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A Behavioral and Electrocorticographic Comparison of Diazepam and Pentylenetetrazol in Rat Pups

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SMYTHE, J. W., C. L. RYAN AND B. A. PAPPAS. *A behavioral and electrocorticographic comparison of diazepam and pentylenetetrazol in rat pups.* PHARMACOL BIOCHEM BEHAV 30(2) 479–482, 1988.—This experiment assessed the possibility suggested by previous research that benzodiazepines cause convulsions in infant rats. Seven-day-old Wistar rats were randomly assigned to receive either diazepam (DZP) (0, 0.5 or 2.5 mg/kg), the convulsogen pentylenetetrazol (PTZ) (50 mg/kg), or DZP followed 30 minutes later by PTZ. The amount of paddling and wall progression and head and body tremors was recorded for each group. Both DZP and PTZ elevated paddling and wall progression, but only PTZ elevated head and body tremor scores. DZP antagonized the PTZ-induced increases in head and body tremors. In a second experiment, seven-day-old pups were implanted with cortical electrodes. The following day, baseline electrocorticograms (ECoGs) were taken for each animal. Each pup subsequently received either DZP vehicle, 0.5 mg/kg DZP, 50 mg/kg PTZ, or 0.5 mg/kg DZP followed 30 minutes later by 50 mg/kg PTZ. Neither the vehicle nor the DZP injections altered ECoG activity. In contrast, PTZ-treated pups showed continuous, high-amplitude, spiking activity. Pretreatment with DZP eliminated these PTZ-induced alterations in ECoG activity. We conclude that in infant rats, the behavioral and electrophysiological effects of DZP and PTZ are distinct from one another. Furthermore, both the behavioral and the electrocorticographic effects of PTZ are blocked by DZP. It is unlikely that DZP causes seizures in neonatal rats.

Neonatal rat Diazepam Pentylenetetrazol Behavior Convulsion Electrocorticogram

PREVIOUS research has raised the possibility that benzodiazepines cause convulsions in infant rats. This was first suggested by Barr and Lithgow [2] who reported that flurazepam and chlordiazepoxide caused "behavioral convulsions" in young rats. Their conclusion was challenged by Pappas and Walsh [9] who showed that the behavioral effects of benzodiazepine administration to young rats differed from those of the established convulsant pentylenetetrazol (PTZ) and who argued therefore, that these effects probably did not reflect brain seizure activity. The following experiment involved additional behavioral comparisons of the benzodiazepine diazepam (DZP) with PTZ and included a comparison of their effects on cortical electroencephalographic activity. In addition, it examined if DZP blocked the effects of PTZ on behavior and on the electrocorticogram (ECoG), as it does in the adult rat [12].

Chemical convulsants such as pentylenetetrazol elicit behavior of the neonatal rat which differs markedly from that elicited of the adult rat. Vernadakis and Woodbury [11] thoroughly characterized the ontogeny of PTZ's behavioral effects, showing that up to approximately eight days of age, the drug caused hyperkinesia, paddling, running, shaking and hyperextension of the head.

It has been shown by Pappas and Walsh [8] that the benzodiazepines (BZs) cause some of these same behaviors (for example, hyperkinesia, characterized by paddling, wall progression and head and limb movements), in rat pups as old as nine days. PTZ also caused some of these behaviors, and in addition, produced head shaking and limb and body clonus. Head shaking and clonus did not occur after BZ administration however. Based on these differing behavioral effects of PTZ and the BZs, Pappas and Walsh argued that the report [2] that the BZs flurazepam and chlordiazepoxide caused "behavioral convulsions" in neonatal rats could not be interpreted to reflect that they caused brain seizures. Barr and Lithgow had defined these "behavioral convulsions" by the presence of "either unilateral tonic-clonic movements or bilateral tonus in association with one or more of a constellation of convulsion-related behaviors, including Straub tail, jaw tremor, full body spasm, limb paralysis, loss of righting reflex, and 'swimming' movements." Like Pappas and Walsh, File and Wilks [3] observed different effects of BZs and the convulsant, picrotoxin. They suggested that the BZs may cause a form of seizure different from that of picrotoxin. Recent reports of differing behavioral effects of flurazepam and the proconvulsant beta carbolines [6,7] have raised further doubt regarding the convulsogenic character of the BZs for neonatal rats.

