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Comparison of the Effects of the Benzodiazepine Midazolam and Three Serotonin Antagonists on a Consummatory Conflict Paradigm

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BECKER, H C *Comparison of the effects of the benzodiazepine midazolam and three serotonin antagonists on a consummatory conflict paradigm* PHARMACOL BIOCHEM BEHAV 24(4) 1057–1064, 1986 —A consummatory conflict procedure that involves an abrupt reduction in magnitude of an expected reward (negative contrast) has been shown to be particularly sensitive to the effects of anxiolytic agents As previously reported with chlordiazepoxide, another benzodiazepine (BDZ), midazolam released suppressed consummatory performance in a dose-dependent manner This effect was not due to a general appetite stimulatory effect of the drug The effects of three 5-HT antagonists on negative contrast were examined to evaluate the role serotonin may play in the anxiolytic action of BDZ Methysergide was found to be ineffective, cinanserin tended to reduce contrast at two intermediate doses, and cyproheptadine eliminated the contrast effect in a similar fashion as midazolam The effectiveness of cyproheptadine may not be attributed to its anticholinergic or antihistaminergic actions since scopolamine and pyrilamine did not produce similar effects The results are discussed in terms of the role serotonin may play in the anti-conflict action of BDZ, as well as possible interactional effects of GABA

Consummatory contrast Benzodiazepines Anti-conflict action Midazolam Serotonin Methysergide Cinanserin Cyproheptadine

WHILE a great deal of research has been directed toward characterizing the behavioral effects of benzodiazepines (BDZ), the mechanism of action by which they produce anti-anxiety or conflict-attenuating effects remains to be elucidated. One proposed mode of action implicates the involvement of serotonin (5-HT). BDZ have been shown to reduce 5-HT turnover and decrease activity of central 5-HT neurons [17, 34, 41, 42, 55, 61, 62, 73]. In addition, presynaptic antagonism of the 5-HT system by a variety of agents has been found to mimic the anti-conflict effects of BDZ For example, the 5-HT synthesis inhibitor parachlorophenylalanine (PCPA) increases rates of punished responding [25, 54, 62, 70] Moreover, the release of suppressed behavior induced by PCPA treatment was reported to be closely correlated with the pharmacological time course of 5-HT depletion [60,69]. Intraventricular administration of the serotonergic neurotoxin 5,6-dihydroxytryptamine (5,6-DHT) [61] and bilateral application of 5,7-dihydroxytryptamine (5,7-DHT) to the ventromedial tegmentum [69] were found to also increase punished responding. A variety of postsynaptic 5-HT antagonists have been shown to possess anti-conflict activity as well, including methysergide [28, 29, 57, 61, 62, 72], bromolysergic acid [29], cyproheptadine [28,58], and cinanserin [13,26].

Conversely, the serotonin precursor 5-HTP was found to reverse the effects of PCPA [25] and cinanserin [26]. The long lasting, centrally acting 5-HT agonist α-methyltryptamine has also been reported to reverse the conflict-attenuating action of cinanserin [26] Additionally, Stein *et al.* [62] showed that intraventricular application of 5-HT attenuated the anti-conflict action of 10 mg/kg oxazepam. These results are further supported by studies showing that behavior suppression produced by stimulation of the raphe nuclei is antagonized by the systemic administration of oxazepam [61,62]. Finally, the serotonin reuptake inhibitor, fluoxetine, was found to further suppress punished responding as well as attenuate the anti-conflict effects of chlordiazepoxide (unpublished data cited in Feldman and Quenzer, [18]).

