessions for which a 15-sec waiting period was imposed in the large-reward arm. Buspirone (2–4 mg/kg, IP, T-30 min) was injected according to the experimental schedule described in Fig. 1. $\star\star p<0.02$; $\star\star\star p<0.01$ compared with rats' choice during the last two training sessions (ANOVA).

animal models of anxiety are numerous, at least as many studies fail to replicate the release of behavioral inhibition after a reduction of 5-HT transmission [6, 25, 28, 44, 52]. Differences in the technical procedures used to alter 5-HT transmission may be responsible for the discrepancies between such studies. Indeed, reduced 5-HT functioning has been achieved by either electrolytic lesions, lesions by neurotoxins such as 5,6- or 5,7-dihydroxytryptamine (5,6- or 5,7-DHT) or para-chloroamphetamine (pCA), inhibition of 5-HT biosynthesis by para-chlorophenylalanine (pCPA), or 5-HT receptor antagonists. Enhanced 5-HT transmission has been obtained by either inhibition of 5-HT uptake, administration of 5-hydroxytryptophan (5-HTP) or 5-HT itself, or with 5-HT receptor agonists. Moreover, the real efficacy and/or specificity of most of these tools for central 5-HT systems are quite rightly questionable and the respective roles of neither 5-HT_1 and 5-HT_2 receptors nor of the 5-HT_{1A} and 5-HT_{1B} subclasses [29,31] have yet been considered. The effects in animal models of anxiety of the newest and more "specific" 5-HT_2 antagonists such as ritanserin or of drugs such as 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) which is reported to specifically bind to 5-HT_{1A} receptors [19] are observed in only a narrow dose-range and fail to reach the magnitude of those produced by BZP [5,11].

The fact that manipulations able to almost completely block 5-HT neuronal activity induce, if any, only a modest release of response suppression whereas BZP, at doses which probably only marginally reduce 5-HT transmission, produce a more salient release of punished responding, implies that the effects of 5-HT blockade and those of BZP probably derive from processes which are poorly overlapping.

EVIDENCE AGAINST THE INVOLVEMENT OF 5-HT IN THE ANXIOLYTIC ACTIVITY OF BZP

When considering the connections between 5-HT and BZP, there are few studies to indicate that 5-HT neurons have a central role in mediating the effects of BZP on punished responding. Tye *et al.* [56] reported that a lesion of ascending 5-HT pathways blocked the action of parenteral chlordiazepoxide (CDP) in a conflict paradigm. However, these authors specified that despite a raised shock level, lesioned rats were responding in conflict at a higher rate than controls; therefore, that "CDP did not reinstate responding in these animals to the same degree seen in controls" may be an artefact: under CDP the lesioned rats reached their higher possible rate of responding sooner and thus the response-releasing effect of CDP could not reach a normal magnitude.

Other studies clearly show that destruction or inactivation of 5-HT neurons or 5-HT receptor blockade fail to impair the release of punished behavior induced by peripheral BZP [7,52]. The injection into the raphe dorsalis of the BZP

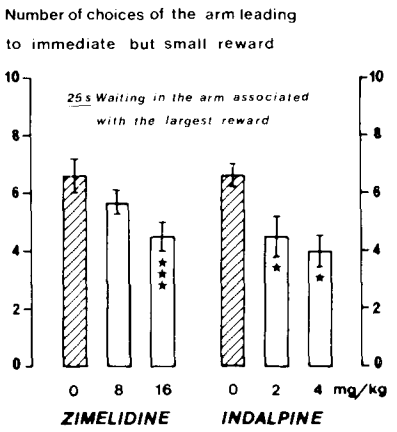

FIG. 3. Effects of two serotonin uptake blockers on the frequency with which the arm leading to immediate-but-small reward was chosen during sessions for which a 25-sec waiting period was imposed in the large-reward arm. Separate groups of rats (n=8–10) were injected IP with either zimelidine (8–16 mg/kg) 60 min or indalpine (2–4 mg/kg) 30 min before each of two consecutive test-sessions (5 trials each) performed 24 hr apart. Columns represent the number of choices (mean±SEM) of the small-reward arm over ten trials. Open columns represent performances of treated rats during the two sessions. Hatched columns represent performances of the rats of the same groups during the last two training sessions (for clarity of the figure, the performances of the two groups are combined, but statistics were performed on individual variations across sessions). $*p<0.05$; $***p<0.01$ compared with rats' choice during the last two training sessions (ANOVA).

