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BRIEF COMMUNICATION

Quantification of the Depressive Effects of Diazepam on the Guinea Pig Righting Reflex

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SCOTT, S. J., P. F. SMITH AND C. L. DARLINGTON. *Quantification of the depressive effects of diazepam on the guinea pig righting reflex.* PHARMACOL BIOCHEM BEHAV 47(3) 739–741, 1994.—The effects of 5 mg/kg/day diazepam (IP for 21–39 days) on righting reflex latency (RRL) and neuronal activity in the medial vestibular nucleus (MVN) were investigated in guinea pigs. Diazepam treatment increased the RRL relative to vehicle-injected controls ($p < 0.05$, ANOVA); although the average RRL in the diazepam-treated animals did decrease over time, this decrease was not statistically significant and therefore evidence of tolerance was not obtained. MVN slices were removed from diazepam-treated animals and recordings were made from MVN neurons in vitro. The average resting activities for MVN neurons in slices from diazepam-treated animals and uninjected animals from a previous study were not significantly different.

Diazepam Righting reflex Tolerance Benzodiazepines Medial vestibular nucleus

THE abolition of the righting reflex is sometimes used as an index of the degree of sedation induced by CNS depressant drugs (10,12). However, few studies have quantified the effects of sedatives on the righting reflex (9); usually it is measured only qualitatively, noting whether or not the reflex occurs for a particular drug dose (10). The righting reflex is a useful behavioral variable to quantify because it is generated by a simple, well-defined reflex pathway (8) and therefore it is easy to interpret and relate to other measurements involving electrophysiological and pharmacological techniques. To our knowledge, currently there are no quantitative data available on the acute or chronic effects of diazepam on the righting reflex. In some studies using rats (4), chronic administration of diazepam (5 mg/kg/day, IP, for 2 weeks) has been reported to induce tolerance to its sedative effects on maze and open field activity. However, many studies using rats have reported that tolerance to the anticonvulsant (5), anxiolytic (1), and muscle relaxant effects (7) of diazepam develops within 2–3 weeks only when high daily IP doses [e.g., 20 mg/kg/day (1)] or a method permitting continuous release of diazepam are used [e.g., the SC silastic capsule method (1)]. One reason for the difficulty in obtaining diazepam tolerance on some behavioral measures in the rat is that this species metabolises benzodiazepines very rapidly [the elimination half-life of diazepam is approx. 1.5 h following a 5 mg/kg IP injection (2)]; therefore, it is difficult to maintain specific blood plasma concentrations of diazepam (1). We were interested in addressing three questions. First, what are the acute effects of a 5 mg/kg IP injection of diazepam on the latency to generate a righting reflex (righting reflex latency, RRL) in the guinea pig [5 mg/kg IP was chosen following previous studies in rats that showed this dose produces marked sedation (1,4)]? Second, will behavioral tolerance develop if the RRL is significantly increased by 5 mg/kg/day IP diazepam and this dose is administered chronically for 3–4 weeks [studies in the rat using the same treatment regimen have shown that tolerance may develop to the sedative effects of diazepam within 2 weeks (4) and that a reduction in the sensitivity of dorsal raphe neurons

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to GABA may also develop within 3-4 weeks (3)]? Third, if diazepam depresses the righting reflex by increasing its latency, will this correlate with a decrease in the resting activity of medial vestibular nucleus (MVN) neurons in the brain stem, which contribute to the generation of the righting reflex?

METHOD

Data were obtained from a total of 12 guinea pigs (250–500 g). They were housed in groups of two in a room maintained at 22°C with a 12 L : 12 D cycle; food and water were available ad lib. Ten animals were used in the RRL study: six of these received 5 mg/kg/day IP diazepam (Valium 10, Roche, New Zealand) in a 1 ml/kg volume for 21–39 days; four animals received 1 ml/kg/day IP vehicle injections (i.e., 3% sodium benzoate, 8% ethanol, 89% NaCl) for the same period of time. To study the resting activity of MVN neurons, slices of the MVN were removed from seven guinea pigs that received diazepam injections as described above; five of these animals were used in the behavioral study and two were additional animals.

Animals were used for slice experiments between 22–26 h following the final diazepam injection so that a withdrawal syndrome was not induced. Diazepam or vehicle injections were administered at approximately the same time each day in the same environment. Measurements of RRL were made every day for at least 29 days according to a method described in detail previously (9). Briefly, an animal was placed in the supine position on a semicylindrical platform on a set of modified kitchen scales; the pointer on the face of the scales indicated the animal's weight and two Hall effect switches were positioned on either side of the pointer at an angle of about 20°. The Hall effect switches were connected to a digital timer that was started with a foot pedal when the animal was placed in the supine position and stopped automatically when the pointer on the face of the scales crossed one of the switches during the performance of a righting reflex. The timer therefore provided an RRL measurement in seconds. RRL was always measured before a drug or vehicle injection and then again 20–30 min following the injection.

The measurement time postinjection was based on pilot studies indicating that this was the approximate time of the maximal effect of diazepam on the RRL. The preinjection RRL was then subtracted from the postinjection RRL to provide an RRL difference (in s), which controlled for any daily fluctuation in the preinjection RRL. The mean RRL difference for each day was calculated for the diazepam and vehicle groups and the data were analysed using a two-way analysis of variance (ANOVA) with repeated measures on time. One animal in the diazepam group died on day 17 and another had to be used for slice experiments on day 21 due to poor health; therefore, from day 22 there were four animals remaining in the diazepam group. Since other animals were used for slice experiments after day 29, only RRL data up to and including day 29 were analysed.

