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Behavioral Tolerance to Flurazepam

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LAU, C. E., S. DOLAN, M. TANG AND J. L. FALK. *Behavioral tolerance to flurazepam.* PHARMACOL BIOCHEM BEHAV **38**(4) 823–827, 1991.—Rats were trained to earn 180 food pellets in daily, fixed-interval 1-min sessions. When performance had stabilized, a Before group was given a weekly 16 mg/kg flurazepam injection IP for 3 weeks immediately before the sessions, while an After group received their weekly injections immediately after the sessions. Then, the After group received 3 such weekly injections before the sessions. Behavioral tolerance developed by the 2nd flurazepam injection for the Before group, but for the After group, the 3 postsession flurazepam injections resulted in subsequent tolerance to presession flurazepam injection for session lever presses, but not for the time taken to earn 180 pellets. Dispositional tolerance to the serum elimination rate of flurazepam did not develop over the course of 3 injections. Behavioral suppression still evident in the initial portion of sessions with the 2nd and 3rd presession injection coincided with the duration of rising and high levels of serum flurazepam.

Flurazepam, behavioral tolerance Dispositional tolerance Benzodiazepine and spontaneous activity
Benzodiazepine and fixed-interval behavior Flurazepam elimination half-life Flurazepam pharmacokinetics, rat

FLURAZEPAM hydrochloride is a benzodiazepine widely prescribed for its sedative-hypnotic action (4,5). It has complex stimulant effects on behavior at low doses and depressant effects at high doses in rats and other species (12). The pharmacokinetics of flurazepam differ for rats and humans. In the human case, following the administration of a single dose, flurazepam and some of its minor, active metabolites disappear rapidly from the systemic circulation, while the active metabolite, desalkylflurazepam, has a long elimination half-life of 40–150 h (5,6). In the rat, both flurazepam and desalkylflurazepam have short half-lives of 1.69 and 1.12 h, respectively (9). One of the aims of the present research was to relate the serum concentration-time profile for flurazepam to the duration of behavioral effects of a flurazepam injection. Tolerance to the sedative effects of benzodiazepine administration can occur after a single dose, and complete tolerance may be evident after only a few doses (3,11). A second aim was to determine whether such tolerance development might be associated with changes in drug disposition, although most reports find little evidence of dispositional changes with chronic benzodiazepine dosage within the clinical range (7). While tolerance to the sedative effects of the benzodiazepines can develop rapidly, tolerance to other effects, such as their anticonflict action, often does not occur (1, 10, 11). The present study examined two aspects of fixed-interval schedule performance (response rate and time taken to complete a session) to determine whether tolerance would develop to both aspects.

Finally, the development of behavioral tolerance to many drugs is often contingent upon the criterion behavior having occurred during the periods when the drug doses were acting (16). In order to explore this possibility, two groups of animals were used. For the Before group, the animals were injected with a flurazepam dose immediately before the appropriate behavioral sessions. The After group received comparable injections immediately after each of the corresponding sessions, and were then given sessions with presession doses to determine whether the series of postsession drug administrations had resulted in the development of tolerance.

EXPERIMENT 1: FIXED-INTERVAL BEHAVIOR

METHOD

Animals

Six male albino rats of the Holtzman stain with a mean initial body weight of 383 g (range: 380–385 g) were used. They were housed in a temperature-regulated room with a 12-h light-dark cycle (lights on 0700–1400 h).

Drug

Flurazepam dihydrochloride was dissolved in nanopure water and administered intraperitoneally (IP) in an injection volume of 1 ml/kg body weight. Flurazepam dose was calculated as the salt.