receptor antagonist, Ro 15-1788, did not counteract the antipunishment effect of systemic diazepam [52] while this treatment was able to completely antagonize the reduction in 5-HT release induced by systemic BZP [46]. This behavioral result, however, may be questioned as intra raphe Ro 15-1788 has been reported to partially antagonize the "anxiogenic" effects (reduction in social interactions in rats) of the BZP receptor inverse agonist β-carboline-3-carboxylate (β-CCM) administered intraperitoneally [21]. The implication of 5-HT neurons in "anxiogenic" effects of β-carbolines remains, however, to be established.

The direct stimulation of 5-HT receptors by 5-methoxy-N,N-dimethyltryptamine (5-Me-O-DMT) is not able to counteract the releasing effect of BZP in a conflict procedure [44] and administration of 5-HTP (+MAOI) has been reported to potentiate the anti-conflict effect of diazepam [25].

All these data give evidence that, in certain experimental conditions, a reduction in 5-HT transmission undoubtedly mimics the effects of BZP. However, it is not the case in other conditions and most of the effects of BZP seem not to be directly dependent on the integrity of 5-HT processes. Therefore, it might be interesting to elucidate whether some unitary component(s) of behavior may be critical target(s) for the effects of both BZP and reduced 5-HT transmission and whether or not these behavioral components are linked to the emotional characteristics of the situations. Indeed, born of a relatively simple (but anthropomorphic) concept, the punishment- (or novelty-) models of anxiety require behavioral tasks which lack purity, and explore composit phenomena among which the salient role of anxiety may be questioned.

The discrepancies found between the various findings from animal experiments just reviewed are also found in clinical research. Indeed, most of the antiserotonergic drugs are not claimed to have real anxiolytic effects in humans. Moreover, metergoline, a 5-HT receptors blocker, has been reported to increase subjective anxiety in healthy volunteers [16] and low concentrations in the cerebrospinal fluid of 5-hydroxyindole acetic acid (5-HIAA), the main metabolite of 5-HT, taken as an index of 5-HT neurons activity, are often associated with increased anxiety and/or low threshold of anxiety tolerance [2,37].

Thus, the direct involvement of 5-HT neurons in the control of anxiety and in the anxiolytic activity of BZP may be questioned.

INTERPRETATIONS OF THE EFFECTS OF BZP AND/OR 5-HT ON BEHAVIOR IN ANIMAL MODELS OF ANXIETY

Concerning BZP, several alternatives to a reduced fear or anxiety have been proposed to explain the ability of these drugs to release behavioral inhibition: these compounds may alter arousal and discrimination processes, thus leading to a neglect of the extero and/or interoceptive cues associated with responding. They have also been claimed to increase perseveration processes: the most probable or the more recently learned response being preferentially emitted by the animals. Finally, a preferential increase in low rates of responding has been suspected for anxiolytics since BZP are much more effective in enhancing behavior which has been suppressed than schedule-induced increases in response rate ([24,41], for review, see [9, 10, 17]). However, such analyses have not been performed with regard to the effects of alterations of 5-HT transmission. The possibility that BZP may interfere with the ability of animals to wait for an expected reward has not yet been hypothesized. During punished periods of numerous conflict schedules, however, not responding for food- or water-reward enables the animals to avoid electric foot-shocks but it also delays the possibility of obtaining food or water. Thus it is conceivable that punishment-induced delay of reward may be as critical a target as shock-induced fear or anxiety for the effects of BZP and/or of reduced 5-HT functioning to be achieved.

According to this hypothesis, the similarities observed between the effects of BZP and of reduced 5-HT transmission in various experimental conditions could be explained by an alteration of the waiting capacities of the animals.

