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Level of Punishment Determines Anticonflict Activity of Ondansetron in Pigeons: Comparison With Buspirone and Diazepam

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CASTEJÓN, A. M. AND L. X. CUBEDDU. *Level of punishment determines anticonflict activity of ondansetron in pigeons: Comparison with buspirone and diazepam.* PHARMACOL BIOCHEM BEHAV **61**(4) 451–457, 1998.—The anticonflict effect of the selective 5-HT_3 receptor antagonist, ondansetron, was investigated employing an operant conflict task in pigeons. Behavior (key pecking) was stimulated by food presentation. A fixed-interval program of alternated punished (electrical shocks) and unpunished responding was employed. The effects of drugs were evaluated at two levels punishment intensity; i.e., baseline responding during the punished interval was 5% (higher punishment) or 10% (lower punishment) of the unpunished responding rate. Ondansetron released responding suppressed by punishment only when pigeons were working at the lower levels of punishment. Under these conditions, ondansetron (100 μg/kg, IV), increased key pecking by 119% above control and vehicle values, and doubled the number of shocks received by the pigeons during the punished intervals. Similarly to ondansetron, the anticonflict effects of buspirone (0.3 and 1 mg/kg) and diazepam (1 and 1.5 mg/kg) were strongly dependent on the intensity of the punishing stimulus. When punished responding was suppressed to 5% of unpunished responding by applying shocks of higher intensity, diazepam and buspirone had negligible anticonflict action. However, at lower levels of punishment, diazepam and buspirone produced much greater anticonflict effects than ondansetron ($p < 0.001$). These results indicate that ondansetron exhibits a modest effect in releasing behaviors suppressed by punishment (anxiolytic-like action), which was highly dependent on the intensity of punishment applied. It is proposed that the anxiogenic response to punishment is less sensitive to 5-HT_3 antagonists than the behavior induced by aversive, unpunished situations, where 5-HT_3 antagonists have shown comparable efficacy to benzodiazepines. © 1998 Elsevier Science Inc.

Serotonin Conflict test 5-HT_3 receptors Ondansetron Buspirone Benzodiazepines Anxiety

SEROTONIN is known to play an important role in the behavioral response to aversive stimuli. Early studies indicated that in conflict tests, behaviors suppressed by punishment were released after inhibition of serotonin synthesis, lesion of serotonin pathways, and/or by administration of serotonin antagonists (4,8,14,18–21). Further, benzodiazepines and 5-HT_{1A} agonists, known to reduce serotonergic activity, are effective in animal models of anxiety, and in human patients (7,20–22).

The role of 5-HT_3 receptors in animal models of anxiety has been explored (2–4,9). 5-HT_3 receptor antagonists have been shown to be effective in some models predictive for anxiolytic activity, such as the light–dark exploration test in the mouse, the social interaction test in the rat, and the aggressive behavior of marmosets and of cynomolgus monkeys triggered by the exposure to an unknown observer (4,9,16). In these models, the 5-HT_3 antagonists were as effective as benzodiazepines in suppressing aversive behaviors, suggesting a role for 5-HT_3 receptors in anxiety. However, 5-HT_3 antagonists were shown to be ineffective in the water-lick conflict test in the rat (3,9). In this model, animals were water deprived, and licking for water was suppressed by means of electrical shocks (24). Ondansetron, a 5-HT_3 antagonist, was unable to increase response rates in this model, whereas benzodiazepines were effective in the water-lick conflict test (3,9). The reason for these differences is unknown. It is possible that punishment may trigger pathways and mechanisms insensitive to 5-HT_3 receptor antagonists, and hence, different from those mediating behavioral responses to aversive, unpunished, situations.

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Based on the above-described findings, it appeared of interest to investigated the role of serotonin and of 5-HT_3 receptors in an operant conflict task, where behavior was suppressed by punishment. In this model, key pecking (responding) for food was stimulated by food deprivation, and electrical shocks were applied to suppress the key pecking behavior. Nonspecific drug effects were controlled by studying the effects of the drugs under punished and unpunished conditions during the same experiment. The effect of the 5-HT_3 antagonist ondansetron, was compared with that of the benzodiazepine, diazepam, and with the 5-HT_{1A} agonist, buspirone; drugs with well-known anticonflict activity on this experimental model (1,11,25). Because behaviors and drug effects may depend on the magnitude of the punishment (11), the effects of these drugs were studied at two levels of shock intensity.

