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Discriminative Stimulus Properties of Buspirone Compared to Central Nervous System Depressants in Rats[1]

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HENDRY, J. S., R. L. BALSTER AND J. A. ROSECRANS. *Discriminative stimulus properties of buspirone compared to central nervous system depressants in rats.* PHARMACOL BIOCHEM BEHAV **19**(1) 97–101, 1983.—Two groups of rats were trained to discriminate either IP buspirone from vehicle or IP oxazepam from vehicle using a two-lever FR-10 schedule of sweetened milk presentation. The discrimination in the buspirone group was difficult to establish due to potent response rate decreasing effects. Oxazepam was a very effective stimulus and the discrimination in the oxazepam group was readily established. Tests with oxazepam and pentobarbital in the buspirone group provided no evidence for generalization of the stimulus properties of buspirone to either drug. Tests with buspirone in the oxazepam group also provided no evidence of generalization to buspirone, although tests with pentobarbital indicated almost complete generalization. Finally buspirone and oxazepam were tested in a group of rats previously trained to discriminate pentobarbital from vehicle. The findings supported the data obtained in the buspirone and oxazepam groups, with no indication of generalization from pentobarbital to buspirone, but with complete generalization to oxazepam. These data suggest that buspirone does not share discriminative stimulus properties which are common to other CNS depressants.

Drug discrimination Buspirone Oxazepam Pentobarbital Antianxiety agents Rats

BUSPIRONE is a relatively new nonbenzodiazepine compound which has been found to be an effective antianxiety agent in humans [8]. In animal studies, buspirone has been found to have some behavioral effects similar to those of benzodiazepines. Buspirone increases rates of punished responding in rats and monkeys in a conflict procedure [7] and attenuates aggressive behavior in monkeys in a pole-prodding procedure [13]. In general, however, the pharmacological profile of buspirone differs from CNS -depressant antianxiety agents [1,11].

The purpose of the present study was to determine if buspirone possesses discriminative stimulus effects similar to the CNS-depressants oxazepam and pentobarbital. Generally, drugs from the same pharmacological class that have similar acute subjective effects in humans share discriminative stimulus properties in animals (see [3,12] for review). Most studies of the discriminative stimulus properties of CNS depressants show generalization among barbiturates, benzodiazepines and ethanol [4, 5, 6] which is consistent with the similar acute intoxication in humans produced by drugs from this class. A comparison of buspirone to representative drugs of this class will help establish whether or not anxiolytic effects, and behavioral pharmacological effects in animals which are characteristic of anxiolytics, can be separated from CNS depressant-like discriminative stimulus effects. It will also provide evidence concerning whether or not high doses of buspirone would be expected to produce a CNS-depressant intoxication in humans which would bear upon the abuse potential of this drug if it becomes available for clinical use.

METHOD

Subjects

Thirty experimentally naive Sprague-Dawley male rats were used, 25 of which were obtained from Harlan Laboratories (Indianapolis, IN) and 5 of which were obtained from Flow Laboratories (Dublin, VA). They weighed between 298 and 314 grams at the beginning of the experiment. The rats were housed individually and gradually food deprived to approximately 85% of their free-feeding weights. Eighteen additional male Sprague-Dawley rats (Flow Laboratories) that had been used in a previous experiment [14] in which they had been trained to discriminate 5 mg/kg pentobarbital from vehicle were also used.

Apparatus

The apparatus consisted of standard operant chambers

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(Model E10–10, Coulborun Instruments, Lehigh Valley, PA) housed within light- and sound-attenuating outer chambers. One wall of the chambers contained a dipper that delivered approximately 0.01 ml of sweetened milk. The recessed area in which the dipper was located was illuminated with a white light when the dipper was activated. Two levers were located on the wall containing the dipper, 5 cm to the left and to the right of the dipper. Illumination of the operant chamber was provided by a 28V houselight. Solid State and electromechanical equipment located adjacent to the chambers programmed session events and recorded data.

Procedure

Initial training. The naive rats were initially trained to press both the right and left levers for sweetened milk on a continuous reinforcement schedule until 100 responses had been made on each lever. The rats were subsequently divided into two groups with 15 subjects each in the oxazepam group and the buspirone group.