BZP, 5-HT AND WAITING CAPACITIES

A behavioral situation without any nociceptive component, aiming at studying the animals tolerance to delay of reward, has been designed. It consists of subjecting hungry rats to a choice, in a T-maze, between delayed access to a large quantity of food, and immediate access to a small quantity of food [53]. After training in this experimental procedure, the amount of preference for the arm associated with small reward is directly related to the waiting time fixed in the arm leading to the large-reward. With a 15-sec waiting period, control rats choose the arm allowing an immediate access to small reward in 20–30% of their runs. In rats given various BZP, the preference for the immediate-but-small reward is significantly enhanced (Fig. 1), thus suggesting that these drugs render the animals less prone than controls to

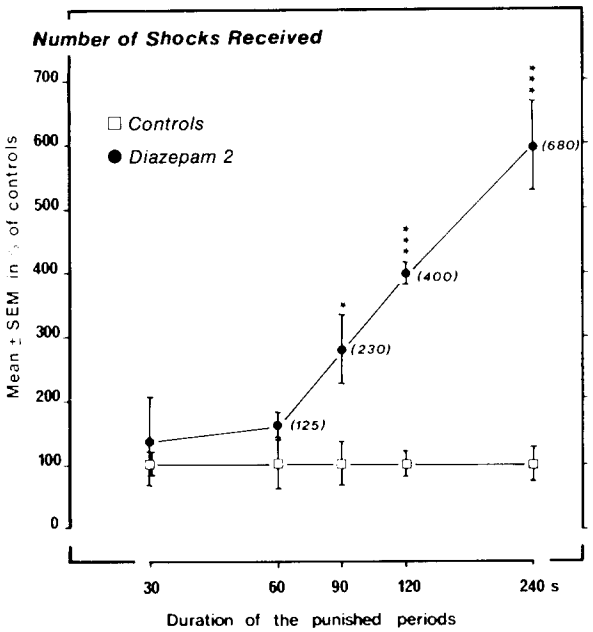

FIG. 4. Effects of diazepam on the number of shocks received (mean±SEM as % of controls) as a function of the duration of the punished periods. Rats of each group (□ controls or ● diazepam 2 mg/kg, IP, T-30 min, n=10–16) were subjected to either 30- and 60-sec, 30 and 90-sec or 120- and 240-sec punished periods interspersed with 1-min FR4 non-punished periods. In parentheses are the mean number of shocks received (% of controls) by diazepam-injected rats during the first 30-sec of the corresponding punished period. The mean total number of shocks received by control rats (100%) during the punished periods was: 0.80±0.16 for 30 sec; 1.00±0.37 for 60 sec; 1.36±0.45 for 90 sec; 0.89±0.35 for 120 sec and 0.78±0.38 for 240 sec.

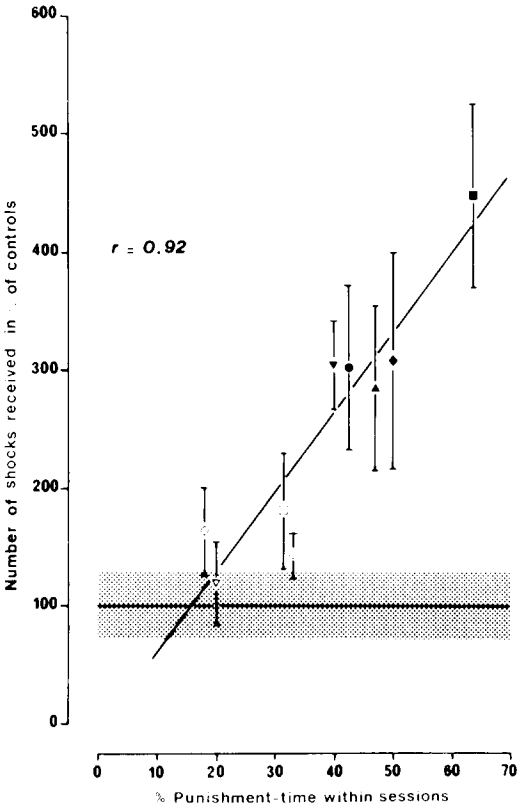

FIG. 5. Relationship between punished time within the various experimental procedures, and the effects of diazepam (2 mg/kg, IP) on the number of shocks received. Punished time was calculated as 100 ×[B/A+B)], in which A=total duration of FR4 non-punished periods and B=total duration of punished periods within a given experimental procedure. The mean number (±SEM) of shocks received by rats of 9 different experimental groups is expressed as % of shocks received by their respective controls (n=8–16 per group). The shaded area represents the mean±SEM number of shocks received by the rats of the 9 control groups. Filled symbols: significantly different from corresponding controls at least at 0.05 level (ANOVA).