Apparatus

Each experimental chamber consisted of a sound-attenuating exterior shell housing a Plexiglas chamber (25.5 × 26 × 30 cm) with a stainless-steel rod floor. A rat lever (G6312, Ralph Gerbrands Co., Arlington, MA) was located on the right side of the front panel of the chamber 1 cm from the adjacent wall and 4.5 cm from the floor. A dispenser delivered 45-mg Rodent Diet food pellets (P. J. Noyes Co., Lancaster, NH; Traditional Formula A) into a stainless-steel receptacle mounted 16.5 cm to the left of the lever on the same wall. An exhaust fan provided ventilation and masked extraneous sounds. The chamber was illuminated by two

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GE 306 incandescent bulbs mounted behind the front panel.

Procedure

Animals were reduced to 80% of their free-feeding body weights by limiting food intake. They were trained to lever press by a shaping procedure. Presses then were reinforced on a fixed-ratio 1 schedule (every lever press delivered a food pellet) and 120 pellets were permitted on this schedule. The schedule of reinforcement was changed to fixed-interval 1 min (FI 1 min; the first response that occurred 1 min after the last reinforced response was reinforced). Daily sessions were terminated after 180 pellets had been delivered. After behavior had stabilized, three rats were give a 16 mg/kg flurazepam injection IP once per week for 3 weeks immediately before the behavioral sessions (Before-Session animals). The remaining three rats were given a total of six such injections, except the initial three injections were administered immediately after the session and the latter three doses immediately before the session. All injections were separated by seven days. For the Before-Session animals, another series of three 16 mg/kg flurazepam doses was given two months after the third injection in order to evaluate a possible shift in serum-drug concentration profile resulting from repeated drug administration.

Determination of Serum Flurazepam Concentration

Tail blood samples were obtained at various times by cutting approximately 1 mm from the tip of the tail with a guillotine. Subsequent samples were obtained by removing a suture that had been tied around the tail tip. Blood samples were centrifuged in an Autocrit Ultra 3 centrifuge (Clay-Adams, Towson, MD) for 5 min at 13,700 × g. The clear serum layer (50 μl) was used for analysis. Flurazepam concentrations were determined by high-performance liquid chromatography with a UV detector (9). Serum samples were obtained at 0.25, 0.5, 1, 3, 5 and 7 h after the first and third flurazepam injections of the second injection series given to the Before-Session animals.

RESULTS

Figure 1 shows the development of tolerance to the weekly presession administration of flurazepam for the Before-Session animals with respect to both the number of lever presses and the time taken to complete the session (solid bars). Compared to vehicle injection, the first flurazepam injection resulted in fewer lever presses ($p<0.05$) and longer session times ($p<0.05$) for this group. For the 2nd and 3rd injections, neither the lever presses nor session times were significantly different from values after vehicle injection.

For the After-Session group, the flurazepam injections given postsession had no effects on lever presses or session times the following day (not shown in Fig. 1). After receiving the series of three after-session injections, the effects of the weekly presession flurazepam doses on the number of lever presses were not significantly different from the effect of the vehicle dose. This is also reflected by the fact that the suppressive effect of the first presession flurazepam dose was attenuated significantly compared to the suppressive effect of the first presession dose on the lever presses of the Before-Session animals, $t(4)=4.70$, $p<0.01$. However, with respect to the session-time measure, the After-Session group value was significantly ($p<0.01$) elevated by the first presession flurazepam injection compared to vehicle. The two groups were not differentially affected with respect to the session-time measure.