More recently, however, the effectiveness of 5-HT depletion and receptor blockade in mimicking the anti-conflict action of BDZ has been questioned. For example, Blakely and Parker [7] and Cook and Sepinwall [13] have indicated that anti-conflict effects of PCPA treatment were weak and

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transient Additionally, Winter [72] found cinanserin to lack anti-conflict activity and in a conditioned suppression procedure where punishment is not contingent on responding (CER paradigm), methysergide failed to release suppressed behavior [48] Furthermore, LSD and mescaline, which are generally regarded as 5-HT agonists at postsynaptic receptors were shown to possess anti-conflict activity [56] Finally, Kilts *et al* [35] failed to mimic the effects of diazepam or potentiate its effects with administration of a variety of presynaptic and postsynaptic 5-HT antagonists Thus, the literature is somewhat contradictory as to whether 5-HT plays a primary role in mediating the anti-conflict activity of BDZ

Over the past several years, this author has investigated the effects of a variety of anxiolytic agents in an appetitively-motivated conflict task Specifically, the task involves an abrupt reduction in the magnitude of an expected reward (sucrose solution) Under such conditions, the consummatory behavior of animals shifted from a preferred (32%) to a less preferred (4%) sucrose solution declines to a level substantially below that of animals that have only experienced the less preferred 4% solution This decrement in consummatory performance is termed a negative contrast effect (for review see Flaherty [19]) The negative contrast that occurs in subjects downshifted in sucrose concentration is highly reliable, with lick frequency of shifted animals often 50% or less than that of the unshifted controls This effect appears to be transitory, diminishing in a systematic fashion over a period of three to five postshift days (when consumption in shifted animals reaches the level of unshifted controls)

Recovery from suppressed consummatory performance (i e , negative contrast) is facilitated by several drugs that have anxiolytic action in humans For example, the benzodiazepine chlordiazepoxide (CDP, 6 and 8 mg/kg) greatly reduces or eliminates negative contrast when administered on the second postshift day [3,22] This effect is not due to a non-specific appetite stimulating effect of the drug since the increase in consumption was much more pronounced in shifted rats than unshifted controls In addition, control groups were run such that state dependency and rate dependency explanations for the results could by ruled out

Ethanol (ETOH, 0 75 and 1 0 g/kg) also greatly reduces negative contrast when administered on the second postshift day [2] Moreover, fairly ineffective doses of CDP (4 mg/kg) and ETOH (0 50 g/kg) additively reduced contrast when administered together [3] Finally, the barbiturate amobarbital sodium (ABS, 17 5 mg/kg) has been found to be effective in reducing negative contrast as well [20,21]

The contrast paradigm appears to be particularly sensitive to these agents since the neuroleptics haloperidol (0 1, 0 5, and 1 0 mg/kg) and chlorpromazine (1, 3, and 5 mg/kg) do not produce similar effects [1] In addition, acute administration of the antidepressant imipramine (8 and 16 mg/kg) had no effect on recovery from negative contrast (unpublished data) This apparent selective sensitivity to anxiolytic drugs suggests the contrast procedure may be useful in further pharmacological analysis of these agents

The purpose of this study was twofold First, to investigate the effectiveness of BDZ in the negative contrast paradigm by studying another BDZ drug, midazolam (MDZ) As previously mentioned, the anxiolytic or anti-conflict activity of BDZ may be mediated by a serotonergic mechanism It might therefore be expected that antiserotonergic agents may produce effects on negative contrast similar to the BDZ (i e , a reduction in negative contrast) Thus, a second purpose of this study was to investigate the potential contrast-reducing properties of three 5-HT antagonists methysergide (METHY), cinanserin (CIN), and cyproheptadine (CYPRO) Since CYPRO possesses anticholinergic and antihistaminergic activity [63,71], to control for these nonspecific effects the anticholinergic agent, scopolamine hydrobromide (SCOPH) and the potent histamine H1 receptor blocker, pyrilamine (PYRIL) [36] were studied in this conflict paradigm as well

METHOD

Subjects

Experimentally naive male Sprague-Dawley rats (Blue Spruce Breeding Farms, Altamont, NY) were used as subjects The animals, approximately 75 days old at the start of the experiment, were maintained at 82% of their free-feeding body weight throughout the experiment by single daily feeding The subjects were individually housed under a 14/10 hour light/dark cycle with water continuously available in the home cage

Apparatus

Subjects were tested in five identical metal cages (24 5×17 5×18 cm) A centrally located hole 1 cm in diameter and 7 cm above the floor was present on one side of each of the cages A graduated cylinder was placed outside the chamber such that the orifice of the drinking spout was centered in the hole and flush with the outside wall of the cage Licking responses were monitored and recorded by standard electromechanical circuitry interfaced with Commodore PET microcomputers