METHOD

Subjects

Adult Columba libia pigeons were maintained at 80% of their free-feeding body weights. The pigeons had free access to water and oyster shell grit. All animals were experimentally naive and were housed in separate living cages in a vivarium. The pigeons had to be entrained in a preliminary procedure (see below).

Apparatus

The experimental chambers (22 × 27 × 31 cm), were similar to those described by Ferster and Skinner (5). The chambers contained a translucent response key, which was mounted in the center of the front panel 23 cm above the wire mesh floor, and could be transilluminated with a red or white 7-W lamp. When a minimum force was applied to the key, activation of a feed-back relay occurred that was recorded as a response. A rectangular opening, approximately 5 cm from the floor of the front panel and below the response key, allowed the presentation of mixed grain by a solenoid activated feeder for 3 s. Food availability was signaled by the illumination of the food hopper and by extinguishing the keylight during the 3-s grain presentation. Electric shocks (120 V, AC, 60 Hz, 200 ms) could be delivered through stainless steel electrodes implanted around the pigeon's pubic bone. The pigeons were connected to the shock source through a swivel and cable mounted in the ceiling of the chamber. The impedance of the electrodes was measured daily to ensure constancy of stimulus presentation. The current was adjusted individually for each pigeon to produce suppression of responding to 5% of the nonpunished levels to the first set of experiments (high levels of suppression) and to 10% on the second set of experiments (lower level of suppression) (see the Procedure section). Experimental events were scheduled and recorded with electromechanical switching circuitry located in a separate room. Cumulative response recorders were employed to provide detailed summaries of each daily performances.

Preliminary Procedure

After food deprivation, key pecking was established according to the method of successive approximations. When food was presented, the grain hopper was illuminated and the key-light was extinguished. Initially each response was reinforced with grain in a single key chamber. Over several sessions the number of key-peck responses required to produce food was gradually incremented from 1–30 (fixed-ratio 30 or FR schedule). Responding was then maintained under a multiple FR30 FR 30 schedule in which key-light colors (red or white) alternated every 3 min. These alternating components were separated by a 30-s time out, during which the key-light was extinguished and responses had no programed consequences.

Conflict Test

When the response rate under the preliminary procedure was stable, the birds were introduced into the experimental chamber for a daily scheduled session. Daily sessions were conducted Monday through Friday. The conflict test consisted of three components: a pure reward (unpunished) component, a time-out component, and a conflict (punished) component. Responses during the nonpunished and punished components were rewarded with food. A fixed interval program was employed consisting of ten intervals of 5-min duration each of alternated punished (electrical shocks) and unpunished responding (5 min each). Fixed interval cycles were separated by a 30-s time-out period during which the chamber was dark and the responding had no scheduled consequences.

During the pure reward component (unpunished) the key-light was white. Responses made during the pure reward (unpunished) component were reinforced by a single food presentation. Under this schedule, the first response after 5 min produced food presentation; followed by the time-out period. If no response occurred within 60 s after the elapse of the interval, food was not delivered and the time-out component began.

The punished interval was signaled by a red key-light. During this interval, the pigeons received an electric shock after each 15th response; consequently, responses were suppressed during this interval. The intensity of the electric shocks was adjusted to produce a desired level of response suppression. Responding was suppressed to 5% of unpunished response rate (high level of suppression) or to 10% of unpunished response rate (low levels of suppression). The effects of drugs were studied at both levels of punished responding.

Drug Procedure

Buspirone HCl , diazepam, and ondansetron, were administered to the pigeons, at the desired dose, immediately before the experimental session. Buspirone was dissolved in sterile 0.9% NaCl solution, and given via intramuscular injections in a volume of 1 ml/kg·b.wt. Ondansetron was dissolved in sterile 0.9 NaCl, and given intravenously, in a volume of 2 ml/kg·b.wt. Diazepam (Valium) or its vehicle was administered intramuscularly. Diazepam vehicle consisted of 8% propylenglycol, 1.6% ethanol, 1% sodium benzoate, 0.3% benzyl alcohol (w/v), and adjusted to pH 6.5 with NaOH. Vehicle injections (saline or diazepam vehicle) were administered on Tuesdays, and the respective drug was administered on Fridays, provided the baseline performances were stable on the preceding day. The criterion for stable performance was that daily performance rates in the respective schedules did not deviate more than 10% of the average of the 10 most recent nondrugged sessions.

