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Lack of Effect of Lamotrigine Against HPNS in Rodent and Primate Models

P. C. PEARCE,* M. J. HALSEY,* C. J. MACLEAN,* E. M. WARD,* H. K. SHERGILL,* G. TINDLEY* AND B. S. MELDRUM†[1]

*Anaesthesia/HPNS group, MRC Clinical Research Centre, Watford Rd., Harrow, Middlesex, HA1 3UJ, UK
†Institute of Psychiatry, De Crespigny Park, London SE5, UK

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PEARCE, P. C., M. J. HALSEY, C. J. MACLEAN, E. M. WARD, H. K. SHERGILL, G. TINDLEY AND B. S. MELDRUM. *Lack of effect of lamotrigine against HPNS in rodent and primate models.* PHARMACOL BIOCHEM BEHAV **48**(1) 259–263, 1994.—The neurophysiological effects of the novel anticonvulsant lamotrigine on the high pressure neurological syndrome, HPNS, were investigated in the rat and nonhuman primate *Papio anubis*. Rats were exposed to pressure at a rate of 3 ATA per min in a helium/oxygen environment. They were pretreated with either lamotrigine isethionate 15, 30, or 60 mg/kg IP or control vehicle. After 15 and 30 mg/kg there were no changes in onset pressures for any of the grades of tremor or myoclonus. After 60 mg/kg, tremor was much slower, at 7–9 Hz, than the 15–20 Hz seen in controls. Four baboons were exposed to pressure at 0.33 ATA per min in the same environment and treated with lamotrigine isethionate at 7.5 mg/kg/h IV. Each animal underwent a control and a drug-treated exposure. No changes in the onset or severity of HPNS behavioural signs were observed. However, an increase in alpha wave amplitude of the EEG was almost prevented. In both species sustained myoclonic jerking occurred at pressures similar to those at which seizure activity was observed in control exposures. It is concluded that although lamotrigine is protective in several models of neuronal excitation, it is ineffective in protecting against behavioural signs associated with high atmospheric pressure.

HPNS Primates Rats Lamotrigine Glutamate EEG Behavioural observations

ANTAGONISTS at excitatory amino acid receptors (of both NMDA and non-NMDA subclasses) delay the onset and alleviate the signs of the high pressure neurological syndrome (HPNS) in several animal models. HPNS is the collective term for the series of neurological disturbances that occur when man or animals are exposed to increasing ambient pressures of helium and oxygen (as in deep sea diving operations). Signs and symptoms include tremor, myoclonus, motor incoordination, sleep disturbances, EEG changes, and, in animals exposed to very high pressures, convulsions (2,4,5).

Competitive antagonists at the NMDA receptor such as 2-amino-7-phosphonoheptanoic acid (AP7) (8,21) and 3-((+)-2-carboxypiperazine-4-yl)-propyl-1-phosphonate (CPP) (15) significantly delay the onset of HPNS signs in rats and reduce the severity of signs in baboons. Noncompetitive NMDA antagonists are more complex, with dizocilpine (MK801) either showing no change or worsening signs of HPNS in rats (22). By contrast, in baboons, MK801 is positively beneficial (13). Competitive antagonism at non-NMDA receptors, using GYKI 52466, has also been shown to ameliorate signs of HPNS in both rats and baboons (16A).

As blockade of glutamate receptors can ameliorate, although not completely prevent, the HPNS it was decided to test a compound that is believed to decrease the release of excitatory amino acid neurotransmitters (glutamate and aspartate). Lamotrigine (3,5-diamino-6-(2,3 dichlorophenyl)-1,2,4-triazine) probably achieves this by prolonging the inactivation of voltage-sensitive sodium channels (3,7).

If successful, this single compound would have the advantage of suppressing the overstimulation of the two principal excitatory amino acid receptors thought to be involved in HPNS. The known safety of the compound in man (18) would make lamotrigine a suitable candidate for testing against HPNS in clinical trials.

