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Onset and Offset of the Diazepam Stimulus Complex

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HAUG, T. AND K. G. GÖTESTAM. *Onset and offset of the diazepam stimulus complex.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1171–1174, 1982.—Rats were trained to discriminate 3.0 mg/kg diazepam from saline in a two lever operant procedure. The time from injection to test session was 30 minutes. The diazepam discrimination consisted of initial responses on the lever paired with saline, but after training shifted to the lever paired with diazepam (onset). When tested with saline immediately after injection, animals responded consistently on the saline lever throughout the test. A shift from the drug lever to the saline lever at a later time point was also observed (offset). In addition, it was not possible to establish a peripheral diazepam drug stimulus complex. The results show that diazepam exerts discriminative control from 10 to 210 minutes after intraperitonal injections, confirming a central action of the diazepam drug stimulus complex. The method might be useful in experimentation on drug control of lever selection.

Drug discrimination Diazepam Drug stimulus complex Onset Offset Rats

SEVERAL years ago it was established that the benzodiazepine, diazepam, was an effective agent for discriminative control of behavior [2,4]. Since discriminative control of behavior can be exerted by either peripheral or central stimuli, it should be determined which kind of control a certain drug has in the operant task. It has been shown that when the drug does not have total discriminative control on behavior 5–10 minutes after administrations (IP), the effect is due to a central drug stimulus complex [9,11]. On the other hand, when rats made their initial responses on the drug lever immediately after IP injections [10] a peripheral locus for the drug stimulus complex was suggested. The bulk of available data on diazepam suggests that the drug stimulus complex is centrally acting. This question is important to assess, especially in experiments where agonist-antagonist effects are studied. The purpose of the present study was to examine the onset and offset of the diazepam drug stimulus complex more closely and to see if it is possible to establish a peripheral drug stimulus complex with diazepam. In addition, this particular study was an evaluation of a new experimental paradigm based on the repeated test procedure.

METHOD

Animals

Sixteen male Wistar rats (Møllegaard-Hansen Avls-laboratorium, Skensved, Denmark), reduced to 80% of free feeding weight were housed in living cages, in a windowless room maintained at 22±1°C. The room was artificially illuminated from 7 p.m. to 7 a.m. The training and testing started at 9 a.m.

Experimental Cages

The rats were placed in individual experimental cages, 30×30×40 cm, during the entire experimental session. Two levers and a food pellet dispenser were positioned on one wall of the cage. Pellets were delivered as a consequence of lever pressing according to the current reinforcement schedule. Lever impulses and pellet delivery were controlled and registered on electronic equipment (Lehigh Valley Electronics, Fogelsville, PA). The experimental cages, with dispensers were placed in a sound proof case with constant ventilation and white noise (Campden Instruments Ltd, London), throughout the training and experimental sessions.

Drug

Diazepam suspended in dimethylacetamide and cremophor EL was used. Physiological saline was used for control conditions. Dimethylacetamide and cremophor EL were separately tested for the possibility of cueing properties in this experiment.

General Procedure

All rats were shaped to bar press for food reinforcement (food pellets, 45 mg, Astra, Södertälje, Sweden) and shifted to a fixed ratio schedule requiring ten lever presses for each reinforcement (FR 10).

Experiment 1

Training procedure. The procedure used was based on the two lever fixed ratio 10 (FR 10) drug discrimination protocol described by Colpaert *et al.* [4,5] and Haug and

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Götestam [7,8]. Thirty minutes prior to the 14 minutes session, diazepam (3.0 mg/kg) or an isovolumetric dose of saline (1 ml/kg) was administered IP. Depending on whether the rat received drug or saline, reinforcement was programmed exclusively on either left or right lever. The lever assignments were "drug lever" (DL) left and "saline lever" (SL) right for 7 animals, and the reverse for the remaining 5 animals. Sessions were conducted Monday through Friday under the alternating drug sequence used by Colpaert *et al.* [4,5] and Haug and Götestam [7,8]; DSSDD (D=drug, S=saline) and SDDSS. The animals were placed in the experimental cage in 10 different orders (i.e., the same order occurring every 10th training and testing session). This was carried out to control for a possible olfactory cue.

