EFFECTS OF BENZODIAZEPINES ON LOW RATE RESPONDING FOR LOW CURRENT BRAIN STIMULATION REWARDS

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Antianxiety drugs have often been evaluated by noting their effects on tasks in which the animal's rate of responding is held by the differential reinforcement of low rate (DRL) schedule (1) or the "conflict" procedure (2). It is well known that benzodiazepine derivatives and another antianxiety drugs exert a facilitating effect on the low rate responding of operant behavior contingent on food reward (3-6). These effects must be attributed to some sort of disinhibitory action of antianxiety drugs (7). These tests, however, have been taken after long-term hunger or in a thirsty condition which may create certain problems on the maintenance of a healthy condition and the gastrointestinal absorption of drug in the animals (8). On the other hand, we reported that the "conflict" situation (9) and DRL responding (10) were maintained by intracranial self-stimulation (ICSS) in satiated rats, and the responses in the punished period of "conflict" situation and DRL respondings were increased by benzodiazepines. On this ICSS, it is advantageous that the reward's efficacy is easily and flexibly changeable by varying current intensity.

The purpose of the present study was to investigate the effects of antianxiety drugs on the low rate response produced by attenuation of the intensity of stimulating current for ICSS.

Twenty-eight male Wistar strain rats weighing between 250-300 g at the time of brain surgery were used in this experiment. All animals were housed with 2 or 3 in each home cage of 34 x 28 x 18 cm with a plastic wall and were given food and water ad libitum. The animals were maintained at a room temperature of 21-25°C and relative humidity of 60%. The animals were anesthetized with 40 mg/kg pentobarbital-Na, i.p., and were stereotaxically and chronically implanted with bipolar stainless steel electrodes (tip diameter, 0.25 mm; polar distance, 0.5 mm; uninsulated length, 0.2 mm) in the lateral posterior hypothalamus (A: 5.4, L: 1.8, H: -3.0) according to de Groot's coordinates (11). All animals were given 150,000 units of penicillin subcutaneously after the surgery. At least 10 days were allowed for recovery before commencing the training for ICSS.

The experiments were carried out using a Skinner box (30 x 27 x 25 cm) which was constructed of transparent Plexiglas. The floor consisted of a stainless steel grid, 5 mm diameter space 1.5 cm apart to allow urine and feces to fall to the tray. A swivel was mounted in the ceiling of the chamber holding the electrode lead, allowing the animal free movement. The metal lever in the Skinner box was placed 4.5 cm above the grid floor and protruded 2.5 cm into the box. A lever press activated a counter and resulted in a brain stimulation. The schedule of reinforcement was programmed automatically, and response records were obtained on an automatic counter and a Gerbrand's cumul-
Following recovery from the implantation surgery, each animal was placed in the Skinner box, and a stimulating cable was connected to the electrode plug mounted on the animal's head. Each animal was trained to press a lever for the brain stimulation reward which was obtainable on a continuous reinforcement (crf) schedule. Each lateral hypothalamic stimulation reward consisted of 60 Hz sinusoidal current lasting for 0.2 sec individually adjusted for each rat. The stimulation current was gradually increased until the animal began to respond at a heightened activity level. Five to 10 daily training sessions (15 min) were given to each animal. The stimulation current intensity was determined to be the approximate level that would support maximum high response rate (high rate) of ICSS without producing gross motor disturbances or convulsion. After the respondings for ICSS reached a high rate under the crf-schedule and were stable through three successive days, the current intensities were gradually reduced until the responses were decreased to under half of the high rate responses. The drugs were administered when the responses were stabilized with 10% of variation through two successive days.

The drugs used were chlordiazepoxide hydrochloride (Hoechst) and diazepam (Kodama). Both of the drugs were suspended in 0.5% carboxymethylcellulose-Na (CMC) solution and administered orally. Control administration of 0.5% CMC was also given. In each test, we recorded the animal's response for 15 min before drug administration and then again for four 15 min periods beginning 1, 2, 4 and 24 hr after drug administration. At least 2 weeks elapsed between each drug administration.