In the studies reported here, the effects of lamotrigine against the HPNS were investigated in both rats and baboons.

[1] Requests for reprints should be addressed to Prof. B. S. Meldrum, Department of Neurology, Institute of Psychiatry, De Crespigny Park, London, SE5 8AF, UK.

METHOD

Drugs

Lamotrigine (Wellcome Research Laboratories) (MW 256.1) was supplied as the isethionate salt (MW 382.5) (which is more soluble in water than the base compound).

Rats

Male Sprague-Dawley rats, weighing 180–220 g were injected IP with either lamotrigine isethionate (15, 30, or 60 mg/kg) or water for injections, six animals per group, 20 min before compression. An additional group received lamotrigine isethionate 30 mg/kg 3 h before compression. Following 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 inserted to allow monitoring of temperature and the animal placed in a small cage mounted over a strain gauge. The gauge was connected to an amplifier outside the chamber and the signal displayed on an oscilloscope (1). This enabled the accurate monitoring of tremor onset. The rat was then placed in a 25 litre steel pressure chamber, rated to 400 ATA. Oxygen was added to a partial pressure of 0.4 ATA and then compression with helium, at 3 ATA/min, commenced. Carbon dioxide was removed with a soda lime absorber and a small fan ensured adequate gas mixing. The high thermal conductivity of helium meant that chamber temperature had to be maintained at 33°C, to maintain the rat's rectal temperature in the 36–38°C range. Compression was halted when seizure activity or continuous myoclonus were observed.

The rats were observed from a video camera mounted at a porthole and the pressures of onset of the behavioural signs of HPNS noted (tremor T1–T5, myoclonus and seizure). The five grades of tremor were, T1—onset of mild tremor, T2—mild to moderate tremor, T3—continuous tremor, T4—moderate to severe continuous tremor, T5—severe continuous tremor. These gradings were based on electromechanical and visual data from two experienced observers who were blind to treatment and precise pressure. This system of analysis has previously been validated and used [e.g., (20)]. Once a seizure had occurred, the rats were deeply anaesthetised with nitrous oxide and killed by rapid decompression.

Baboons

Four adolescent baboons (*Papio anubis*), three male and one female, weighing 6.5–7.5 kg, were implanted with SC monitoring electrodes, for the monitoring of EEG, ECG, EMG, and EOG (electroocculogram), and arterial and venous catheters. Anaesthesia was provided by 1% halothane and a breathing mixture of oxygen : nitrous oxide 1 : 2. The surgical techniques, postoperative care, and the system of backpack, umbilical, and swivel have been described previously (16). Each animal had freedom of movement within its cage while intravenous drug administration and monitoring of EEG and other parameters took place.

The 1200 litre pressure chamber and its ancillary equipment have also been previously described in detail elsewhere (17). Atmospheric conditions could be kept constant, by the recycling of gases through filters, and temperature optimal (31–33°C), depending on the pressure. Food and drinking water were available ad lib and a funnel below the cage was flushed daily to remove excreta via a pressure lock.

Protocol

Individual baboons, like humans, vary in their responses to pressure; hence, each animal was used as its own control. Two exposures from each animal were, therefore, required, and so recovery from each in good condition was necessary. The protocol for accustoming each animal to the chamber environment and the setting up of intravascular fluid supplies has been described in an earlier publication (16). Twenty-four hours before compression the environmental partial pressure of oxygen was raised to 0.4 ATA and control observations began. The compression rate was 0.33 ATA/min with helium, which continued until either the animal showed signs of seizure activity or sustained myoclonus, or 91 ATA was achieved. When the highest pressure was reached, decompression began immediately and proceeded over the following 70 h. The partial pressure of oxygen during decompression to 3 ATA was raised to 0.7 ATA. The baboon was then allowed 3 weeks to recover before the procedure was repeated. During this time the animals' behaviour and EEG were monitored to ensure that no overt effects from the first exposure were apparent before the second commenced.