Behavioral observation by itself, however, may be an inappropriate criterion for seizures, especially for the infant rat whose skeletal behaviors are still poorly differentiated. Furthermore, there may be several differing skeletal motor

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manifestations of brain seizure activity, one of which is characteristic of the BZs. If the BZs cause seizures in neonatal rats, there are theoretical and clinical significances. In the first instance, Barr and Lithgow [2] have suggested that it could reflect the differential functional ontogeny of type I and II receptors. This possibility was further elaborated by File and Wilks [3] who suggested that this differential ontogeny could allow for the discovery of the behavioral relevances of the type I and II receptors. The clinical significance is due to the therapeutic use of the BZs during pregnancy and the early postnatal period [5]. If the BZs are convulsogenic for the fetus/neonate, such use could have adverse and long-lasting neural consequences.

METHOD

Subjects

Wistar rat pups, bred in our colony, served as subjects. Pups with their mothers were housed in standard maternity cages, on a reverse light cycle (off at 08:00, on at 20:00 hr). All testing took place between 13:00 and 17:00 hr.

Behavioral Testing

At seven days of age, pups were taken from their mothers and randomly assigned to receive either saline, vehicle, 0.5 or 2.5 mg/kg DZP, 50 mg/kg PTZ or 0, 0.5 or 2.5 mg/kg DZP followed 30 minutes later by 50 mg/kg PTZ (n=15/group). The water-based vehicle for DZP was the same as that used by Pappas and Walsh [8]. All injections were given in the nape of the neck with 26 gauge needles. After drug administration, pups were placed in circular chambers (15 cm in diameter, 17 cm high), and videotaped using a time-lapse RCA recorder and camera. The recording unit was set to scan at 12 frames per second. Test trials lasted 50 minutes. The videotapes were later scored by an experimenter who was blind to the drug regimes. Scoring was done using a time sampling procedure (15 seconds out of each minute of the test trial). Three measures were chosen beforehand as dependent variables: paddling and wall progression, head tremors and body tremors. Paddling and wall progression consisted of combinations of limb movements that resembled swimming motions, or continued forward crawling when forward locomotion was impeded by the chamber walls. Head tremors consisted of rapid lateral saccade-like movements of the head, or quick up and down motions. Body tremors consisted of twitching motions and trembling of the hind-quarters, which would sometimes result in loss of righting.

