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Interaction of Buspirone and Dopaminergic Agents on Punished Behavior of Pigeons

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WITKIN, J M AND J E BARRETT *Interaction of buspirone and dopaminergic agents on punished behavior of pigeons* PHARMACOL BIOCHEM BEHAV **24**(3) 751–756, 1986 —The non-benzodiazepine anxiolytic buspirone was studied alone and in combination with either haloperidol or apomorphine Drug effects were evaluated under a baseline of punished and unpunished keypeck responses of pigeons, every 30th response produced food (no punishment) in the presence of a white keylight and, when the keylight was red in alternate 3 min periods, every 30th response produced both food and a brief electric shock (punishment) Buspirone (0 03–3 mg/kg, IM) increased the low rates of punished responding to a maximum of 1000% of control at doses of 0 1–1 mg/kg Unpunished responding was only marginally affected at lower doses and dose-dependent decreases were obtained from 1 to 10 mg/kg Although less potent, chlordiazepoxide (1–100 mg/kg IM) produced effects which were similar to those of buspirone, a finding which contrasts with the greater efficacy of benzodiazepines for increasing punished behavior in mammals Dose-effect functions for buspirone were unchanged by haloperidol administration (0 01 and 0 03 mg/kg, IM, 5 min prior) or by concurrent treatment with a behaviorally-ineffective dose of apomorphine (0 003 mg/kg, IM) Rate-decreasing doses of apomorphine (0 01–0 1 mg/kg) reversed the increases in punished responding produced by lower doses of buspirone (0 03 and 0 1 mg/kg) and the apomorphine-induced decreases in unpunished responding were antagonized by buspirone at doses which had little affect when given alone The ability of buspirone to reverse the rate-decreasing effects of apomorphine on unpunished responding suggests that buspirone does exhibit dopaminergic antagonist properties *in vivo* However, effects of buspirone on punished responding of pigeons do not appear to be due to dopaminergic mechanisms Punished behavior of pigeons provides a unique model for further investigations of the mechanism of action of the potent anxiolytic buspirone

Buspirone Punished behavior Haloperidol Apomorphine Dopamine Keypeck Pigeons

BINDING of benzodiazepines to specific recognition sites within the central nervous system appears to initiate events leading to the anxiolytic activity of these compounds Non-benzodiazepine drugs such as the barbiturates may also produce clinical relief from anxiety by altering binding at benzodiazepine receptors (cf [12, 13, 18, 25, 26]) Although mechanisms involving ligand binding to benzodiazepine receptors may be sufficient to account for anti-anxiety activity of drugs, these mechanisms may not be necessary Buspirone, an azaspirodecanedione, is structurally unrelated to the benzodiazepines [34] and does not bind to benzodiazepine receptors [20], however, recent clinical trials demonstrate buspirone to be an effective anxiolytic devoid of a number of side-effects indigenous to the 1,4-benzodiazepines [7, 11, 16, 21]

Behavior suppressed by response-produced electric shock (punishment) is a well-established pre-clinical baseline against which to predict anxiolytic drug activity (cf [24]) Buspirone, like benzodiazepine compounds, increases punished behavior [2, 10, 20] although buspirone appears to be much less efficacious than benzodiazepines [28,32] In contrast to benzodiazepines, effects of buspirone on punished behavior are not antagonized by the benzodiazepine antagonists Ro 15-1788 or CGS 8216 [32] indicating that distinct pharmacological actions of buspirone may be reponsible for its behavioral effects

Buspirone interacts with dopamine receptors *in vitro* [20,33], and has pharmacological properties in common with both dopaminergic agonists and antagonists [15, 20, 29] Based on these observations, Stanton *et al* [27] and Taylor *et al* [30] have suggested that buspirone's antianxiety activity may be dopaminergically mediated The present study was undertaken to provide a direct assessment of this possibility Punished behavior of pigeons was examined since, in this species, buspirone is at least as equi-efficacious as the benzodiazepines [2]

METHOD

Subjects

Adult male White Carneaux Pigeons (Palmetto Pigeon Plant, Sumter, SC) were maintained at 80% (409–504 g) of their free feeding body weights The pigeons were experimentally-naive and were housed in separate living