Data Analysis

The response rate (responses per second) average of all animals after drug administration were compared to the average of non injection control performances (Thursday's sessions) and to the average response rates after administration of drug vehicle. For each subject the control response rate is

the average of between 10 and 14 sessions. In addition to response rates, the number of shocks received by each pigeon during the punished interval, were also quantitated as an index of anticonflict activity. A total of nine pigeons were used in these experiments. Student's *t*-test was used to evaluate the effects of shock intensity (level of punishment) on punished and unpunished response rates, and on the number of shocks received by the pigeons (data on Fig. 1). The effect of each of the drug doses tested was evaluated against their respective vehicle-control rates, by means of a paired *t*-test (data on Figs. 2–4). Comparisons between drugs and/or drug doses were assessed by ANOVA followed by the Duncan's test (data on Fig. 5).

RESULTS

Control response rates during the unpunished intervals averaged 0.69 ± 0.04 (responses/s). Unpunished responding rate was not affected by the intensity of the electric shocks applied during the punished intervals (Fig. 1). The control response rates of punished responding and the number of shocks received during the punished interval were both dependent on the intensity of the punished stimulus applied. Higher levels of punishment were associated with lower response rates and lesser number of shocks received by the pigeons (Fig. 1).

The effects of ondansetron on punished and unpunished responding are shown in Fig. 2. Ondansetron, in doses of 1, 10, and 100 μg/kg did not modify unpunished responding (Fig. 2). The effects of ondansetron on punished responding were dependent on the intensity of punishment employed (Fig. 2). Ondansetron, at 100 μg/kg, released responding suppressed by punishment only when pigeons were working at the lower levels of punishment. Under these conditions, key-pecking was increased by 119% above control values, and the number shocks received by the pigeons increased from 5.6 ± 0.8 (vehicle) to 11.6 ± 3.1 with $p < 0.01$. Neither the rate of responding nor the number of shocks received by the pigeons (vehicle: 1.6 ± 0.5; ondansetron 100 μg/kg: 1.67 ± 0.7) (NS) were affected by 1, 10, and 100 μg/kg ondansetron, in animals working under high levels of response suppression induced by high shock intensity (Fig. 2).

Similarly to ondansetron, the effects of buspirone (0.3 and 1 mg/kg) on punished responding was dependent on the intensity of the punishing stimulus. With the lower level of punishment (low response suppression), buspirone increased the rate of punished responding, as well as, the number of shocks received by the pigeons. With 0.3 and 1 mg/kg of buspirone, the number of shocks received by the pigeons increased from 5.3 ± 0.9 (control) to 32.4 ± 16.4 and to 24 ± 10, respectively. Buspirone (0.3 and 1 mg/kg) showed a small, but not significant, anxiolytic-like effect, at higher levels of punishment (basal: 1.7 ± 0.3; 0. 3 mg/kg buspirone: 2.4 ± 0.5; and 1 mg/kg buspirone: 3.5 ± 1.3) (NS). Buspirone in doses of 0.3 and 1 mg/kg had no effect on unpunished responding (Fig. 3). Higher doses of buspirone (1.5 mg/kg) reduced unpunished responding by 30% (data not shown).

Similarly to ondansetron and buspirone, the effects of diazepam on punished responding were dependent on the intensity of the punishing stimulus. With the lower level of punishment (lower shock intensity), diazepam (1 and 1.5 mg/Kg) increased the number of shocks received by the pigeons from 2 ± 1 (vehicle) to 21.3 ± 9.3 and to 48.6 ± 16, respectively ($p < 0.01$). The response rates were increased by 3- and 6.5-fold above vehicle levels with 1 and 1.5 mg/kg of diazepam, respectively ($p < 0.01$). Similar to ondansetron and buspirone, diaz-