Procedure

Half the rats were randomly assigned to a group that received access to a 32% sucrose solution for 10 days (designated as the preshift period) and then a 4% solution for four postshift days The remaining animals served as unshifted controls, receiving 4% sucrose on all days of the experiment It is important to note that during the postshift period, *all* animals received the *same* reward (4% sucrose) The difference between shifted and unshifted animals was that only the former group had prior experience with a more preferred reward (32% sucrose). The 14 daily sessions consisted of five minutes access to the appropriate sucrose solution, timed from the first lick Following the first postshift day session (day 11 of testing), shifted (32-4) and unshifted (4-4) groups were matched with respect to their first postshift day lick rates and then further separated on the basis of drug treatment In Experiment 1, shifted and unshifted subjects were divided into six groups (N=5–7) and injected with either 0 25, 0 50, 1 0, 1 25, or 2 0 mg/kg MDZ, or isotonic saline Similarly, in Experiment 2, shifted and unshifted rats (N=5–7) were injected with either METHY (3, 6, or 12 mg/kg), CIN (5, 10, 15, or 20 mg/kg), CYPRO (3, 6, or 12 mg/kg), SCOPH (0 25 or 0 50 mg/kg), or PYRIL (3 or 6 mg/kg) Each of these drug conditions were run separately with the appropriate vehicle control groups included in each part of the experiment Vehicle injections were always volume-equivalent to the drug injections Data from each part of the experiment were analyzed separately Additionally, since the 5 and 20 mg/kg CIN groups were run after the other CIN conditions were completed, an additional

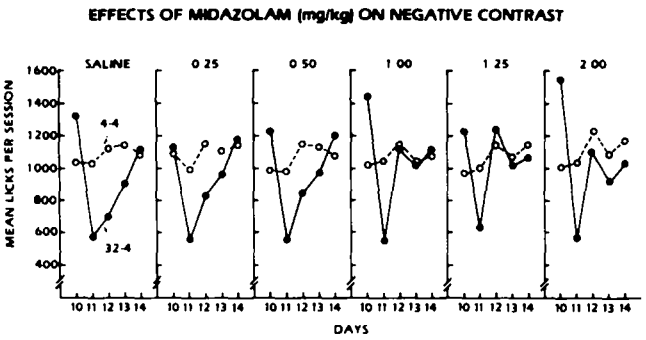

FIG 1 Mean lick rate per 5 minute session for shifted (32-4) and unshifted (4-4) subjects as a function of drug dose (mg/kg) and day of test Day 10 is the last preshift day Saline and MDZ injections were given on the second postshift day (day 12)

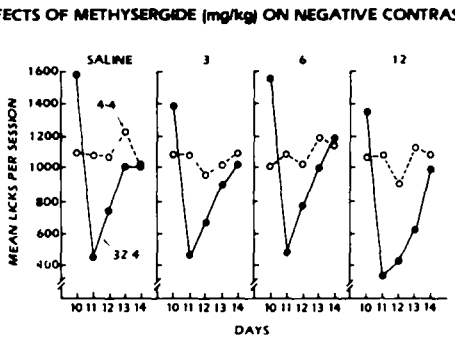

FIG 2 Mean lick rate for 32% and 4% sucrose as a function of drug dose (mg/kg) and day of test Saline or METHY injections were given on the second postshift day (day 12)

saline group was run with these animals. These data were analyzed separately as well

All injections were administered intraperitoneally prior to the start of the second postshift day trials No injections were given on any other day of the experiment. Doses of MDZ (midazolam maleate) were administered 15 minutes prior to the test session; CIN was administered 60 minutes prior to testing, while pretreatment times for all other drugs were 30 minutes. METHY (methysergide maleate) was donated by Sandoz Pharmaceuticals (East Hanover, NJ), CIN (cinanserin hydrochloride) was obtained from E R. Squibb & Sons (Princeton, NJ), CYPRO (cyropheptadine hydrochloride) was obtained from Merk, Sharp & Dohme (West Point, PA), and SCOPH (scopolamine hydrobromide) and PYRIL (pyrilamine maleate) were purchased from Sigma Chemical Company (St. Louis, MO) CYPRO was dissolved in a 5% polyethylene glycol (PEG) solution in a concentration of 1 mg/ml The vehicle was prepared by mixing 1 ml of 95% PEG with 19 ml of distilled water. All other drugs were dissolved in 0.9% saline. Sucrose solutions (w/w) were prepared from commercial grade cane sugar and tap water, 24 hours before each session.