Following completion of the behavioral measurements, animals were anesthetised, decapitated, and slices of the MVN were prepared for neuronal recording as described in detail previously (11). The resting activity of single MVN neurons was recorded extracellularly using glass micropipettes. Action potentials were amplified using a Dagan single electrode amplifier, displayed on an Iwatsu digital storage oscilloscope and monitored using a Grass audiomonitor. Spike frequency was analysed on-line either using the computational facilities of the oscilloscope or by using a MacLab data acquisition system (40 kHz sampling frequency) to display action potentials on a Macintosh LC (Chart program).

RESULTS

Vehicle-injected or uninjected guinea pigs typically generated a righting reflex within 1-2 s each time they were placed in the supine position; this meant that there was virtually no difference in RRL before and after the vehicle injection (Fig. 1). By contrast, a 5 mg/kg IP injection of diazepam increased the RRL to between 300 and 400 s in some cases, resulting in large RRL differences between measurements before and after the drug injection [$F(1, 6) = 11.83, p < 0.05$, ANOVA; Fig. 1]. After approximately 3 weeks of diazepam injections, animals were showing some signs of tolerance to the diazepam: the postinjection RRLs were very short compared to those generated during the first 3 weeks, resulting in only small RRL differences between the pre- and postinjection tests (Fig. 1); qualitative observations indicated that animals experienced fewer sedative and anxiolytic effects. Nonetheless, the diazepam effects on the RRL were still variable and the ANOVA showed no significant interaction ($p > 0.05$) between drug and time, which would be indicative of the development of tolerance.

Comparison of the resting activity of MVN neurons in slices from diazepam-treated animals and MVN neurons in slices from uninjected animals from a previous study (11) showed no significant difference (16.0 ± 9.1 spikes/s, n = 23 neurons, compared to 14.9 ± 8.6 spikes/s, n = 41, respectively; $p > 0.05$, two-tailed unpaired t-test).

DISCUSSION

The present study indicates that a 5 mg/kg IP injection of diazepam significantly increases the RRL in guinea pigs relative to vehicle-injected controls. The average effect of diazepam on the RRL does decrease after about 3 weeks of injections; however, the effects were still variable between animals

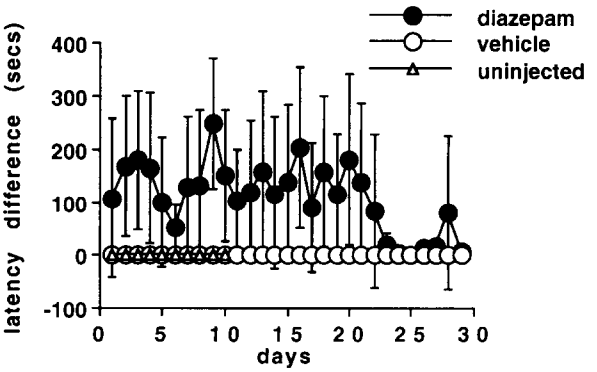

FIG. 1. Effects of 5 mg/kg/day (IP) diazepam on the righting reflex latency in guinea pigs (diazepam; filled circles, n = 6) compared to vehicle-injected animals (1 ml/kg/day, IP, vehicle; open circles, n = 4) and uninjected animals [uninjected; open triangles; data from (9); n = 4]. All symbols represent the mean latency difference in seconds (i.e., postinjection minus preinjection in the case of the diazepam- and vehicle-injected groups). Bars represent ± 1 SD of the mean. Note that the data points for the vehicle-injected and uninjected groups overlap during the first 10 days and that for both groups the SD bars are smaller than the mean symbols.

up to 4 weeks, and the reduction in the effect of diazepam on the RRL was not statistically significant. Therefore, tolerance did not develop despite the large increases in RRL that diazepam injections produced during the first 3 weeks (100–200 times the normal RRL in most cases; Fig. 1). The average resting activity of MVN neurons in vitro was not significantly different in slices from diazepam-treated and uninjected guinea pigs (11); Gallager et al. (3) have also reported that chronic diazepam treatment does not significantly alter the resting activity of rat dorsal raphe neurons. In some previous studies, tolerance has been reported to develop following 5 mg/kg/day IP diazepam for 2–4 weeks (4); in other studies, IP doses 3–4 times greater than this have been necessary for tolerance to develop (1,6). It may be that, similar to the rat, the guinea pig metabolism of diazepam is rapid and therefore multiple IP injections or continuous release of diazepam are necessary to achieve steady blood plasma concentrations (1). Single, daily, 5 mg/kg IP diazepam injections probably produce only short-term benzodiazepine receptor occupation; Davis and Gallager (1) have suggested that continuous occupation of benzodiazepine binding sites may be necessary for tolerance to develop.

It is clear from the data obtained in the present study that the extent to which a 5 mg/kg IP injection of diazepam increases the RRL is highly variable between animals (see Fig. 1). Given this variability, what is the advantage of using a quantitative analysis of RRL over qualitative analyses that are simpler to perform? The main advantage is the additional information that is available regarding the variability in response between animals. Using a qualitative analysis of the data presented in this study, it is possible to conclude that tolerance developed after 3 weeks of diazepam treatment. However, a quantitative analysis, using parametric statistics (e.g., ANOVA) to evaluate the drug effect over time in relation to the variability between animals, indicated that the reduction in RRL was not statistically significant.

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