Discrimination training. Following initial lever-press training, the animals were introduced to a fixed-ratio (FR) schedule of reinforcement by gradually increasing the number of responses required to obtain reinforcement each day until all animals were responding on a FR-10 schedule. The session duration was 15 min and sessions were conducted five days a week. Forty-five minutes prior to each session the oxazepam group was injected IP with either 20 mg/kg oxazepam or vehicle on a random schedule with the exception that no more than two consecutive sessions with the drug or vehicle could occur. The buspirone group was injected IP 15 min prior to each session with either buspirone or vehicle, presented on a double alternation schedule. A 3.0 mg/kg dose of buspirone was initially administered to this group, however, this dose severely disrupted responding. After six drug training sessions, the dose was reduced to 1.5 mg/kg which also produced response disruption and little evidence for acquisition of the discrimination. The subjects were then divided into three subgroups of five rats each with one subgroup receiving 2.25 mg/kg buspirone, another subgroup receiving 0.75 mg/kg and the third subgroup continuing to receive 1.5 mg/kg buspirone. After 12 drug sessions for each group it became apparent that only 0.75 mg/kg could be administered without continued substantial response rate disruption. All rats were subsequently administered 0.75 mg/kg buspirone prior to the drug training sessions for the remainder of the experiment.

For half of the animals in each group, presses on the right lever produced reinforcement following drug administration and presses on the left lever produced reinforcement following vehicle administration. For the other half, presses on the left lever produced reinforcement after drug and presses on the right lever produced reinforcement after vehicle.

Generalization testing. Generalization testing was conducted under extinction conditions. Subjects were placed in the chamber until 10 responses were made on one of the levers and then were immediately removed. If the animals had not made 10 responses on a single lever within 5 min, the test session was terminated. At least one drug training session and one vehicle training session intervened between each test session. Data obtained during generalization testing were compared with baseline drug and vehicle sessions. These baseline data are the means of the drug and vehicle training sessions preceding all the doses tested of a given drug.

The buspirone group was tested initially with oxazepam at doses from 1.75 to 160 mg/kg. Subsequently, this group was tested with pentobarbital at doses from 2.5 to 10 mg/kg. The oxazepam group was initially tested for generalization to buspirone at doses from 0.09 to 0.75 mg/kg. This group was subsequently tested with pentobarbital at doses from 0.5 mg to 15 mg/kg. In addition, generalization testing was conducted with buspirone and oxazepam in a group of rats previously trained to discriminate 5 mg/kg of pentobarbital from vehicle under the same conditions [14]. This group was initially tested with buspirone at doses from 0.5 to 5 mg/kg and then with oxazepam at doses from 1.5 to 100 mg/kg. Test sessions were conducted identically to those in the buspirone and oxazepam groups. Within each group, the order of test drug doses was randomized. Buspirone was administered 15 min pre-session; oxazepam was administered 45 min pre-session; and pentobarbital was administered 10 min pre-session.

Drugs. Buspirone HCL (supplied by Mead Johnson) and Na pentobarbital (obtained from Abbott Laboratories) were prepared by dissolving the drugs in 0.9% saline. Oxazepam free base (supplied by Mead Johnson) was prepared by suspending the drug in distilled water with 2.5% carboxymethylcellulose. Drug doses of buspirone and pentobarbital refer to the salt while doses of oxazepam refer to the free base. All drugs and vehicle were injected IP in a volume of 1 ml/kg except 80 and 160 mg/kg of oxazepam which were injected in a volume of 2 ml/kg.

Data analyses. Percent of responding on the drug lever was obtained for each subject by dividing the number of responses occurring on the drug lever by the total number of responses occurring on both levers prior to obtaining the first reinforcement during training and prior to obtaining a total of 10 responses on a single lever during testing. This value was then multiplied by 100. The means and standard errors for all subjects were than calculated. Response rates during acquisition of the buspirone and oxazepam discriminations were expressed as the total number of responses per second on both levers during the 15-min sessions averaged across subjects.

