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BRIEF COMMUNICATION

Rapid Tolerance to the Depressive Effects of Diazepam on Guinea Pig Motor Control Using Divided Doses

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SMITH, P. F. AND C. L. DARLINGTON. *Rapid tolerance to the depressive effects of diazepam on guinea pig motor control using divided doses.* PHARMACOL BIOCHEM BEHAV **48**(2) 535–538, 1994.—The effects of acute and chronic IP injections of diazepam on the guinea pig righting reflex latency (RRL) were measured using an automated measurement system known as a "tolerometer." Single IP injections of 2.0, 6.0, 18.0, and 20.0 mg/kg diazepam significantly increased the RRL compared to no injection (naive animals), diazepam vehicle injections, or 1.0 mg/kg diazepam injections. The effects of chronic IP injection schedules on the RRL were compared: 18 or 20 mg/kg in a single, once daily injection for 5 days; 6 mg/kg in a single, once daily injection for 5 days; and 6 mg/kg, three times a day, for 5 days. Neither 20, 18, nor 6 mg/kg/day for 5 days resulted in significant tolerance to the depressive effects of diazepam on the righting reflex. By contrast, when 6 mg/kg was administered three times a day for 5 days, tolerance developed by the third day of treatment. There were no differences between the three groups in the amount of exposure to the measurement apparatus or the testing situation. These results support the view that species like guinea pig and rat that metabolise diazepam rapidly, develop tolerance more quickly if diazepam is administered in divided doses or by continuous release; this may be because the duration of the occupation of CNS benzodiazepine recognition sites is a critical factor in the development of tolerance.

Benzodiazepines Diazepam Tolerance Dependence Righting reflex Vestibular reflexes

THE development of tolerance to benzodiazepine (BDZ) tranquilizers is of importance both to clinical practice and to understanding the neurochemical mechanisms by which these drugs act on CNS neurons [see (29) for a review). Nonetheless, there is still much disagreement about whether, and how quickly, tolerance develops for some of the effects of BDZs [see (29) for a review]. This is particularly true in the case of diazepam. Although there is general agreement that tolerance to the anticonvulsant effects of diazepam occurs in less than a week (6,7,9,10,18), there is little agreement regarding the rate at which tolerance develops to its sedative and muscle relaxant effects (12,20–22) and whether tolerance occurs at all to its anxiolytic effects [cf., (1,3,12,24–27)]. It is probable that many of the apparent discrepancies in the literature are due to differences in diazepam dose, route, and frequency of administration [see (3) for a review].

Davis and Gallager (3) suggested that many studies have failed to obtain tolerance to the effects of diazepam in rats because this species eliminates diazepam very rapidly [the elimination half-life is approximately 1.1 h; (8,16,18)] and, therefore, BDZ recognition sites are occupied only transiently following a single administration. In fact, most lower mammals metabolize diazepam at least three times as rapidly as humans (16). The hypothesis proposed by Davis and Gallager (3) was supported by their observation that tolerance to the effects of diazepam on the acoustic startle reflex developed in 5 days when 5 mg/kg/day diazepam was released continuously by an SC silastic capsule, but did not develop within 3 weeks when 5 mg/kg/day was administered in single, once daily IP injections (3).

We were interested in testing the hypothesis that diazepam tolerance occurs more rapidly with divided doses by examining

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the effects of diazepam on the guinea pig righting reflex. The righting reflex is a useful behavioral variable to measure in chronic drug studies because it is generated by a fast, vestibular reflex pathway and usually occurs within 1 s in an untreated animal (4,22). Although some previous studies have reported tolerance to the sedative and muscle relaxant effects of diazepam in 2–3 days (20,21), tolerance to the depressive effects on the righting reflex has been reported to occur only slowly, if at all, with single, once daily injections (14,21,23). We, therefore, compared the chronic effects of diazepam on the righting reflex latency when it was delivered in single, once daily, IP injections or three IP injections per day, spaced throughout the day.

METHOD

Subjects

The subjects were 32 male, pigmented guinea pigs weighing between 250 and 450 g. Animals of the same gender and similar age were chosen because these factors are known to influence the effects of diazepam (11,15). The animals were housed in pairs in a room maintained at 22°C with a 12 L : 12 D cycle; food and water were available ad lib.

Drug Injections

Diazepam (Valium 10, Roche, New Zealand) was administered IP in all cases. The IP route was chosen because this route is subject to first-pass metabolism (2) and, therefore, is more similar to oral administration than other injection routes.

