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# Effects of four common anticonvulsants on the high pressure nervous syndrome in the rat

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Wardley-Smith B, Doré C, Hudson S, Wann K. Effects of four common anticonvulsants on the high pressure nervous syndrome in the rat. Undersea Biomed Res 1992; 19(1):13–20.— This study reports results from experiments designed to test common, clinically useful anticonvulsants for their effectiveness, if any, against the high pressure nervous syndrome (HPNS) in rats. Phenytoin, carbamazepine, phenobarbitone, or diazepam were administered orally to rats before compression. Endpoints used to assess the progression of the HPNS were $T_1$, $T_3$, and $T_5$ (onset of, continuous, and severe tremor), myoclonus, and seizures. Of the four drugs tested, only phenobarbitone increased the onset pressure for tremor and seizures by: $T_1$ 33%; $T_3$ 11%; $T_5$ 14%; seizures 10%. Neither phenytoin, carbamazepine, nor diazepam had any significant effect on any of the endpoints studied. High dose chronic pretreatment with phenytoin also had no effect on the HPNS. These data suggest that conventional anticonvulsant treatment would be of limited value for HPNS in man, and the lack of effect also suggests that HPNS seizures are of an unusual type.

rats — tremor
anticonvulsants — seizures
high pressure nervous syndrome

The high pressure nervous syndrome (HPNS) has been extensively studied in recent years, both for interest in its mechanisms (for review *see* 1, 2) and for its practical implications for deep sea divers (3, 4). The underlying causes of HPNS remain unclear, although a variety of studies using animal models of HPNS suggest that disturbances in neurotransmission involving excitatory amino acids may contribute to the tremor and convulsion components of HPNS (5, 6).

However, studies of the mechanisms of the HPNS have not greatly contributed to its practical prevention. Manipulation of both speed of compression and breathing mixture (e.g., by adding nitrogen to a heliox mixture) can reduce the severity of the HPNS in some, but not all, divers (4). These changes may produce additional problems, such as prolonging the duration of a dive or causing inert gas narcosis in susceptible subjects if nitrogen is used in the breathing mixture.

Several studies have tested in animal models clinically useful anticonvulsants for their activity against the HPNS. However, these reports are mostly qualitative or descriptive and have used different animal models and different experimental conditions (7–9).

We thus believed that a systematic quantitative study of the effects of some common anticonvulsant drugs on all the behavioral aspects of the HPNS in rats was appropriate. The very wide anticonvulsant profiles of some of the clinically useful anticonvulsants suggest that these drugs should have some effect on HPNS. Definitive data obtained in a well-established animal model of the HPNS (10) will in the future enable comparisons with other experimental seizure models. If effective, these drugs would have practical advantages for treating the HPNS, because at anticonvulsant doses there are few behavioral side effects, and there is an acceptable safety record for the majority of drugs used in the treatment of epilepsy.

## METHODS

Male Sprague-Dawley rats (weight range 250–400 g) were used in all experiments. They were supplied by the Clinical Research Centre SPF Unit, and were maintained in pathogen-free conditions until the day of the experiment. All the drugs were suspended in 0.5% tragacanth in water and administered orally using a 10 × 0.15-cm stainless steel tube with a rounded end attached to a syringe. The following doses and pretreatment times were used: phenytoin (diphenylhydantoin), 50 mg/kg 2 h before compression; carbamazepine, 50 mg/kg 30 min before compression; phenobarbital sodium, 20 mg/kg 2 h before compression; diazepam, 15 mg/kg 1 h before compression, all from Sigma Ltd. Control rats received 0.5% tragacanth 1 h before compression. None of the drugs at these doses produced any overt behavioral changes, and there was no evidence of any sedation or motor incoordination after either phenobarbital or diazepam.

After pretreatment, the rats were returned to their home cages until 10 min before compression was due to start. Each rat was exposed to pressure individually. A rectal probe was fitted to allow temperature monitoring, and the rat was lightly restrained and placed in a small cage. The cage was mounted over a strain gauge, connected to an amplifier outside the chamber, and the signal displayed on an oscilloscope. This enabled us to monitor accurately tremor onset.