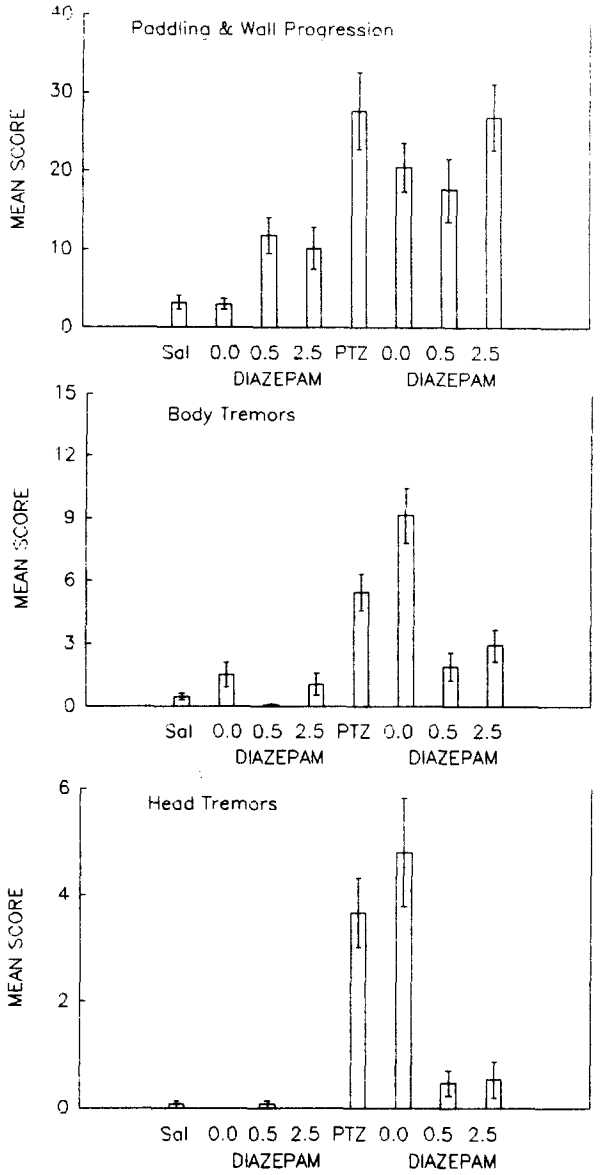

FIG. 1. The effects of saline, diazepam (0.0, 0.5 or 2.5 mg/kg), PTZ and PTZ plus diazepam (0.0, 0.5 or 2.5 mg/kg) on scores for paddling and wall progression (upper panel), body tremors (middle panel) and head tremors (lower panel). Group means ±S.E.M. are plotted. Note that the ordinal scales vary for the different measures.

ECoG Recording

Electrode implants were done at seven days of age in naive rat pups. The construction of the electrodes and implantation procedure were adapted from Gilbert and Cain [4] and Schickerova *et al.* [10]. Electrodes were constructed of Teflon-coated electrode wire, bent into an elbow shape. Approximately 0.5 mm of the electrode tip was scraped clean of Teflon. The electrode shaft was about 3.5 mm in length, while the distal bent portion was about 1 mm.

Surgical implantation of the electrodes was done under metofane (Pitman-Moore, Mississauga) anesthesia. A midline incision was made on the top of the skull, exposing bregma and lambda. Holes were drilled bilaterally, approximately 2.5 mm lateral to the midline suture and halfway between bregma and lambda. Electrodes were slipped under the skull on each side, leaving exposed 3.5 mm shafts which lined up along the midline. A reference electrode was placed under the skin along the nasal bone. The electrodes were anchored in place using Durelon cement (Klink-Pulver, W. Germany), to form a firm base on the skull. The headcap was reinforced using Nuweld dental cement (Caulk Co., Canada). The incisions were sutured around the edges of the headcaps. The pups were placed on heating pads for 1–2 hours. Recovery from anesthesia was relatively quick and the pups were fully responsive when returned to dam.

The following day pups were removed from their dams and individually placed in a cardboard container (15×15×5 cm), located inside a Faraday cage. The electrodes were connected to a Beckman Dynograph with an amplification to 0.2 mV/cm and a 30 Hz high frequency rolloff. Initially,

