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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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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.

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.

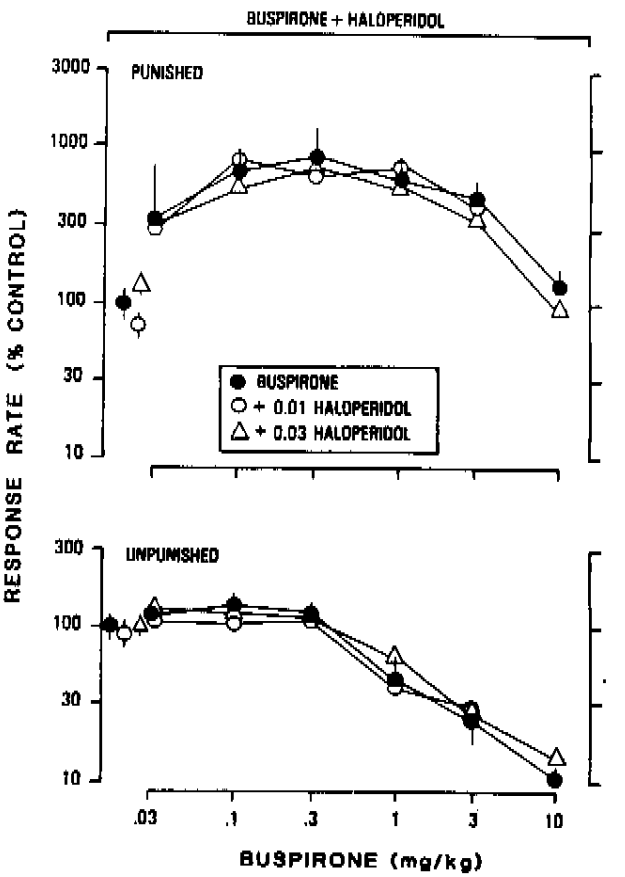

FIG. 3. Effects of buspirone alone (filled circles) and in combination with haloperidol (open symbols). Each point represents the mean effect determined in three pigeons. Vertical lines denote ±S.E.M. around the control mean (unconnected, filled circles), effects of haloperidol alone (unconnected, unfilled 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.

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

[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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