RESULTS

Discrimination training

The data obtained during the first 24 sessions of discrimination training (12 with drug and 12 with vehicle) for five subjects in the buspirone group are presented in Fig. 1. These five subjects were previously administered 3.0 and then 1.5 mg/kg as the training dose of buspirone, before being switched to 0.75 mg/kg for which the data are presented here. The data in the left panel, which represent the percent of drug-lever responding, reveal that the buspirone discrimination was not readily acquired. The percent drug-lever responding was highly variable across sessions and between subjects during discrimination training after both buspirone and vehicle injections. However, there was a tendency for the percent of drug-lever responding to gradually increase after buspirone injections. During the last sessions presented in the figure, 82% of the responses occurred on the drug lever after buspirone and 0% after vehicle. The data in the right panel of Fig. 1 represents the response rates during these training sessions. These data reveal that even after 0.75 mg/kg buspirone, the rate of responding was generally lower compared to that after vehicle. Response rates

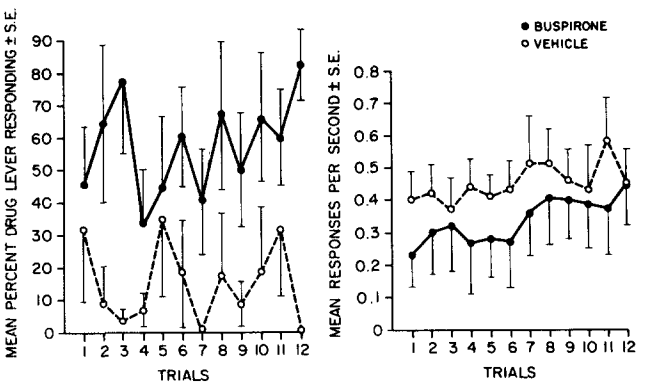

FIG. 1. Initial acquisition of a discrimination between 0.75 mg–kg of buspirone (closed circles) and vehicle (open circles) by five rats. The left panel represents the percent drug lever responding after 12 buspirone and after 12 vehicle administrations. The right panel represents the response rate during the same sessions. Vertical lines represent the S.E.M.

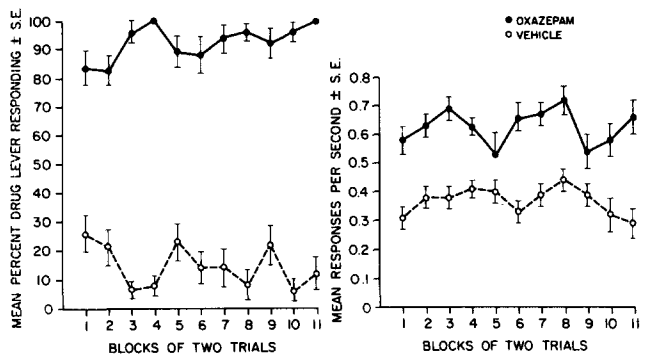

FIG. 2. Acquisition of a discrimination between 20 mg–kg of oxazepam (closed circles) and vehicle (open circles). The left panel represents the percent drug lever responding after oxazepam and vehicle administration for 11 blocks of two trials. The right panel represents the response rate during the same sessions. Vertical lines represent the S.E.M.

after both drug and vehicle increased during discrimination training, however, and converged at 0.45 response per second on the last session presented in the figure. After these data were obtained the remaining 10 subjects in the buspirone group were switched from their respective doses of buspirone to 0.75 mg/kg. After 14 additional training sessions each with drug and with vehicle, generalization testing was initiated even though in 10 of the subjects the mean percent of drug lever responding over the last 10 training sessions was 80% with buspirone and 28% with vehicle. The buspirone discrimination improved, however, through the period of generalization testing with oxazepam and pentobarbital.

The data obtained during discrimination training in the oxazepam group are presented in Fig. 2. The data in the left panel, representing the percent of drug-lever responding after all animals were responding on the FR-10 schedule (which required four drug sessions and three vehicle sessions), indicate that the oxazepam discrimination was readily acquired. The percent of drug-lever responding after oxazepam rapidly approached 100%, while the percent of drug-lever responding after vehicle was consistently low. In addition, there was very little between subject variability after either drug or vehicle. The data in the right panel of Fig. 2, representing responses per second during these same sessions, indicate that response rates were consistently higher after oxazepam than after vehicle during discrimination training.