Lamotrigine does not remain in solution in the presence of sodium chloride, and so during compression the physiological saline keeping the intravascular lines patent was changed to an isotonic solution of 4% glucose. Doses of the drug or control vehicle, water for injections, were injected into the intravenous line in volumes of 0.5 ml at 30 min intervals, starting 30 min before compression began and continuing until maximum pressure was achieved, such that the animal received an infusion of 7.5 mg/kg/h of salt (19.5 μmol/kg/h). The total dose of lamotrigine isethionate, assuming 91 ATA was achieved, was 37.5 mg/kg (97.5 μmol/kg). The sequence of control drug-treated or drug-treated control was randomised among animals.

Observations

Two observers, who were blind to both treatment and precise pressure and who were looking through separate portholes, made continuous behavioural notes throughout compression.

Two ways of evaluating the behavioural signs were employed, as previously described (14). In brief, firstly the pressures of onset of the tremor and nontremor components were recorded and, secondly, the degree of tremor or frequency of nontremor signs were graded and a score given that described the overall severity of the behavioural signs at a given pressure.

EEG Analysis

Polygraph and tape recordings were made of the EEG and other signals before and during the exposure period. Fast Fourier transform analysis was carried out on the EEG data as previously described (14).

RESULTS

Rats

Figure 1 shows the onset pressures for tremor grades 1, 3, and 5, and myoclonus. There were no significant differences, using the Mann-Whitney *U*-test between values from control animals and those receiving lamotrigine 15 and 30 mg/kg, whether at 20 min or 3 h. The end point for the lamotrigine-treated exposures was not a tonic-clonic seizure as seen in

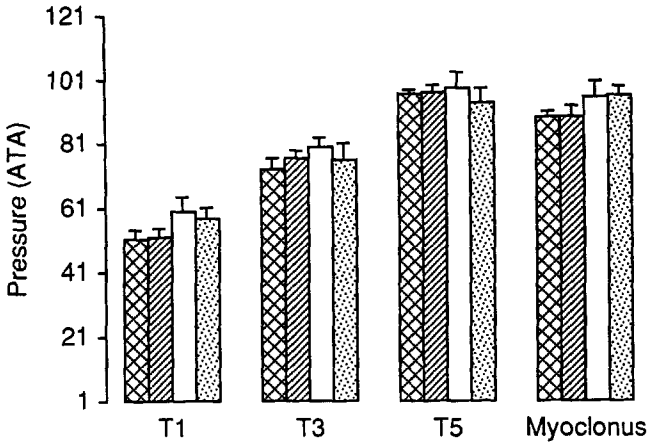

FIG. 1. Mean onset pressures for three grades of tremor (described in the text) and myoclonus. Cross-hatched bars represent control exposures, diagonal lined bars represent lamotrigine 15 mg/kg at 20 min, open bars represent lamotrigine 30 mg/kg at 20 min, and dotted bars represent lamotrigine 30 mg/kg at 3 h. Error bars are SEMs.

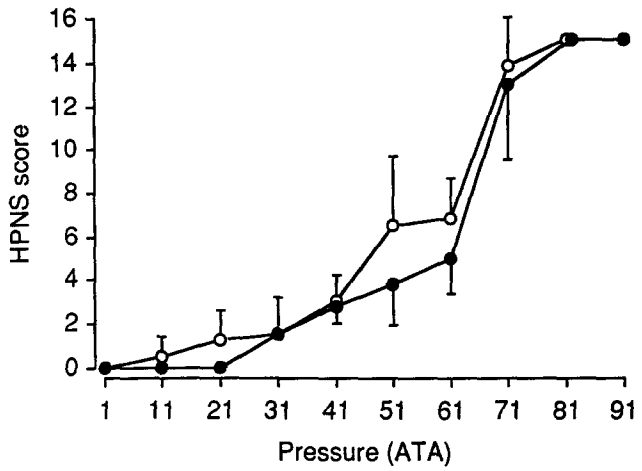

FIG. 2. Mean HPNS score values plotted at increments in pressure of 10 ATA. Open circles refer to control compressions, closed circles refer to lamotrigine treated compressions. Error bars are SEMs.

control exposures, at 94.4 ± 1.4 ATA, but the sudden appearance of sustained whole-body myoclonic jerking. This occurred, for all doses, at pressures not significantly different from the seizure activity observed in control animals.