RESULTS

Experiment 1 Effects of Midazolam on Negative Contrast

Six subjects were discarded from the experiment because they failed to lick the 4% sucrose solution during the preshift period.

Mean lick rates as a function of sucrose and drug conditions are illustrated in Fig 1. On the last preshift day (day 10) animals receiving 32% sucrose generally licked at a higher rate than rats receiving 4% sucrose. This impression was supported by a reliable sucrose concentration × day interaction, $F(4,328)=50.92$, $p<0$ 01, and subsequent analysis with Fisher's Least Significant Difference (LSD) test ($p<0.05$)

On the first postshift day (day 11), negative contrast occurred in all drug groups, i e , unshifted control animals (4-4) licked at a reliably greater rate than shifted subjects (32-4) On the second postshift day, however, negative contrast was reliable only in rats that received either saline, 0 25 or 0.50 mg/kg MDZ Doses of 1.0, 1.25, and 2 0 mg/kg MDZ effectively eliminated the contrast effect, i e , there was no reliable difference between the lick rates of shifted and unshifted animals This was reflected in a significant sucrose concentration × drug × day interaction, $F(20,328)=1$ 74, $p<0.05$, with subsequent LSD tests ($p<0.05$) In addition, while all doses of MDZ reliably increased licking of shifted animals on postshift day 2 compared to the first postshift day (this was not true for saline-injected subjects), only shifted subjects that received the 1.0, 1 25, or 2 0 mg/kg doses of MDZ licked reliably more than the saline animals on the second postshift day (LSD test, $p<0.05$). Lick rates of unshifted rats did not reliably differ between the first and second postshift days for all drug conditions.

These results demonstrate a dose-dependent effect of MDZ on negative contrast. It is important to note that MDZ had no effect on unshifted controls, i.e , (4-4 MDZ) and (4-4 Saline) groups did not reliably differ for all doses of the drug. This fact rules out the possibility that the drug's effect on the shifted groups was due to a generalized excitation effect Moreover, the differential effect MDZ had on shifted and unshifted animals rules out the possibility that the drug increased consumption merely due to its appetite-stimulatory effects (a characteristic property of benzodiazepines).

Experiment 2 Effects of Serotonin Antagonists on Negative Contrast

The effects of methysergide on negative contrast are illustrated in Fig. 2 As can be seen, animals that had prior experience with 32% sucrose consumed substantially less 4% sucrose during the postshift period than unshifted subjects that received only the 4% solution on all 14 test days. This negative contrast effect was significant on the first three postshift days as indicated by a reliable sucrose concentration × day interaction, $F(3,81)=15$ 81, $p<0.01$, followed by LSD tests ($p<0$ 05) It is also apparent that the contrast effect was uninfluenced by the drug treatment. ANOVA supported this impression in that the shift × drug term was not statistically reliable ($F<1$ 0). Similarly, the shift × drug × day interaction was not significant either ($F<1$ 0).

Data for the 10 and 15 mg/kg CIN groups and for the 5 and 20 mg/kg doses of CIN are shown in the top and bottom panels of Fig. 3, respectively. In both cases negative contrast was reliable on all postshift days, $F(3,66)=19$ 62, $p<0.01$ and $F(3,72)=7.70$, $p<0.01$, respectively, followed by LSD tests ($p<0.05$) As can be seen in the top panel, drug administra-

