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Effects of Chronic Treatment With Triazolam on Operant Responding in Rats

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COHEN, C. AND D. J. SANGER. *Effects of chronic treatment with triazolam on operant responding in rats.* PHARMACOL BIOCHEM BEHAV **49**(3) 455–461, 1994. – The aims of the present study were to investigate the effect of the benzodiazepine antagonist, flumazenil, on operant responding in rats treated chronically with the short-acting hypnotic triazolam and to study the consequence of chronic triazolam treatment on the time course of effects of triazolam and zolpidem. Zolpidem is an imidazopyridine with a pharmacological and behavioral profile that differs from that of the benzodiazepine hypnotics. Rats were treated with saline or triazolam (1 or 3 mg/kg) twice daily for 5 days and were tested daily 1, 3, 5.5 or 7.5 h after injection. In addition, on the 5th day of chronic treatment all rats were injected with flumazenil (10 mg/kg) 10 min before session. The time course of effects of triazolam and zolpidem was determined after cessation of repeated saline or triazolam treatment. Tolerance to the depressant effect of 1 mg/kg of triazolam developed during long-term administration. Flumazenil decreased operant responding in rats pretreated with triazolam. The effect was statistically significant when rats had received 1 mg/kg of triazolam 3 h before the session or 3 mg/kg of triazolam 3, 5.5 or 7.5 h before the session. After cessation of chronic treatment, rats pretreated chronically with 3 mg/kg of triazolam displayed decreased sensitivity to triazolam and to 10 mg/kg but not 3 mg/kg of zolpidem. The present results indicate that chronic treatment with triazolam induces tolerance to the rate-decreasing effect of the drug and dependence as measured by flumazenil-induced disruption of operant responding. The limited degree of cross-tolerance between zolpidem and triazolam may suggest that their pharmacological mechanisms of action are distinct.

Zolpidem Triazolam Tolerance Cross tolerance Dependence

DISRUPTION of operant responding on cessation of long-term drug treatment has been documented for a range of drugs and has been taken to be a sign of a withdrawal syndrome (7). However, such an effect has not often been observed on cessation of long-term treatment with benzodiazepines (4,11, 15), although response disruption has been documented when withdrawal was precipitated by the benzodiazepine antagonist, flumazenil (4,15,29,30). Flumazenil-induced decreased operant responding has been observed in rats treated chronically with chlordiazepoxide (100 mg/kg/day) (30). In this study, the benzodiazepine antagonist was administered 24 h after the agonist. A similar effect was observed in diazepam-treated rats (5 mg/kg twice daily) when flumazenil was injected 1 or 3 h, but not 18 h, after the last treatment with diazepam (15). Flumazenil produced response rate decreases in squirrel monkeys when administered 24 h but not 1 h after a single dose of chlordiazepoxide, diazepam, or *N*-desmethyldiazepam, reflecting acute benzodiazepine dependence (29). Flumazenil-induced operant disruption has also been described in rats treated with the short-acting hypnotic, triazolam (4). However, in this study triazolam was continuously infused to the animals via osmotic pumps.

One aim of the present experiment was therefore to complement these studies on the activity of flumazenil in rats after chronic benzodiazepine treatment. A second aim was to assess the degree of cross tolerance to the depressant effect of zolpidem, an imidazopyridine hypnotic that binds to the ω (BZ) modulatory site of the γ-aminobutyric acid ($GABA_A$) receptor but possesses a pharmacological and behavioral profile that differs in several respects from that of the benzodiazepine hypnotics (2,5,13,21,22,25–28,32). Evidence from neurochemical studies and recent knowledge of the molecular biology of ($GABA_A$) receptors has indicated that the ω (BZ) sites exist in at least two subtypes, ω_1/ω_2 (BZ_1/BZ_2), in the

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brain with distinct regional distributions (2,6,16). A variety of novel compounds including zopidem, CL 218,872, abecarnil, and alpidem display selectivity for the ω_1 (BZ_1) sites (2,16). Therefore, differences between ω (BZ) site ligands in their pharmacological properties may be related to their selectivity for subtypes of ω (BZ) sites (13,25,32). Results from studies of cross tolerance may help to draw conclusions about the mechanisms involved in the differential behavioral profiles of ω (BZ) receptor ligands.