For each session, the number of incorrect responses emitted prior to receiving the first food pellet (FFP) was the measure of discrimination accuracy.

The training criterion was reached when the animal's FFP did not exceed 12 on at least 10 consecutive training sessions. Thereafter the animal participated in the experiment.

Experimental procedure. The rats were placed in the experimental cage at different time intervals and the selected lever and the FFP were recorded. After 10 responses on one lever, the rats were immediately taken out of the experimental cage without obtaining reward. Each rat was tested up to eight time intervals per day (i.e., after a single injection) and always with a minimum time spacing of five minutes.

For both onset and offset tests the rats received no pretraining in the experimental cage on that particular test day.

Experiment 2

This group of rats (n=4) was trained differently from the group in Experiment 1. The rats were placed in the experimental cage immediately after injections (IP or SC) and the sessions were terminated 5 minutes later. The daily alternating schedule was DSDSD (D=drug, S=saline). This experiment was terminated after 138 training plus experimental sessions. During the first 100 sessions the injections were IP. Thereafter the injection procedure was shifted to SC on the rat's back and the training continued for 38 sessions. The data were taken from the last 20 sessions of the IP injections and another set of data were taken from the 10 last sessions of the SC sequence.

RESULTS

Experiment 1

The percentage of responses on the diazepam lever (percent cue detection) for each time interval is shown in Fig. 1.

When injected with diazepam the rats immediately selected the saline lever (SL) and there was no significant drug lever (DL) selection until 5 minutes after diazepam administration. When injected with saline, the rats responded appropriately for saline administrations throughout the entire test (i.e., selection of saline lever, SL). The FFP value did not vary significantly from the training procedure, that is the rats were either selecting the drug lever (DL) or the saline lever (SL) consistently without any lever shifts within the same test interval.

The offset was consistently observed between 100 and 330 minutes after administration, with the least square method crossing the abscissa at 329 minutes. When pretreated with saline the rats always selected the saline lever (Fig. 1).

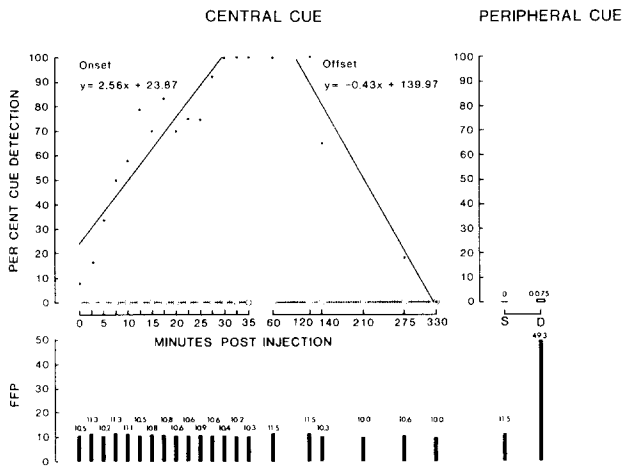

FIG. 1. Time course is illustrated on the abscissa and FFP and percent cue detection (drug lever selection) are illustrated on the ordinate. FFP (first food pellet) values are computed for each time interval selected. Percent drug lever selection is expressed as the percentage of animals found to select the drug lever, at onset and offset of a centrally acting cue. The least square method lines are calculated (excluding the three surplus 100% points in the middle) for the onset and offset of this centrally acting cue (left). Percent drug lever selection immediately after a peripherally acting saline (S) or diazepam (D) (right). These data were calculated on the basis of sessions 81–100.

Both vehicles consistently produced selection of the SL lever.

Experiment 2

Prolonged training did not establish diazepam, nor the vehicles (cremophor EL or dimethylacetamide) as peripheral cues for lever selection. During the training procedure, the rats, without exception, increased the SL selection independent of drug or saline administrations, and during the test sessions (session 81 to 100) there was always a consistent SL selection despite the systematic variation in administration of drug and saline. As the rats selected SL consistently, this resulted in low FFP values for saline (when SL was reinforced) and high FFP values for diazepam administrations (when SL was not reinforced) (Fig. 1). The data from session 81 to 100 did not differ significantly from the data from the sessions 129 to 138.