tolerate a delay before having access to the reward. This effect seems to result from neither a discrimination impairment nor an increased hunger drive since no crucial variation in choice strategy appears when delay no longer impairs the access to the large reward [53]. That the delay is introduced before the access to the food and its consumption seems to be of crucial importance since up to a 8 min waiting period in the goal-box after the consumption of the large reward fails to alter the preference of the rats for this large-reward goal box, a choice strategy that is not sensitive to BZP treatment either (unpublished results). A non-BZP drug, buspirone, claimed to exhibit BZP-like effects in some animal models of anxiety, to displace ^{3}H-5HT binding with nanomolar affinity and to dose-dependently reduce 5-HIAA concentrations [30], similarly lessens the rats' preference for the large-but-delayed reward alternative (Fig. 2).

Conversely, when a 25-sec delay is imposed in the arm leading to the large reward goal box, rats choose the other arm in 60–70% of their runs. The preference is shifted towards the large but delayed alternative when the rats are given either zimelidine or indalpine, two 5-HT uptake inhibitors (Fig. 3). The implication of 5-HT neurons in the ability of animals to wait for food in the T-maze is also suggested by current experiments suggesting that rats whose 5-HT neurons have been destroyed by an injection of the neurotoxin 5,7-DHT into the nucleus raphe dorsalis are less prone than controls to tolerate a delay before reaching food reward (unpublished results).

Therefore, not only do BZP fail to enhance the animals' tolerance to an aversive event such as confinement, but on the contrary, they impair the rats' ability to wait for an expected reward. The fact that a similar phenomenon (decreased waiting ability) can be observed after lesion of the 5-HT neurons of the dorsal raphe and that increased 5-HT transmission by 5-HT uptake inhibitors produce opposite effects lead us to propose that 5-HT neurons may play an important role in the animals' waiting capacities, i.e., in impulse control, according to Herrnstein [20].

WAITING ABILITY AND BZP EFFECTS IN ANIMAL SCHEDULES OF BEHAVIORAL INHIBITION

In the light of the consideration that suppression of ongoing behavior also delays the possibility of obtaining food or water reward, it is conceivable that BZP-induced release of punished behavior is due, not only to a decrease in shock-induced fear or anxiety, but also to a lessened tolerance to a delay of reward, this latter effect being putatively mediated by a 5-HT process. Therefore, factors able to modulate the animals' ability to wait for food should differentially affect

BZP-induced release of behavior independently of punishment itself.

A series of conflict paradigms in Skinner boxes have been used to test this hypothesis. They consisted of an alternation of punished and non-punished periods of lever pressing for food reward. The respective durations of these periods were fixed throughout the experimental procedure, each being associated with an appropriate conditional light-stimulus maintained throughout the period. During non-punished components, rats were required to press the lever for food pellets under a fixed ratio 4 (FR4) schedule and during punished components, each lever press resulted in the delivery of both one pellet and one non-painful electric foot shock. The rats in different groups were subjected to schedules differing in the ratio between the respective duration of punished and non-punished periods by manipulating the durations of the periods *per se* and/or the pattern of presentation of punished periods throughout the experimental sessions.

After extensive training in one of these paradigms, rats were able to associate each warning signal with a given duration of punished period. They displayed a substantial rate of non-punished responding but almost completely blocked their presses during the punished periods regardless of their duration.

When rats were subjected to signalled punished periods of different durations (30, 60, 90, 120 or 240 sec) the typical response-releasing effect of diazepam could be observed during the longest periods (1.5, 2 and 4 min) but not when punished periods were short-lasting (30 or 60 sec) (Fig. 4). This suggests that diazepam-induced release of punished behavior may vary (probably) without any modification of the anxiogenic factors associated with the experimental situation, in particular of the characteristics of the foot shocks.