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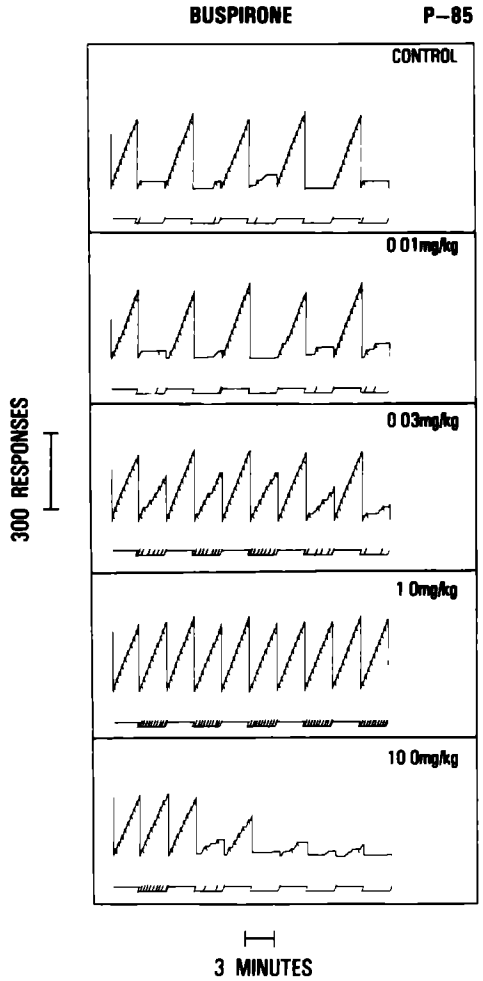

FIG 1 Cumulative response records of a pigeon showing representative control performance under the multiple FR 30, FR 30 plus punishment schedule (top panel) Successive panels illustrate effects of increasing doses of buspirone The response pen was incremented with each response Diagonal slashes of the response pen indicate food delivery (unpunished responding) or the simultaneous presentation of food and shock (punished responding) The lower pen was deflected downward during the punishment component, shock presentation is marked by a momentary upward tracing

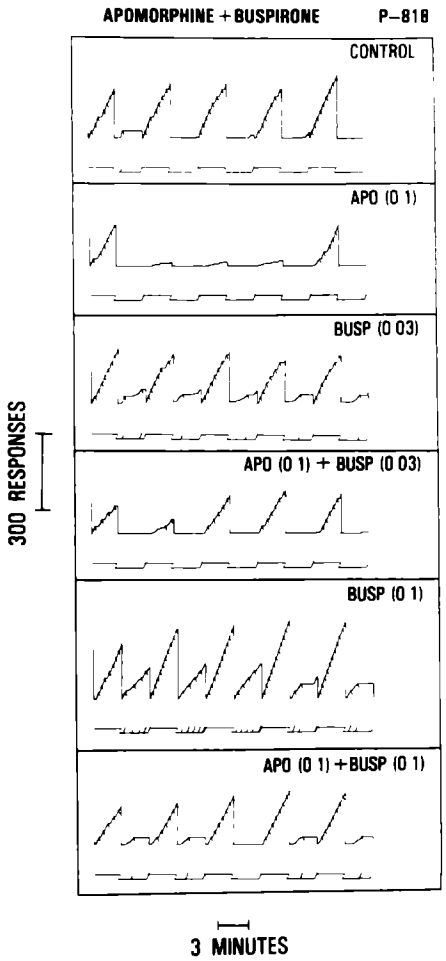

FIG 2 Cumulative response records of a pigeon showing representative control performance under the multiple FR 30, FR 30 plus punishment schedule (top panel) and the effects of apomorphine alone and in combination with buspirone Recording details as in Fig 1

cages within a temperature- and light-controlled vivarium (12 hr light-dark cycle) where they were given continuous access to water and oyster shell grit

Apparatus

The experimental chamber (22×27×31 cm), similar to that described by Ferster and Skinner [8], contained a translucent response key (2 cm diameter, R Gerbrands, Co , Arlington, MA) located in the center of the front panel, 23 cm above a wire mesh floor The key could be transilluminated with red or white light from a pair of 7 W lamps A minimum normal force of 0 15 N (15 g) applied to the key produced the click of a relay mounted behind the front panel and defined a response A rectangular opening was located below the response key through which mixed grain could be made available for 3 sec by the operation of a solenoid-activated feeder The experimental chamber was located within a sound- and light-attenuating enclosure that was ventillated and which provided white noise to further mask extraneous sounds Electric shock (120 V, AC, 60 Hz) was delivered to stainless steel electrodes implanted around each pubis bone [1] for 200 msec The birds were connected to the shock source via a vest-mounted plug The impedance of the electrodes was measured daily to ensure a constancy of stimulus presentation Experimental events were scheduled and recorded with electromechanical switching circuitry located in a separate room

