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Buspirone Blocks the Discriminative Stimulus Effects of Apomorphine in Monkeys[1]

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KAMIEN, J. B. AND W. L. WOOLVERTON. *Buspirone blocks the discriminative stimulus effects of apomorphine in monkeys.* PHARMACOL BIOCHEM BEHAV 35(1) 117–120, 1990.—Three rhesus monkeys were trained to discriminate apomorphine (APO) from saline in a two-lever, food-reinforced drug discrimination procedure. After acquisition of the discrimination, the monkeys were given various doses of APO in combination with saline or buspirone before test sessions in which responses occurring on either lever were reinforced. Combinations of APO (0.01–0.08 mg/kg, IV) and saline resulted in a dose-related increase from 0 to 100% in the percentage of responses that occurred on the APO-appropriate lever. When buspirone (0.04–0.16 mg/kg, IV) was combined with APO, reductions from 100% to 0% APO-appropriate responding were seen following at least one dose combination in all three monkeys. A parallel shift to the right of the APO dose-response curve with buspirone was evident in 2 monkeys, indicating surmountable antagonism. In one case, a further increase in buspirone dose resulted in an insurmountable antagonism, i.e., increasing APO dose still resulted in primarily saline-appropriate responding. These results suggest that buspirone can function as a D_2 dopamine (DA) receptor antagonist at behaviorally relevant doses.

Drug discrimination Apomorphine Buspirone D_2 receptors Dopamine Behavior Rhesus monkeys

BUSPIRONE (8-[4-[4-(2-pyrimidinyl)-1-piperazinyl] butyl]-8-aza-spiro [4,5]-decane-7,9-dione) is a nonbenzodiazepine drug that possesses anxiolytic activity in man (3) and is active in some animal models of anxiety (13). Buspirone does not interact with GABA or benzodiazepine receptors as do most typical anxiolytics (10,15). Buspirone does, however, have high affinity for 5-HT_{1A} serotonin binding sites (7,15) and evidence suggests that the antianxiety effects of buspirone are due primarily to interactions with serotonin systems (12, 14, 15). Interestingly, buspirone also binds to D_2-dopamine (DA) binding sites (1,4) and has biochemical effects similar to those of D_2 DA antagonists (8). The behavioral significance of these DA antagonist effects is largely unknown.

The results of behavioral studies with buspirone are generally consistent with the in vitro results. Investigations of the discriminative stimulus (DS) effects of buspirone revealed that buspirone did not generalize to oxazepam, a benzodiazepine anxiolytic (5,9). Mansbach and Barrett (9) reported that buspirone's DS effects were shared by gepirone (a structural analog of buspirone) and the 5-HT_{1A} binding ligand 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), implicating interactions with 5-HT_{1A} receptors as its possible mechanism of action. On the other hand, Mansbach and Barrett (9) reported that neither the DA agonist apomorphine nor the DA antagonist haloperidol substituted for buspirone as a discriminative stimulus. It is curious that haloperidol did not substitute for buspirone in buspirone-trained pigeons given the interactions of buspirone with D_2 DA binding sites in vitro. It may be that DA antagonist properties are poorly reflected by their DS effects (6).

The purpose of the present study was to examine further the behavioral relevance of the DA antagonist properties of buspirone by testing it as an antagonist of the DS effects of apomorphine in rhesus monkeys. The DS effects of apomorphine are probably mediated by interactions with D_2 DA receptors in the CNS of both rats (16) and monkeys (17). Thus, the DS effects of apomorphine

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provide a behavioral bioassay for the effects of drugs at D_2 receptors. The results demonstrate that buspirone can block this effect of apomorphine and suggest that its action at D_2 receptors is behaviorally relevant.

METHOD

Animals and Apparatus

The subjects were three rhesus monkeys (two males: 0034 and 3196, and one female: 3012) that weighed between 4.0 and 10 kg at the beginning of the experiment. All had extensive experience with IV drug self-administration and studies of drug effects on schedule-controlled behavior. These three monkeys also participated in a previous study of the DS effects of APO (17) and the experimental chamber, apparatus and housing conditions are described in detail in that report. Food intake was restricted to 1-gram banana-flavored pellets (P. J. Noyes Co., Lancaster, NH) received in experimental sessions and postsession Purina Monkey Chow sufficient to maintain stable body weight and behavioral performance. In addition, each animal was given a chewable multiple vitamin tablet every day. Water was continuously available in the home cage.