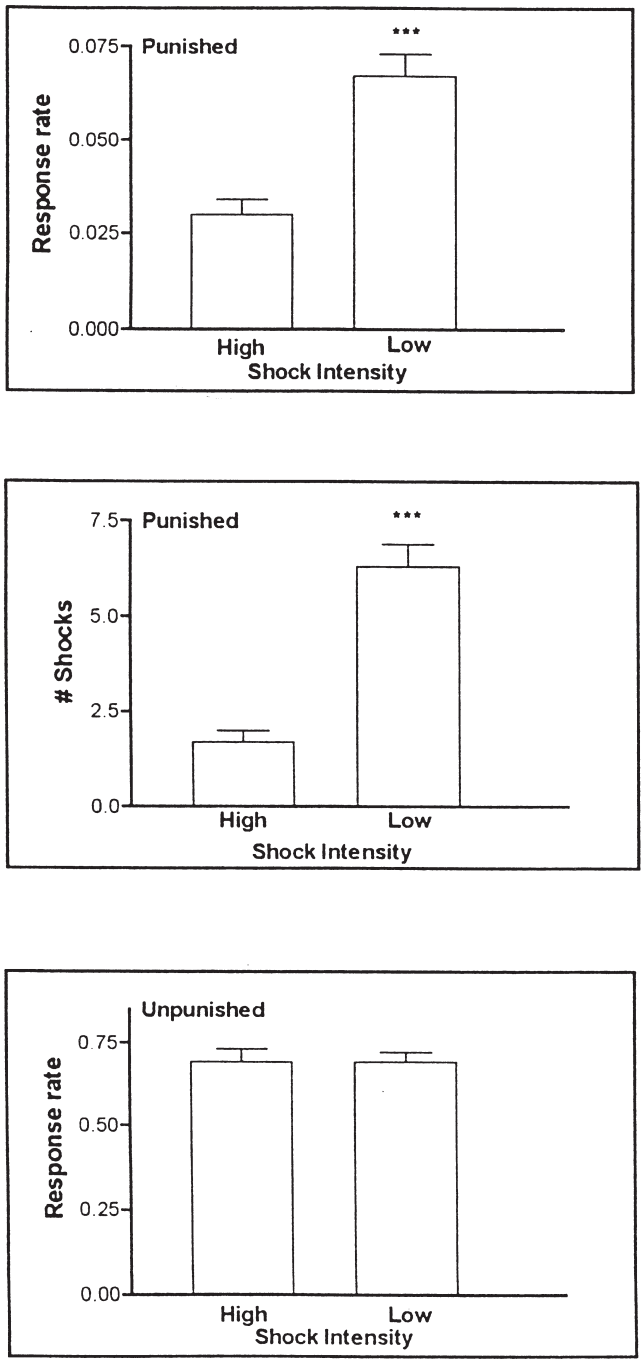

FIG. 1. Shock intensity determines punished responding in an operant conflict test in pigeons. Abscissae: high: high shock intensity (shock intensity was adjusted so that punished responding rate was 5% of unpunished responding rate). Low: low shock intensity (shock intensity was adjusted so that punished responding rate was 10% of unpunished responding rate). Ordinates: top: response rate (responses/s) during punished intervals. Middle: No. of shocks received during punished intervals. Bottom: response rate (responses/s) during unpunished intervals. Shown are mean values ± SEM of at least six trials per pigeon, in nine pigeons. ***$p < 0.001$.