The rat was then placed in a 25-liter steel pressure chamber, rated to 400 atm abs. Compression was with helium, at a rate of 3 atm abs/min; oxygen partial pressure was kept constant throughout at 0.6 atm abs. Carbon dioxide was removed with a soda lime absorber, and a small fan ensured adequate gas mixing. Chamber temperature was increased from 28° to ≈33°C, which maintained the rat's rectal temperature in the range 36°–38°C.

Development of tremor, myoclonus, and convulsions was used to assess the severity of the HPNS. Progression of tremor was estimated by dividing it into five stages: $T_1$—onset of mild tremor, $T_2$—mild-moderate tremor, $T_3$—continuous tremor, $T_4$—moderate-severe continuous tremor, $T_5$—severe continuous tremor. However, $T_2$ and $T_4$ proved less reproducible endpoints and thus results are reported only for $T_1$, $T_3$, and $T_5$. Once a seizure had occurred, all rats were deeply anesthetized with nitrous oxide and killed by rapid decompression.

Results were analyzed using a one-way analysis of variance for all 5 groups (4 drugs and control). Each drug was compared with the control group using the pooled estimate of variability from the analysis of variance. Residuals from the analysis of variance were tested for normality using Shapiro and Wilk's W test, and equality of residual variances between groups was tested using Bartlett's test.

A second series of experiments was carried out where phenytoin at a higher dose was added to the normal diet of rats for 4 wk before exposure to pressure. This protocol was designed to ensure a very high plasma level of phenytoin in the rats, given their relative resistance to the effects of phenytoin in comparison with other species. Rats were randomly allocated to 2 groups (weight range 200–250 g) and housed singly. Powdered rat diet was made up with, initially, 200 mg/kg phenytoin in 25 g food every 24 h. After 1 wk the dose of phenytoin was increased to 500 $mg^{-1} \cdot kg^{-1} \cdot 24\ h^{-1}$ mixed with the diet. Control rats were fed the same powdered diet without phenytoin. Preliminary experiments showed that 25 g of powdered food represented 80–90% of normal food consumption, and thus both groups ate all the food. This treatment regimen has been shown to give phenytoin plasma levels of 30–40 μg/ml plasma. After 4 wk treatment the rats were exposed to pressure in a 20-liter pressure chamber rated to 100 atm abs. In these experiments they were unrestrained and could move freely in a space 22 × 7 × 12 cm. This enabled any additional behavioral events such as hyperactivity resulting from the relatively high dose of phenytoin to be observed. Since rat temperature was not monitored, chamber temperature was increased from 28° to 32°C as the pressure increased to 100 atm abs. Observations of tremor, myoclonic jerks, and seizures, if present, were made by an observer who was unaware of the pretreatment. The maximum pressure limit of this chamber (100 atm abs) meant that not all rats had seizures by the maximum pressure, although all were exhibiting severe jerking.

Results from these experiments were analyzed using the Mann-Whitney U test to compare the groups. Five rats were used in each group.

## RESULTS

Results for the onset pressures for the endpoints used in the single-dose pretreatment group of experiments are shown in Table 1. Only phenobarbital at a dose of 20 mg/kg gave any significant protection against the HPNS. All the endpoints for tremor, $T_1$, $T_3$, and $T_5$, were significantly increased by 33, 11, and 14%, respectively. Myoclonus was not affected by phenobarbital, but seizure threshold was increased by 10%. It is clear that the most potent effect of phenobarbital is in delaying the onset of tremor, and that the continuation to other events of the HPNS is less affected.

Results for the second group of experiments involving chronic pretreatment with a high dose of phenytoin are shown in Table 2. None of the five HPNS endpoints used were affected by pretreatment with phenytoin. The limited maximum pressure of the chamber (100 atm abs) meant that 1 animal in each group did not have a seizure, i.e., there were two censored observations. However, it is unlikely that the overall significance of the results was affected by this. No additional behavioral differences, such as hyperactivity, between phenytoin and control groups were noted. Rats fed the diet containing phenytoin tended to gain less weight than controls, although food consumption was the same in both groups. However, all rats appeared healthy.