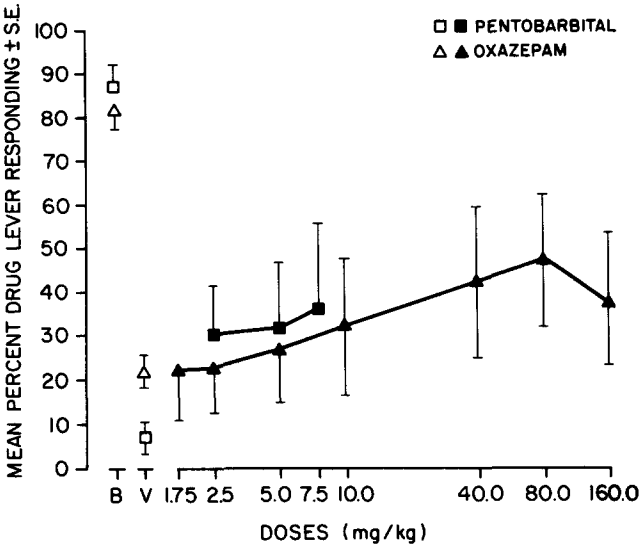

FIG. 3. Generalization tests with oxazepam (closed triangles) and pentobarbital (closed squares) in rats trained to discriminate 0.75 mg/kg of buspirone from saline. The open symbols represent the percent drug lever responding after buspirone (B) and after vehicle (V) administration obtained during training sessions that intervened between tests of oxazepam (open triangles) and tests of pentobarbital (open squares). Verticle lines represent the S.E.M.

Generalization testing

The data obtained for the buspirone group during generalization testing with oxazepam and pentobarbital are presented in Fig. 3. Unless noted otherwise, these data are based on 10 of the original 15 subjects; three rats having died prior to testing and the data of two rats were eliminated because of poor baseline performance. There were a number of deaths throughout this study most likely due to a chronic respiratory disease problem in the rodent colony and not to any direct effects of the drugs. As the data for buspirone and vehicle obtained during generalization testing with oxazepam and pentobarbital in Fig. 3 reveal, the buspirone-vehicle discrimination tended to improve during testing. During tests with oxazepam, drug lever responding was 82% after buspirone and 22% after vehicle. During the subsequent tests with pentobarbital, drug lever responding was 87% after buspirone and 7% after vehicle.

The generalization curve obtained with oxazepam in the buspirone group was relatively shallow (Fig. 3). Although responding on the drug lever tended to increase slightly as the dose of oxazepam increased, the maximum observed was after administration of 80 mg/kg oxazepam, with only 47% of

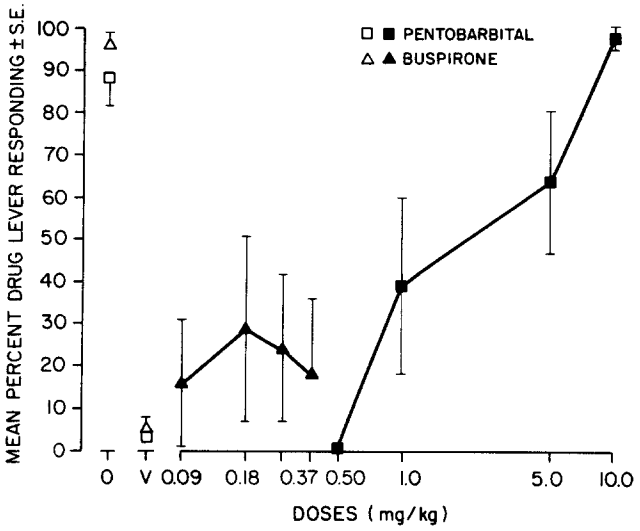

FIG. 4. Generalization tests with buspirone (closed triangles) and pentobarbital (closed squares) in rats trained to discriminate 20 mg/kg of oxazepam from vehicle. The open symbols represent the percent drug lever responding after oxazepam (O) and vehicle (V) administration obtained during training sessions that intervened between tests of buspirone (open triangles) and tests of pentobarbital (open squares). Vertical lines represent the S.E.M.