METHOD

Animals

Male Wistar rats (Charles River, France) were used. They weighed 180–200 g when obtained from the suppliers (285–345 g when chronic treatment was started) and were individually caged under standard laboratory conditions. Food intake was restricted to the food pellets obtained during the sessions and a small fixed quantity of chow (15–20 g) given at the end of each day and over the weekend. Water was always available in the home cages.

Apparatus

The experiments were carried out in standard, two-lever operant test chambers (Med Associates Inc.). Reinforcement was provided by 45-mg food pellets (Bio-Serv), which were delivered into a food cup centered between the two levers.

Training

Rats were trained to press the left lever to obtain 45-mg food pellets. Initially, sessions were 30 min and every response produced a pellet. In addition, a pellet was automatically delivered every minute. The schedule requirement was then gradually increased until 10 lever presses were necessary for each pellet (FR10) and pellets were delivered only upon completion of the schedule requirement. At this stage, the daily session duration was reduced to 15 min and rats were given injections of saline 15 min before sessions.

Chronic Treatment

As soon as a stable rate of response was achieved for a rat, it was allocated to a chronic treatment group and repeated injections were started. Rats were divided so that groups were similar in their range of response rates. There were three types of chronic treatment that consisted of two daily intraperitoneal (IP) injections of either saline, triazolam 1 mg/kg, or triazolam 3 mg/kg. All rats were injected daily for 5 days from Monday to Friday at 0845–1000 and 1645–1800. Twenty-eight rats were included in each chronic treatment. On the 5 days of chronic treatment, and for each type of chronic treatment, four separate groups of seven rats were tested daily 1, 3, 5.5, or 7.5 h after the morning injection.

Flumazenil Administration

On the 5th day of chronic treatment (i.e., Friday), all rats were tested as normal 1, 3, 5.5, or 7.5 h after their morning injection of saline, triazolam 1 mg/kg, or triazolam 3 mg/kg. In addition, all rats were injected with flumazenil (10 mg/kg, IP) 10 min before the session (i.e., 0 h 50 min, 2 h 50 min, 5 h 20 min, or 7 h 20 min after the morning injection of saline or triazolam).

Time Course of Effect of Triazolam and Zolpidem

The time course of the acute effect of triazolam at 1 and 3 mg/kg and zolpidem at 3 and 10 mg/kg was studied in rats from the three types of chronic treatment (saline, triazolam 1 mg/kg, and triazolam 3 mg/kg). Tests were carried out on Monday, Wednesday, and Friday—that is, 3–7 days after the end of the chronic dosing. Each time course study included 36 rats, with 12 rats from each type of chronic treatment. For each time course study, four rats from each chronic treatment were tested on Monday, four on Wednesday, and four on Friday. Rats were used in a maximum of three time course studies. On a test day, the animal was injected IP with the drug and was placed in the test chamber for a 15-min session 1 min after injection. Rats were then given 5-min trials at 30, 60, and 120 min after injection, and for the dose of 3 mg/kg of triazolam rats were last tested 180 min after injection. On Tuesday and Thursday, rats were only given a 15-min session.

Drugs

Sources of drugs were as follows: flumazenil (Hoffmann La Roche, Basel, Switzerland) and triazolam (Profarmaco, Italy). Zolpidem (tartrate) was synthesized at the Chemistry Department, Synthelabo Recherche, Paris. All doses are expressed in terms of the bases. All drugs were prepared as solutions or suspensions in saline to which two drops of Tween-80 had been added and were given by IP injection in a volume of 2 or 5 ml/kg.

Data Analysis

The effects of chronic treatment were analyzed separately for each session time (i.e., 1, 3, 5.5, and 7.5 h after injection). Response rates obtained during the first 4 days of chronic treatment were analyzed by separate two-way analysis of variance with the chronic treatment group serving as the between-subjects factor and day as the within-subject factor. Follow-up comparisons were conducted using Student-Newman-Keuls test and Dunnett's test. The effects of flumazenil on response rates were analyzed for data expressed as the differences from the previous day's response rates by two-way analysis of variance, with the factors of chronic treatment group and session times. Follow-up comparisons were conducted using Student-Newman-Keuls test. The effects of chronic treatment on the time course of triazolam and zolpidem were analyzed separately for each dose on the area under the curves with an analysis of variance and Dunnett's test or Student-Newman-Keuls test. The day of testing was included as a factor in the analysis of variance.

