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"Conflict" Situation Based on Intracranial Self-Stimulation Behavior and the Effect of Benzodiazepines

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GOMITA, Y. AND S. UEKI. *"Conflict" situation based on intracranial self-stimulation behavior and the effect of benzodiazepines.* PHARMAC. BIOCHEM. BEHAV. **14**(2) 219–222, 1981.—Based on lateral hypothalamic self-stimulation behavior of the rat in a Skinner box, a "conflict" situation was established by combining foot shock punishment with brain stimulation. Diazepam (10–20 mg/kg, PO) caused a marked increase in the lever pressing response in the punished period without affecting the unpunished response. Bromazepam (10–20 mg/kg PO) also caused an increase in the lever pressing response in the punished period and a decrease of the punished response. These results indicate that a "conflict" situation based on self-stimulation behavior is useful for the evaluation of antianxiety action.

"Conflict" situation   Hypothalamic self-stimulation   Benzodiazepines

ESTES AND SKINNER [2] reported that behavioral suppression was obtained by the presentation of a stimulus preceding an unavoidable foot shock in food-maintained behavior. Geller and Seifter [4] also showed that a "conflict" situation was established by combining the punishment of response-contingent foot shock with the lever pressing behavior based on hunger drive. The "punishment" or "conflict" situation has been utilized in the measurement of the antianxiety effect of drugs. Benzodiazepine derivatives and other minor tranquilizers greatly reduce the suppressive effect of punishment on operant behavior contingent on food reward [1, 6, 8]. These methods, however, have been undertaken after long-term hunger conditions which create certain problems in assessing the activity of antianxiety drugs. That is, the maintenance of a healthy condition in the animals is somewhat difficult and gastrointestinal drug absorption may be altered in such a deprived state.

The purpose of the present study was to create a "conflict" situation based on intracranial self-stimulation, where the rewarding behavior does not entail deprivation, and to investigate the possible effects of antianxiety drugs on this situation.

METHOD

Animals

Twenty-four male Wistar strain rats weighing between 200 and 400 g at the time of brain surgery were used in this experiment. All animals were housed 2 or 3 each in a plastic cage with dimensions of 34×28×18 cm and were given food and water ad lib. The animals were maintained at room temperature (23–24°C) with a relative humidity of 60%.

Surgery and Histology

Surgery was performed on the animals under pentobarbital anesthesia (40 mg/kg, IP). After rats were placed on a stereotaxic instrument, bipolar stainless steel electrodes (250 μ in diameter, insulated except at the tip) were chronically implanted into the lateral hypothalamus (A: 5.8, L: 1.8, H: −2.5) according to the stereotaxic coordinates of König and Klippel's brain atlas [7]. All animals were given penicillin of 150,000 units subcutaneously after the surgery. One week was allowed for recovery before training for self-stimulation behavior.

At the end of the experiment all animals were given an overdose of sodium pentobarbital. The head was perfused through the heart with 0.9% saline and 10% Formalin. The brain was immersed in the Formalin-saline solution for at least one week. Each brain was frozen, and 40 μ slices were made and mounted on glass slides followed by staining with cresyl-violet. The location of the implanted electrodes in the brain was determined for each rat.

Apparatus

The experiments were carried out in a Skinner box which was constructed of transparent Plexiglas with inside dimensions of 30 cm wide, 27 cm high and 25 cm deep. The floor consisted of a steel grid. A swivel was mounted in the ceiling of the chamber holding the electrode lead and allowing the animal free movement. The lever was placed 4.5 cm above the grid floor and protruded 2.5 cm into the box. A small lamp was provided near the lever for a cue light. A lever press activated a counter and resulted in a brain stimu-

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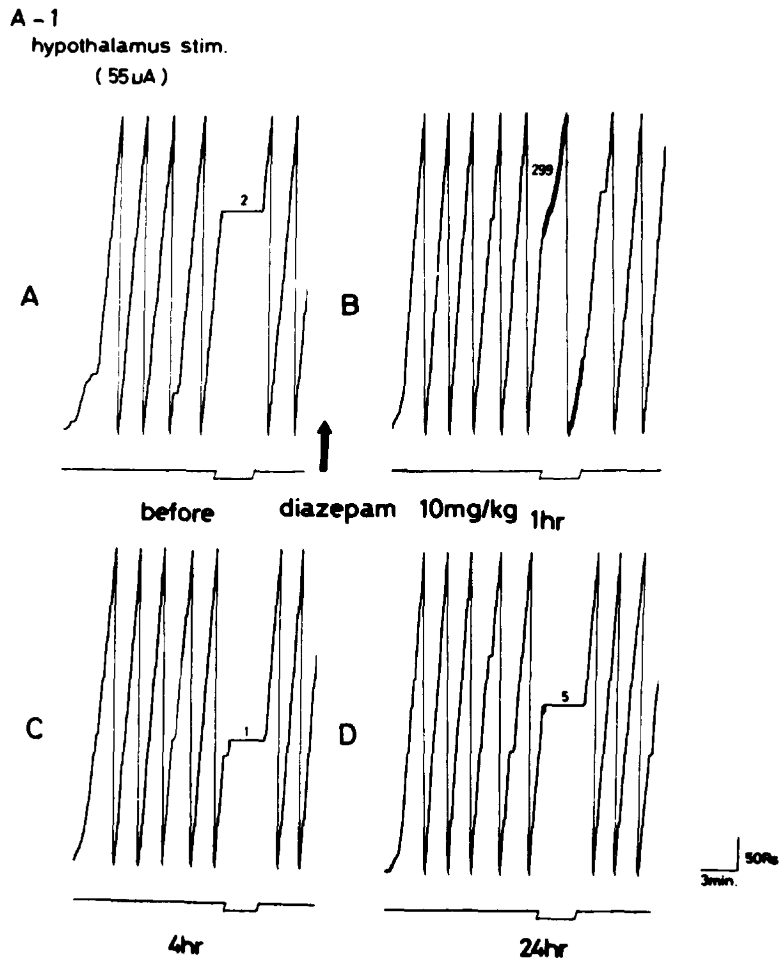