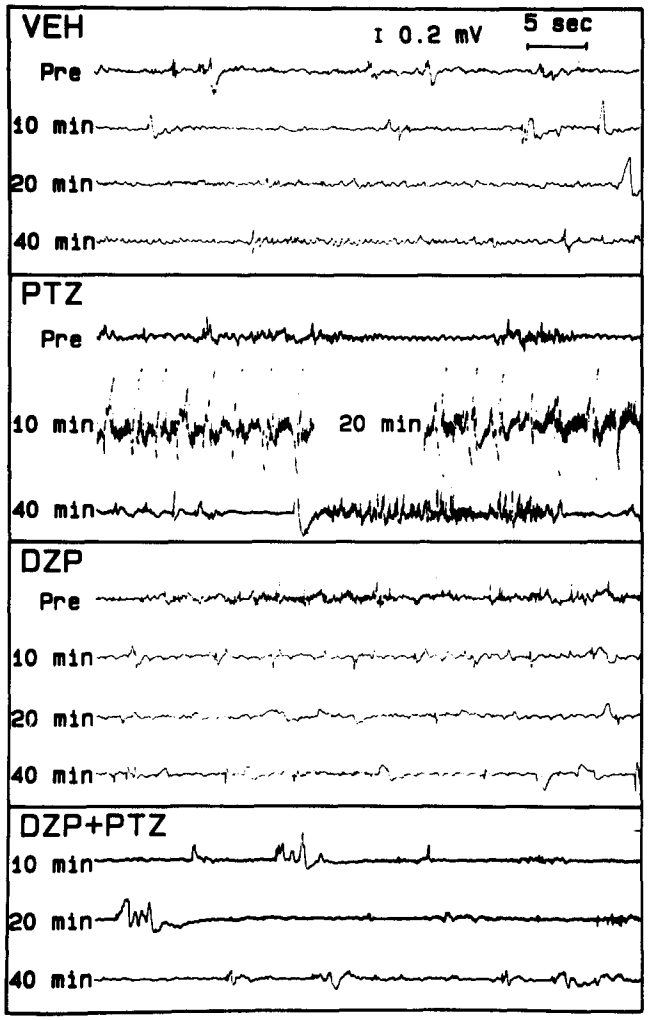

FIG. 2. Representative electrocorticograms from rat pups treated with vehicle, 50 mg/kg PTZ, 0.5 mg/kg DZP or 0.5 mg/kg DZP, followed 30 minutes later by 50 mg/kg PTZ. The voltage and time calibration for all traces is shown in the top panel.

spontaneous ECoG activity was recorded for each pup over a 10-minute trial. Following this, three different groups of pups received a subcutaneous injection of one of the following: vehicle (n=6), 50 mg/kg PTZ (n=6) or 0.5 mg/kg DZP (n=8). Ten minutes following this injection, ECoG activity was recorded for 40 minutes. A fourth group of pups was pretreated with 0.5 mg/kg DZP, followed 30 minutes later by an injection of 50 mg/kg PTZ (n=6). Ten minutes following PTZ administration ECoG activity was recorded for 40 minutes.

RESULTS

Behavior

Separate ANOVAs were performed for DZP-, PTZ-, and DZP+PTZ-treated rats. Subsequently, pairwise comparisons were performed using Newman-Keuls procedure. ANOVAs examining paddling and wall progression scores showed a significant effect of DZP, $F(2,42)=5.03$, $p<0.01$, PTZ, $F(1,28)=23.65$, $p<0.001$, and DZP+PTZ, $F(3,56)=9.06$, $p<0.001$. As shown in Fig. 1 (top panel), the PTZ, DZP and DZP+PTZ groups all showed more paddling and wall progression than their respective control groups ($p<0.05$). There was no attenuation of the PTZ effect by DZP.

TABLE 1

AVERAGE TOTAL DURATION OF BOUTS OF LARGE AMPLITUDE WAVE DISCHARGE

Group	Duration (sec)
Vehicle	27.3 ± 10.9
PTZ	1133.5 ± 284.1*
DZP	14.4 ± 3.1
DZP and PTZ	74.5 ± 16.6

*Significantly different from other three groups ($p<0.001$).

ANOVAs looking at the body tremor scores showed no effect of DZP, $F(2,42)=2.65$, n.s., but significant effects of PTZ, $F(1,28)=31.73$, $p<0.001$, and DZP+PTZ, $F(3,56)=16.35$, $p<0.001$. From Fig. 1 (middle panel), it can be seen that PTZ elevated body tremor compared to the saline group ($p<0.05$). DZP produced no effect on this measure by itself but antagonized the PTZ-elevated tremor ($p<0.05$).

ANOVAs of the head tremor data showed no effect of DZP, $F(2,42)=1.0$, n.s., a significant effect of PTZ, $F(1,28)=30.15$, $p<0.001$, while overall, DZP+PTZ groups differed significantly, $F(3,56)=16.69$, $p<0.001$. Figure 1 (bottom panel) shows that only PTZ elevated head tremor ($p<0.05$), while DZP alone produced no effect. Additionally, DZP blocked the PTZ effect on head tremor ($p<0.05$), as it did for body tremor.