When lamotrigine 60 mg/kg was injected, the type of tremor was different from that normally associated with HPNS. From a mean of 24 ATA, the animals showed mild to moderate tremor at a frequency of 7-9 Hz. This was much slower than the tremor at 15-20 Hz, which started at a mean of 51.3 ATA, seen in the control exposures. The severity did not worsen until the sustained myoclonus occurred.

Baboons

Pressure onset. Table 1 presents the median and range of onset pressures for the behavioural signs. It shows that there were no changes in tremor onset produced by pretreatment with lamotrigine. Myoclonus occurred at a later stage in the drug-treated exposures and in three out of four animals the first sign was a whole-body jerk. Vomiting occurred in three out of four lamotrigine-treated exposures and only in one out of four controls. In each lamotrigine-treated exposure it was the appearance of sustained myoclonus that triggered the decision to halt compression. This occurred at higher pressures than seizure activity in control exposures.

HPNS score. The HPNS score quantifies the overall severity of behavioural signs at given pressures, and Fig. 2 illustrates the mean score at each 10 ATA interval throughout compression. It can be seen that severity increased with pressure in both groups and that there were no evident differences between them. The score was also split into the tremor and nontremor components (data not shown), and when treated

TABLE 1

MEDIAN AND (RANGE) OF PRESSURE ONSET (ATA) OF HPNS SIGNS IN FOUR BABOONS, WITH OR WITHOUT LAMOTRIGINE

	Control		Lamotrigine	
	Median	Range	Median	Range
Tremor				
Hand/foot	34	(12-45)	34	(28-50)
Limb	34	(13-45)	34	(28-54)
Head	45	(23-52)	51	(31-54)
Body	69	(67-85)	75	(73-78)
Face	>91	(52->91)	>91	(>91->91)
Myoclonus				
Muscle	49	(44-59)	75	(59-81)
Head	65	(48-72)	75	(67-81)
Body	65	(48-72)	75	(67-81)
Vomit	>91	(49->91)	74	(39->91)
Seizure/	72	(67-85)		
cont myoclonus			83	(76-91)

If a sign was not observed, then it is listed as occurring at >91 ATA.

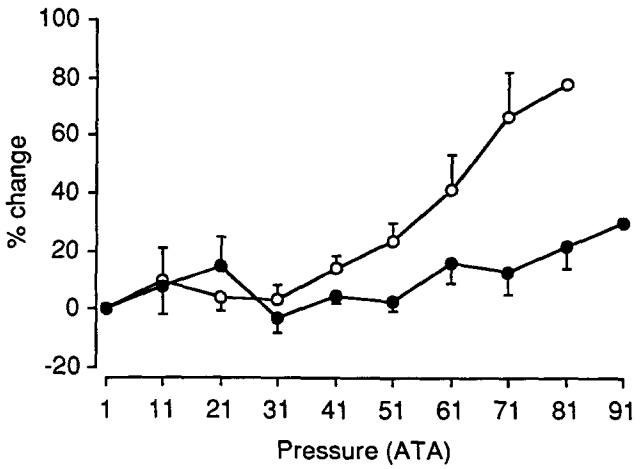

FIG. 3. Mean percentage change in amplitude of the alpha frequency band from the EEG of three animals, compared to initial baseline values at 1 ATA and plotted against increasing pressure. Open circles refer to control compressions and closed circles represent data from lamotrigine treated compressions. Error bars are SEMs.

separately, there were still no differences between the two groups.