RESULTS

Rates of response during chronic treatment with saline or triazolam are shown in Fig. 1 separately for each of the four session times. Values from the three daily sessions immediately preceding chronic dosing are also displayed. The SEMs are not indicated; however, they ranged between 5 and 19. During the chronic treatment period, the rate-decreasing effects of triazolam were dependent on the time after the last injection. Both doses of triazolam decreased response rates when given 1 h before the session but not when given 3, 5.5, or 7.5 h before testing. Analysis of variance showed significant chronic treatment [$F(2, 18) = 13.11$; $p < 0.001$] and day [$F(3, 54) = 4.88$; $p < 0.001$] effects for the 1-h time session. As illustrated in the upper left panel of Fig. 1, the effect of 1 mg/kg triazolam at 1 h after injection decreased over days of

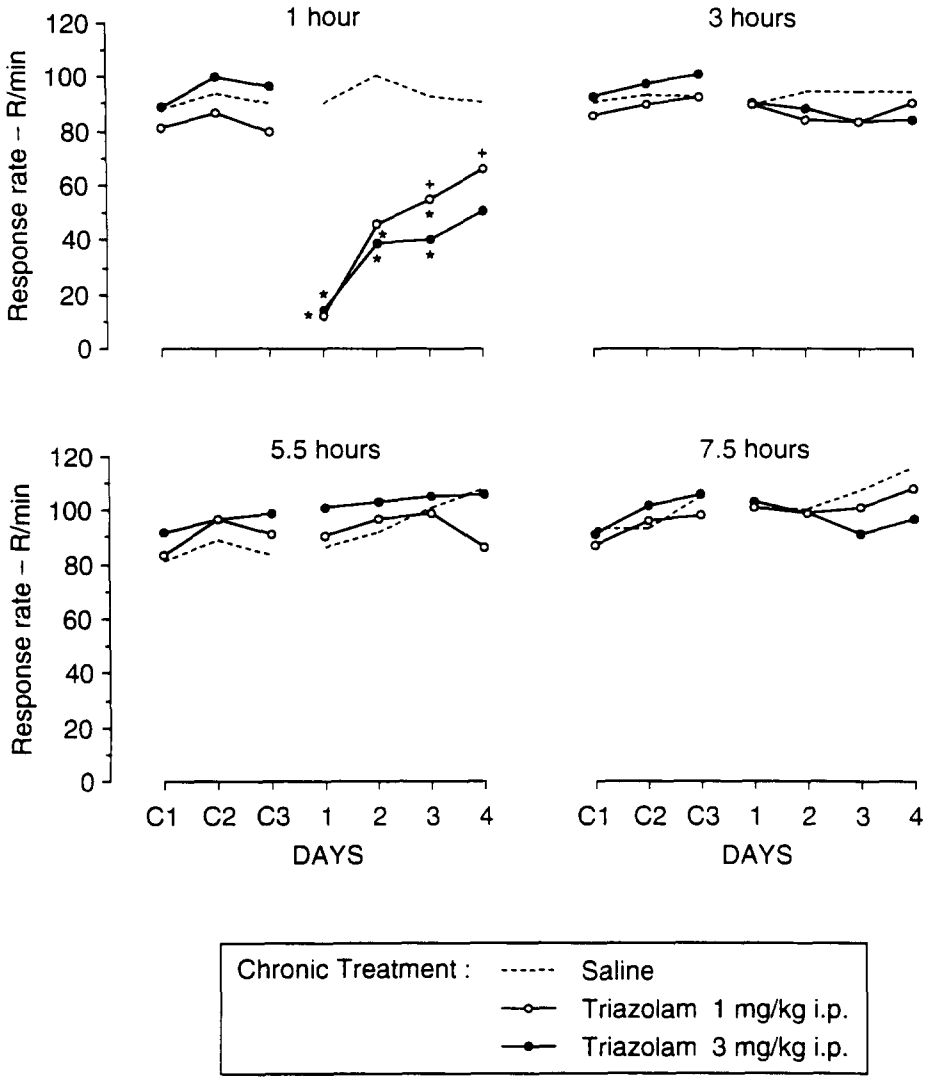

FIG. 1. Rates of FR 10 responding during the three sessions immediately preceding chronic treatment and during the first 4 days of chronic treatment with saline, triazolam 1 mg/kg, or 3 mg/kg twice daily. Sessions were conducted at different times (1, 3, 5.5, and 7.5 h) after injection, with each rat being tested once daily at the same time after injection. Mean values are shown separately for each time. $n = 7$. *Significantly different from corresponding response rates in saline group ($p < 0.05$); +significantly different from response rates on day 1 ($p < 0.05$)