At the end of the experiment, all animals were given an overdose of pentobarbital-Na. The head was intracardially perfused with 0.9% saline and 10% formalin. The brain was immersed in formalin-saline solution for at least 10 days. Each brain was put on the platform of freezing microtome and was cut in 40 μm sections, followed by staining with cresylviolet. The localization of implanted electrode tips in the lateral hypothalamus was verified by inspection of the stained sections.

The statistical analysis of the experimental results was made by means of the two-sample t-test (12).

In most of the rats, ICSS with a high current of 25–100 μA caused a high response rate of lever pressing. Several days after commencing the training, the stimulus current was gradually attenuated. Then the response rates were current-dependently reduced.

![Fig. 1. Effect of chlordiazepoxide (10 mg/kg, p.o.) on the low rate response of hypothalamic self-stimulation behavior in rat. A: pre-drug control response (with 17 μA); B, C and D: 1, 2 and 24 hr, respectively, after administration of 10 mg/kg chlordiazepoxide, p.o.](image-url)
The intensity of stimulating current that maintained 1/4 to 1/2 of the high response rate was individually determined for each animal. When the intensity of the stimulating current was attenuated to 37.4±4.60% (mean±S.E.M.) of that in high rate responding, the response rates were decreased to 42.8±4.55% (mean±S.E.M.) of the high response rate. The stable respondings were obtained at the 14th to 30th session with low stimulating current.

Thereafter, the effects of benzodiazepines, chlordiazepoxide and diazepam, were investigated in 20 rats showing the most stable performance in this low rate responding. The effect of chlordiazepoxide at a dose of

![Diagram of Fig. 2](image)

**Fig. 2.** Effects of chlordiazepoxide (CDP) or diazepam (DZP) on low rate responding for low current brain stimulation reward in rats. ( ) number of animals, pre: pre-drug administration, †: P<0.10, *: P<0.05 and **: P<0.01.
10 mg/kg in representative rat is shown in the cumulative records of Fig. 1. The increases of response rate were noted at 1 and 2 hr after the administration of chlordiazepoxide. Figure 2 shows the effects of chlordiazepoxide (CDP) and diazepam (DZP) on lever press responses. Significant differences from the CMC treated group were observed at 1 and 2 hr after administration of chlordiazepoxide at a dose of 10 mg/kg (df=11, t=2.675 and 2.604, P<0.05, respectively). Chlordiazepoxide at a dose of 20 mg/kg conversely decreased response rates. The significant difference from the CMC treated group was observed at 1 hr after drug administration (df=1, t=5.372, P<0.01); at this high dose, marked muscular relaxation was observed. On the other hand, at doses of 1 and 2 mg/kg of diazepam, the response rates were increased. At 1 hr after administration of 1 mg/kg of diazepam, the significant difference from the CMC treated group was observed (df=18, t=3.987, P<0.01); and at 1 hr after administration of diazepam 2 mg/kg, the response rate was slightly increased, but this was not significant (df=13, t=1.954, P<0.10). At a dose of 10 mg/kg of diazepam, there was no difference of response rate from the CMC treated group, and a slight muscular relaxation was observed.

Our previous reports have demonstrated that a DRL-responding and a "conflict" situation, which is useful for assessing antianxiety drugs, is able to induce ICSS (9, 10). In this experiment, we also noted the low rate response induced by the attenuation of intensity of brain stimulation current for ICSS and the facilitating effects of chlordiazepoxide and diazepam at low doses on this situation. The effective doses of benzodiazepines on this brain stimulation reward were markedly lower in comparison with the results of DRL-respondings or the "conflict" situation for conventional reinforcement such as food and milk (13, 14). In addition, we noted the decreasing effect of chlorpromazine and the facilitating effect of methamphetamine on this low rate response in another experiment (15). Therefore, the facilitating effect may be characterized by the drugs with disinhibitory action such as antianxiety drugs or central stimulant action such as sympathomimetic amines. These drugs may influence the intensity of brain stimulation. At high doses of these drugs, however, a depressive effect was observed on this low rate response. The depressant action may be depend upon their effects such as sedation or muscular relaxation.

References


