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Lack of Tolerance or Withdrawal Effects in Mice After Chronic Administration of the Non-Sedating Anxiolytic, CGS 9896

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BOAST, C A AND S C GERHARDT *Lack of tolerance or withdrawal effects in mice after chronic administration of the non-sedating anxiolytic, CGS 9896* PHARMACOL BIOCHEM BEHAV **26**(3) 601–606, 1987 —CGS 9896, a non-sedating anxiolytic, was compared to diazepam with respect to the development of tolerance and withdrawal. Both compounds were administered daily to mice at various doses (3, 10 or 30 mg/kg) for periods of up to 4 weeks Measures of sedation/muscle relaxation, motor activity and anticonvulsant effects were then assessed When administered acutely, CGS 9896 increased motor activity, had no effect on traction reflex, and elevated the threshold for PTZ-induced convulsions After chronic administration of CGS 9896, no changes in these parameters were observed compared to the effects seen after acute treatment Acute administration of diazepam reduced motor activity, impaired traction reflex and increased PTZ-induced convulsion threshold Tolerance developed to the effects of diazepam in all three measures Following a four week dosing period with 30 mg/kg of either CGS 9896 or diazepam, the drugs were withdrawn and similar behavioral measures obtained at various withdrawal intervals up to 15 days In separate groups of mice, precipitated withdrawal was also assessed by the administration of the benzodiazepine inverse agonist, CGS 8216. No effects were observed after any period of withdrawal from CGS 9896 By contrast, withdrawal from diazepam resulted in significant alterations of motor activity and convulsion threshold These results indicate that CGS 9896 is likely to be free of undesirable tolerance and withdrawal effects typically associated with the benzodiazepines

CGS 9896 Diazepam Chronic administration Tolerance Withdrawal effects

BENZODIAZEPINES, such as diazepam, are widely prescribed for anxiety and often are taken for extended periods They are known to cause dependence in some patients [16,23], a condition that may be linked to the emergence of tolerance to the therapeutic effects of the drug, and/or to subsequently increased dosage taken to maintain consistent therapeutic effects [23]. Studies in animals have demonstrated tolerance to the sedative [11,15], anxiolytic [6,24], and anticonvulsant [12, 14, 15] actions of benzodiazepines Similarly, chronic administration of benzodiazepines and subsequent withdrawal can result in overt effects in rodents [8,22], dogs [20], cats [8], squirrel monkeys [8], and baboons [19]. It would be a major clinical advantage to have an anxiolytic agent devoid of tolerance and dependence/withdrawal liability

CGS 9896 [26] is a pyrazoloquinoline anxiolytic lacking sedative properties [2,3]. CGS 9896 also has anticonvulsant activity [2,3] Because of the lack of sedation produced by CGS 9896, and the biochemical characterization of this drug as a mixed agonist/antagonist at benzodiazepine sites [25], it was of interest to determine whether CGS 9896 would share the liability of the benzodiazepines to cause tolerance. Since CGS 9896 does not cause sedation/muscle relaxation, tolerance to the anticonvulsant effects of the drug was of particular interest A further issue was the question of whether CGS 9896 would induce withdrawal effects, suggesting dependence liability A previous study in baboons suggests that CGS 9896 may not have dependence liability [17] In that study no independent measure of efficacy was obtained.

In the present investigations, mice, chronically treated with either CGS 9896 or diazepam, were assessed in a traction test to evaluate sedation/muscle relaxation, a motor activity test to detect more subtle motor effects, and a pentylenetetrazol convulsion threshold test to assess anticonvulsant activity In a second series of experiments, chronically treated mice were tested after varying periods of drug withdrawal to assess spontaneous or precipitated (by the benzodiazepine inverse agonist CGS 8216) withdrawal [9, 20, 25].

METHOD

Subjects

Male CF-1 mice (Crl.CF1BR, Charles River, Kingston, NY) weighing 25–35 g were used in these experiments. The animals were group housed (10/cage) in an environmentally

controlled room with 12 hr of light (on at 7 00 a.m.) during the chronic drug administration or drug free periods. Mice were brought to the laboratory on the day of behavioral testing.