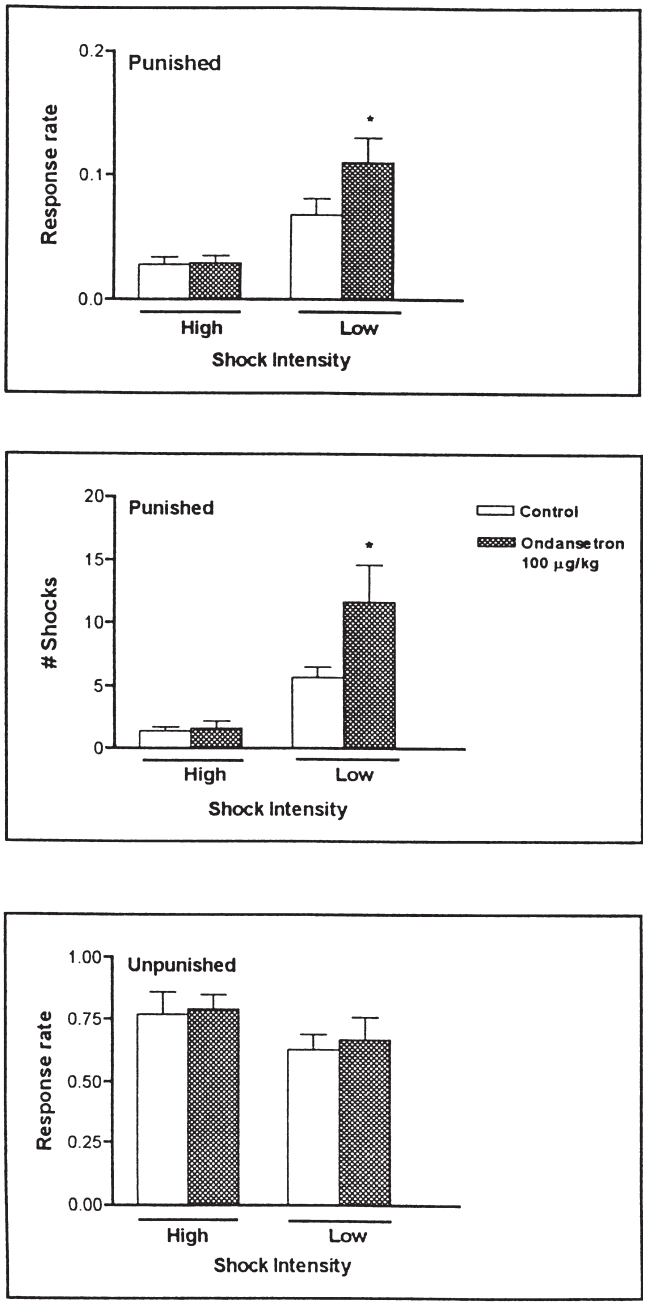

FIG. 2. Shock intensity determines antipunishment effects of ondansetron in an operant conflict test in pigeons. Abscissae: high: high shock intensity (shock intensity was adjusted so that punished responding rate was 5% of unpunished responding rate). Low: low shock intensity (shock intensity was adjusted so that punished responding rate was 10% of unpunished responding rate). Ordinates: top: response rate (responses/sec) during punished intervals. Middle: No. of shocks received during punished intervals. Bottom: response rate (responses/s) during unpunished intervals. Shown are mean values ± SEM of at least two trials per pigeon in nine pigeons. $*p < 0.05$.