Acute Experiment

In the acute experiment, 28 animals were used to examine the acute effects of different doses of diazepam on the righting reflex latency (RRL). Four animals were randomly assigned to each of the following seven groups: a) naive (no injection); b) diazepam vehicle injection (1.2 ml/kg of 3% sodium benzoate, 8% ethanol, 89% saline); c) 1.0 mg/kg diazepam; d) 2.0 mg/kg diazepam; e) 6.0 mg/kg diazepam; f) 18.0 mg/kg diazepam; g) 20.0 mg/kg diazepam. No animal received more than one injection.

Chronic Experiment

The chronic experiment consisted of the following four groups, each with four animals: a) 6.0 mg/kg diazepam, three times a day for 5 days (injections administered between 0800–0900 h, 1200–1300 h, 1700–1800 h); b) 6.0 mg/kg diazepam in a single, once daily injection for 5 days; c) 18.0 mg/kg diazepam in a single, once daily injection for 5 days; d) 20.0 mg/kg diazepam in a single, once daily injection for 5 days. The animals in the latter three groups had been used in the acute experiment and their injections were then continued according to the protocol described. The RRLs of the animals in these groups were measured only once a day. The animals receiving three 6 mg/kg diazepam injections per day were exposed to the testing apparatus only once daily, as for the other groups.

Our previous studies have shown that daily diazepam vehicle injections have no significant effect on the RRL, even over periods as long as 6 weeks (14,23). Therefore, we did not examine the effects of vehicle injections in the current chronic experiments.

Measurements

All measurements were made 30 mins following the IP injections because pharmacokinetic studies indicate that peak blood plasma concentrations of diazepam are attained approximately 20 min following a 5 mg/kg IP diazepam injection (13). Our own behavioral studies have also confirmed that the maximal behavioral effects of diazepam are observed approximately 20–30 min following an IP injection (14,23). In the case of the groups that received 6 mg/kg/day and 6 mg/kg three times a day, measurements were also made the day before the drug injections began ("Day 0"), to determine the baseline RRLs for these animals.

Daily RRL measurements were made using a device called a "tolerometer"; a detailed description of this device and the method employed in obtaining RRL measurements has been given elsewhere (4). Briefly, the tolerometer consists of a semicylindrical platform positioned on a 2 kg load cell, which is connected to a strain gauge amplifier (Radio Spares Ltd, New Zealand). The amplifier is connected to one channel of a MacLab data acquisition system (Analog Digital Instruments, New Zealand), controlled by a MacClassic computer. The MacLab Chart program (V2.52) is used to display the output from the MacLab system, which samples from the strain gauge amplifier at 20 Hz, providing a resolution of 0.05 s. When the animal is placed on the tolerometer platform in the supine position, the generation of a righting reflex produces a characteristic waveform on the computer screen. The latency (in s) to completion of the righting reflex can be measured on the screen using cursors avaliable in the Chart program (4).

Statistical Analysis

Analyses were carried out using a one-way analysis of variance (ANOVA) for the acute experiment and linear regression ANOVAs for the chronic experiment (excluding the data for day 0) (28). The significance level was set at 0.05 for all comparisons.

RESULTS

In the acute experiment, diazepam significantly increased the RRL with increasing dose, $F(6, 21) = 8.8, p \leq 0.005$; see Fig. 1). At 1.0 mg/kg, the RRL was similar to uninjected (naive) animals and vehicle-injected animals, which generated a righting reflex in less than 1 s. For 2.0 and 6.0 mg/kg, the

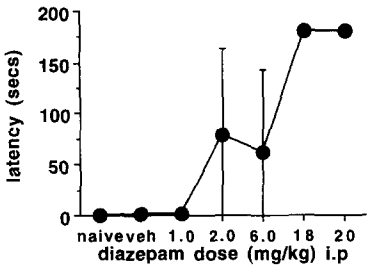

FIG. 1. Acute effects of single, IP diazepam or vehicle injections on the righting reflex latency. Naive (no injection; $n = 4$ animals). Veh (diazepam vehicle injection, 1.2 ml/kg; $n = 4$). 1.0 (1.0 mg/kg diazepam; $n = 4$); 2.0 (2.0 mg/kg; $n = 4$); 6.0 (6.0 mg/kg; $n = 4$); 18.0 (18.0 mg/kg; $n = 4$); 20.0 (20.0 mg/kg; $n = 4$). Symbols indicate means. Bars represent ± 1 SD. Note that the SD bars are smaller than the mean symbols for the naive, vehicle, and 1.0 mg/kg diazepam groups.