Behavioral Procedure

The pigeons, after being trained to eat out of the food magazine, were trained to peck the response key [8] When food was presented, the grain hopper was illuminated and

TABLE 1

EFFECTS OF CHLORDIAZEPOXIDE ON PUNISHED AND UNPUNISHED RESPONDING*

Dose (mg/kg)	Unpunished	Punished
0	2 11 ± 0 10	0 04 ± 0 01
1	114 60 ± 17 60	398 20 ± 178 90
3	121 70 ± 20 40	478 00 ± 243 70
5 6	111 90 ± 10 90	1380 20 ± 452 50
10	116 80 ± 13 50	1217 60 ± 372 30
100	26 70 ± 4 70	955 40 ± 314 40

*Values are given as a percentage of control response rates ±S E M (shown at 0 mg/kg in responses/sec, N=14) from duplicate determinations made in two pigeons Significant increases in punished responding were obtained with doses from 3 to 100 mg/kg in each bird tested

the keylight extinguished The number of responses required to produce food was gradually incremented from one to thirty (fixed-ratio 30 or FR 30 schedule) in the presence of white or red keylights Responding was next established under a multiple FR 30 FR 30 schedule in which every thirtieth response in the presence of red or white keylights produced food Keylight colors alternated successively every 3 min for 5 cycles, schedule components were separated by a 60-sec timeout period during which the chamber was dark and responding had no scheduled consequences Experimental sessions began with the white keylight and lasted 39 min When responding stabilized under the multiple FR 30 FR 30 schedule, an FR 30 schedule of shock delivery was programmed conjointly with the FR 30 food-presentation schedule in the presence of the red keylight Shock intensity (1 5–4 0 mA) was adjusted for each pigeon in order to suppress food maintained responding by at least 80% Thus, under the baseline upon which behavioral effects of drugs were assessed, responding was maintained by food (unpunished responding) in the presence of a white keylight and was simultaneously maintained by food and suppressed by shock (punished responding) in the presence of a red keylight

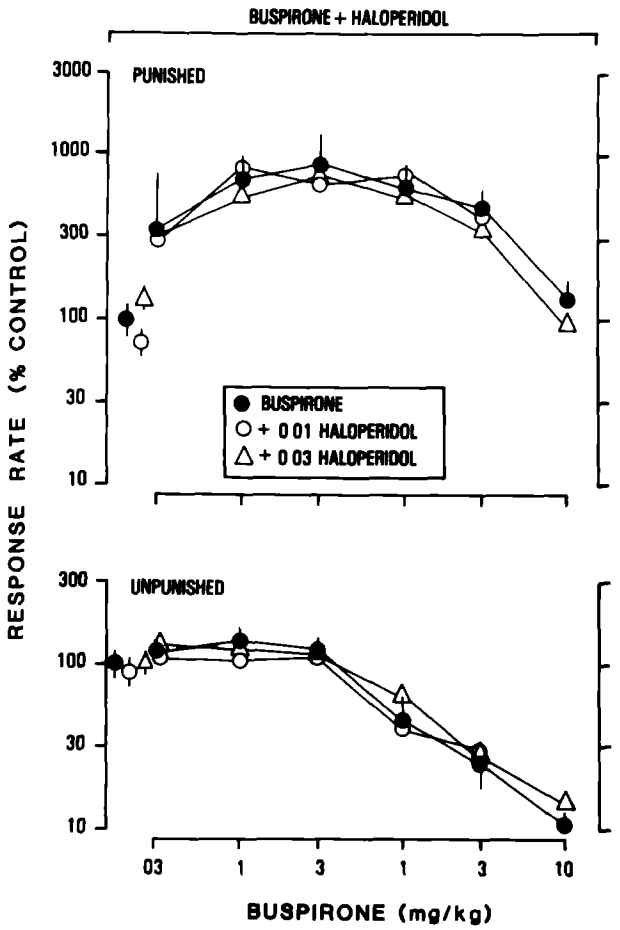

FIG 3 Effects of buspirone alone (filled circles) and in combination with haloperidol (open symbols) Each point represents the mean effect determined in three pigeons Vertical lines denote ±S E M around the control mean (unconnected, filled circles), effects of haloperidol alone (unconnected, unfilled symbols) and the effects of buspirone alone Mean control response rates were 2 34±0 34 (unpunished) and 0 07±0 01 (punished) responses per sec