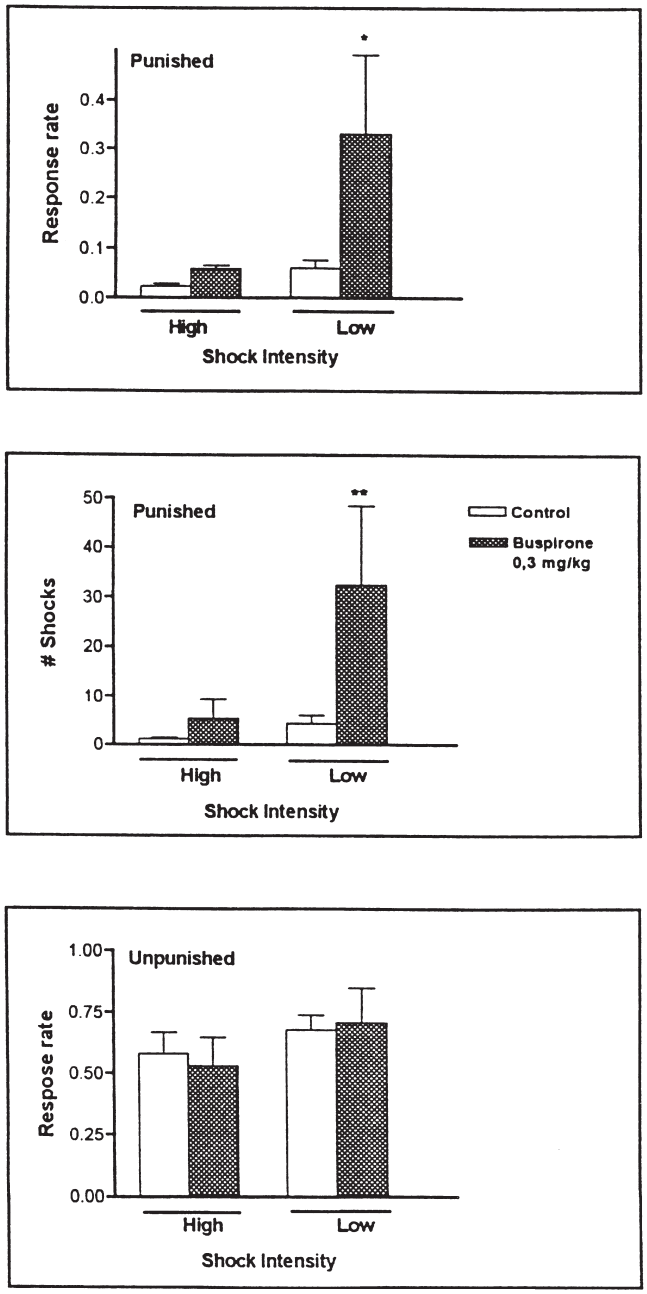

FIG. 3. Shock intensity determines antipunishment effects of buspirone in an operant conflict test in pigeons. Abscissae: high: high shock intensity (shock intensity was adjusted so that punished responding rate was 5% of unpunished responding rate). Low: low shock intensity (shock intensity was adjusted so that punished responding rate was 10% of unpunished responding rate). Ordinates: top: response rate (responses/s) during punished intervals. Middle: No. of shocks received during punished intervals. Bottom: response rate (responses/s) during unpunished intervals. Shown are mean values ± SEM of at least two trials per pigeon in nine pigeons. $*p < 0.05$, $**p < 0.01$.

epam show no anticonflict activity when punished responding was suppressed to 5% of unpunished responding rate (response rates in responses/s were vehicle: 3.2 ± 0.4; 1 mg/kg diazepam: 2.4 ± 0.9; 1.5 mg/kg diazepam: 3.6 ± 0.6) (NS). Diazepam in doses of up to 1.5 mg/kg failed to modify the rate of unpunished responding (Fig. 4).

Figure 5 shows the comparative effect of effective doses of ondansetron, buspirone, and diazepam in releasing punished

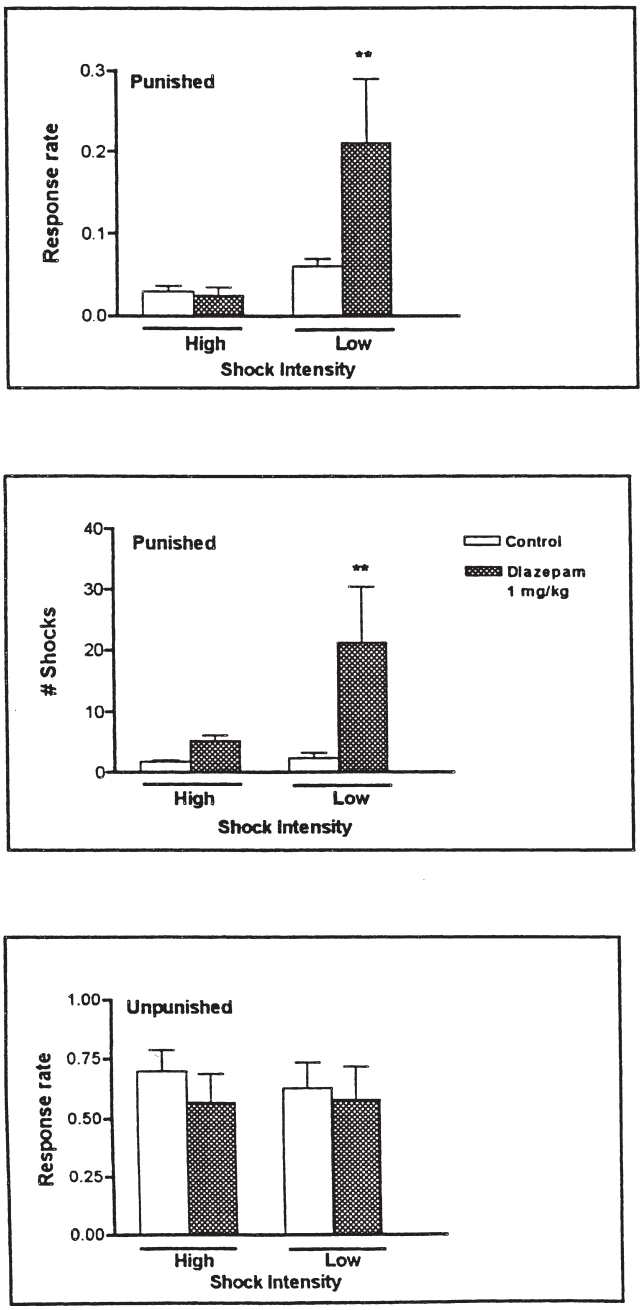

FIG. 4. Shock intensity determines antipunishment effects of diazepam in an operant conflict test in pigeons. Abscissae: high: high shock intensity (shock intensity was adjusted so that punished responding rate was 5% of unpunished responding rate). Low: low shock intensity (shock intensity was adjusted so that punished responding rate was 10% of unpunished responding rate). Ordinates: top: response rate (responses/s) during punished intervals. Middle: No. of shocks received during punished intervals. Bottom: response rate (responses/s) during unpunished intervals. Shown are mean values ± SEM of at least two trials per pigeon in nine pigeons. $^{**}p < 0.01$.

responding, without affecting unpunished responding. Diazepam and buspirone were more effective than ondansetron in increasing response rates and number of shocks received by the pigeon under similar experimental conditions (Fig. 5).