Procedure

Training. The training procedure was described in detail by Woolverton *et al.* (17). Briefly, training sessions were conducted once a day, 5 or 6 days/week, in a double alternation sequence; that is, 2 consecutive sessions of drug pretreatment alternated with 2 consecutive sessions of saline pretreatment. The final response requirement on both levers was 30 responses per food pellet (fixed ratio:FR 30). An additional contingency was in effect such that an inappropriate response reset the response requirement of the lever appropriate to the injection. Sessions ended with food delivery or after 10 minutes, whichever occurred first. The route of drug administration was IV (saphenous vein, 3–5 sec injection), and the pretreatment time was 5 minutes. Under the final training conditions, the dose of APO was 0.04 mg/kg for monkeys 3012 and 0034. For monkey 3196, a training dose of 0.08 mg/kg was used because stable discrimination was not maintained at 0.04 mg/kg. When at least 80% of the responses before food delivery occurred on the appropriate lever for at least 7 of 8 consecutive sessions, the discrimination was considered to be acquired.

Testing. When the training criterion was achieved, every third session became a test session. A drug and a saline training session were conducted between test sessions to maintain and affirm stimulus control of behavior. If a monkey's responding fell below criterion in these training sessions, test sessions were not conducted and the monkey was returned to the double alternation training sequence until its performance again reached criterion. Test sessions were identical to training sessions with two exceptions: 1) a dose of buspirone (or saline), was injected IV 10 minutes (monkeys 0034 and 3196) or 25 minutes (monkey 3012) before a dose of APO or saline and 2) food was available following 30 responses on either lever, whether the responses were consecutive or not. A pretreatment time of 25 minutes was used in 3012 since maximum blockade of the training dose of apomorphine was seen at this time point. Thus, each test treatment consisted of two injections: buspirone or saline followed by either APO or saline. Test treatments were studied in an irregular order. Each dose or dose combination was tested twice, once with a saline training session the preceding day and once with a drug training session the preceding day. The percentage of APO-appropriate responding and the rate of responding from these two sessions were averaged.

Drugs

Drugs injected before test sessions were apomorphine (APO) HCl (Sigma Chemical Co., St. Louis, MO) and buspirone HCl (Bristol-Myers, Evansville, IN) and the doses refer to these salts. Both drugs were dissolved in sterile saline at concentrations that allowed injections to be given at a volume of 1.0 ml/10 kg.

RESULTS

When APO was tested in combination with saline, a dose-dependent increase occurred in the percentage of APO-appropriate responding of all three monkeys (Fig. 1, top panels, open circles). The lowest dose of APO tested in each monkey occasioned only saline-appropriate responding during both tests, while the highest dose was followed by 100% APO-appropriate responding in both tests. An intermediate dose of APO engendered nearly 100% APO-appropriate responding during one test and nearly 0% APO-appropriate responding during the other test for all three monkeys. Neither the type of training session on the previous day nor the order of the tests were predictive of these results. The average rates of responding during saline training sessions from the first test session to the last were calculated for each monkey (Fig. 1, lower panels, stippled bars). Monkey 0034 averaged 3.6 ($\pm$0.6 S.D., range = 2–4.6, n = 36) responses/second during saline training sessions, while monkeys 3012 and 3196 averaged 5.0 ($\pm$0 S.D.; range = 0; n = 20) and 4.3 ($\pm$0.4 S.D.; range = 3.7–5.0; n = 22), respectively. APO in combination with saline reduced response rates in two monkeys (0034 and 3196) compared to rates during saline training sessions, but not in monkey 3012 (Fig. 1, lower panels).

