Water Intake and Time Course of Drinking After Single or Repeated Chlordiazepoxide Injections

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Abstract. Chlordiazepoxide (5.0, 10.0 or 15.0 mg/kg) was given to rats either acutely or for 9 consecutive days. Its effects were examined in a 15-min drinking test in which latency to drink, volume of water consumption and the time-course of drinking were measured. Chlordiazepoxide (10.0 mg/kg) produced the strongest stimulant effect on drinking and enhanced the frequency of occurrence of drinking both at the beginning and at the end of the test period. Chlordiazepoxide (15.0 mg/kg) delayed the onset of drinking and its peak effect was observed later than for other injection conditions. Single and repeated administration of chlordiazepoxide had the same effects on the measures taken in the test. Initial sedation to the drug treatment and subsequent tolerance to this effect were not, therefore, factors influencing the drug effects observed in this experiment. Possible mechanisms underlying the stimulation of drinking by chlordiazepoxide are considered.

Key words: Chlordiazepoxide - Chronic administration - Time-course of drinking - Water intake.

Many studies show that benzodiazepines facilitate feeding responses in a variety of mammalian species (Cooper, 1978; Dantzer, 1977). Less attention, however, has been paid to the effects of benzodiazepine treatment upon drinking responses. In rats which have been thoroughly adapted to a 23-h water deprivation schedule, chlordiazepoxide (CDP) (3.75 - 20.0 mg/kg) increases the volume of water intake (Maickel and Maloney, 1974; Miczek and Lau, 1975), with a peak effect appearing at 10.0 mg/kg (Miczek and Lau, 1975). Thus, CDP can stimulate water intake under conditions which are thoroughly familiar to the animals. Soubrié et al. (1976) demonstrated that benzodiazepines (CDP, diazepam, lorazepam) increase the time devoted to drinking in a 10-min test period in the rat and the mouse, irrespective of whether the animals are naïve to the test situation or experienced. The first aim of the present experiment was to examine the effects of CDP on drinking in rats which were relatively unfamiliar with the test situation, and to describe their behaviour in terms of both the amount of water consumption and the time-course of drinking within the 15-min test period.

There are few studies of the effects of chronic treatment with benzodiazepines on feeding and drinking responses. Repeated administration of diazepam or CDP increases daily food intake in the dog, leading to an increase in body weight (Heilman et al., 1974; Randall et al., 1960). Wise and Dawson (1974) reported that food intake increased in a daily 45-min feeding test, particularly over the first week of treatment with diazepam. They interpreted their results in terms of the development of tolerance to diazepam's sedative action. Cooper and Francis (1979) measured the effect of CDP on the rate of food intake in a short feeding test and found that after a single injection, it depressed the rate of feeding in a dose-related manner. However, after nine daily injections of CDP the depression in feeding rate was significantly alleviated. Cooper and Francis (1979) suggest that the depressed rate of feeding reflects a sedative action of chlordiazepoxide, and that tolerance develops to it within the course of nine daily injections.

There are less data on the effects of repeated benzodiazepine treatment affecting drinking responses. Wise and Dawson (1974) failed to find stimulation of water intake with chronic diazepam treatment (2.5 mg/kg given on alternate days) in non-deprived rats. Falk and Burnidge (1970) observed no change in water...
intake with daily administration of CDP (15.0 mg/kg) in rats adapted to a 23-h water-deprivation schedule. In both studies, dose-effect data were not obtained, and comparisons were not made between the effects of single or repeated drug administrations.

The design of the present experiment, therefore, included comparisons between the effects of CDP on drinking responses after a single injection and after nine daily injections. The study was designed to match the drug administration procedures used in a separate study on feeding responses in the rat (Cooper and Francis, 1979), so that CDP was given at three dose levels (5.0, 10.0 and 15.0 mg/kg), under single and repeated injection conditions.

Materials and Methods

Animals. The subjects were 72 naive male Sprague-Dawley rats supplied by Olac Southern Limited. On arrival in the laboratory, they were allocated to groups of three per cage (Bowman’s MRC-type grill cages). In the home cages, standard chow pellets (Diet 41B) and tap water were continuously available. Room temperature was maintained at 21 – 23°C, and humidity kept at > 50%. Lighting operated on a 12-h dark-light cycle (lights on at a.m.). The rats weighed 240 – 280 g at testing.

Apparatus. The drinking test was conducted in a wooden, enclosed box (30 x 20 x 23 cm) equipped with a grill floor. A drinking spout attached to a 50 ml calibrated burette, mounted outside the box, protruded into the box 25 mm above the floor. Water volume was read to the nearest 0.1 ml.

Procedure. For the first week after arrival, the rats were handled daily as a taming procedure. Each rat was then left overnight, alone in a drinking box, to learn to drink from the spout. Food pellets were placed on the grill floor. Other than this experience of the drinking box, in which the animals had not yet been subjected to any water deprivation experience, there was no further exposure to the test situation until at least 10 days later. The single, overnight exposure to the drinking box was sufficient for all animals to locate and drink from the drinking spout provided in the box.

The rats were then allocated at random to acute and chronic treatment groups. In the chronic treatment group, each rat was injected daily for 9 days at one of three dose levels of CDP (5.0, 10.0 or 15.0 mg/kg) or with isotonic saline as a control injection procedure. After injection, the animals were returned immediately to the home cage. In the acute treatment group, each rat was handled briefly each day for 8 days to match the handling experience of the chronic treatment group. On day 9, each rat was injected for the first time with either isotonic saline control or with one of the three dose levels of CDP. Altogether, there were eight treatment groups, with nine rats allocated to each group. At 5 p.m. on day 8, water was removed from the home cages for the first time, and each rat was run in the drinking test on day 9 (10 a.m. – 12:30 p.m.). The drinking tests were restricted to a short period in the morning to avoid any diurnal variation in drinking behaviour.

All injections were given 30 min before the start of the drinking test. CDP HCl was injected IP at one of three dose levels (5.0, 10.0 or 15.0 mg/kg). Isotonic saline served as a control injection solution. CDP solutions were made up freshly each day that the drinking tests were run. In the drinking test itself, the rat was transferred from the home cage to the drinking box for a 15 min test period. An observer recorded the latency to start drinking (s) and also the volume of water consumed. The following procedure was adopted to follow the time course of drinking. At 15-s intervals from the beginning of the test session, an observer scored whether or not the rat was engaged in drinking. Afterwards the test session was divided into five 3-min blocks, and the total number of occasions on which each rat was drinking was calculated for each block. Hence, each rat could score a maximum of 12 occasions on which it was observed drinking for each 3-min block of the test. This time-sampling procedure provides a valid estimate of the overall time course of drinking (Cooper, 1975).

Five out of the nine animals in each treatment condition were run. In the drinking test itself, the rat was transferred from the home cage to the drinking box for a 15 min test period. An observer recorded the latency to start drinking (s) and also the volume of water consumed. The following procedure was adopted to follow the time course of drinking. At 15-s intervals from the beginning of the test session, an observer scored whether or not the rat was engaged in drinking. Afterwards the test session was divided into five 3-min blocks, and the total number of occasions on which each rat was drinking was calculated for each block. Hence, each rat could score a maximum of 12 occasions on which it was observed drinking for each 3-min block of the test. This time-sampling procedure provides a valid estimate of the overall time course of drinking (Cooper, 1975).

Results

Latency to Begin Drinking. Figure 1 shows the results for latency to begin drinking. An Anova revealed that there was no significant effect of repeated drug treatment as compared with single injection treatment (F < 1.0), but that there was a significant drug dose