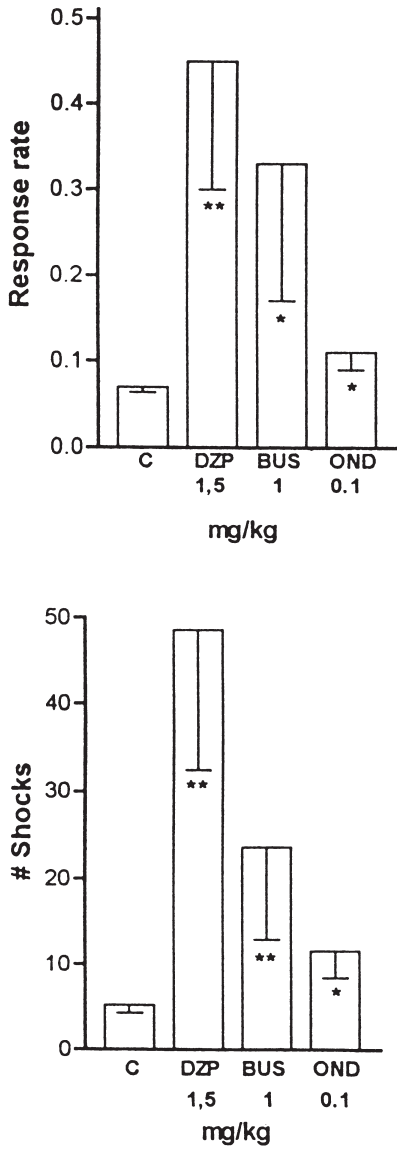

FIG. 5. Comparative effects of maximally effective doses of ondansetron, buspirone, and diazepam on punished responding under lower suppression. In these experiments, shock intensity was adjusted so that punished responding rate was 10% of unpunished responding rate). Ordinates: top: response rate (responses/s) during punished intervals. Bottom: No. of shocks received during punished intervals. C = Control (vehicle), DZP = diazepam, BUS = buspirone, OND = ondansetron. Shown are mean values ± SEM of at least two trials per pigeon in nine pigeons.$^{*}p < 0.05$, $^{**}p < 0.01$.

DISCUSSION

Serotonergic antagonists and lesions of serotonergic systems have been shown to increase punished responding (2–4, 8,14,16,17). The anticonflict effect of benzodiazepines and of buspirone may be mediated by inhibition of serotonergic neurotransmission (2,18,21). Recent studies have suggested that 5-HT$_3$ receptors may play a role in certain models of anxiolytic activity (3,4,6,9,16). In this investigation we evaluated the anticonflict effect of ondansetron, in pigeons.

Operant behavior in pigeons has been extensively employed for the study of drugs with anxiolytic (antipunishment) activity (1,11,20). An anxiolytic-like response would be reflected during the conflict component in a responding above baseline levels (i.e., more key-pecking or greater anticonflict effect). Employing this test, we demonstrated that ondansetron, a selective antagonist of 5-HT_3 receptors, released suppressed responding induced by punishment. Ondansetron, increased the rate of responding and the number of electrical shocks received during the punished interval; without affecting unpunished responding as in the vehicle-treated pigeons. The effects of ondansetron, were qualitatively similar to those of clinically effective anxiolytics, i.e., diazepam and buspirone. However, much greater enhancement of punished responding was observed with diazepam and buspirone than with ondansetron. The smaller quantitative anticonflict effect of ondansetron may account for its weaker clinical anxiolytic action (10,13, 17). In addition, it is of importance to indicate that the effects of the three agents were highly dependent on the magnitude of punishment and, hence, on the degree of suppression of responding induced by the painful stimulus. When responding during the punished intervals was 5% of unpunished responding, none of the three drugs tested showed significant anxiolytic-like activity; although, a small effect was observed with diazepam and buspirone. When the intensity of the painful stimulus was reduced, so that punished response rate was 10% of the unpunished responding, then all three agents effectively enhanced responding suppressed by punishment.