EEG analysis. In one of the animals it was impossible to measure the EEG because of technical problems, and so the results were calculated from three animals. They were expressed as percentage change from the preexposure reading of the mean amplitude of each of the conventional frequency bands, delta (2–4 Hz), theta (4–8 Hz), alpha (8–13 Hz), and beta (13–40 Hz). This was carried out in the frontal, parietal, and occipital regions of the left cortex. In the delta, theta, and beta bands there were no differences between control and drug-treated exposures (data not shown). However in the alpha band, frontal channel shown in Fig. 3, there was a gradual increase with pressure in control exposures that was not present in the lamotrigine-treated exposures. Similar differences, though to a lesser degree, were seen in the parietal and occipital regions.

DISCUSSION

The initial experimental design of the primate study was to use six baboons, randomly allocated to receive either lamotrigine or vehicle on the first exposure, and carry out full statistical analysis on the results. It became apparent, however, after four animals that, other than the sustained myoclonus, there were no differences in the behavioural responses between treatments and so the study was curtailed. The lack of apparent effects could not be due to the drug precipitating out once injected or an inability to cross the blood–brain barrier, as there were definite differences noted in the EEG between control and drug-treated exposures. In addition, the doses used are known to be effective against other types of seizures (9). The EEG results came from six epochs at each 10 ATA interval on each exposure from the three animals, and so it was decided that this was sufficient to give credence to the results.

The results from both species show that neuronal membrane stabilisation and postulated inhibition of glutamate release by pretreatment with lamotrigine failed to provide protection from the effects of exposure to high ambient pressures. Full seizure activity may have been delayed or prevented but was replaced by sustained whole-body myoclonic jerking. This activity was generalised, and may have been a form of seizure, but was different from that normally associated with HPNS. Once started, it was almost continuous and no postictal phase was observed either behaviourally or on the EEG in baboons. The signs were considered severe enough to halt compression and start decompression to alleviate the condition.

Analysis of the EEG produced some surprising results in that, despite having no effects on the behavioural signs, lamotrigine appeared to drastically reduce and almost prevent the major change, a linear increase in alpha wave amplitude. In previous experiments, this result has been associated with a dramatic amelioration of the level of tremor and myoclonus; for example, that produced by the NMDA receptor antagonist CPP (15). The present study would suggest that the EEG and behavioural signs may not be related, and further selected pharmacological interventions may dissect out the different components of the HPNS.

The behavioural results were also surprising. Antagonism at the excitatory amino acid receptors, both NMDA and non-NMDA, has been shown to ameliorate and delay the HPNS in rat and baboon models. In past experiments, doses of the compounds affording protection against high pressure were very similar to those that provided protection against sound-induced seizures in rats and photically induced seizures in photosensitive baboons, for example, CPP (12,15) and abecarnil (14,19). Lamotrigine was effective in both the latter models but not in the high pressure model of neuronal hyperexcitation. The potency of lamotrigine, however, can vary depending on the species and the insult against which it is being tested. For example, in the gerbil model of global ischaemia, the dose to provide protection against hippocampal CA1 damage is approximately six times the anticonvulsant ED_{50} in the same species (6). It could, therefore, be possible that to protect against high pressure-induced hyperexcitability a much higher dose is required, which would be in the range showing unacceptable side effects, such as the early appearance of 7–9 Hz tremor in rats receiving 60 mg/kg.

In addition to inhibiting glutamate release, lamotrigine also inhibits GABA release but is much less potent in this effect in vitro (10). It is possible that under high pressure conditions in vivo this effect on GABA may become more important. It is known that agents enhancing GABA-mediated inhibition such as abecarnil (a partial agonist at the benzodiazepine receptor) provide protection against the effects of high pressure (14). It is not known, however, whether this course of action is attacking the source of the problem or masking an increase in excitation.