Procedure

Animals received oral (10 ml/kg) diazepam (Hoffman-LaRoche, Nutley, NJ) or CGS 9896 (CIBA-Geigy, Summit, NJ) once daily for periods of 1 to 4 weeks. Mice that received drugs for less than 4 weeks were given vehicle (3% colloidal cornstarch containing 5% Tween 80 and 0.34% polyethyleneglycol 400) for the preceding weeks in order to equate handling, weight and number of intubations over all treatment groups.

Thirty minutes after the last dose, motor activity was assessed in Digiscan motor activity monitors (Omnitech, Columbus, OH) housed in a dark, sound-attenuated chamber. Mice were tested individually in 15 minute test sessions divided into 5 segments of 3 minutes each (i e., 0–3 min, 3–6 min, 6–9 min, 9–12 min, and 12–15 min). Preliminary data analysis indicated that the distance travelled parameter reflected significant changes

Motor activity data were statistically analyzed by testing, first, for inequality of group variances [18] When inequality was found, the Games-Howell test [13] was used to compare dosing intervals to acute administration for each dose. When variances were not unequal, analysis of variance was used followed by Dunnett's test [21] for multiple comparisons

Sixty minutes after the last dose, animals were tested for loss of traction reflex Each mouse was permitted to grasp, by its front paws, a thin wire stretched horizontally Any animal unable to bring its hind paws up to the wire within 10 seconds was considered to have a loss of traction. These data were statistically evaluated using Fisher's exact probability test [10]

Immediately after the traction test, threshold for pentylenetetrazol (PTZ)-induced convulsions was determined PTZ (10 mg/ml—for tolerance experiment or 2 5 mg/ml—for withdrawal experiment) was injected via a tail vein in 0 05 ml increments, 10 seconds apart, until a clonic convulsion was observed. The total dose (mg/kg) of PTZ required to induce a convulsion was calculated and used as a measure of sensitivity to PTZ In some cases data were subjected to a log transformation because of unequal variances. Data were subjected to analysis of variance followed by multiple comparisons using Tukey's [5] or Dunnett's tests [21]

Procedures for all behavioral testing were thoroughly counterbalanced for dose and duration of treatment. A given mouse was used only in one condition (i e , tolerance or withdrawal, dose, duration of chronic dosing, withdrawal time), however, the battery of behavioral tests was applied to each mouse. At the time of behavioral testing the experimenters involved were "blind" to the treatment condition of a given animal

Tolerance Experiment

Mice were treated once daily with vehicle, diazepam (3, 10, or 30 mg/kg PO) or CGS 9896 (3, 10, or 30 mg/kg PO) for 1, 2, 3 or 4 weeks Thirty min following the last drug treatment, distance travelled was recorded for 15 min At one hour after treatment, traction reflex and PTZ convulsion threshold were assessed Eight to ten mice were used for each dose and duration condition

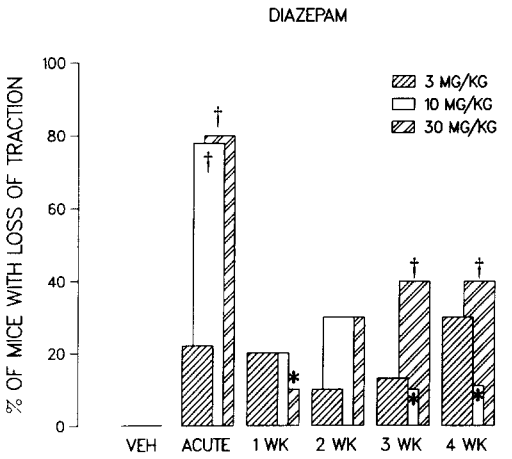

FIG 1 Effects of acute and chronic diazepam treatment on traction reflex in mice *$p<0$ 05 vs acute diazepam treatment, †$p<0$ 05 vs vehicle treatment (Fisher's test) CGS 9896 did not cause a loss of traction reflex at any dose or time tested