DISCUSSION

The results show that the onset and offset of the diazepam drug stimulus complex are neither instantaneous nor rapid. The drug is apparently "detectable" from 10 to 15 minutes after administration, while 100% cue detection was not present until about 30 minutes after administration (y=100 for x=29.0). The variation in detection may be related to characteristics in the training procedure, absorbtion, transport, and CNS-activity as well as other factors known to occur in the operant paradigm.

Some rats were relatively late in changing levers (i.e., from SL to DL), a fact which may be dependent on training procedure, absorbtion, transport, and CNS-activity as well as attentional factors: some rats selecting the DL during the first 17.5 minutes (i.e, up to three times consecutively) happened to select the SL during the following 12.5 minutes. As the injection to session interval during training was 30 min-

utes this may account for the fact that 100% cue detection was not observed before this particular time point. This phenomenon may also be controlled by other factors than the diazepam drug stimulus complex itself. Firstly, failures in attention to the drug stimulus complex can account for missed DL selections. Attentional failures are observed in animals trained to a 100% correct criterion, and the failures are not results of sudden losses of conditioning effects [6]. In trained animals it was shown that this attentional failure occurred intermittently rather than continuously, and operate phasically on post-training performance [6]. Due to limited number of time intervals (i.e. test intervals), this phasic occurrence is difficult to assess in our experiment. Secondly, one factor governing the lever shift may be the absence of reinforcement during repeated sessions in the experimental chamber within one experimental session. Response probing is known to occur on both levers in the absence of reinforcement, and the percentage of drug appropriate responses does not directly reflect the discriminative stimulus control excerted by the drug [3]. Due to characteristics of the method used in this experiment, however, this factor can not be used as a general explanation. The occurrence of the lever shifts in the drug test sessions and the lack of occurrence of lever shifts in the saline test sessions point to attentional failures as an explanation for the missed DL selections. On the other hand, each test at a single time interval (requiring only 10 lever presses) should be too short to induce response probing, and up to 8 tests (with 10 lever presses in each) should not be enough non-rewarded lever presses to induce response probing.

In the offset test the time intervals between lever selection tests were longer (15–30 minutes) and the frequency of lever shifts observed was much lower. Although this is difficult to explain, one suggestion is that several tests with short time spacing more easily absorbed the intermittent and phasic occurrence of the attentional failures. In this sense the offset graph more correctly reflects the stimulus control excerted by the drug diazepam.

The finding that dimethylacetamide and cremophor EL did not control lever selection similar to diazepam is well in line with earlier data where diazepam was the drug stimulus complex and dimethylacetamide and cremophor EL the vehicle [7,8].

Within 138 training sessions it was impossible to establish a peripheral cue with the drug diazepam and the vehicles. To eliminate the possibility of a systematic central component in the training, the sessions were always stopped after five minutes (i.e. five minutes after injection). The consistent SL selection independent of administration and route of administration was evident after 30 to 60 training sessions with diazepam and saline alternating (mean for the group: 45 sessions). This observation is difficult to explain. It could not be explained by a position preference, as the lever assignments were reversed for two of the rats in this experiment and there were no differences between rats with opposite assignments with relation to SL preferences. One possible explanation may be the variations in the diazepam drug effect both peripherally and centrally during the experimental period: In addition to variations from session to session with relation to stimulus intensity and quality, the variation within the experimental period (5 minutes with the possibility of an increasing central action) may have varied considerably, as a contrast to the saline post-treatment period.

This group of rats was not transferred to weekly alternating sequences since no discrimination was observed during the daily alternating sequence (required criterion).

The difference in training time between the two groups (45 percent less for the group in Experiment 2) should be of minor importance since the number of training sessions seems to be more important than the length of the sessions (within some limits) in this kind of paradigm [1].

Our conclusion is that the diazepam drug stimulus complex is centrally acting. After intraperitonal administrations some time is required both for the onset and offset of the drug stimulus complex. Lever selection after administrations of diazepam can not be controlled by peripheral effects produced either by the drug itself and/or the vehicles used in this experiment.

The method used in this experiment might be useful for further experimentation on central versus peripheral effects of different drugs and dose concentrations.

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