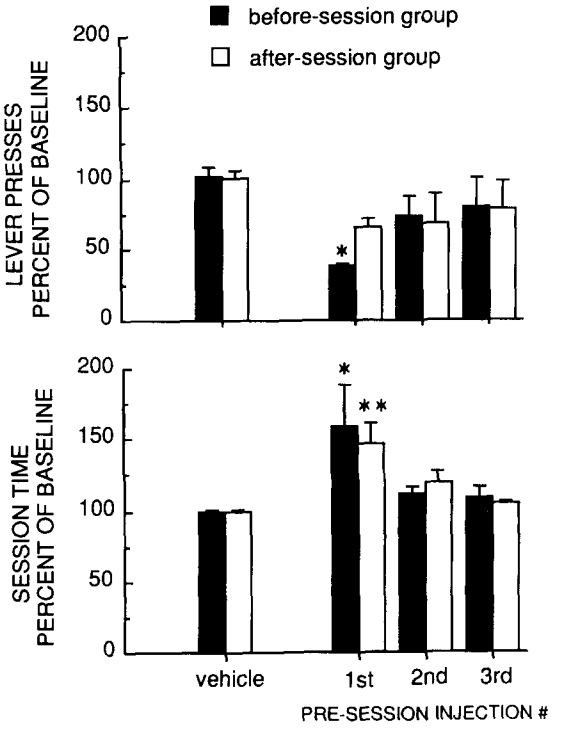

FIG. 1. Mean (SE) effects of 3 weekly presession injections of flurazepam (16 mg/kg, IP) on lever presses (upper panel) and time to earn 180 pellets (lower panel) on a FI 1-min schedule for rats that had received no prior drug injections (Before-Session group, N = 3) or had a history of 3 weekly postsession injections of flurazepam (After-Session group, N = 3). *$p<0.05$; **$p<0.01$ when compared to vehicle levels (Student-Newman-Keuls comparison).

Figure 2 shows the cumulative FI 1-min lever-pressing records for one Before-Session animal over the series of three flurazepam injections. Session time, with respect to vehicle injection, was increased 205% for the first flurazepam injection, whereas the second and third flurazepam injections had much less effect: 111% and 103%, respectively. Total lever presses were decreased to 38%, 89% and 94% of the vehicle-injection level for the 1st, 2nd and 3rd flurazepam injections, respectively.

Figure 3 shows the mean serum concentration-time profiles for flurazepam after the first and third doses from the second series of three doses given to the Before-Session animals two months after the last presession dose. The two profiles are similar. The half-life for flurazepam was 1.90 h for the first injection and 1.87 h for the third injection.

EXPERIMENT 2: SPONTANEOUS ACTIVITY

METHOD

Animals

Four male albino adult rats of the Holtzman strain with a mean initial body weight of 389.5 g (range: 386–395 g) were used. They were housed individually in stainless-steel cages in a temperature-regulated room. Body weights were reduced to 80% of their ad lib weights over a 2-week period by limiting daily food