Another factor that may have contributed to the failure of lamotrigine to protect against HPNS is the fact that it inhibits glutamate release by acting on the voltage-sensitive sodium channels only and it has no effect on potassium-evoked release (10). It is possible that potassium may have an important role in HPNS as the same behavioural signs in rats have also been produced by the potassium channel blocker 4-aminopyridine (4-AP) (23). By comparing interactions of anaesthetics and NMDA receptor antagonists with high pressure and 4-AP, these authors suggested that a potassium ion channel dysfunction contributes to the symptoms seen in HPNS. Further work will be required to establish the importance of potassium in the genesis and expression of HPNS.

Finally, although lamotrigine is chemically unrelated to antiepileptic drugs in current use, its anticonvulsant pharmacological profile is similar in most respects to phenytoin and carbamazepine (11). Neither of these drugs have any effect on the HPNS in rats (20) although they differed from lamotrigine in that the seizures observed were no different from those in control animals. Further support is, therefore, given to the suggestion that HPNS seizures are not of the same type as those associated with other experimental models of epilepsy.

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REFERENCES

1. Baker, J. A.; Halsey, M. J.; Wardley-Smith, B.; Wloch, R. T. Assessment of the high pressure neurological syndrome (HPNS): A new method of measuring tremor in an animal model. In: Bachrach, A. J.; Matzen, M. M., eds. Underwater physiology VII. Bethesda: Undersea Medical Society, Inc.; 1981:415–420.
2. Brauer, R. W. The high pressure nervous syndrome: Animals.