Pharmacological Procedure

Buspirone HCl (donated by Dr L Riblet, Bristol-Myers Co , Evansville, IN), apomorphine HCl (Sigma Chemical Co , St Louis, MO), chlordiazepoxide HCl (donated by Hoffmann-LaRoche, Inc , Nutley, NJ), and haloperidol (McNeil Pharmaceutical, Spring House, PA) were dissolved in 0 9% NaCl All drugs were given by intramuscular injection in 1 0 cc/kg body weight Buspirone and apomorphine were given immediately prior, haloperidol 5 min prior, and chlordiazepoxide 60 min prior to experimental sessions These pretreatment times, based both on preliminary research and previously published data [2], were used to study effects of the drugs alone as well as in combination with buspirone Dose-effect curves for buspirone were determined prior to the drug-interaction experiments Doses of the drugs and drug-combinations were studied in a mixed order and the effects of the drugs alone were determined on at least two occasions Injections were made on Tuesdays and Fridays providing that baseline performances were within the range of control values Except for haloperidol, drug doses are expressed as the salt

Data Analysis

Rates of responding were computed separately for each multiple schedule component by dividing the total number of responses by the total elapsed time in the components This measure correlates directly with the rate of food or shock delivery Response rates after drug administration were compared to non-injection control performances (Thursdays) and to response rates after administration of saline for each individual pigeon, each pigeon served as its own control Composite dose-effect functions were obtained by averaging mean percentage changes from control values, for each bird, across animals. Drug effects with individual animals were considered significant if responding deviated more than two standard deviations from control levels or from the effects of a drug alone Drug effects noted in the text are discussed in relation to this criterion Changes in

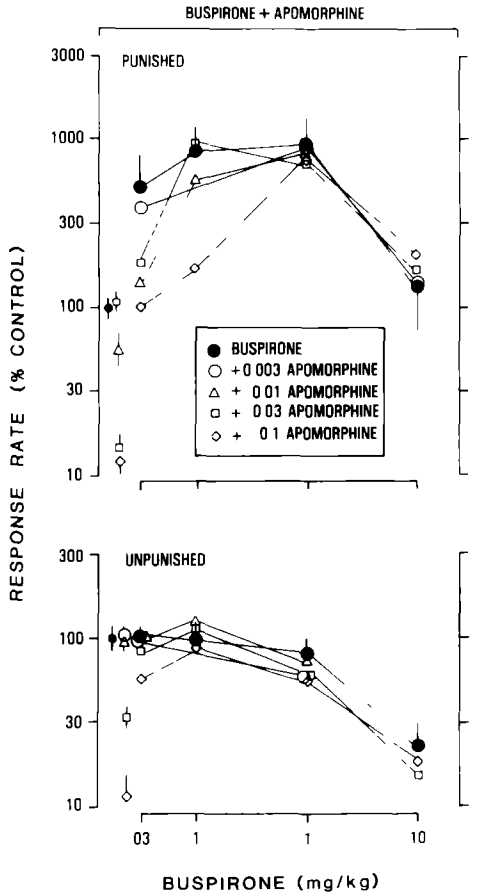

FIG 4 Effects of buspirone alone (filled circles) and in combination with apomorphine (open symbols) Each point represents the mean effect determined in two pigeons Vertical lines denote ±S E M around control values (unconnected, filled circles), effects of apomorphine alone (unconnected, unfilled symbols) and the effects of buspirone alone Mean control response rates were 1 70±0 25 (unpunished) and 0 14±0 01 (punished) responses per sec

performance were also evaluated by inspection of cumulative response records (Gerbrands recorders, R Gerbrands Co , Arlington, MA)

RESULTS

Representative control performances under the multiple schedule are shown in Figs 1 and 2 (top panels) Unpunished responding was characterized by brief pauses after food delivery followed by high steady rates of responding Under the punishment component, relatively few responses occurred during control conditions and rates of food and shock delivery were quite low

Buspirone produced significant increases in punished responding of all 5 animals across a wide range of doses from 0 1 to 3 mg/kg Higher doses (3 and 10 mg/kg) decreased or eliminated unpunished responding in all animals but, overall, did not reduce punished responding below control levels (Figs 1, 3, and 4) Increases in punished responding at doses less than 3 0 mg/kg lasted for at least 30 min (Figs 1 and 2) Initial increases in punished behavior after 10 mg/kg buspirone were followed several minutes later by profound suppression of punished and unpunished responding (Fig 1)

Although less potent, chlordiazepoxide produced effects on punished and unpunished behavior comparable to those of buspirone (Table 1) Maximal rate-increasing effects of either buspirone or chlordiazepoxide resulted in similar rates of punished and unpunished behavior As with buspirone, chlordiazepoxide produced significant increases in punished responding at doses that did not affect or which decreased unpunished responding