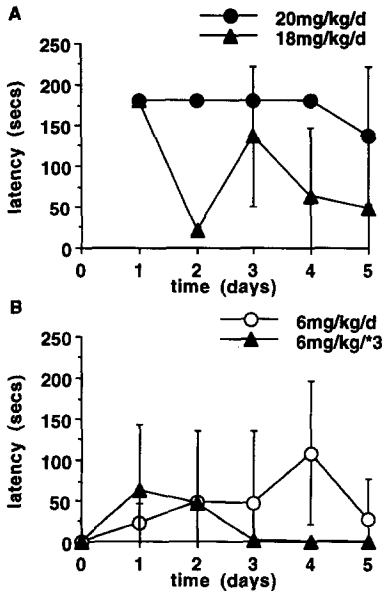

FIG. 2. Chronic effects of IP diazepam injections; (A) 20 mg/kg/day: 20 mg/kg in a single, once daily injection for 5 days (n = 4); 18 mg/kg/day: 18 mg/kg in a single, once daily injection for 5 days (n = 4); (B) 6 mg/kg/day: 6 mg/kg in a single, once daily injection for 5 days (n = 4); 6 mg/kg/*3: 6 mg/kg, three times a day for 5 days (n = 4). Symbols represent means. Bars represent ± 1 SD. Note that where no SD bar can be seen, they are smaller than the mean symbols.

increase in the RRL was variable between animals but the average increase was similar (about 60–70 s). At 18 and 20 mg/kg, none of the animals generated a righting reflex in less than 3 min.

In the chronic experiment, the animals that received 18 mg/kg/day diazepam for 5 days showed highly variable RRLs (see Fig. 2A). The mean RRL decreased over the 5 days of treatment; however, there was no significant linear regression and, therefore, no evidence of tolerance, $F(1, 18) = 3.0$, $p > 0.05$. The animals that received 20 mg/kg/day for 5 days showed very long RRLs (see Fig. 2A). During the first 4 days, none of these animals generated a righting reflex within 3 min; over 5 days there was no significant linear regression, $F(1, 18) = 2.1, p > 0.05$.

Animals that received 6 mg/kg/day showed a variable increase in the RRL over the 5 days of treatment; there was no evidence of the development of tolerance over time, $F(1, 18) = 0.4, p > 0.05$ (see Fig. 2B). By contrast, the animals that received 6 mg/kg, three times a day, showed significant tolerance over the 5 days of treatment, $F(1, 18) = 4.4, p \leq 0.05$; mean slope = −16.8 ± 8.0 (SE). Even by day 3, the average RRL was less than 1 s (see Fig. 2B).

DISCUSSION

The present data indicate that tolerance to the depressive effects of diazepam on the righting reflex does not occur with a single, once daily IP injection of a high dose (e.g., 18 or 20 mg/kg) for 5 days. By contrast, if 6 mg/kg IP is administered three times a day using spaced injections (i.e., 18 mg/kg/day IP in total), complete tolerance develops within 3 days. However, a single, once daily 6 mg/kg IP injection of diazepam for 5 days does not produce significant tolerance.

It is not possible that tolerance developed more rapidly in the divided dose group due to greater exposure to the measurement apparatus and testing situation, because these animals were exposed to the laboratory testing room only once a day as for the other groups. The animals in the divided dose group and the 6 mg/kg/day group received exactly the same dose of diazepam in the same vehicle volume, 30 min before their RRLs were measured; therefore, the rapid development of tolerance in the divided dose group can be explained only by the additional 6 mg/kg injections, which they received between 0800–0900 h and 1700–1800 h each day. The total amount of diazepam administered to the divided dose group cannot account for their rapid development of tolerance because the 18 mg/kg/day group received the same total dose of diazepam in a single, once daily injection and yet did not develop tolerance over the 5 days of treatment. Even a higher, single daily dose (20 mg/kg) did not induce tolerance over 5 days.

These results are in complete agreement with Davis and Gallager's (3) hypothesis that in species in which diazepam is metabolised rapidly, tolerance develops more quickly with continuous release or divided doses, even if the peak blood plasma levels that are achieved are lower than following a single large dose. Davis and Gallager (3) suggested that tolerance may develop more rapidly and completely when BDZ recognition sites are occupied more continuously. The present data are consistent with this suggestion; however, we cannot exclude the possibility that the rapid tolerance we observed with divided doses was due to pharmacokinetic factors such as increased metabolism of diazepam. Nonetheless, other studies have demonstrated that tolerance to the sedative effects of BDZs such as lorazepam and triazolam can occur in as little as 3 days, and that this rapid tolerance is not attributable solely to pharmacokinetic parameters [e.g., (5,17)]. The possible contribution of pharmacokinetic changes to the rapid development of tolerance to the effects of diazepam on the RRL will require further investigation.

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