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Development of Physical Dependence on Midazolam by Oral Self-Administration[1]

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FALK, J. L. AND M. TANG. *Development of physical dependence on midazolam by oral self-administration.* PHARMACOL BIOCHEM BEHAV 26(4) 797–800, 1987.—Groups of rats drank either a solution of the ultrashort-acting benzodiazepine midazolam or water under schedule-induced polydipsia conditions in chronic, daily, 3-hr sessions. In Experiment 1, the physical dependence status of animals was tested after 9 months by the precipitated withdrawal method using the benzodiazepine-blocking agent Ro 15-1788 and by exposure to a brief audio stimulus at 1.5, 12 and 24 hr following drug withdrawal. Ro 15-1788 failed to produce withdrawal signs, while the audio stimulus plus withdrawal did. In Experiment 2, similar groups were periodically tested for susceptibility to audiogenically-induced seizures at 3, 6, 12, 15, 18, 21, 24 and 26 weeks 90 minutes after their drug or vehicle intake sessions. In the midazolam-drinking group, physical dependence developed at about 12 weeks and increased in severity over the course of the study. Serum measures confirmed that continuous elevation of drug and active metabolite levels are not necessary for the development of physical dependence. A chronic, daily, single elevation of a few hr was sufficient.

Midazolam Benzodiazepine dependence Oral self-administration Midazolam serum level Schedule-induced polydipsia

REPORTS of physical dependence development in conjunction with therapeutic use of the long-acting benzodiazepines have received increasing attention in recent years [14,18]. With the development of shorter-acting benzodiazepines, studies of rebound anxiety and insomnia upon abrupt drug discontinuation [6] suggest that physical dependence can develop to these short-acting agents as well. Recent experimental evidence indicates that these short-acting agents can produce withdrawal signs in animals similar to those of the long-acting benzodiazepines when the daily dose is distributed evenly over each 24-hr cycle [9, 10, 19]. Midazolam, an ultrashort-acting benzodiazepine [15,16] is used therapeutically for the treatment of insomnia and is administered as a single dose at bedtime [4,12]. What may be directly relevant to the situation in humans is whether a single administration episode per day can produce frank signs of physical dependence. Accordingly, single, daily exposures to midazolam using the oral self-administration technique of food-schedule-induced polydipsia was used. The schedule-induced polydipsia method is an experimental arrangement under which food-limited animals fed small food pellets on an intermittently-spaced food-delivery schedule concurrently drink large amounts of fluid [2]. If a drug solution is made available for drinking under this condition, oral self-administration of large, daily doses of psychoactive drugs may be attained (e.g., midazolam [3]).

METHOD

A total of twenty-two male, albino, Holtzman rats with an initial mean body weight of 381.1 g (range: 372–389 g) were housed in standard Acme stainless-steel cages. They were reduced slowly to 80% of their ad lib body weights by limiting their food rations. They were transferred into individual Plexiglas chambers (30×26×23 cm) and housed under continuous illumination (Day 1). Each chamber was equipped with a stainless-steel pellet receptacle and a drinking fluid reservoir which consisted of a stainless-steel, ball-bearing spout attached to a 250-ml Nalgene graduated cylinder. At 1000 hr each day all animals were weighed, their overnight intakes recorded and the appropriate fluid placed on each cage. For the next 3 hr a 45-mg Noyes food pellet was automatically delivered into a food receptacle every 60 sec (FT 1 min), thus giving a total of 180 pellets during each feeding session. At the end of the 3-hr session, fluid intakes were recorded, the session fluids were removed and distilled water provided as the overnight fluid. Any food rations necessary for maintaining the animals at 80% body weight were given at that time.

Midazolam maleate (Ro 21-3981) and the benzodiazepine receptor-blocking agent Ro 15-1788 were both gifts from Hoffmann-LaRoche (Nutley, NJ). Orally self-administered midazolam solution was prepared fresh daily by dissolving

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TABLE 1

MEAN WITHDRAWAL SCORES AND NUMBER OF RATS REACTING FOR GROUPS POLYDIPSIC ON EITHER MIDAZOLAM SOLUTION OR WATER

	Weeks on Midazolam (0.005%) or Water							
Groups	3	6	12	15	18	21	24	26
Midazolam (N=5)								
Score (0–3)	0.2	0.2	0.8	0.8	1.0	1.2	1.8	1.8
Duration (sec)	0.6	1.0	4.0	1.8	2.3	3.0	6.4	7.4
Reacting Animals	1	1	4	3	3	3	4	4
Water (N=4)								
Score (0–3)	0	0	0	0	0	0.2	0	0
Duration (sec)	0	0	0	0	0	0.2	0	0
Reacting Animals	0	0	0	0	0	1	0	0