FIG. 1. Effect of diazepam on a "conflict" situation in lateral hypothalamic self-stimulation behavior. Cumulative recording of the lever pressing before (A) and 1 hour (B), 4 hours (C), and 24 hours (D) after administration of diazepam 10 mg/kg PO. The punished period (3 min) is indicated on the lower line in each panel. The number of lever presses in the punished period is included in the figure.

lation. The schedule of reinforcement was programmed automatically and response records were obtained with an automatic counter and a Gerbrands cumulative recorder.

Self-stimulation and Procedure

Following recovery from the implantation surgery, each animal was placed in a Skinner box and the stimulating cable was connected to the electrode plug mounted in the head. Each hypothalamic stimulation reward consisted of a 60 Hz sinusoidal AC lasting for 0.2 sec individually adjusted for each rat. The stimulation current was then gradually increased until the animal began to show self-stimulation behavior, accompanied with sniffing and a heightened activity level. A number of daily training sessions was given to each animal. Training was performed on a continuous reinforcement (CRF) schedule.

After the lever pressing for self-stimulation under the CRF schedule reached its maximum response rate, the reinforcement contingency was gradually altered until all rats performed stable responding for self-stimulation under the fixed ratio (FR) 5 schedule without decreasing the maximum lever press rate. Thereafter, foot shock punishment (0.1–1.5 mA, 0.2 sec in duration) was combined with the self-stimulation. The self-stimulation reward-punishment (conflict) procedure was the same as the method of Geller and Seifter [4], except that they used food reward. Each rat was tested for 30 min a day. The test consisted of 2 sessions of each 15-min period, in which a 12-min unpunished period was followed by a 3-min punished period. The rat responded to a brain stimulation reward under the FR 5 schedule in the unpunished period. The punished period was accompanied by a tone of 1,850 Hz and a cue light near the lever, and every response in this period was rewarded with brain stimulation and concurrently punished with a brief electric foot shock. The intensity of the foot shock was gradually increased and adjusted in each animal until the response rate in the punished period was suppressed to less than 10 responses, while the unpunished response under the FR 5 schedule remained at relatively high levels.

The drugs used were diazepam and bromazepam. Both of the drugs were suspended in a 0.5% carboxymethylcellulose (CMC) solution and administered orally. Control administration of 0.5% CMC was also given. Two pre-drug sessions were observed for 30 min before drug administration.