Withdrawal Experiment

Animals were treated once daily with vehicle, diazepam (30 mg/kg PO) or CGS 9896 (30 mg/kg PO) for 4 weeks. Since CGS 9896 has been shown to be equipotent with diazepam with respect to anxiolytic effects [2,3], matching doses were selected. A relatively high dose was selected in order to facilitate the observation of any physical dependence. Separate groups of mice were tested at 1, 3, 8, or 15 days after the last drug treatment At each time period, separate groups of mice were treated with vehicle (spontaneous withdrawal), or 30 mg/kg (PO) of the benzodiazepine inverse agonist, CGS 8216 (precipitated withdrawal) [20] Thirty min following this treatment, distance travelled was recorded for 15 min At one hour, traction reflex and PTZ convulsion threshold were assessed. Eight to ten mice were used for each treatment condition

RESULTS

Tolerance Experiment

In animals previously treated only with vehicle, acute diazepam treatment (10 and 30 mg/kg) produced a loss of the traction reflex which was significant ($p<0.05$) by comparison with animals that received vehicle After one week of daily treatment with 30 mg/kg diazepam, this loss of traction after a challenge dose (30 mg/kg) of diazepam was significantly reduced ($p<0$ 05) compared with the acute effects (Fig 1) Similarly, mice receiving three or four weeks of daily treatment with 10 mg/kg diazepam did not show a loss of traction after a challenge dose (10 mg/kg) of diazepam ($p<0$ 05 compared with acute effects) Following these longer treatment regimens, a challenge dose of 30 mg/kg diazepam caused a reduced but still significant ($p<0.05$) loss of traction. CGS 9896 did not cause a loss of traction reflex at any dose or time tested (data not shown).

Distance travelled was not significantly altered by acute diazepam treatment After 2 weeks (data not shown) or 3 weeks (Fig 2A) of chronic treatment with 30 mg/kg of diazepam, the distance travelled measure was significantly ($p<0$ 05) increased relative to the acute effect at the 3, 6, and 9 min intervals

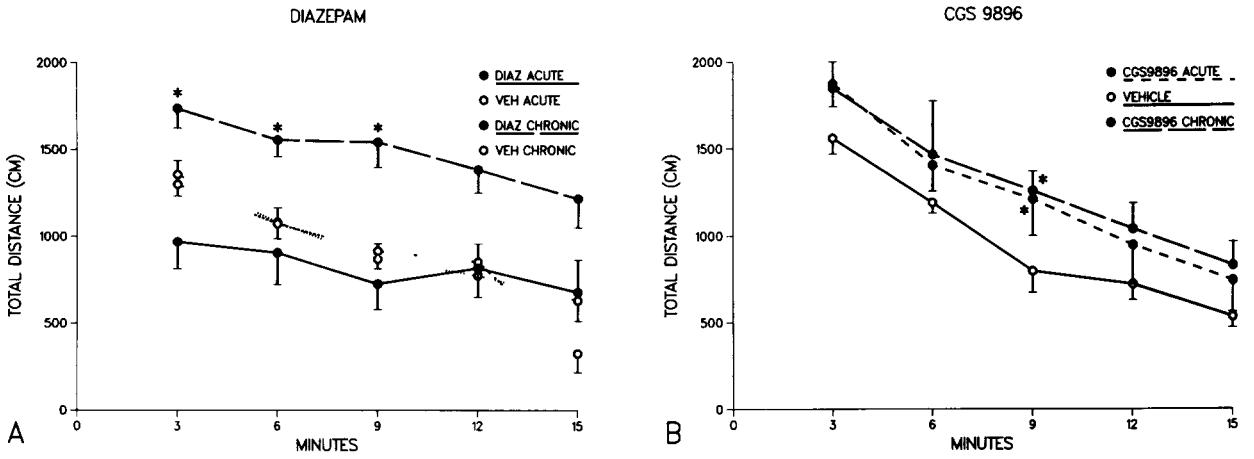

FIG 2 Effects of acute or chronic (3 week) diazepam (A) or CGS 9896 (B) treatment (30 mg/kg) on distance travelled by mice during a 15 min motor activity session *$p<0$ 05 vs (A) acute diazepam treatment, or (B) vehicle (Games-Howell test)