The relative amplitude of the behavioral release induced by diazepam seems not to be a consequence of the lengthening of the punished periods since, when it does exist, the effect of diazepam appears as early as during the first 30 sec of the punished periods whatever their total duration (Fig. 4). These results are consistent with the fact that classical punishment procedures used to study the anxiolytic activity of BZP involve punished periods ranging from 2 to 5 min [7, 13, 22, 32, 56]. It is also noteworthy that chlordiazepoxide has been reported to release punished behavior during 1 min punished periods that were followed by a 4 min "time-out" period thus further delaying the access to non-punished rewards [27]. A strong correlation ($r=+0.92$, $p<0.01$) is observed when the response-releasing action of diazepam is plotted against the relative punishment time (whatever the internal organization of punished and non-punished periods) within a session (Fig. 5). The point which seems critical for the effect of diazepam to be observed corresponds to sessions during which punished periods represented at least 40% of the total duration of the sessions.

The predictability of the duration of the punished periods seems to be an additional critical factor for the effects of BZP to be achieved. Indeed, when diazepam was given to rats subjected for the first time to a conflict schedule including short (1 min) punished periods, it released punished pressing whereas it did not when given to experienced rats (13th conflict session) [54]. This suggests that, when the duration of the punished periods is not predictable, i.e., in "acute" punishment paradigms such as those used by Vogel *et al.* [59] and Thiébot *et al.* [52], diazepam-treated rats behave as though they were exposed to punished periods of long duration.

These findings suggest that at least some of the effects of BZP on behavioral suppression induced by punishment may be related to a reduction in the ability of the animals to delay a response giving access to food reward. This is consistent with the reports that BZP increase premature responses in DRL procedures, a situation in which animals are required to let a specified time elapse between successive response to obtain reward [4,40]. Diazepam has also been reported to shift the behavior of hamsters from hoarding to eating [3].

Although other intervening variables may contribute to the phenomenon, a comparative study of the characteristics of various experimental situations could show that BZP and reduced 5-HT transmission exert similar effects in situations including a marked waiting component, whereas the two kinds of brain manipulations induce different effects in procedures which do not include such a waiting component. The weight of this characteristic is evident in conflict schedules but remains to be established for various other situations such as those in which novelty- or non-reward-induced behavioral blockade can be observed (see [45]).

Further experiments are required before definite conclusions can be drawn, however, the effects of blockade of 5-HT transmission in animal models of anxiety are probably not associated to a reduction of fear or anxiety produced by the situation, but more likely to a reduced impulse control. This hypothesis is compatible with the growing amount of clinical and animal data suggesting that impulsiveness or disinhibition is related to decreased 5-HT transmission [42, 45, 58]. A reduced 5-HT functioning should disrupt behavior, inducing a shift from passivity to activity.

This point raises a fundamental problem: in punishment models of anxiety, during the course of which animals are required to stop responding to avoid punishment, are we really studying the anxiolytic properties of BZP, or rather their ability to reduce the capacity of the animals to wait for a food reward? It should be noted, however, that these two proposals are not necessarily mutually exclusive in that the ability of BZP to alter waiting capacities and to facilitate active behavior may contribute to their efficacy as anxiolytics. Indeed, under certain circumstances, a behavioral blockade can be regarded, at least in humans, as an anxiety-producing situation.

Finally, the advisability of BZP treatment administered to people with poor control of impulsivity can be questioned.

REFERENCES

1. Ansseau, M., A. Doumont, D. Thiry and Y. Gelders. Pilot study of a specific serotonergic antagonist, pirenperone, in the treatment of anxiety disorders. *Acta Psychiatr Belg* **83**: 517–524 1983.
2. Banki, C. M., G. Molnar and M. Vojnik. Cerebrospinal fluid amine metabolites, tryptophan and clinical parameters in depression. Part 2. Psychopathological symptoms. *J Affective Disord* **3**: 91–99, 1981.
3. Birk, J. and R. G. Noble. Naloxone antagonism of diazepam-induced feeding in the Syrian hamster. *Life Sci* **29**: 1125–1131, 1981.
4. Canon, J. G. and A. S. Lippa. Use of DRL in differentiating anxiolytic and neuroleptic properties of CNS drugs. *Pharmacol Biochem Behav* **6**: 591–594, 1977.