The effects of different shock intensities on anticonflict drug activity have been previously studied for pentobarbital and diazepam (11). It was demonstrated that the magnitude of increase in the rate of punished responding induced by pentobarbital or diazepam was highly dependent upon the intensity of the electrical shocks applied to the pigeons. Our results support these previous observations, and indicate that the level of punishment and/or the behavioral suppression induced by the intensity of the punishing stimulus is a strong determinant of the magnitude of the anticonflict drug action, and may be responsible for the lack of drug effects observed in certain experimental paradigms.

Selective 5-HT_3 receptor antagonists have been shown effective in models predictive for anxiolytic activity, such as the light–dark exploration test in the mouse, the social interaction test in the rat, and the aggressive behavior triggered in marmosets and in cynomolgus monkeys by the exposure to an unknown observer (4,9,16). These models are characterized by exposure of the animals to a novel environment. In these models the 5-HT_3 antagonists were as effective in suppressing aversive behaviors as the benzodiazepines; suggesting a role for 5-HT_3 receptors in anxiety. However, ondansetron was reported ineffective in the water-lick conflict test in rats; a test where the benzodiazepines showed efficacy (9). In addition, ondansetron did not produce a behavioral profile consistent with anxiety reduction, in the elevated plus-maze paradigm in mice, a procedure involving aspects of defensive behavior (15). The water-lick conflict test has some similarities with the experimental paradigm employed in this work. In the water-lick test, rats are deprived of water for 24 h, and licking for water is automatically punished with electrical shocks applied at the grid floor and at the spout, either every five licks or every 0.75 s of contact with the spout. In our study, pigeons were food deprived and key-pecked for food. The pigeons worked under a fixed-interval program consisting of 10 intervals of 5-min duration each, of altered punished and unpunished intervals (5 min each). On the punished intervals the key-light was red, while it was white during the unpunished intervals. Punishment consisted in delivering an electric shock after the 15th response. Several factors may explain the differences observed between both studies; namely, the animal species employed (rats vs. pigeons), the absence or presence of a discriminative stimulus (red key-light) and the intensity of punishment employed. The intensity of the painful stimulus is a fundamental determinant of drug action [(11), present study], in particular, for drugs with modest antipunishment effect. It is possible that the shock intensity employed in the water-lick conflict was high enough to inhibit the anticonflict action of ondansetron, without affecting that of benzodiazepines. In addition, 5-HT_3 receptors antagonists may be less effective than benzodiazepines in releasing behavior inhibited by punishment. The possibility that pigeons are more sensitive to the effects of ondansetron cannot be ruled out.

The cerebral topology of the antiaversive activity of 5-HT_3 antagonists has been investigated in mice. Selective 5-HT_3 receptor antagonists have been shown effective in models predictive for anxiolytic activity, such as the light–dark exploration test in the mouse and the social interaction test in the rat. Studies on direct drug injection into brain structures suggest that attenuation of aversive responses induced by 5-HT_3 antagonists is mediated by actions on the dorsal raphe nucleus and the amygdala (3,6). Direct injections of selective 5-HT_3 agonists into these structures produced aversive, anxiogenic-like, effects, which were opposite to those of 5-HT_3 antagonists (3,6).

In addition to antagonizing 5-HT_3 receptors, recent evidence suggests that ondansetron may have actions at other receptor sites. Blockade of voltage-gated potassium channels on human neuroblastoma cells, TE671 (23), and activation of imidazoline receptors in a mouse neuroblastoma N1E-115 cell line (12), have been described. Although, the anxiolytic-like actions of ondansetron are shared by other structurally different 5-HT_3 receptor antagonists, the above described effects were not produced by other 5-HT_3 antagonists (12,23).

In summary, ondansetron exhibited an anxiolytic-like effect qualitatively similar but quantitatively smaller than that produced by diazepam or buspirone in pigeons. We propose that the anxiogenic response induced by punishment is less sensitive to 5-HT_3 antagonists, than the behavior induced by aversive, unpunished, situations, where 5-HT_3 antagonists have shown comparable efficacy to benzodiazepines. The anticonflict effect of the three drugs was strongly dependent on the level of punishment applied, indicating that the intensity of the punishing stimulus must be carefully controlled in experiments of anxiolytic-drug efficacy.

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