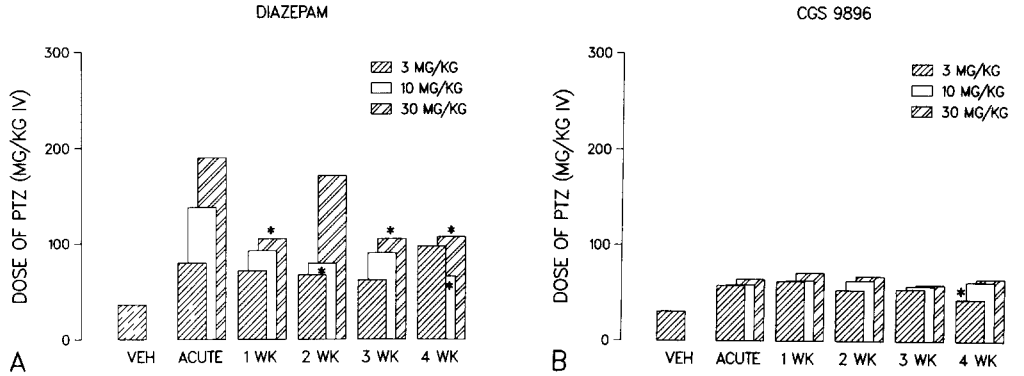

FIG 3 Effects of acute or chronic diazepam (A) or CGS 9896 (B) treatment on PTZ convulsion threshold *$p<0$ 05 vs acute treatment (A) or NS vs vehicle treatment (B) (Tukey's test)

After acute administration, 10 mg/kg of CGS 9896 increased distance travelled (significant at the 9 min interval) (Fig. 2B). After 3 weeks of chronic treatment this trend was still apparent (significant at the 9 min interval).

Acute administration of diazepam produced an apparent dose dependent increase in the PTZ convulsion threshold (Fig 3A). A challenge dose of 30 mg/kg diazepam given after 1, 3 or 4 weeks of chronic dosing with 30 mg/kg of diazepam resulted in PTZ convulsion thresholds that were reduced relative to the acute effect, although they were still significantly higher than in the vehicle group. Similarly, a challenge dose of 10 mg/kg diazepam given after 2 or 4 weeks of chronic treatment with 10 mg/kg diazepam, resulted in PTZ convulsion thresholds that were reduced relative to acute effects

Acute administration of CGS 9896, consistent with a previous report [3], produced a statistically significant ($p<0.05$) increase in PTZ convulsion threshold which was smaller in magnitude than that seen after acute diazepam treatment, and which appeared to be asymptotic over the range of doses assessed (Fig. 3B) This anticonvulsant effect of CGS 9896 was not reduced after chronic treatment with either the 10 or 30 mg/kg doses of CGS 9896. After 4 weeks of dosing with 3 mg/kg of CGS 9896, the increase in PTZ convulsion threshold was not statistically significant. This could reflect a slight degree of tolerance to the anticonvulsant effect of CGS 9896, or could be due to the weak anticonvulsant effect seen at this low dose of the compound. The reduction in convulsion threshold at the 3 mg/kg dose after 4 weeks of dosing was not significant relative to the acute effect of this dose

Withdrawal Experiment

Changes were seen in motor activity at 24 hr ($F=4.99$–$9\ 88$, $p<0.01$) and 72 hr ($F=2.34$–$4\ 49$, $p<0.05$ at 3, 12, 15 min, $p=0.06$ at 9 min, ns at 6 min) of withdrawal, but not at 8 and 15 days of withdrawal. Specifically, at 24 hr the distance travelled measure for the diazepam treated, precipitated withdrawal group showed statistically significant decreases ($p<0.05$) at each of the three minute intervals tested (Fig. 4B). No other changes were seen at 24 hr (Fig. 4A and B). At 72 hr the distance travelled measure for the diazepam treated, precipitated withdrawal group was significantly de-