Buspirone completely blocked the DS effects of at least one dose of APO in all 3 monkeys (Fig. 1, top panels). The reduction in APO-appropriate responding caused by buspirone was dose-dependent in two monkeys (0034 and 3196), with 0.08 mg/kg buspirone reducing APO-appropriate responding occasioned by 0.08 mg/kg APO from 100% to 50%, while 0.16 mg/kg further reduced the percentage of APO-appropriate responding to 0%. The blockade engendered by 0.08 mg/kg buspirone could be overcome in monkeys 0034 and 3012 by increasing the dose of APO, resulting in an approximately 2-fold parallel shift to the right of the APO + saline dose-response curve. A higher dose of buspirone (0.16 mg/kg) completely blocked the DS effects of up to 0.32 mg/kg APO (4 times the training dose) in monkey 0034. In monkey 3196, 0.16 mg/kg buspirone reduced the percentage of APO-appropriate responding to 0% in both tests. An attempt to overcome this antagonism with a dose of 0.16 mg/kg APO completely eliminated responding (Fig. 1, lower right-hand panel).

Response rates following combinations of buspirone and APO were either slightly higher than (monkeys 0034 and 3196) or the same as (monkey 3012) rates subsequent to injections of the same dose of APO + saline (Fig. 1, bottom panels). The dose of buspirone that completely blocked APO-appropriate responding was also tested in combination with saline in each monkey (Fig. 1). In two monkeys (0034 and 3012), virtually all responding after this combination occurred on the saline-appropriate lever at rates comparable to those seen during saline training sessions. Responding by monkey 3196 was eliminated by the combination of 0.16 mg/kg buspirone and saline (Fig. 1, bottom right-hand panel), but was restored when this dose of buspirone was combined with 0.08 mg/kg APO.

DISCUSSION

The results of the present experiment demonstrate that buspirone can block the DS effects of APO in rhesus monkeys. Two lines of evidence suggest that this blockade was due to antagonist actions at D_2 DA receptors. First, previous research has indicated that the DS effects of APO are shared by other D_2 DA agonists and