treatment [$F(3, 54) = 4.90$; $p < 0.01$]; response rates on days 3 and 4 were significantly different from those observed on the 1st day of triazolam treatment ($p < 0.05$). Statistical analysis also indicated that response rates of the 1 mg/kg triazolam group were significantly below the levels of response rates of the saline group at days 1, 2, and 3 ($p < 0.05$) but not at day 4. The effect of 3 mg/kg of triazolam decreased over days of treatment, but the decrease was not statistically significant. Response rates of the 3 mg/kg triazolam group were significantly different than those of the saline group on days 1, 2, and 3.

Figure 2 shows the effects of flumazenil (10 mg/kg) on day 5 of chronic treatment. Data are expressed as the differences from the response rates on the previous day. Analysis of variance indicated significant effects for chronic treatment [$F(2, 72) = 9.16$; $p < 0.001$] and for time session [$F(3, 72) = 7.94$; $p < 0.001$]. According to Student-Newman-Keuls test, the effects of flumazenil were significantly different between the rats treated with saline and those treated with triazolam 1 mg/kg ($p < 0.05$) or triazolam 3 mg/kg ($p < 0.01$). Compared with the response in saline-treated rats, flumazenil decreased response rates when rats were injected with triazolam 1 mg/kg 3 h before the session or with triazolam 3 mg/kg 3, 5.5, or 7.5 h before the session.

The time course of the acute effect of triazolam 1 and 3 mg/kg and zolpidem 3 and 10 mg/kg in rats that had received chronic treatment with saline and triazolam 1 or 3 mg/kg are presented in Fig. 3. Rates per minute were calculated for each minute during the first 15-min test and thereafter for each complete 5-min test. The upper left panel depicts the pattern

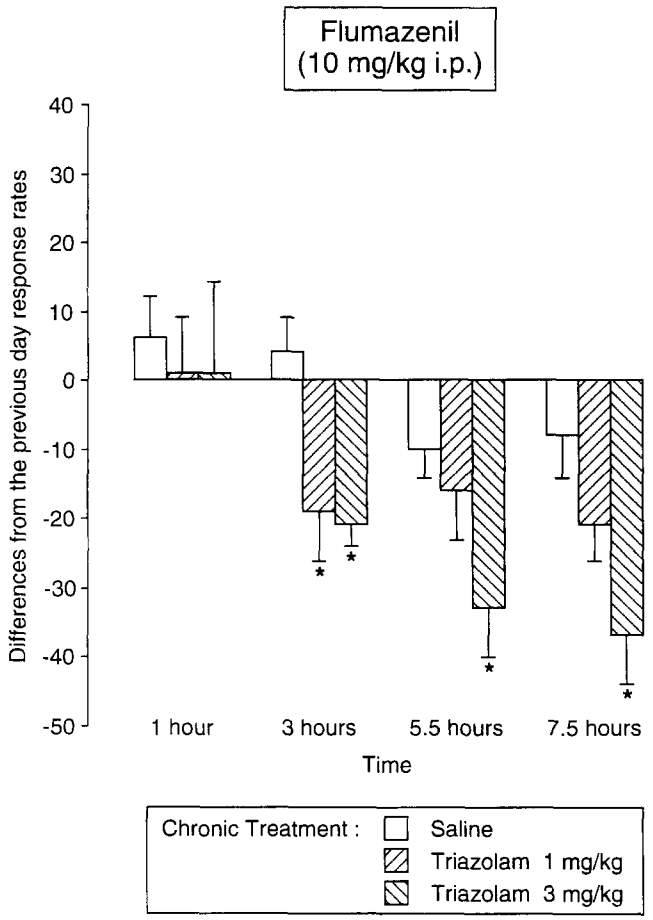

FIG. 2. Effects of flumazenil (10 mg/kg, IP) on rates of FR 10 responding in rats chronically treated with saline, triazolam 1 mg/kg, or 3 mg/kg twice daily. Flumazenil was administered on day 5 of chronic treatment, 10 min before experimental sessions. Sessions were conducted at the indicated times after the regular morning dose of triazolam or saline. Data are expressed as the differences from the previous day's response rates. The values shown are means and SEM $n = 7$. *Significantly different from corresponding values in saline group ($p < 0.05$)