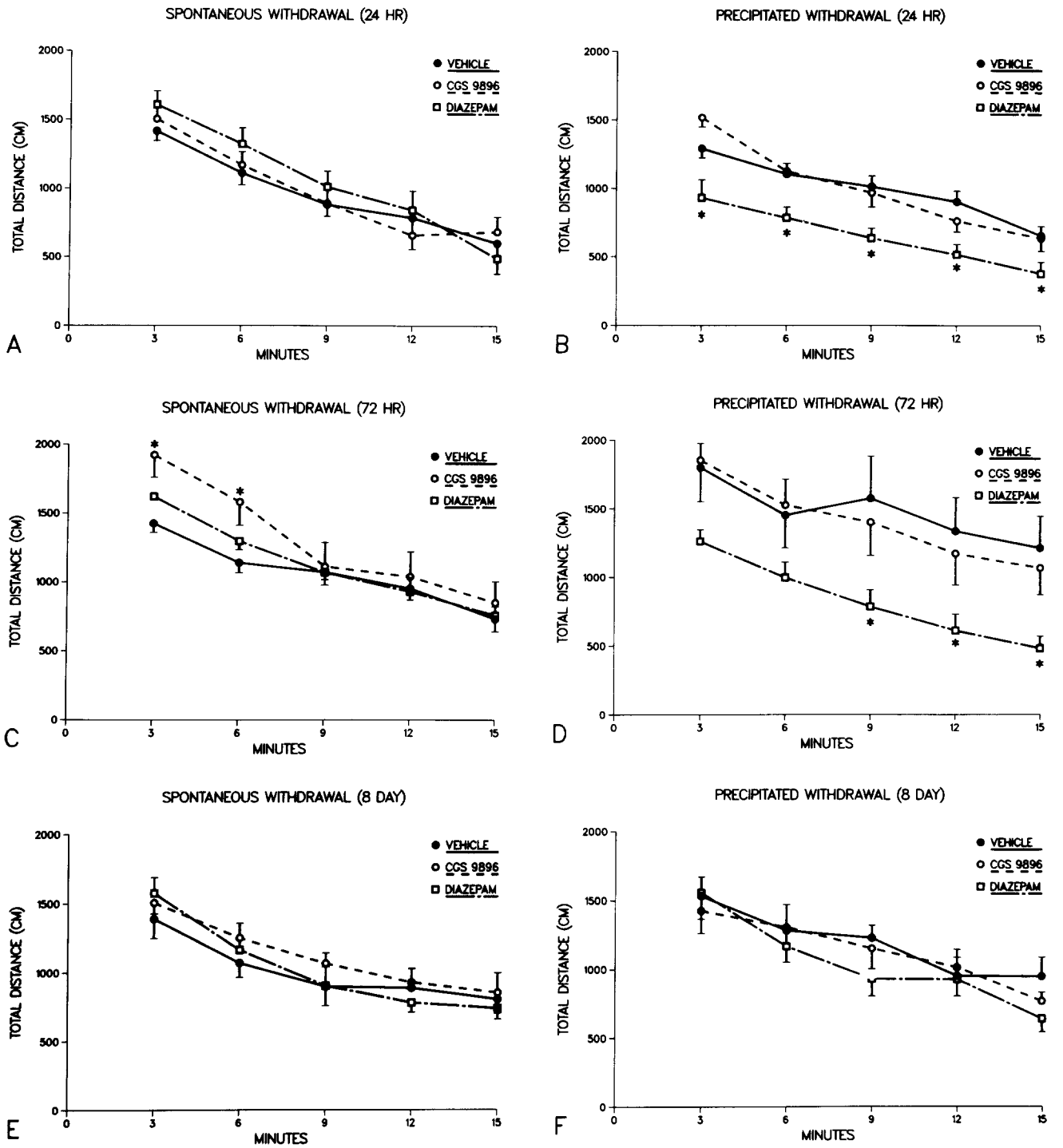

FIG 4 Effects of spontaneous (A, C, E) or precipitated (CGS 8216, 30 mg/kg) (B, D, F) withdrawal on distance travelled by mice in a 15 min session 24 hr (A, B), 72 hr (C, D), or 8 days (E, F) after 4 weeks of treatment with diazepam or CGS 9896 (30 mg/kg) *$p<0.05$ vs vehicle (Dunnett's test)