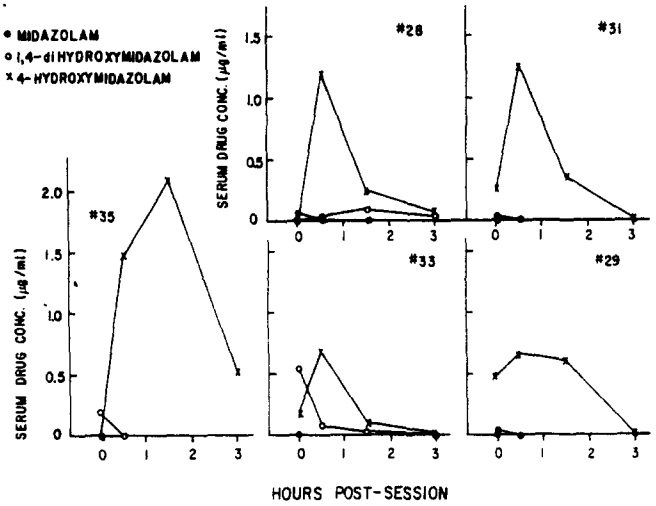

FIG. 1. Serum levels of midazolam and two metabolites following a 3-hr midazolam solution polydipsia session for 5 rats.

the drug in distilled water. Ro 15-1788 was suspended in a vehicle consisting of 1 mg of Agent K (Bio-Serv, Frenchtown, NJ) per ml of distilled water.

Experiment 1: Physical Dependence on Midazolam

Thirteen animals were assigned randomly to one of 2 session-fluid groups: Midazolam (N=7) and water (N=6). On the first 12 days of exposure to the 3-hr intermittent feeding condition distilled water was the session fluid for both groups. Thereafter, a midazolam solution was the session fluid for the Midazolam group. The concentration of the midazolam solution was set initially at 2.5 mg of midazolam maleate per 100 ml distilled water and was progressively increased to a final concentration of 5.0 mg/ml (0.005%) over the next 2 months. Once session fluid intakes were stabilized in both groups, the interpellet time (FT interval) was changed (0, 0.5, 3.0 or 5.0 min) on particular days in order to evaluate its effect on both session midazolam solution acceptance (Days 64–159) and preference (Days 170–259). During the preference testing phase of the experiment, both distilled water and 0.005% midazolam solution were available to all animals during the daily feeding session. At the end of the preference phase the animals were re-adapted to the original midazolam one-tube condition. After 3 weeks the animals were tested for precipitated withdrawal. Four midazolam-drinking animals were given a subcutaneous dose of the benzodiazepine receptor-blocking agent Ro 15-1788 (N=3 for 100 mg/kg and N=1 for 30 mg/kg dose) and 3 water-drinking animals were also given the blocker (N=1 for 100 mg/kg and N=2 for 30 mg/kg) 30 min post-session and the animals were observed for the next 60 min for withdrawal signs. One day later all animals were tested for susceptibility to audiogenic seizures at 1.5, 12 and 24 hr post-session by exposing them to a 30-sec key-shaking stimulus.

Experiment 2: Time Course of Midazolam Dependence Development

Nine rats were used to characterize the temporal development of physical dependence to orally self-administered midazolam. The maintenance condition used in this study

was identical to that of the acceptance phase of Experiment 1. The session fluid was midazolam (0.005%) for 5 animals and distilled water for the remaining 4 rats. The development of physical dependence on midazolam was assessed by testing the susceptibility of all animals to audiogenic seizures after the midazolam group had been on the final concentration of the midazolam solution for 3, 6, 12, 15, 18, 21, 24 and 26 weeks. Physical dependence testing involved placing each animal into a styrofoam container (59×59×119 cm) 90 min after a drinking session. The response of the animal to the 30-sec audio stimulus was given a score of 0 to 3 (0=no reaction; 1=running with all 4 limbs on the floor; 2=running fit often accompanied by 2 or more paws off the floor as the animal caroms from wall to wall; 3=running fit with all paws off the floor plus presence of post-seizure depression). The duration of the response was also recorded. The entire testing procedure was video taped and a second scoring was performed by a different person using the video recordings. Both rating procedures were performed by individuals who were blind to the group identification of the animals. After the animals had been exposed to daily midazolam for 2 months, tail-tip blood samples (100 μl/sample) were obtained immediately, 0.5, 1.5, 3 and 21 hr post-session for drug level analysis. The serum concentrations of midazolam and two of its major metabolites were determined with HPLC using a reversed phase column and a UV detector [11].

RESULTS

Experiment 1

Exposure of animals to the FT 1-min schedule resulted in polydipsic levels of session intake for both the Water (105.7 ml) and Midazolam (111.4 ml) groups of animals. The mean daily intake of midazolam was 17.5 mg/kg for the 3-hr session. A detailed analysis of the acceptance and preference data has been reported elsewhere [3]. None of the water polydipsic animals showed any observable reaction to the 30-sec audio stimulus. All except 2 animals in the midazolam group reacted with either a sustained running fit or severe clonic convulsions at every time point tested. One of the 2 unaffected animals in the drug group was the one with the lowest daily midazolam intake. Of the animals injected with 30 or 100 mg/kg Ro 15-1788, none of them showed any signs of pre-convulsive or convulsive behaviors.