Haloperidol (0 01 and 0 03 mg/kg) had no effect on the buspirone dose-effect functions (Fig 3) Higher doses of haloperidol (0 1 mg/kg) markedly suppressed punished and unpunished responding and were not tested in combination with buspirone Behaviorally-inactive doses of apomorphine (0 003 mg/kg) did not alter the effects of buspirone (Fig 4) Rate-decreasing doses of apomorphine (0 01–0 1 mg/kg) on the other hand, reversed the effects of lower doses of buspirone on punished behavior Furthermore, the rate-decreases produced by apomorphine (0 03 and 0 1 mg/kg) on unpunished behavior were reversed by behaviorally-ineffective doses of buspirone (Figs 2 and 4) However, a rate-decreasing dose of buspirone (10 mg/kg) did not reduce the rate-suppressant effects of apomorphine

DISCUSSION

Buspirone produced large, dose-dependent increases in punished behavior of pigeons in the present study Increases of 1000% of control values were obtained at optimal doses At the same time, unpunished responding was only marginally affected at lower doses while dose-dependent decreases occurred at higher doses The effects obtained with buspirone were comparable to those obtained with chlordiazepoxide The similar efficacy of buspirone and chlordiazepoxide contrasts with the relatively weak efficacy of buspirone in rats and squirrel monkeys reported earlier [28,32] Under baselines and behavioral performances similar to those used here, buspirone only modestly increased punished responding, whereas midazolam produced 20-fold increases in responding of the same squirrel monkeys [32] Although Geller and Hartmann [10] reported comparable increases in punished responding with buspirone and diazepam in rats and cynamologous monkeys, details of the data render ambiguous conclusions For example, neither buspirone nor diazepam produced sizeable increases in punished responding in either species The baselines of suppressed behavior did not recover for several days after drug administration Due to the large increases in punished behavior which occur similarly with buspirone and chlordiazepoxide pigeons may provide a useful model of the anxiolytic actions of buspirone and, perhaps, other anxiolytic compounds as well

Buspirone is a relatively potent displacer of radiolabeled dopamine receptor ligands from brain tissue *in vitro* [20,33] In a number of systems, the pharmacology of buspirone reflects its dopamine receptor binding characteristics, presenting a dopaminergic agonist or antagonist profile [15, 20, 29] The ability of buspirone to reverse the rate-decreasing effects of apomorphine reported here may indicate a dopamine-antagonist component to buspirone's spectrum of activity However, the results of the present study showed that alteration in dopaminergic neurotransmission by buspirone is not relevant to its punishment-attenuating effects Neither the dopamine receptor antagonist haloperidol (i e ,

[4]) nor the agonist apomorphine (i e , [3]) increased punished behavior Moreover, neither of these compounds specifically antagonized this action of buspirone MJ 13805, a structural analog of buspirone, increases punished responding and shares other pharmacological properties with buspirone but has no significant influence on central dopamine systems [5, 14, 31] The direct role of dopaminergic neurotransmission in the anxiolytic activity of drugs (cf [27,30]) and of buspirone in particular is limited and appears to be of no general significance

The mechanisms responsible for the anxiolytic activity of buspirone remain obscure Buspirone is atypical in a number of systems traditionally used to evaluate anti-anxiety activity For example, buspirone, unlike other anxiolytics does not depress firing of the locus coeruleus, sometimes held to be an important anti-anxiety mechanism [19, 22, 23] Although buspirone does not influence GABA-inhibition of neuronal firing, unlike the benzodiazepines [15], the significance of the facilitation of benzodiazepine binding in brain by buspirone [9, 17, 32] requires further study However, in view of the fact that buspirone does not affect either *in vivo* or *in vitro* benzodiazepine binding in pigeon brain, and since the benzodiazepine-receptor antagonist Ro 15-1788 does not alter buspirone's effects in the pigeon, the role of the GABA-benzodiazepine complex in buspirone's effects appears minimal (Barrett, Witkin, Mansbach, Skolnick and Weissman, submitted manuscript) The influence of buspirone on serotonin binding may have important relationships to its effects on punished behavior [5, 6, 20, 33], involvement of serotonin neurotransmission has also been implicated in anticonflict actions of benzodiazepines (cf [25]) Investigations along these lines are currently under way Elucidation of the mechanism of action of buspirone promises to significantly clarify current understanding of anxiety and its pharmacological control

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