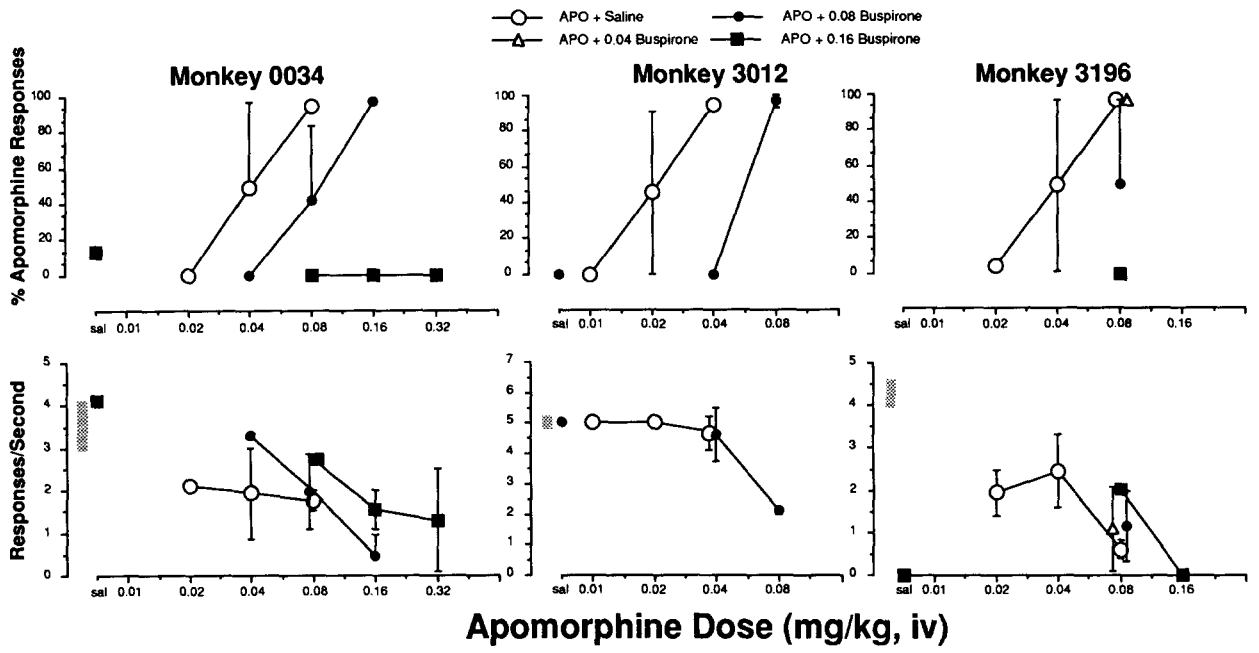

FIG. 1. Effects of combining buspirone with APO in monkeys trained to discriminate APO (0.04 or 0.08 mg/kg, IV) from saline. Upper panels: Percentage of responses during test sessions that occurred on the APO-appropriate lever as a function of APO dose. Lower panels: Response rate during test sessions as a function of APO dose. The stippled bars represent the average ± S.D. of response rates during the saline training sessions which were interspersed with the test sessions. Otherwise, each point is the average of two determinations and the vertical lines represent the range of those values. Where no vertical lines appear, the range is contained within the point. APO or saline was injected 5 min before the session and buspirone (0.04–0.16 mg/kg, IV) or saline was injected 10 min (monkeys 0034 and 3196) or 25 min (monkey 3012) before the APO injection.

are blocked by D_2 antagonists (2, 11, 16, 17), suggesting that the DS effects of APO are primarily mediated through interactions with D_2 receptors. Second, the dose-dependent decreases in APO-appropriate responding and the parallel shift to the right in the APO dose-response function caused by buspirone is characteristic of competitive antagonism at the receptor level. It is interesting to note that the complete blockade of APO's DS effects in two of the three monkeys occurred at doses of buspirone that neither reduced response rates nor increased the percentage of APO-appropriate responding when administered in combination with saline. Thus, the APO DS was blocked by doses of buspirone that had few overt behavioral effects of their own. Moreover, there was evidence for a mutual antagonism of the effects of these two drugs on response rate. Blockade of APO-induced response rate decreases by buspirone has been reported previously (14), as has the antagonism of APO's rate reducing effects by pimozide (17), a selective D_2 antagonist. Taken together, these results suggest that buspirone can function as a D_2 DA antagonist in behavioral tasks.

The current finding that buspirone acts as a D_2 DA antagonist in a drug discrimination experiment might be intepreted as being at odds with the Mansbach and Barrett (9) report concerning the discriminative stimulus effects of buspirone. In that study, buspirone was established as a discriminative stimulus in pigeons and drugs from several classes, including DA agonists and antagonists, were tested for their capacity to substitute for the buspirone stimulus. Neither apomorphine nor the DA antagonist haloperidol substituted for buspirone. On the other hand, two drugs with actions at 5-HT_{1A} sites, gepirone (a buspirone analog with less DA activity than buspirone) and 8-OH-DPAT substituted completely for buspirone. These results implicated serotonergic mechanisms in the discriminative stimulus properties of buspirone. However, the current study did not directly evaluate the discriminative stimulus properties of buspirone, other than finding that buspirone does not substitute for APO. This finding is not surprising given that Mansbach and Barrett (9) reported that APO does not substitute for buspirone. It is likely that although buspirone may function as a DA antagonist in blocking APO's discriminative stimulus effects, it is not this mechanism that is salient in buspirone's own discriminative stimulus effects.

The class of nonbenzodiazepine anxiolytic drugs typified by buspirone has generated great interest. This interest is primarily due to the relative dearth of side-effects of buspirone in comparison to benzodiazepines (12). The current study shows that the D_2 DA antagonist effects of buspirone can play an important role behaviorally. It would be of great interest to test buspirone's congeners gepirone and ipsapirone, which are reported to have less dopaminergic activity than buspirone (9,15) as possible antagonists of the APO DS. If these drugs failed to block APO's DS effects, it would strengthen the argument that the blockade produced by buspirone in the current study was due to interactions with D_2 DA receptors.

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