Experiment 2

As in Experiment 1, the intermittent-feeding schedule (FT 1 min) produced large session intakes in both the Midazolam and Water groups (103.0 and 111.0 ml, respectively). The mean daily midazolam intake was 17.1 mg/kg. Figure 1 shows the serum concentrations of midazolam and two metabolites for each animal at various intervals following a 3-hr intake session. In every case the levels of serum midazolam are negligible even immediately after the drinking period. Although 1,4-dihydroxymidazolam was detected in 3 animals during the first 3 time points (0–1.5 hr post-session) the serum level of this metabolite dropped to insignificant values by the third post-session hr. The major metabolite present in these animals was 4-hydroxymidazolam.

The physical dependence testing data are summarized in Table 1. Each data point represents the mean score of the two observers who showed almost complete concordance. The midazolam self-administering group showed a progressive increase in response to the audiogenic stimulus as a function of the length of time the animals drank the 0.005% midazolam solution. This is reflected in a steady increase in both the withdrawal score, its duration, as well as the number of animals that reacted. The one animal (No. 35) that did not respond to the audio stimulus even by the 26th week is the same animal with the highest serum concentration of 4-hydroxymidazolam at 1.5 hr post-session. In contrast, similarly treated water-drinking controls did not show an increase in susceptibility to audiogenic seizures. In all but the 21-week test point, water control animals did not show any discernable convulsive or pre-convulsive signs during the audio test. The positive finding at week 21 was produced by one animal showing a 1-sec running episode. This animal did not react to the stimulus at any other time.

DISCUSSION

Both Experiments 1 and 2 showed that chronic, single, daily, oral midazolam self-administration episodes resulted in the development of physical dependence. With the drug intake regimen used, dependence developed in about 12 weeks and, with the exception of one animal, increased in severity throughout the 26 weeks of the study. It should be noted that technically the dependence testing in experiment 2 did not occur after animals had been withdrawn from midazolam. There was no termination or interruption of the drug-exposure regimen; animals were simply tested at one point (90 min) post-session. This was possible owing to the rapid elimination rate of midazolam and its active metabolites, and is analogous to the temporal considerations in humans receiving a nightly midazolam dose for the treatment of chronic insomnia who reveal a rapid development of rebound insomnia and anxiety [7, 8, 13].

Most of the experimental studies on the short- and ultrashort-acting agents have used methods that result in the exposure of animals to a rather continuous level of drug. Baboons continuously-infused intragastrically with lorazepam showed both spontaneous withdrawal signs upon abrupt drug cessation and precipitated withdrawal when the benzodiazepine antagonist Ro 15-1788 was administered [9]. In a similar study, precipitated withdrawal was also observed in baboons infused with the ultrashort-acting agent triazolam [10]. Rhesus monkeys given 3 divided gastric doses per 24 hr of the ultrashort-acting agent brotizolam showed withdrawal signs upon abrupt drug cessation [19]. Studies by the Upjohn Company (described in [5]) in which rats and mice were given continuous access to diets adulterated with triazolam showed stimulus-provoked seizure indications of physical dependence when the drug was removed from the diet.

In the present study, drug exposure was episodic (3-hr session/day) rather than continuous, but physical dependence nonetheless developed. Aside from this study, the only experiment we are aware of that tested for physical dependence after chronic, episodic exposure to an ultrashort-acting benzodiazepine is an unpublished study by Griffiths and his associates described in their review [5]. Two baboons orally self-administering triazolam in daily 3-hr sessions showed mild withdrawal signs upon drug termination. Alcohol, another drug with a short half-life, also produces a mild to moderate physical dependence when withdrawn following chronic, daily, 3-hr schedule-induced polydipsia sessions [20].

Long- and short-acting agents differ in both their duration of bioavailability and their rate of disappearance. Since both

continuous and episodic drug exposure regimens can produce physical dependence, a long, daily duration of bioavailability is clearly not necessary. On the other hand, the rate of disappearance of the drug and its active metabolites determines the latency to onset of withdrawal symptoms; abrupt discontinuance of ultrashort-acting agents producing symptoms within hours while long-acting agents are in the order of several days [1,19]. This, in turn, could be due to: (a) the elimination rate itself, or (b) the resulting decrease in absolute level of the drug and its active metabolites. Current experiments do not allow these two possibilities to be differentiated. However, it is of interest to note that those humans withdrawn from diazepam who had slower elimination of the drug or its active metabolite desmethyldiazepam also had mild to no withdrawal reactions [17]. Animal No. 35 in the present experiment with the greatest level of 4-hydroxymidazolam 3 hours post-session was also the animal that failed to show any withdrawal signs.

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