of action of acute triazolam at 1 mg/kg. The SEMs ranged from 1-16. At 4 min after injection the drug markedly reduced response rates in control rats. This depressant effect was still present at 60 min but not at 120 min. Rats that had received long-term treatment with triazolam at 3 mg/kg were less sensitive to the effect of the benzodiazepine than were control rats ($p < 0.05$). In these animals, the maximal reduction of response occurred at 5-9 min after injection. The duration of action of triazolam was ≤ 60 min. The time-effect curve for rats pretreated with 1 mg/kg triazolam was between those for control rats and 3 mg/kg triazolam rats.

The upper right panel of Fig. 3 presents the pattern of action of acute triazolam at 3 mg/kg. The SEMs ranged from 8-19. In control animals, response rates were markedly decreased from 2-120 min after injection. The figure indicates that rats that had received repeated treatment with triazolam 3 mg/kg were less sensitive to the rate-suppressant effect of the benzodiazepine; however, statistical analysis did not show a significant chronic treatment effect.

The lower left panel of Fig. 3 shows the pattern of action of acute zolpidem at 3 mg/kg. The SEMs ranged from 0-12. In control animals, response rates were suppressed from 1-30 min after injection. Rats recovered quickly between 30 and 60 min after injection. Statistical analysis did not show a significant chronic treatment effect.

The effects of zolpidem at 10 mg/kg are illustrated in the lower right panel. The SEMs ranged from 0-10. In control rats, response rates were suppressed 1-60 min after injection with a recovery in the following hour. The rats that had received long-term treatment with triazolam 3 mg/kg were less sensitive to the effect of 10 mg/kg zolpidem than were control rats ($p < 0.01$).

DISCUSSION

When rats were tested daily after injection of 1 mg/kg of triazolam, tolerance to the depressant effect of the drug was observed. Daily assessment of tolerance indicated that the phenomenon developed rapidly. Previous studies have observed tolerance to the behavioral effects of triazolam in rodents and monkeys (1,4,8,12,28), and it is known that tolerance to the effects of benzodiazepines such as lorazepam, chlordiazepoxide, or diazepam occurs after a few doses (8,9). When rats were injected daily with a high dose of triazolam (3 mg/kg), some tolerance developed but the change was not statistically significant. Clearer tolerance to the depressant effect of this dose of triazolam would probably have occurred if the chronic treatment had been continued.

When tolerance was reassessed after the end of the chronic treatment, rats chronically pretreated with 3 mg/kg but not 1 mg/kg of triazolam displayed tolerance to the rate-decreasing effect of the benzodiazepine. The figures that depicted the time course of effect of triazolam clearly indicated that decreased sensitivity to triazolam was expressed by decreased maximal depressant effects and a shortened duration of action of triazolam.

Previous experiments have observed differential and asymmetrical cross tolerance between benzodiazepines (10,24) and between benzodiazepines and other ω (BZ) site ligands (4,17). In particular, a previous experiment showed that chronic treatment with chlordiazepoxide or CL 218,872 produced tolerance to the sedative effect of chlordiazepoxide but not to that of CL 218,872 (17). Using an operant behavioral baseline, it was found that long-term treatment with triazolam via subcutaneously implanted osmotic pumps conferred less cross tolerance to the rate-decreasing effect of zolpidem than to those of the benzodiazepines triazolam or lorazepam (4). These results were consistent with observations that zolpidem and CL 218,872 possess pharmacological and behavioral profiles that differ from that of benzodiazepines (5,14,21,22,25-28,32). However, in another study, mice made tolerant to the depressant and anticonvulsant effects of midazolam were also tolerant to the similar effects of zolpidem, although tolerance did not develop to the effects of zolpidem itself (22). In the present study, pretreatment with triazolam (3 mg/kg) conferred some cross tolerance to the depressant effects of 10 mg/kg but not to 3 mg/kg of zolpidem. It has been shown that several subtypes of ω (BZ) sites exist in the brain and that zolpidem, but not triazolam, displays a selectivity for the ω_1 (BZ_1) sites (2,6,16). The observation that zolpidem and other ω_1 (BZ_1)-selective ligands induced little or no tolerance to their depressant effects, whereas repeated administration of triazolam or midazolam produced tolerance to their effects, when tested in the same conditions, has suggested that ω_1 (BZ_1) sites are not