**TABLE 1**

MEAN ONSET PRESSURES IN ATM ABS FOR EACH GROUP OF RATS[a]

| HPNS Event | Control | Phenytoin | | Carbamazepine | | Phenobarbital | | Diazepam | | Pooled SD |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | *P* | Mean | *P* | Mean | *P* | Mean | *P* | |
| $T_1$ | 40.0 | 40.4 | 0.9 | 38.5 | 0.5 | 53.1 | <0.001 | 42.8 | 0.2 | 4.2 |
| $T_3$ | 68.5 | 65.7 | 0.4 | 71.0 | 0.4 | 75.9 | 0.04 | 71.0 | 0.4 | 6.3 |
| $T_5$ | 85.1 | 87.7 | 0.4 | 87.3 | 0.5 | 97.3 | 0.001 | 84.3 | 0.8 | 6.2 |
| Myoclonus | 92.2 | 85.7 | 0.17 | 90.5 | 0.7 | 89.3 | 0.6 | 89.5 | 0.5 | 7.1 |
| Seizure | 98.9 | 98.9 | 0.98 | 103.0 | 0.3 | 108.5 | 0.04 | 106.2 | 0.08 | 7.9 |
| *n* | 9 | 8 | | 7 | | 5 | | 7 | | |

[a] $T_1$ = tremor onset; $T_3$ = continuous tremor; $T_5$ = severe continuous tremor. Seizures were either clonic or combined tonic-clonic type. *P* values obtained from analysis of variance, those where $P < 0.05$ are underlined.

**TABLE 2**
MEAN ONSET PRESSURES FOR HPNS IN ATM ABS FOR RATS TREATED WITH PHENYTOIN FOR 4 WK BEFORE EXPOSURE TO PRESSURE COMPARED WITH CONTROLS[a]

| HPNS Event | Control | | Phenytoin | | |
|---|---|---|---|---|---|
| | Median | Range | Median | Range | *P* value |
| $T_1$ | 43.5 | 39.5–56.5 | 46.3 | 36.7–56.5 | 0.8 |
| $T_3$ | 68.0 | 66.0–74.2 | 69.4 | 61.2–74.2 | 0.8 |
| $T_5$ | 93.9 | 81.7–95.3 | 88.5 | 81.7–96.6 | 0.5 |
| Myoclonus | 92.5 | 77.6–95.3 | 82.0 | 80.3–90.5 | 0.22 |
| Seizure | 98.7 | 91.2–>100 | 100 | 88.5–>100 | 0.99 |

[a]HPNS events as in Table 1. *P* values obtained using the Mann Whitney U test.

Mean weights at the time of exposure to pressure were phenytoin group 280 (SD 39) g and control group 403 (SD 29) g. Weights before pretreatment were in the range 200–250 g.

## DISCUSSION

The results of this study indicate that of the four drugs tested, phenytoin, carbamazepine, phenobarbital, and diazepam, only phenobarbital significantly delayed the onset of the HPNS, increasing the threshold for tremor by 33% and that for seizures by 10%. This protection provided by phenobarbital has been reported by others, although previous experiments have been confined mainly to mice (8, 11). The dose of phenobarbital used in our study (20 mg/kg) was without any obvious sedative effect. This is perhaps surprising: it is possible that a degree of sedation was present, but was not significantly pronounced to be detected by visual observation and response to handling. Thus the protective effect on the HPNS is unlikely to be primarily associated with its sedative or "anesthetic" properties.

Neither phenytoin nor carbamazepine had any protective effect on the HPNS. This result for phenytoin confirms the findings of others, who have also shown little effect with this drug. In our rats there was no difference in the type of seizure seen after phenytoin pretreatment. The hyperbaric seizure seen in rats is a combined clonic-tonic seizure, in contrast to mice, which show a distinct clonic followed by a tonic phase. In mice, phenytoin delays and abolishes the tonic seizures without affecting the clonic phase.

Similar results were obtained in the rats receiving chronic, high dose phenytoin treatment, where again the clonic-tonic combined seizures remained unchanged. This also confirms that the absence of effect of phenytoin on the HPNS is not simply caused by too low a dose. A single dose of phenytoin at 500 mg/kg will produce plasma levels of 10–20 μg/kg; thus chronic oral treatment with 500 mg/kg should produce plasma levels substantially higher than the minimum plasma level required for anticonvulsant activity (12).