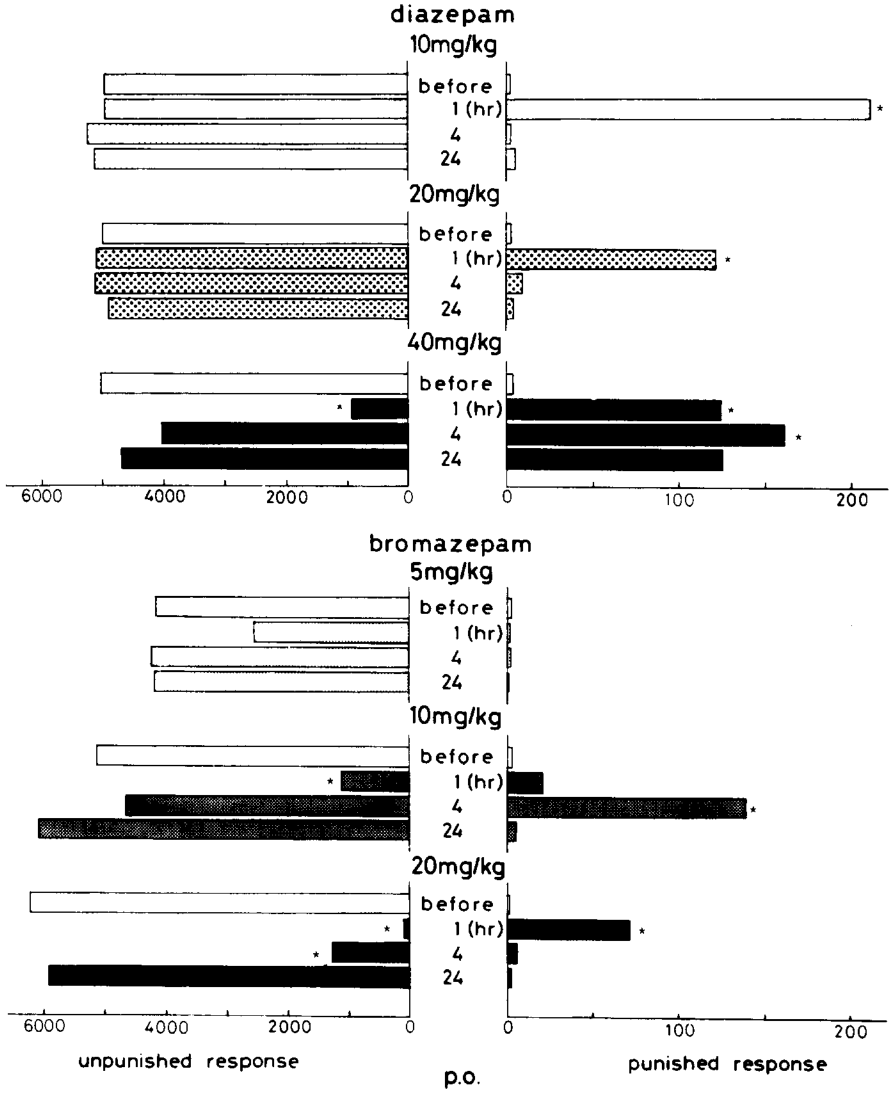

FIG. 2. Effects of diazepam and bromazepam on the punished (3-min period) and unpunished responses (12-min period). Asterisk shows a significant difference from the value of the saline group ($p<0.05$).

Twenty minutes after the test drug or CMC was administered, the post-drug sessions were started, and the unpunished and punished responses were recorded. Six rats were used for each dose of the drugs, and at least 2 weeks elapsed between each drug administration.

Results were evaluated statistically by means of the Mann-Whitney U test [9].

RESULTS

In most of the rats, self-stimulation of the lateral hypothalamus with a high current of approximately 50 μA caused a high response rate of lever pressing. After several days of training, a stable high response rate was obtained under the FR 5 schedule. Thereafter foot shock punishment was combined with self-stimulation under a CRF schedule, and the stimulus current for foot shock in the punished period was gradually increased. Then in the punished period, approach-avoidance behavior was observed and the lever pressing was extremely reduced; i.e., a "conflict" situation was established.

Thereafter the effects of benzodiazepines were investigated in 16 rats showing the most stable performance in the conflict situation. The effect of diazepam 10 mg/kg, PO in the representative rat is shown in the cumulative records of Fig. 1. Diazepam caused a marked increase of the lever pressing in the punished period without affecting the unpunished response. The effect appeared within 20 min, reached its maximum at about 60 min and lasted for 2 to 3 hr after oral administration. Figure 2 shows the mean lever pressing responses in the punished and unpunished periods after oral administration of diazepam and bromazepam at various doses in each group of 6 rats. Diazepam at a dose of 5 mg/kg, PO showed no significant effect, but the drug at doses of 10 and 20 mg/kg, PO caused a marked increase of the lever pressing in the punished period without affecting unpunished responding. At a dose of 40 mg/kg, PO, the response in the punished period markedly increased, although the unpunished response decreased, and the effect lasted much longer.

Bromazepam at doses of 10 and 20 mg/kg, PO caused an increase of the lever pressing response in the punished period and a decrease of the unpunished response. Both the drugs showed a so-called anticonflict action in a "conflict" situation based on intracranial self-stimulation behavior.

DISCUSSION

Gellar and Seifter [4] reported that a "conflict" behavior was established by combining foot shock punishment with food reward. Geller *et al.* [3] also indicated that tranquilizers attenuated this "conflict" behavior based on hunger drive. In a conflict experiment, it has been recognized that a drug-induced increase in the rate of punished response is taken as an index of antianxiety activity, and the effect on the conflict behavior on the rat appears to be correlated with the clinical potency of the drug [10,11].

Our present results have demonstrated that a "conflict" situation based on self-stimulation behavior is inducable.

The schedule in the punished period was the same as in the method of Geller and Seifter [4]. In this "conflict" situation based on self-stimulation behavior, both diazepam and bromazepam at doses over 10 mg/kg, PO caused a marked increase of the lever pressing response in the punished period. The results obtained here in this experiment were similar to those reported for a conditioned suppression maintained by punishment based on hunger drive [3,4]. The effective dose of diazepam for attenuating the suppression of behavior in the punished period with this self-stimulation reward was slightly lower in comparison with the results of Gomita *et al.* [5] with food reward.

The present results indicate that a "conflict" situation based on intracranial self-stimulation behavior may be as useful for the evaluation of antianxiety drugs as that based on hunger drive. In this conflict experiment, it is not necessary to maintain the animal in a deprivation state and thus it is easy to maintain a healthy condition in the animals throughout the experimental period. This is an advantage of this method over the conflict experiment based on hunger drive.

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