5. Colpaert, F. C., T. F. Meert, C. J. E. Niemegeers and P. A. J. Jansen. Behavioral and 5-HT antagonist effects of ritanserin: a pure and selective antagonist of LSD discrimination in rat. *Psychopharmacology (Berlin)* **86:** 45–54, 1985.
6. Commissaris, R. L., W. H. Lyness and R. H. Rech. The effects of d-lysergic acid diethylmide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM), pentobarbital and methaqualone on punished responding in control and 5,7-dihydroxytryptamine-treated rats. *Pharmacol Biochem Behav* **14:** 617–623, 1981.
7. Cook, L. and J. Sepinwall. Behavioral analysis of the effects and mechanisms of action of benzodiazepines. In: *Mechanisms of Action of Benzodiazepines*, edited by E. Costa and P. Greengard. New York: Raven Press, 1975, pp. 1–28.
8. Corrodi, H., K. Fuxe, P. Lidbrink and L. Olson. Minor tranquilizers, stress and central catecholamine neurons. *Brain Res* **29:** 1–16, 1971.
9. Dantzer, R. Behavioral effects of benzodiazepines: a review. *Biobehav Rev* **1:** 71–86, 1977.
10. Dantzer, R. Dissociation between suppressive and facilitating effects of aversive stimuli on behavior by benzodiazepines. A review and reinterpretation. *Prog Neuropsychopharmacol* **2:** 33–40, 1978.
11. Engle, J. A., S. Hjorth, K. Svensson, A. Carlsson and S. Liljequist. Anticonflict effect of the putative serotonin receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). *Eur J Pharmacol* **105:** 365–368, 1984.
12. Geller, I. and K. Blum. The effects of 5-HTP on para-chlorophenylalanine (pCPA) attenuation of "conflict" behavior. *Eur J Pharmacol* **9:** 319–324, 1970.
13. Geller, I., J. T. Kulak and J. Seifter. The effects of chlordiazepoxide and chlorpromazine on a punishment discrimination. *Psychopharmacologia* **3:** 374–385, 1962.
14. Graeff, F. G. and R. I. Schoendfeld. Tryptaminergic mechanisms in punished and non-punished behavior. *J Pharmacol Exp Ther* **173:** 277–283, 1970.
15. Graeff, F. G. and N. G. Silveira Filho. Behavioral inhibition induced by electrical stimulation of the median raphe nucleus of the rat. *Physiol Behav* **21:** 477–484, 1978.
16. Graeff, F. G., A. W. Zuardi, J. S. Giglio, E. C. Lima Filho and I. G. Karniol. Effects of metergoline on human anxiety. *Psychopharmacology (Berlin)* **86:** 334–338, 1985.
17. Gray, J. A. Précis of the neuropsychology of anxiety: an enquiry into the functions in the septo-hippocampal system. *Behav Brain Sci* **5:** 469–534, 1982.
18. Haefely, W., L. Pieri, P. Polc and R. Schaffner. General pharmacology and neuropharmacology of benzodiazepine derivatives. In: *Handbook of Experimental Pharmacology*, vol 55, part II, edited by F. Hoffmeister and G. Stille. Berlin: Springer-Verlag, 1981, pp. 13–262.
19. Hall, M. D., S. El Mestikawy, M. B. Emerit, L. Pichat, M. Hamon and H. Gozlan. ^{3}H-8-hydroxy-2-(di-n-propylamino)tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J Neurochem* **44:** 1685–1696, 1985.
20. Herrnstein, R. J. Self control as response strength. In: *Quantification of Steady-State Operant Behavior*, edited by C. M. Bradshaw, E. Szabadi and C. F. Lowe. Elsevier: North-Holland Biomedical Press, 1981, pp. 3–20.
21. Hobbs, A., I. A. Paterson and M. H. T. Roberts. The effects on social interaction of microinjections of Ro 15-1788 into the nucleus raphe dorsalis of the rat. *Br J Pharmacol* **82:** 241P, 1984.
22. Hodges, H. M. and S. Green. Evidence for the involvement of brain GABA and serotonin systems in the anticonflict effects of chlordiazepoxide in rats. *Behav Neural Biol* **40:** 127–154, 1984.
23. Iversen, S. D. 5-HT and anxiety. *Neuropharmacology* **23:** 1553–1560, 1984.
24. Iwasaki, T., K. Ezawa and S. Iwahara. Differential effects of chlordiazepoxide on simultaneous and successive brightness discrimination learning in rats. *Psychopharmacology (Berlin)* **48:** 75–78, 1976.