ECoG

ECoG recordings for all baseline (pre-drug) measures in pups were the same for all groups. The predominant wave pattern consisted of a low to moderate amplitude (20–40 μV) with an 8–12 Hz frequency. Occasional higher amplitude lower frequency waveforms were interspersed with the usual alpha pattern.

Figure 2 shows representative pre- and post-drug traces from all groups. Rats injected with vehicle exhibited no change in the predominant waveform between baseline and post-treatment intervals. The DZP-treated rats demonstrated periodic, infrequent bouts of larger amplitude waves, these bouts lasting approximately 8–12 sec. These had the appearance of sleep spindles and they disappeared by the end of the recording period when the ECoG progressively flattened. In contrast, PTZ caused a dramatic change in the ECoG. Large amplitude, long duration waves occurred at about one-second intervals. These spike bouts were interspersed with moderate to high amplitude fast waves. Those rats that were pretreated with DZP prior to receiving PTZ did not show these bouts of spike activity.

In order to quantitate these impressions, the ECoG records were scored for the duration of bouts of waves which were a minimum amplitude of 150 μV and minimum duration of 0.5 sec. Termination of a bout of these waves was defined when there was a period of 2 sec during which a criterion wave did not occur. The total postinjection recording time that was characterized by such bouts was then calculated for each rat. The group averages are shown in Table 1. One-way ANOVA of these data indicated a significant difference among groups, $F(3,22)=17.37$, $p<0.01$. Further examination

using Tukey's HSD test showed that the total duration of spike bouts for the PTZ-treated rats significantly (p's<0.01) differed from those for the other three groups. There were no significant differences among these three groups.

DISCUSSION

The results clearly showed that the behavioral effects of DZP and PTZ are different. While both drugs increased the incidence of paddling and wall progression, only PTZ increased the incidence of head and body tremors. DZP counteracted the PTZ-induced increase of head and body tremors. These results suggest that DZP is not convulsogenic, at least not in the same way as PTZ. This agrees with Pappas and Walsh [8] in contraindicating the contention that BZs cause convulsions in young rats as Barr and Lithgow [2] suggested. It should be noted that whereas Barr and Lithgow used much higher doses of BZ, they also reported that the dose range used here had convulsogenic effects.

Since skeletal motor behavior may be an unreliable indicator of brain seizures, it is significant that our ECoG recordings also indicated that DZP does not cause seizures. PTZ profoundly increased the incidence of cortical spikes while DZP did not affect their incidence. Furthermore, DZP counteracted the effects of PTZ on the ECoG.

The motor and ECoG manifestations of convulsogenics are sometimes dissociated in young rats [10]. This would be the case when the seizure focus is subcortical. If diazepam causes subcortical seizures that fail to affect the ECoG of young rats, then there should have been some seizure-like behavioral consequence. This was not the case, however, as the behavioral effects of DZP were different from those for PTZ and, as well, DZP antagonized the tremorigenic effects of PTZ. We conclude therefore that the behavioral and ECoG effects of DZP and PTZ are different. Thus, it is improbable that DZP causes brain seizures in infant rats despite its behavioral effects which when not explicitly compared to those of a known convulsogen, appear to be seizure-like.

The BZ's are not unique with respect to their contrary effects on the activity of infant and adult rats. The alpha agonist clonidine also suppresses activity in the adult but causes hyperactivity in the neonate [8]. The activating effects of the BZ's on the rat pup may reflect two effects, sleep induction and the disinhibition of the motor suppression that normally occurs during sleep. File and Wilks [3] have suggested that they may cause sleep and that the occurrence of sleep-jerks could mistakenly convey the impression of seizures. Our present and earlier [8] observations are that BZ-induced activity is more than sleep-jerks, however, and that relatively complex motor behaviors such as swimming and wall climbing are also characteristic. Nevertheless, as File and Wilks suggested, the pups may be asleep. This is consistent with our impression that the ECoG's of DZP-treated pups showed what seemed to be sleep spindles.

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