involved in the mechanisms of benzodiazepine tolerance (4,22,25,27,28). This hypothesis is in agreement with the present observation that triazolam conferred some cross tolerance to the effects of zolpidem, only at a high dose of zolpidem—that is, at a dose that may involve ω_2 (BZ_2) sites. However, previous observations that midazolam produced cross tolerance to a large range of doses of zolpidem in mice has suggested that quantitative aspects, such as widespread rather than selective enhancement of GABAergic transmission, are more important than qualitative differences in receptor activation (22,25,32). It is also known that drug efficacy (in addition to selectivity) at subtypes of ω (BZ) sites is important in determining the potential to induce tolerance (25,32). Further experimental investigations of cross tolerance between ω (BZ) site ligands are warranted to relate differential cross tolerance to differences in mechanisms of action.

In the present study, flumazenil administered on the 5th day of chronic dosing decreased response rates of triazolam-treated rats. This effect may be interpreted as a sign of precipitated withdrawal, indicating that the period of 5 days' treatment with triazolam had been sufficient to induce some physiological dependence. Previous experiments have observed that flumazenil can precipitate a benzodiazepine withdrawal syndrome similar to that observed after cessation of chronic benzodiazepine administration (31). Flumazenil-induced operant disruption has been described in rats pretreated with benzodiazepines such as diazepam, chlordiazepoxide, or triazolam (4,15,29,30). In a previous study, decreased response rates upon flumazenil administration was observed in rats treated with triazolam (3 mg/kg per day) via subcutaneously implanted osmotic pumps (4). The antagonist was administered on the 11th day of chronic treatment at