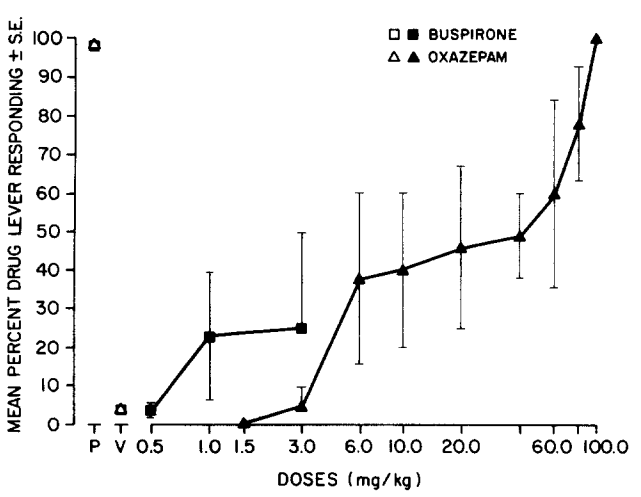

FIG. 5. Generalization tests with buspirone (closed squares) and oxazepam (closed triangles) in rats trained to discriminate 5.0 mg/kg of pentobarbital from vehicle. The open symbols represent the percent drug lever responding after pentobarbital (P) and vehicle (V) obtained during training sessions that intervened between tests of buspirone (open squares) and tests of oxazepam (open triangles). Vertical lines represent S.E.M.

the responses occurring on the drug lever. After 160 mg/kg oxazepam, responding on the drug lever decreased to 38%. One rat died prior to testing 80 and 160 mg/kg of oxazepam so these data, as well as the data obtained during generalization testing with pentobarbital, are based on an N of 9.

The generalization curve obtained with pentobarbital in the buspirone group was also extremely shallow with a maximum of 36% drug lever responding after 7.5 mg/kg of pentobarbital (Fig. 3). A dose of 10 mg/kg of pentobarbital was also tested in this group and all but two subjects failed to complete 10 responses on a single lever within 5 min.

The data obtained for the oxazepam group during generalization testing with buspirone and pentobarbital are presented in Fig. 4. These data are based on a N of 10 unless otherwise noted. Of the original 15 subjects, 3 subjects died during discrimination training and two subjects were not tested due to very low response rates during baseline sessions. There was very little responding on the drug lever after any of the four doses of buspirone presented in the figure. The maximum effect observed was after 0.18 mg/kg of buspirone with only 29% of the responses occurring on the drug lever. An additional test with 0.75 mg/kg of buspirone disrupted responding in all but two subjects. During generalization testing with buspirone, three subjects died. Therefore, the data were based on an N of 8 at 0.18 and 0.37 mg/kg of buspirone and an N of 7 at 0.09 and 0.27 mg/kg of the drug.

The generalization curve obtained with pentobarbital in the oxazepam group was very steep, reflecting a dose-related increase in responding on the drug lever, reaching an average of 98% after 10 mg/kg of the drug (Fig. 4). An additional test with 15 mg/kg of pentobarbital disrupted responding. During generalization testing with pentobarbital, three additional subjects died. Consequently, the N was 7 at 1.5 and 10 mg/kg pentobarbital and the N was 4 at 0.5 and 15 mg/kg of drug.

Figure 5 shows the data from the subjects that had been trained to discriminate 5 mg/kg pentobarbital from vehicle. Very good stimulus control had been established as evidenced by the near 100% drug lever responding after pentobarbital during these tests and the near 0% drug lever responding after vehicle. Generalization testing with buspirone and oxazepam in this group of rats is also presented in Fig. 5. Six rats were tested with each dose of buspirone and five rats were tested with each dose of oxazepam except 1.5 and 6 mg/kg oxazepam which were tested with four rats. The generalization curve obtained with buspirone was very shallow with a maximum of 25% drug lever responding after 3 mg/kg. An additional test with 5 mg/kg of buspirone disrupted responding for all but two subjects. Generalization testing with oxazepam in the pentobarbital group demonstrated a dose-dependent increase in responding on the drug lever, reaching a maximum of 100% after 100 mg/kg.