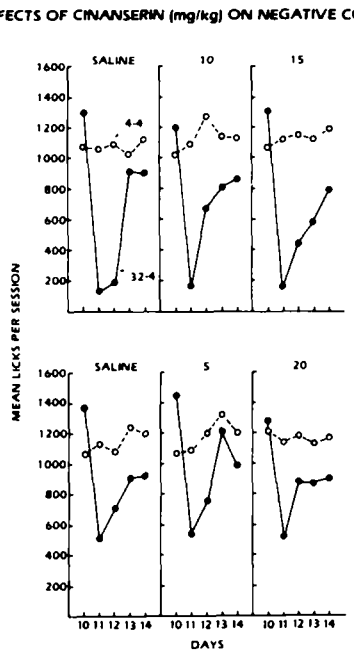

FIG 3 Mean lick rate for 32% and 4% sucrose as a function of CIN dose (top panel 10 and 15 mg/kg, bottom panel 5 and 20 mg/kg) and day of test Saline or CIN injections were given on the second postshift day (day 12)

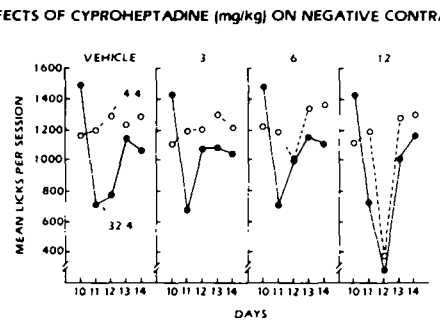

FIG 4 Mean lick rate for 32% and 4% sucrose as a function of CYPRO dose (mg/kg) and test day Vehicle and CYRPO injections were given on the second postshift day (day 12)

tion (10 and 15 mg/kg CIN) attenuated negative contrast Analysis of the first two postshift days for shifted subjects revealed a marginally reliable drug × day interaction, $F(2,11)=3\ 68$, $p=0\ 059$ Further analysis indicated that shifted animals injected with either 10 or 15 mg/kg CIN licked reliably greater on the second postshift day than on postshift day 1. In addition, these animals licked reliably greater than the saline-injected subjects on the second postshift day (by LSD tests, $p<0\ 05$). Similar analysis of unshifted subjects indicated no significant differences between drug groups on either postshift day

As shown in the bottom panel of Fig. 3, drug treatment did not greatly influence the contrast effect relative to the saline control condition. In fact, ANOVA of the first two postshift days for shifted subjects indicated that all animals licked reliably more on the second postshift day than on the first, irrespective of drug treatment, $F(1,12)=12\ 18$, $p<0\ 01$ Analysis of unshifted subjects revealed no reliable differences between postshift day one and postshift day two consummatory performance across all drug conditions

Figure 4 illustrates the data obtained from the CYPRO part of the experiment Negative contrast was reliable on the first postshift day for all dose conditions, $F(3,57)=3\ 96$, $p<0\ 01$. On the second postshift day, negative contrast was only reliable in rats injected with vehicle. In addition, first and second postshift day lick rates did not reliably differ for shifted and unshifted vehicle-injected subjects Although contrast was eliminated in the three drug groups (i.e , no reliable differences between shifted and unshifted lick rates), for some of the doses this effect was due to the drug's action on unshifted as well as shifted animals. Shifted subjects that received the 3 mg/kg dose of CYPRO licked at a reliably greater rate on the second postshift day than on the first postshift day Moreover, these animals licked reliably more than vehicle controls on the second postshift day (by LSD test, $p<0.05$) Importantly, this dose of CYPRO did not influence the consummatory performance of unshifted controls, i.e , there was no reliable difference between postshift day 1 and day 2 lick rates for the unshifted rats receiving 3 mg/kg CYPRO Therefore, the elimination of negative contrast in animals receiving 3 mg/kg CYPRO would appear to be due to the differential effect this dose of CYPRO has on shifted and unshifted subjects—performance was facilitated, or disinhibited, in shifted rats while that of unshifted animals was not significantly influenced