25. Kilts, C. D., R. L. Commissaris, J. J. Cordon and R. H. Rech. Lack of central 5-hydroxytryptamine influence on the anticonflict activity of diazepam. *Psychopharmacology (Berlin)* **78:** 156–164, 1982.
26. Laurent, J. P., M. Margold, V. Hunkel and W. Haefely. Reduction by two benzodiazepines and pentobarbitone of the multiunit activity in substantia nigra, hippocampus, nucleus locus coeruleus and dorsal raphe nucleus of "encéphale isolé" rats. *Neuropharmacology* **22:** 501–512, 1983.
27. Miczek, K. A. Effects of scopolamine, amphetamine and chlordiazepoxide on punishment. *Psychopharmacologia* **28:** 373–389, 1973.
28. Morato de Carvalho, S., J. C. de Aguiar and F. G. Graeff. Effects of minor tranquilizers, tryptamine antagonists and amphetamine on behavior punished by brain stimulation. *Pharmacol Biochem Behav* **15:** 351–356, 1981.
29. Pedigo, N. W., H. I. Yamamura and D. L. Nelson. Discrimination of multiple ^{3}H-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J Neurochem* **36:** 220–226, 1981.
30. Peroutka, S. J. Selective interaction of novel anxiolytics with 5-hydroxytryptamine$_{1A}$ receptors. *Biol Psychiatry* **20:** 971–979, 1985.
31. Peroutka, S. J. and S. H. Snyder. Multiple serotonin receptors: differential binding of ^{3}H-5 hydroxytryptamine, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. *Mol Pharmacol* **16:** 687–699, 1979.
32. Prado de Carvalho, L., G. Grecksch, G. Chapouthier and J. Rossier. Anxiogenic and non-anxiogenic benzodiazepine antagonists. *Nature* **301:** 64–66, 1983.
33. Pratt, J., P. Jenner, E. H. Reynolds and C. D. Marsden. Clonazepam induces decreased serotonin activity in mouse brain. *Neuropharmacology* **18:** 791–799, 1979.
34. Pratt, J., P. Jenner and C. D. Marsden. Comparison of the effects of benzodiazepines and other anticonvulsant drugs on synthesis and utilization of 5-HT in mouse brain. *Neuropharmacology* **24:** 59–68, 1985.
35. Przewlocka, B., L. Stala and J. Scheel-Kruger. Evidence that GABA in the nucleus dorsalis raphe induces stimulation of locomotor activity and eating behavior. *Life Sci* **25:** 937–946, 1979.
36. Robichaud, R. C. and K. L. Sledge. The effects of p-chlorophenylalanine on experimentally induced conflict in the rat. *Life Sci* **8:** 965–969, 1969.
37. Rydin, E., D. Schalling and M. Åsberg. Rorschach ratings in depressed and suicidal patients with low levels of 5-hydroxyindoleacetic acid in cerebrospinal fluid. *Psychiatry Res* **7:** 229–243, 1982.
38. Sainati, S. M. and S. A. Lorens. Intra-raphe benzodiazepines enhance rat locomotor activity: interactions with GABA. *Pharmacol Biochem Behav* **18:** 407–414, 1983.
39. Saner, A. and A. Pletscher. Effects of diazepam on cerebral 5-hydroxytryptamine synthesis. *Eur J Pharmacol* **55:** 315–318, 1979.
40. Sanger, D. J. and D. E. Blackman. The effects of tranquilizing drugs on timing behaviour in rats. *Psychopharmacologia* **44:** 153–156, 1975.
41. Sanger, D. J. and D. E. Blackman. Rate-dependent effects of drugs: a review of the literature. *Pharmacol Biochem Behav* **4:** 73–83, 1976.
42. Schalling, D., M. Åsberg, G. Edman and S. Levander. Impulsivity, nonconformity and sensation seeking as related to biological markers for vulnerability. *Clin Neuropharmacol* **7:** Suppl 1, 746–747, 1984.
43. Sepinwall, J. and L. Cook. Mechanism of action of the benzodiazepines: behavioral aspect. *Fed Proc* **39:** 3024–3031, 1980.
44. Shephard, R. A., D. A. Buxton and P. L. Broadhurst. Drug interactions do not support reduction in serotonin turnover as the mechanism of action of benzodiazepines. *Neuropharmacology* **21:** 1027–1032, 1982.
45. Soubrié, P. Reconciling the role of central serotonin neurons in human and animal behavior. *Behav Brain Sci*, in press, 1986.