In: Bennett, P. B.; Elliott, D. H., eds. The physiology and medicine of diving and compressed air work. London: Bailliere Tindall; 1975:231–247.
3. Cheung, H.; Kamp, D.; Harris, E. An in vitro investigation of the action of lamotrigine on neuronal voltage-acyivated sodium channels. Epilepsy Res. 13:107–112; 1992.
4. Halsey, M. J. Effects of high pressure on the central nervous system. Physiol. Rev. 62:1341–1377; 1982.
5. Hunter, W. L.; Bennet, P. B. The causes, mechanisms and prevention of the high pressure nervous syndrome. Undersea Biomed. Res. 1:1–28; 1974.
6. Leach, M. J.; Baxter, M. G.; Critchley, M. A. E. Neurochemical and behavioural aspects of lamotrigine. Epilepsia 32(Suppl. 2):S4–S8; 1991.
7. Meldrum, B. S. Excitatory amino acid transmitters in epilepsy. Epilepsia 32(Suppl. 2):S1–S3; 1991.
8. Meldrum, B. S.; Wardley-Smith, B.; Halsey, M. J.; Rostain, J. C. 2-Amino-phosphonoheptanoic acid protects against the high pressure neurological syndrome. Eur. J. Pharmacol. 87:501–502; 1983.
9. Meldrum, B. S.; Leach, M. J. The mechanisms of action of lamotrigine. Rev. Contemp. Pharmacol. 5:107–114; 1994.
10. Miller, A. A.; Sawyer, D. A.; Roth, B.; Peck, A. W.; Leach, M. J.; Wheatley, P. L.; Parsons, D. N.; Morgan, R. J. I. Lamotrigine. In: Meldrum, B. S.; Porter, R. J., eds. Current problems in epilepsy: New anticonvulsant drugs. London: John Libbey; 1986:165–177.
11. Miller, A. A.; Wheatley, P. L.; Sawyer, D. A.; Roth, B.; Baxter, M. G. Pharmacological studies on lamotrigine, a novel potential epileptic drug: I. Anticonvulsant profile in mice and rats. Epilepsia 27:483–489; 1986.
12. Patel, S.; Chapman, A. G.; Graham, J. L.; Meldrum, B. S.; Frey, P. Anticonvulsant activity of the NMDA antagonists, D(−)-4-(3-phosphonopropyl)piperazine-2-carboxylic acid (D-CPP) and D(−)(E)-4-(3-phosphonoprop-2-enyl)piperazine-2-carboxylic acid (D-CPPene) in a rodent and a primate model of reflex epilepsy. Epilepsy Res. 7:3–10; 1990.
13. Pearce, P. C.; Dore, C. J.; Halsey, M. J.; Luff, N. P.; Maclean, C. J.; Meldrum, B. S. The effect of MK-801 on the high pressure neurological syndrome in the baboon (*Papio papio*). Neuropharmacology 29:931–941; 1990.
14. Pearce, P. C.; Halsey, M. J.; Maclean, C. J.; Passingham, S.; Pearson, J.; Mehta, R. L.; Meldrum, B. S.; Jordon, C. J.; Ward, E. M. Interactions of the B carboline abecarnil with the high pressure neurological syndrome in a primate model. Psychopharmacology (Berlin) 109:163–171; 1992.
15. Pearce, P. C.; Halsey, M. J.; Maclean, C. J.; Ward, E. M.; Webster, M. T.; Luff, N. P.; Pearson, J.; Charlett, A.; Meldrum, B. S. The effects of the competitive NMDA receptor antagonist CPP on the high pressure neurological syndrome in a primate model. Neuropharmacology 30:787–796; 1991.
16. Pearce, P. C.; Halsey, M. J.; Ross, J. A. S.; Luff, N. P.; Bevilaqua, R. A.; Maclean, C. J. A method of remote physiological monitoring of a fully mobile primate in a single animal cage. Lab. Anim. 23:180–187; 1989.
16A. Pearce, P. C.; Maclean, C. J.; Shergill, H. K.; Ward, E. M.; Halsey, M. J.; Pearson, J.; Meldrum, B. S. Protection from high pressure induced hyperexcitability by the AMPA/kainate receptor antagonists GYK 52466 and LY 293558. Neuropharmacology; in press.
17. Pearce, P. C.; Ross, J. A. S.; Luff, N. P.; Halsey, M. J.; Monk, S. A chamber system for maintaining a hyperbaric environment for long-term animal studies. Undersea Biomed. Res. 18:53–61; 1991.
18. Ramsey, R. E.; Pellock, J. M.; Garnett, W. R.; Sanchez, R. M.; Valakas, A. M.; Wargin, W. A.; Lai, A. A.; Habbell, J.; Chern, W. H.; Allsup, T. Pharmacokinetics and safety of lamotrigine (Lamictal) in patients with epilepsy. Epilepsy Res. 10:191–200; 1991.
19. Turski, L.; Stephens, D. N.; Jensen, L. H.; Petersen, E. N.; Meldrum, B. S.; Patel, S.; Bondo Hansen, J.; Loscher, W.; Schneider, H. H; Schmiechen, R. Anticonvulsant action of the B-carboline abecarnil: Studies in rodents and baboon, *Papio papio*. J. Pharmacol. Exp. Ther. 253:344–352; 1990.
20. Wardley-Smith, B.; Dore, C. J.; Hudson, S.; Wann, K. T. Effects of four common anticonvulsants on the high pressure nervous syndrome in the rat. Undersea Biomed. Res. 19:13–20; 1992.
21. Wardley-Smith, B.; Rostain, J. C.; Meldrum, B. S.; Halsey, M. J. Effect of 2-aminophosphoheptanoic acid on the EEG of rats exposed to high pressure. Undersea Biomed. Res. 13:155–163; 1986.
22. Wardley-Smith, B.; Wann, K. T. The effects of noncompetitive NMDA receptor antagonists on rats exposed to hyperbaric pressure. Eur. J. Pharmacol. 165:107–112; 1989.
23. Wardley-Smith, B.; Wann, K. T. Effects of four drugs on 4-aminopyridine seizures: A comparison with their effects on HPNS. Undersea Biomed. Res. 18:413–419; 1991.