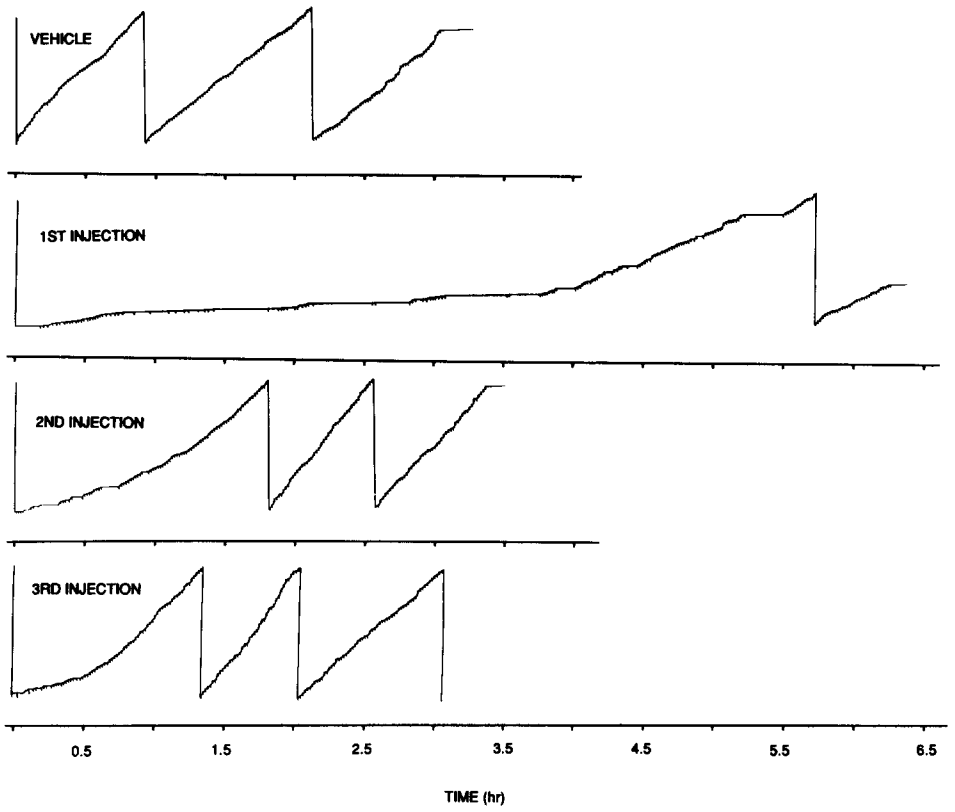

FIG. 2. Cumulative lever-press response records (FI 1-min schedule, 180 food pellets) for a Before-Session rat receiving one IP injection per week: vehicle, followed by 1st, 2nd and 3rd injection of 16 mg/kg flurazepam.

rations and animals were maintained at these weights for the duration of the experiment.

Apparatus

Spontaneous activity was measured in a room isolated from other activities and noise. Each animal's stainless-steel home cage (38.0 × 25.5 × 17.5 cm) was placed on an individual accelerometer platform (E45-01) (Coulbourn Instruments, Allentown, PA). The individual activity monitors (E52-01), which were located in an adjacent room, were adjusted to record on separate counters two kinds of activity during each session: small movements (grooming movements) and large movements (locomotion).

Procedure

After weights were stabilized, 12 daily 5-h activity-monitoring sessions were conducted. Immediately before each session, animals were weighed, replaced in their home cages from which the water bottles were removed, and transported to the experimental room. Food necessary to maintain body weights at the 80% level was given after each session. On day 13, a vehicle injection was administered IP immediately before the session and a 16 mg/kg flurazepam dose was administered on day 14.

RESULTS

The attenuating effects of the acute 16 mg/kg dose of flurazepam on both large- and small-movement activity rates are presented in Fig. 4. The attenuating effects at 0.25 and 0.5 h postinjection are consonant with the period of elevated levels of serum flurazepam.

DISCUSSION

Serum Flurazepam and Behavioral Effect

An initial aim of this study was to relate the serum concentration-time profile for flurazepam to the duration of behavioral effects of a flurazepam injection. After a 16 mg/kg flurazepam dose (IP), serum flurazepam concentration reached its maximum value in 0.5–1.0 h and then fell logarithmically [(9) and Fig. 3]. The serum elimination half-life for flurazepam of 1.89 h was similar to the 1.69 h value we obtained previously (9). Although Fig. 3 yields no evidence of a change in the pharmacokinetics of flurazepam from the first to the third injection, Figs. 1 and 2 indicate a considerable change in its behavioral effects after the initial dose (Before-Session group). While serum levels of flurazepam, as well as its metabolites, fell to quite low levels by 6 h after injection (9), the depressant effect of the first dose was still evident at this time (Fig. 2). This result yields no evidence for the development of acute tolerance during the duration of the first-dose effect. In contrast, acute tolerance to an initial dose of flurazepam has been reported in humans (6). The 2nd and 3rd flurazepam doses produced neither total lever presses nor session times that differed from those after vehicle injection (Fig. 1), but Fig. 2 indicates that for approximately the first h of these sessions lever