46. Soubrié, P., C. Blas, A. Feron and J. Glowinski. Chlordiazepoxide reduces in vivo serotonin release in the basal ganglia of "encéphale isolé" but not of anaesthetized cats: evidence for a dorsal raphe site of action. *J Pharmacol Exp Ther* **226:** 526–532, 1983.
47. Stein, L., C. D. Wise and B. D. Berger. Anti-anxiety action of benzodiazepines: decrease in activity in serotonin neurons in punishment system. In: *The Benzodiazepines*, edited by S. Garattini, E. Mussini and L. O. Randall. New York: Raven Press, 1973, pp. 299–326.
48. Stein, L., C. D. Wise and J. D. Belluzzi. Effects of benzodiazepines on central serotoninergic mechanisms. In: *Mechanism of Action of Benzodiazepines*, edited by E. Costa and P. Greengard. New York: Raven Press, 1975, pp. 29–44.
49. Stein, L., J. D. Belluzzi and C. D. Wise. Benzodiazepines: behavioral and neurochemical mechanisms. *Am J Psychiatry* **134:** 665–669, 1977.
50. Thiébot, M. H., M. Hamon and P. Soubrié. Attenuation of induced-anxiety in rats by chlordiazepoxide: role of raphe dorsalis benzodiazepine binding sites and serotonergic neurons. *Neuroscience* **7:** 2287–2294, 1982.
51. Thiébot, M. H., M. Hamon and P. Soubrié. The involvement of nigral serotonin innervation in the control of punishment-induced behavioral inhibition in rats. *Pharmacol Biochem Behav* **19:** 225–229, 1983.
52. Thiébot, M. H., P. Soubrié, M. Hamon and P. Simon. Evidence against the involvement of serotonergic neurons in the anti-punishment activity of diazepam in the rat. *Psychopharmacology (Berlin)* **82:** 355–359, 1984.
53. Thiébot, M. H., C. Le Bihan, P. Soubrié and P. Simon. Benzodiazepines reduce the tolerance to reward delay in rats. *Psychopharmacology (Berlin)* **86:** 147–152, 1985.
54. Thiébot, M. H., P. Soubrié and P. Simon. Is delay of reward mediated by shock-avoidance behavior a critical target for anti-punishment effects of diazepam in rats? *Psychopharmacology (Berlin)* **87:** 473–479, 1985.
55. Trulson, M. E., D. W. Preussler, G. A. Howell and C. J. Frederickson. Raphe unit activity in freely moving cats: effects of benzodiazepines. *Neuropharmacology* **21:** 1050–1082, 1982.
56. Tye, N. C., S. D. Iversen and A. R. Green. The effects of benzodiazepines and serotonergic manipulations on punished responding. *Neuropharmacology* **18:** 689–695, 1979.
57. Tye, N. C., B. J. Everitt and S. D. Iversen. 5-Hydroxytryptamine and punishment. *Nature* **268:** 741–743, 1977.
58. Valzelli, L. Animal models of aggression and violence. *Clin Neuropharmacol* **7:** Suppl 1, 752–753, 1984.
59. Vogel, J. R., B. Beer and D. E. Clody. A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia* **21:** 1–7, 1971.
60. Wilbur, R. and F. A. Kulik. Gray's cybernetic theory of anxiety. *Lancet* **II:** 803, 1981.