Although shifted animals that received the 6 mg/kg dose of CYPRO did not lick reliably more than vehicle shifted subjects on the second postshift day, their lick rate reliably increased on postshift day 2 as compared to their lick rate on postshift day one (LSD tests, $p<0\ 05$) This increase eliminated the contrast effect in that shifted and unshifted subjects did not reliably differ in lick rate on the second postshift day However, this elimination of contrast is partly due to a decrease in lick rate of unshifted drug animals Consumption of the 4% sucrose solution by these unshifted animals was significantly less on the second postshift day than it was on the first postshift day (drug × day interaction $F(3,30)=18\ 27$, $p<0\ 01$, LSD test, $p<0\ 05$) Furthermore, these animals licked reliably less than unshifted vehicle-injected rats on the second postshift day Nevertheless, despite the apparent depressant effect this dose of CYPRO had on the performance of unshifted controls, 6 mg/kg CYPRO did reliably increase the performance of shifted animals, thereby attenuating the contrast effect

The depressant effects of CYPRO is most apparent with an even higher dose of the drug (12 mg/kg) While contrast was eliminated with this dose of the drug, it is clear that both shifted and unshifted rats licked substantially less on the second postshift day than on the first postshift day. In addition, shifted and unshifted animals that received 12 mg/kg CYPRO licked reliably less on postshift day 2 than subjects that received injections of the vehicle (LSD test, $p<0\ 05$). The elimination of contrast in animals administered 12 mg/kg CPYRO then, is more a reflection of the general depressant effect this dose had on consummatory performance of shifted and unshifted subjects

SCOPH (data not presented) had a dose-dependent effect of decreasing consumption in unshifted, $F(2,13)=8\ 11$,

$p<0.01$ and shifted, $F(2,15)=5.90$, $p<0.05$, subjects Finally, neither 3 nor 6 mg/kg PYRIL (data not shown) influenced negative contrast relative to controls ($Fs<1.0$ for both shifted and unshifted analyses)

DISCUSSION

This series of experiments has demonstrated that: (1) administration of midazolam on the second postshift day reduces, in a dose-dependent fashion, consummatory negative contrast, (2) the influence of three serotonin antagonists on contrast produced mixed results—methysergide was ineffective while cinanserin and cyproheptadine reduced negative contrast under limited dose ranges, (3) the anticholinergic agent, scopolamine, produced a generalized depressant effect on consummatory performance, and (4) the antihistamine pyrilamine did not reliably influence negative contrast relative to controls

The dose-dependent attenuation of negative contrast by MDZ substantiates previous work with another benzodiazepine, CDP, which also is effective in eliminating negative contrast when administered on the second postshift day [3,22] MDZ appears to be more potent in this contrast procedure than CDP in that the minimal effective dose for the elimination of negative contrast was 1 mg/kg for MDZ as compared to 6 mg/kg for CDP. While MDZ has been found to be roughly equipotent to diazepam in a variety of experimental situations [52], the greater potency of MDZ as compared to CDP has also been observed by others [66].

In addition, for the doses of MDZ that effectively eliminated contrast, when the drug was not administered on the third postshift day, there was a fairly pronounced decrease in lick rate This pattern of results is reminiscent of those found with doses of ETOH and CDP that also greatly reduce or eliminate negative contrast and thus, lend credence to the notion that these drugs may be working in a disinhibitory fashion, with inhibition returning on the third postshift day (when there is no administration of drug) Some of the behavioral effects, of at least the benzodiazepines, have been described as disinhibitory by others as well [22, 43, 62].

The results obtained with the three antiserotonergic drugs were more ambiguous Two of the three 5-HT antagonists (cinanserin and cyproheptadine) tested in this study were effective in reducing negative contrast

The doses of METHY employed in this study are within the range of doses (1–10 mg/kg) that have been reported to produce anti-conflict activity in operant situations, albeit not to the same degree as optimal doses of BDZ (e.g , [28, 57, 72]). While it is unclear as to whether the contrast paradigm is less sensitive to the effects of this 5-HT antagonist, thus requiring higher doses of the drug to produce similar effects seen in the operant conflict tests, some reports indicate that higher doses of METHY may produce a general depressant effect on responding For example, Sepinwall and Cook [57] found 10 mg/kg METHY to decrease unpunished responding while not altering punished behavior. Kilts *et al* [35] found this same dose to actually increase suppression of punished behavior. With respect to the latter observation, although not statistically reliable, data from this experiment indicate that the 12 mg/kg dose of METHY tended to suppress consummatory performance in shifted animals relative to other doses of the drug and the saline control group. In any event, the data clearly demonstrate that METHY does not produce BDZ-like effects in reducing negative contrast.