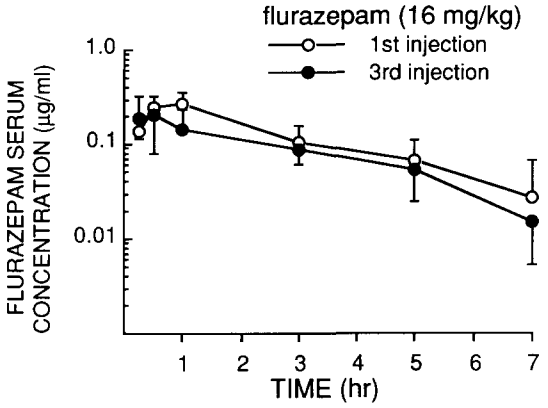

FIG. 3. Mean (SE) serum flurazepam concentration-time profiles for 3 rats after a 1st and 3rd flurazepam injection (16 mg/kg, IP).

pressing was relatively depressed. Tolerance to flurazepam may have been incomplete after three injections, so that further injections may have led to the disappearance of the decreased response rates evident in the initial segments of the sessions preceded by flurazepam injection. Figure 4 shows that baseline activity levels were appreciable only during the first 0.5 h of these sessions. Although flurazepam administration appears to decrease spontaneous activity, this depressive effect cannot be evaluated beyond the first 0.5 h owing to the low baseline activity level for the remaining 4.5-h portion of each session. Figure 2 illustrates that serum levels of flurazepam were not consonant with the prolonged depressive effect resulting from the initial dose. However, the immediacy of the depressive effect evident after all three flurazepam injections and its 1–1.5 h duration for the 2nd and 3rd injections is associated with the period of sharp rise and elevated concentration of serum flurazepam.

Lack of Dispositional Tolerance

Figure 3 reveals no change in the serum elimination rate of flurazepam that could account for the marked tolerance to its depressive effects evident by the 2nd injection. In humans, there was also no change in the pharmacokinetics of desalkylflurazepam after a 2-week exposure to flurazepam dosing (7). In general, serum and brain benzodiazepine levels seem to bear little relation to the development of tolerance (3).

Tolerance as the Attenuation of Sedative Action

The two measures of fixed-interval performance, 1) the number of lever presses in a session, and 2) the time taken to finish the session (i.e., to earn 180 pellets), could be viewed as two aspects of sedation. For the Before-Session group, both measures were significantly altered by the first flurazepam injection in directions that are consistent with a strong sedative effect, while the remaining doses produced effects not significantly different from the vehicle levels (Fig. 1). In previous research, we used a discriminative motor control procedure to evaluate the acute and chronic effects of benzodiazepines (2,15). One of the measures used was work rate. Work rate was defined as the proportion of a session which an animal spent operating a force transducer, the proper holding of which was reinforced by the delivery of food

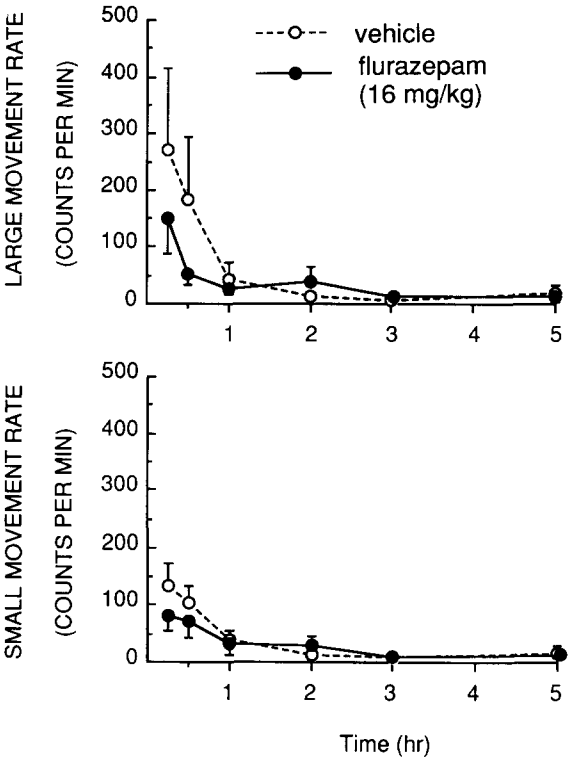

FIG. 4. Mean (SE) hourly spontaneous activity rates for 4 rats receiving vehicle or flurazepam (16 mg/kg, IP) injections.