DISCUSSION

Clear differences in the discriminative stimulus properties of buspirone and the representative CNS depressants oxazepam and pentobarbital were demonstrated in this study. These data also confirm the similarities in the stimulus properties of benzodiazepines and barbiturates previously reported [4, 5, 6]. Generalization testing revealed that the rats trained to discriminate buspirone from vehicle did not generalize to either oxazepam or pentobarbital. In the group trained to discriminate oxazepam from vehicle, generalization to buspirone was not observed, although there was clear generalization to pentobarbital. In the group trained to discriminate pentobarbital from vehicle, generalization to buspirone was not observed, but generalization to oxazepam was virtually complete. Thus, although buspirone has been reported to have some behavioral effects in animals which are similar to those of the benzodiazepines such as increas-

ing the rate of punished responding in rats and monkeys [7] and reducing agression in monkeys [13] and to have clinical anxiolytic properties [8], the interoceptive effects of the drug are clearly not similar to those of the benzodiazepines. Buspirone is a relatively new compound, however, and the role of factors such as species and route of administration in determining pharmacological activity has not been thoroughly examined. It is, therefore important to note that these conclusions apply only to the IP route in rats. Extensions to other species and routes of administration await further research.

Buspirone was also found to differ from oxazepam with respect to its effectiveness as a discriminative stimulus. The data indicate that the oxazepam-vehicle discrimination was more readily acquired and reached a higher level of asymptotic accuracy than the buspirone-vehicle discrimination. It has also been reported that it is difficult to establish discriminations using a two-lever operant procedure with other psychotherapeutic drug classes such as the tricyclic antidepressants [10]. It should be pointed out, however, that a direct comparison of the acquisition data for the buspirone and oxazepam groups cannot be made since the procedures differed somewhat for the two groups during early discrimination training. The dose of buspirone administered during discrimination training to the buspirone group was decreased several times when it became apparent that tolerance was not developing to the response disruption produced by the drug. The highest dose of buspirone that could be administered without severly disrupting responding was ultimately determined to be 0.75 mg/kg. Even at this low dose, however, overall response rates were consistently lower than those obtained following vehicle injections. In addition, at this maximally tolerated dose, considerable between- and within-subject variability was still apparent during training. After 53 training sessions with various doses of buspirone, asymptotic stimulus control for the best 10 subjects surviving the study was only 80% drug lever responding after buspirone and 28% after vehicle. Even at the end of the study, during generalization testing with pentobarbital, there was only 87% drug lever responding after buspirone and 7% after vehicle. In the oxazepam group, however, the training dose was constant throughout discrimination training. Oxazepam, in contrast to buspirone, was already exercising stimulus control by the time the subjects were responding on the FR-10 schedule, as evidenced by the early acquisition data depicted in Fig. 2. Within only 22 additional sessions (11 each with oxazepam and vehicle), nearly perfect accuracy was achieved. Although stimulus control by oxazepam weakened somewhat during generalization testing, it never fell below an average of 88% drug lever responding after oxazepam and 4% after vehicle.

The lack of similarity of the discriminative stimulus properties of buspirone and the reference CNS-depressant compounds oxazepam and pentobarbital may have implications concerning the abuse potential of buspirone if it becomes more widely available. The evidence provided by this and other studies that benzodiazepines share discriminative stimulus properties with frequently abused CNS depressants such as barbiturates and ethanol [4, 5, 6] is consistent with reports that benzodiazepines and depressants produce similar subjective intoxication in humans [9]. This alcohol-like intoxication may be partly responsible for the recreational use of currently available benzodiazepines. The data from the present rodent study predict that buspirone would be unlikely to produce a CNS depressant-like intoxication and thus would not have abuse potential of that type. Buspirone has also been examined for reinforcing properties using IV self-administration procedures in rhesus monkeys [2]. Buspirone failed to maintain responding, providing further pre-clinical evidence for low abuse potential.

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