The pattern of results obtained with CIN appears somewhat similar to that previously reported in the literature. That is, CIN has been shown to exhibit anti-conflict activity under very limited conditions (e.g., [13]). In addition, it should be noted that although negative contrast was reliable in both parts of the experiment, there was a difference in absolute lick rates of shifted rats (e.g , compare top and bottom panels of Fig. 3) Whether this difference in "baseline" or degree of contrast is a significant factor in the analysis of CIN's effects on negative contrast cannot be fully assessed at present. However, there is some evidence to indicate that the effectiveness of the 10 and 15 mg/kg doses in attenuating contrast was not merely due to a rate dependent effect. For example, similar doses of CIN have not been found to alter low rates of responding maintained by a DRL schedule for reinforcement [45].

CYPRO (3 mg/kg) eliminated negative contrast in a similar fashion as BDZ. That is, the drug produced an increase in responding in shifted animals while leaving unshifted performance relatively unaltered. The 6 mg/kg dose of CYPRO effectively increased performance of shifted rats, however, this dose of the drug also decreased responding in unshifted animals The depressant effects of CYPRO was most clearly observed with the 12 mg/kg dose. Similar depressant effects of high doses of CYPRO have been previously noted [35]. The narrow range of effective doses for the anti-conflict activity of CYPRO has been previously reported as well [28], although a wide range of doses have also been found to be effective by others [58].

The contrast-ameliorating effects of CYPRO (particularly the 3 mg/kg dose) may not be attributed to the drug's anticholinergic or antihistaminergic actions. SCOPH generally decreased consumption in all animals This result is similar to that previously reported with a 1 mg/kg dose of SCOPH [23] This effect also seems consistent with previous reports that have shown SCOPH to reduce water intake under a variety of experimentally-induced situations, e.g., water deprivation, hypovolemia, and hypertonicity [8,27]. The consumption of an 8% dextrose solution was also found to be decreased in squirrel monkeys after administration of 0.1 or 0.5 mg/kg SCOPH [46] The fact that responding in shifted subjects was further suppressed by SCOPH also supports findings that SCOPH decreases punished behavior. This effect was observed with doses similar to those used in this experiment (0.3 and 0.5 mg/kg), but was absent when similar doses of the peripherally acting quaternary analogue of SCOPH (scopolamine methyl nitrate) was administered [47].

The antihistamine PYRIL did not significantly influence contrast relative to controls. These results are in agreement with those obtained by Graeff [28] where pyrilamine, as well as two other antihistaminergic drugs, were found to lack anti-conflict activity in an operant situation.

There may be several reasons for the discrepant results obtained with these three putative 5-HT receptor blockers. For example, it might be that CYPRO and CIN are more potent 5-HT antagonists than METHY Cyproheptadine has been found to be a more potent 5-HT antagonist than methysergide in peripheral preparations, i e., rat uterine tissue [30] However, according to many recent binding studies with central serotonin receptors, this has not been the case For example, in a recent study, methysergide was found to be the most potent of the three antagonists [9] Therefore, the lack of consistency of these agents to influence negative contrast does not seem to be related to their differential potencies as 5-HT antagonists.

Recently, two distinct types of 5-HT receptors have been

detected in brain tissue by *in vitro* binding studies [4, 39, 51] The existence of different receptors raises the possibility that the different findings obtained in this study with the three serotonin antagonists may be related to their differential action at the two 5-HT binding sites. However, this does not appear to be the case since the serotonin antagonists employed in the present study bind to both S1 and S2 receptors [38,50]