pellets. The sedative benzodiazepine midazolam sharply reduced work rate, but complete tolerance to this effect occurred by the 4th dose in a chronic-administration series (15). Diazepam, not noted for its sedative effects, did not compromise work rate, nor was there evidence for the development of tolerance with that particular measure (2). (Other aspects of fine motor control were compromised by these benzodiazepines and tolerance to these impairments was incomplete.) The effect of midazolam on work rate was interpreted as a sedative effect and it is of interest that flurazepam, also noted for its sedative action, altered the fixed-interval performance measures in ways interpretable as indicative of sedative action.

Loss of Tolerance: Temporal Aspect

In a series of studies, Rosenberg and his associates (13) explored the duration for which tolerance was maintained after cessation of chronic exposure to flurazepam. Rats were given continuous access to a saccharin-flurazepam solution as their drinking fluid which resulted in the self-administration of up to 150 mg/kg flurazepam daily. After flurazepam was discontinued for 24 h, tolerance, as measured by the degree of ataxia produced by an IP flurazepam dose, had disappeared. The down regulation of brain benzodiazepine receptors produced by this 4-week exposure to flurazepam was also reversed 24 h after flurazepam withdrawal. However, tolerance to the antipentylenetetrazol action produced by the flurazepam exposure schedule was not lost until one week after flurazepam exposure was discontinued. In contrast to this general picture of a rather rapid loss of tolerance, animals

in the present study were exposed to three weekly flurazepam injections, with tolerance being maintained after the initial injection. The mode of exposure to the drug, IP injection versus ingestion over a 24-h period, with a consequent difference in serum concentration profile, may be an important factor determining the institution of tolerance. In this regard, it is of interest that rats given a course of midazolam injections (SC), a benzodiazepine with a serum elimination half-life somewhat shorter but similar to that of flurazepam for the rat (15), did not lose tolerance 4–6 weeks after drug exposure was terminated (14).

Behavioral Tolerance to Flurazepam

Comparing the Before-Session with the After-Session group, tolerance had developed in the After-Session group as a result of its history of receiving postsession doses of flurazepam (Fig. 1, upper panel). However, the results for the two groups were indistinguishable with respect to the session-time measure (Fig. 1, lower panel), indicating that tolerance had not been produced by the postsession doses, i.e., that the development of tolerance was behaviorally contingent. Tolerance to the suppressive effect of midazolam on fixed-ratio behavior in rats developed comparably in Before and After groups, i.e., it was not behaviorally contingent (8). To our knowledge, flurazepam has not been evaluated previously for behavioral tolerance in a Before- versus After-group comparison. However, in summarizing the results of such studies using other benzodiazepines, Wolgin (16) concludes that tolerance can develop from simple pharmacological exposure, and demonstrations of behaviorally contingent tolerance suggest that "behavioral experience may simply augment the rate at which tolerance develops" [(16), pp. 83–84]. In using the discriminative motor control procedure to evaluate tolerance to midazolam, presession drug probes to the After group revealed that, although tolerance to work-rate decrement had developed, no tolerance had developed to midazolam's ability to impair other indices that measured fine motor capacity (15). Thus tolerance to the sedative action of benzodiazepines is perhaps the most prominent behavioral feature to which tolerance develops. However, the way in which "sedation" should be characterized behaviorally is not entirely clear. In the present study, both total session responses and time taken to complete a session were measured, and both measures could conceivably be taken to describe sedation. Behavioral tolerance to flurazepam was observed to occur with respect to the session-time measure but not to the total-responses measure.

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