TABLE 1

MEAN (±SE) PTZ CONVULSION THRESHOLD (mg/kg) SPONTANEOUS WITHDRAWAL

Drug	Withdrawal Duration 24 hr	72 hr	8 Days	15 Days
Vehicle	46 5(2 4)	42 3(2 4)	48 9(3 2)	43 1(2 2)
CGS 9896	44 7(6 3)	44 9(4 0)	39 7(2 7)	39 1(3 4)
Diazepam	36 7(4 6)	42 9(4 7)	41 2(2 7)	30 4(2 5)*

*$p<0$ 05 vs vehicle

Convulsions were produced by injecting pentylenetetrazol (2 5 mg/ml IV) in 0 05 ml increments every 10 sec Groups were chronically treated with vehicle, diazepam (30 mg/kg PO), or CGS 9896 (30 mg/kg PO) for 4 weeks and then allowed drug free periods of the durations indicated The diazepam-treated spontaneous withdrawal (15 day) group showed a reduced PTZ convulsion threshold relative to vehicle treated animals CGS 9896-treated mice showed PTZ convulsion thresholds that were similar to those of vehicle-treated mice at all withdrawal times

TABLE 2

MEAN (±SE) PTZ CONVULSION THRESHOLD (mg/kg) PRECIPITATED WITHDRAWAL

Drug	Withdrawal Duration 24 hr	72 hr	8 Days	15 Days
Vehicle	23 3(3 6)	25 6(4 8)	27 4(4 5)	26.6(2 6)
CGS 9896	23 1(2 4)	27 1(4 3)	24 1(4 1)	22 1(5 4)
Diazepam	16 8(2 1)	22 4(3 1)	18 9(3 0)	18 0(1 9)*

*$p<0$ 05 vs vehicle

As for Table 1, except that all groups were treated with CGS 8216 (30 mg/kg PO) 60 min prior to testing The diazapam-treated precipitated withdrawal groups showed a trend toward a reduced PTZ convulsion threshold relative to vehicle-treated animals, which was statistically significant for the 15 day withdrawal group CGS 9896-treated mice showed PTZ convulsion thresholds that were similar to those of vehicle-treated mice at all withdrawal times

creased at the 9, 12, and 15 min intervals (Fig 4D) In addition, the movement time measure was decreased in this group at the 3 and 6 min time interval (data not shown) In contrast to these effects after diazepam treatment, the CGS 9896 spontaneous withdrawal group showed a statistically significant increase in the distance travelled measure at the 3 and 6 min interval (Fig. 4C) No effects on motor activity were seen at later periods of withdrawal from diazepam or CGS 9896 (Fig. 4E and F)

Statistically significant changes ($F=5$ 98, $p<0.01$) were also seen in the PTZ convulsion threshold measure in the diazepam-treated groups at the 15-day withdrawal interval. PTZ convulsion threshold was reduced ($p<0.05$) for both spontaneous (Table 1) and precipitated (Table 2) withdrawal groups Although trends toward reduced PTZ convulsion threshold were seen at other diazepam withdrawal intervals, these were not statistically significant. PTZ convulsion threshold was not altered at any time after withdrawal from CGS 9896

DISCUSSION

Under the conditions of the present experiment, tolerance developed readily to the sedative/muscle relaxant effects, the motor activity effects, and the anticonvulsant effects of diazepam. These findings are in agreement with previous reports of the development of tolerance to the sedative/muscle relaxant [11,15], and anticonvulsant [12, 14, 15] effects of diazepam In contrast, there was no evidence of the development of tolerance after chronic administration of CGS 9896 It is also known that CGS 9896 and diazepam do not show cross tolerance in tests of muscle relaxation or antianxiety [1,7] To the extent that benzodiazepine tolerance and dependence may be linked, this experimental outcome suggests that CGS 9896 may be relatively free of dependence potential

It is possible that all of the tolerance to the effects of diazepam seen in this experiment was due to a selective reduction in the sedative/muscle relaxant properties of the drug This is, of course, the interpretation for the traction reflex data The observation that motor activity no longer decreased, but actually increased, after chronic administration of diazepam, may also reflect the development of tolerance to sedative/muscle relaxant effects. Increases in motor activity were also associated with lower doses of diazepam which most likely lack sedative/muscle relaxant effects, and with CGS 9896, a nonsedating anxiolytic.