Many 5-HT antagonists have nonspecific effects, influencing neurotransmitter systems other than serotonin For example, as noted earlier, cyproheptadine has both anticholinergic and antihistaminergic activity [63,71] The difference in efficacy of the three 5-HT antagonists to attenuate negative contrast is not likely due to these nonspecific properties of CYPRO, since CIN, which is relatively void of anticholinergic and antihistaminergic activity [38] was also effective in reducing negative contrast. Moreover, neither scopolamine nor pyrilamine greatly influenced contrast Thus, the greater efficacy of CYPRO as compared to CIN in attenuating negative contrast (3 mg/kg CYPRO eliminated contrast while effective doses of CIN reduced contrast) cannot be attributed to the anticholinergic or antihistaminergic activity of the former drug In summary, it is unclear as to why only two of the three tested serotonin antagonists were effective in reducing negative contrast

The inconsistent nature of the results obtained with serotonin antagonists is not unique In reviewing the literature, a variety of possible explanations for the lack of consistency has emerged. First, as posited by Kilts *et al* . [35] and others, it is possible that the wide variety of behavioral tasks employed in studies assessing anti-conflict activity may be differentially sensitive to the rate-enhancing effects of BDZ and other drugs For example, the dependent measure in such studies has varied from instrumental behaviors (e g , rate of lever pressing) to exploratory behavior to consummatory behaviors (e.g , eating, drinking). Although BDZ are effective in releasing suppressed behavior in all these situations, it is quite possible that other agents, particularly those that influence specific neurotransmitter systems, may have differential effects on this myriad of behavioral tasks This point is clearly illustrated in a recent study where methysergide and cinanserin were found to be effective in increasing punished responding in a lick-suppression task, but ineffective in releasing suppressed responding (lever pressing) in a multiple-schedule task [9]

Another possible explanation for the inconsistent results of studies aimed at determining the involvement of 5-HT in the anti-conflict activity of BDZ stems from the fact that many serotonin drugs (particularly the antagonists) have nonspecific effects and their locus of action in the CNS is poorly understood [18]. This is particularly exemplified by the fact that the efficacy of the putative 5-HT antagonists employed in the present study (as well as others) in blocking central 5-HT receptors has been disputed, since they failed to antagonize the synaptic inhibition induced by iontophoretic application of 5-HT in brain areas that receive a dense serotonin input [32,59] In addition, some evidence indicates that many drugs thought to be 5-HT antagonists, including those employed in the present study, may in fact act complexly as mixed agonist-antagonists [12]

Taken together, the lack of a consistent body of evidence in support of the hypothesis that decreased 5-HT activity is involved in mediating the anxiolytic action of BDZ may be attributed to the differential effects of drugs on a wide variety of behavioral paradigms employed, and the lack of an indepth understanding of the complex serotonin system These points are perhaps best illustrated by a recent study in which the relationship between binding affinity for S2 receptors and anti-conflict activity of 5-HT antagonists was investigated [9] As previously described, the effectiveness of these agents to increase punished responding was in many cases task-dependent (i e , licking vs lever pressing behavior) In addition, although a positive correlation between anti-conflict effects and S2 potency was observed, ketanserin, which is a potent serotonin antagonist and binds most selectively to S2 sites with minimal nonspecific activity [38,40] did not exhibit strong anti-conflict activity

It may be then, that BDZ do not produce their behavioral effects through direct interaction with the serotonin system In support of such a notion is the fact that BDZ appear to have little affinity for 5-HT postsynaptic receptors in rat brain [24] In fact, the effects of BDZ on serotonin activity may be secondary to their effects on other neurotransmitter systems Indeed, BDZ have been intimately linked to GABA on both molecular (e g , [44, 53, 68]) and functional (e g , [5, 15, 37, 64, 65]) levels There is also growing evidence supporting an important role for GABA in mediating many of the therapeutic actions of BDZ (e g , [6, 10, 14, 16, 49, 60, 74]) Accordingly, it has been suggested that the BDZ-induced decrease in 5-HT activity may be the result of benzodiazepine facilitation of GABA-mediated presynaptic inhibition at serotonin nerve terminals [31, 58, 60] Moreover, several convergent lines of evidence support the hypothesis that GABA and serotonin may interact in mediating the anti-conflict (or anti-anxiety) activity of BDZ (e g , [11, 33, 67]) Clearly, additional research is needed to further substantiate this possibility Toward this end, investigation of the role of GABA in mediating the contrast-reducing effects of BDZ is currently being pursued

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