In preliminary experiments in mice, carbamazepine lowered the threshold for tremor (7). In this study, the $T_1$ pressure for rats treated with carbamazepine was also slightly lower than for controls (≈4%) but this was not statistically significant.

This lack of significant effect is not surprising, because clinically carbamazepine shows a similar anticonvulsant profile to that of phenytoin (13).

Effects of the benzodiazepines on the HPNS have shown less consistency. In this study we found no effect of 15 mg/kg oral diazepam on any of the HPNS endpoints we studied, although the seizure threshold was increased to a value approaching statistical significance ($P = 0.08$). Possibly a larger group size would produce a significant $P$ value, although our group ($n = 7$) should be adequate. These results are in contrast to Gran et al. (14) who reported that Valium (Roche) injected s.c. at a dose of 7.5 mg/kg protected against the HPNS at 90 atm abs. However, their study used untreated control animals, which are known to differ from vehicle-injected ones (15, 16). Our results are more consistent with those of Rowland-James et al. (8) who found no protection against tremor, with some increase in convulsion threshold pressure at doses of 5 mg/kg and above. However, the incidence of seizures was not affected. Other benzodiazepines which have been tested for anti-HPNS activity include flurazepam (17) and clonazepam (7), both of which provided some protection. The reasons for these discrepancies in the data are not clear. It may be that oral diazepam is less effective than injected forms, although oral administration is the route of choice in man. In any case, the dose we used has been shown to be active against other types of seizure, e.g., pentylenetetrazol (18). The mechanisms for the rather tenuous effects of benzodiazepines on the HPNS are not clear. The ability of these drugs to potentiate γ-aminobutyric acid (GABA)-ergic neurotransmission is well known. Electrophysiologic studies show that this potentiation is due to an enhancement of postsynaptic responsiveness to GABA, and this is likely to be mediated in turn by an increase in the frequency of chloride channel opening in response to GABA (*see*, e.g., 19). In any case the possible role of GABA in the HPNS remains to be defined. Previous studies have not been entirely consistent: Bichard and Little (17) suggested increasing GABA-ergic neurotransmission protected against HPNS, whereas Rostain et al. (16) showed that enhancing GABA-mediated transmission had no effect on the behavioral aspects of HPNS.

Thus, of the four anticonvulsants we tested, only phenobarbital had any protective effect against the motor events seen in the HPNS. The reasons for this remain to be clarified, but it is possible that the anti-HPNS effect of phenobarbital is related to its ability to inhibit responses mediated via excitatory amino acid receptors, although preference is shown for the non-*N*-methyl-D-aspartate (NMDA) type (20). Certainly barbiturates have been shown to potentiate the anticonvulsant effect of MK-801, a noncompetitive NMDA receptor antagonist (21). Although in the rat the noncompetitive NMDA receptor antagonists have had little or no effect on HPNS (22), competitive NMDA antagonists are effective in delaying the onset of the HPNS (5). However, from a practical viewpoint, the sedative effects of phenobarbitone in man have reduced its clinical usefulness, which would probably make it unsuitable for use in divers.

The absence of any beneficial effect of phenytoin, carbamazepine, or diazepam on the HPNS and the relatively small effect of phenobarbitone are perhaps surprising. All these anticonvulsants have a fairly wide spectrum of pharmacologic activity, both against epileptic and experimental seizures (for review, *see* 13). The doses we selected have all been shown to have anticonvulsant activity against one or more types of seizures in rats, and the chronic pretreatment with phenytoin used the highest dose possible without overt toxicity. Since both phenytoin and carbamazepine are thought

to stop seizure spread throughout the brain, the lack of effect of these drugs on the HPNS is hard to understand. This may in itself suggest that the seizures seen in the HPNS are of a highly unusual type. The majority of seizures seen in man respond, at least in part, to one or more conventional anticonvulsant drugs. Since clinically hyperbaric seizures have not been seen in man, it is again hard to speculate on what type such seizures might be or on their sensitivity to drugs. However, the known existence of spike and wave changes in the EEG and the occurrence, although rare, of myoclonic jerking in man (23 and J. C. Rostain, personal communication) suggest that seizures will occur at higher pressures. All the evidence available so far indicates that conventional anticonvulsant treatment would be of limited value in man and that further research is needed to find a clinically acceptable, practical treatment for severe HPNS in man.

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