The diazepam-induced elevations of PTZ convulsion thresholds were attenuated after chronic diazepam treatment, but remained significantly elevated above vehicle treatment levels Since tolerance occurs to the sedative/muscle relaxant properties, it is possible that a contribution to the apparent anticonvulsant effects of diazepam comes from these sedative/muscle relaxant actions [3]. CGS 9896 produced a consistent but smaller degree of convulsion threshold elevation, which did not change with chronic treatment The lack of sedative/muscle relaxant effects of CGS 9896 may account for the lack of tolerance to this anticonvulsant activity

The second experiment explored the question of whether CGS 9896 would produce withdrawal effects upon cessation after chronic treatment. Chronic diazepam treatment and subsequent precipitated withdrawal resulted in motor activity changes It is important to note that this decrease in motor activity occurred in the absence of diazepam. Although the direction of this effect is similar to that seen after acute treatment with diazepam, it is unlikely that it reflects sedative/muscle relaxant properties of the drug, since no effects were seen on the traction reflex measure

Chronic CGS 9896 treatment and subsequent withdrawal had no effect on motor activity except that after 3 days of withdrawal the CGS 9896 treated mice showed an increase in distance travelled relative to controls. This type of effect was also seen after both acute and chronic CGS 9896 treatment. It is clear that withdrawal from CGS 9896 does not produce effects on motor activity similar to those seen after diazepam withdrawal. This suggests that CGS 9896 may not produce physical dependence of a type that is characteristic of benzodiazepines [8, 19, 20, 22, 23] Of course, it should be kept in mind that this conclusion is based on the set of parameters for drug administration used in the present experiments It is possible that a paradigm employing higher doses or more frequent drug administration would reveal physical dependence properties of CGS 9896 Still, the dose of CGS 9896

used was relatively high (e.g., 3 mg/kg will readily produce an anxiolytic or anticonvulsant effect) and equivalent to the diazepam dose since the two drugs show equipotent effects on anxiolytic measures [2,3]. Although one cannot unequivocally conclude that CGS 9896 does not produce physical dependence from the present studies, at least it is clear that CGS 9896 does not produce dependence as readily as diazepam. A more complete comparison of these two drugs could be accomplished by using a "chronically equivalent" dosing method [4]

The convulsion threshold data show further evidence of withdrawal effects after cessation of chronic diazepam treatment. This suggests an altered sensitivity of benzodiazepine receptors opposite to that seen in the presence of the drug. This increased susceptibility to convulsions is consistent with the presence of a withdrawal syndrome following chronic diazepam treatment. There were no effects on convulsion threshold at any withdrawal time following chronic treatment with CGS 9896.

It is clear from the present data that withdrawal effects of diazepam are more readily observed following precipitated than after spontaneous withdrawal. This is consistent with previous reports [17,19]. In the present experiments, motor activity changes were seen only in precipitated withdrawal conditions. Also, there was a clear trend for reduced convulsion threshold effects in the precipitated withdrawal groups although the only clear reduction in the spontaneous withdrawal groups came after 15 days Consistent with previous reports [22,23], the results of the present experiments confirm that withdrawal after chronic benzodiazepine administration can be observed after relatively long drug free periods. For example, up to 3 days after drug withdrawal, effects on motor activity were seen, and after 15 days effects on convulsion threshold were seen

The data from the withdrawal experiment support the hypothesis that CGS 9896 does not show dependence liability. When the tolerance and withdrawal data are considered together, this conclusion is supported further. These findings are consistent with an earlier report showing that CGS 9896 did not produce withdrawal effects in baboons [17]. CGS 9896 may, therefore, prove to be largely free of dependence potential in clinical studies

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