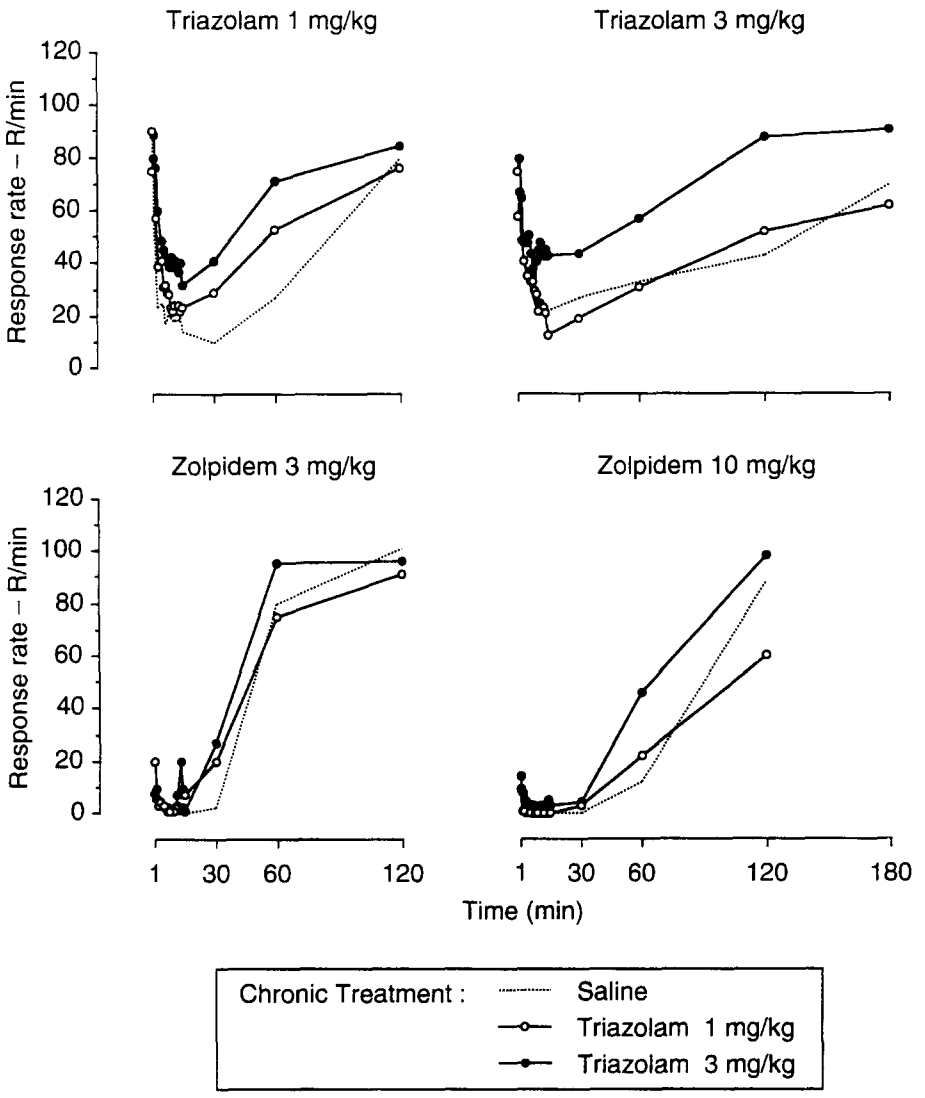

FIG. 3. Time course of effect of triazolam 1 mg/kg (upper left panel), triazolam 3 mg/kg (upper right panel), zolpidem 3 mg/kg (lower left panel), and zolpidem 10 mg/kg (lower right panel) on rates of responding in rats from each chronic dosing group. Tests were carried out on days 3, 5, and 7 after the end of the chronic treatment. Each animal was tested at several times after the injection. Rates per minute were calculated for each 1-min period during the first 15-min trial and thereafter for each 5-min test. The values are means. $n = 12$.

doses of 3.0, 10, and 30 mg/kg using a cumulative dosing procedure. In the present study, the disruptive effect of flumazenil was dependent on the dose of triazolam used for chronic treatment and on the time after the last injection of triazolam. The decrease in operant responding induced by flumazenil was of a greater magnitude in rats treated with 3 mg/kg of triazolam than in those treated with 1 mg/kg. The flumazenil-induced decrease in response rate was not observed when flumazenil was administered to rats pretreated with triazolam 1 h before the session (i.e., 0 h 50 min before the flumazenil injection). Decreases in responding occurred when triazolam at the dose of 1 mg/kg was injected 3 h before the session (i.e., 2 h 50 min before flumazenil) or when 3 mg/kg triazolam was injected 3, 5.5, or 7.5 h before the session (i.e., 2 h 50 min, 5 h 20 min, 7 h 20 min before flumazenil). Results from the present study indicate that flumazenil disrupted operant responding when the rate decreasing effect of triazolam was not detectable. Similar results were obtained in another experiment that studied acute chlordiazepoxide dependence (3). In this study, flumazenil was administered to rats at different times after a single injection of chlordiazepoxide. Flumazenil produced behavioral effects similar to behavioral signs of withdrawal that emerged spontaneously several hours after chlordiazepoxide administration. The duration of flumazenil effects was greater for a high dose than for a low dose of chlordiazepoxide and the onset time was shorter for a low dose than for a high dose of the benzodiazepine agonist. The time of loss of flumazenil effects coincided with the loss of detectable behavioral depression induced by chlordiazepoxide and the emergence of acute spontaneous dependence. Chronic benzodiazepine treatment has often produced a reduction in ω (BZ) site number (18), whereas cessation of benzodiazepine treatment has induced an increase in their number (19). The time course of effects of flumazenil in benzodiazepine-dependent animals observed in the present and previous studies indicates that flumazenil effects may coincide with a state preceding upregulation of ω (BZ) site number. The question remains as to whether flumazenil precipitated some form of central nervous system adaptation or whether the behavioral effects of flumazenil reflected a change in the direct behavioral activity of flumazenil. A number of studies have in fact suggested that chronic administration of benzodiazepine agonists shifts the effects of benzodiazepine ligands toward greater inverse agonist activity (20,23,30).

In conclusion, the present results indicate that chronic treatment with triazolam induced tolerance to the depressant effect of the drug and dependence as measured by flumazenil-induced disruption of operant responding. Both effects were functions of the chronic dosing regimen. Chronic treatment with triazolam produced cross tolerance to the rate-decreasing effect of zolpidem, but only when the imidazopyridine was given at a high dose (10 mg/kg).

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