Antiepileptic Drugs Prevent Seizures in Hyperbaric Oxygen: A Novel Model of Epileptiform Activity

Ivan T. Demchenko,^{1,2,4} Sergei Yu Zhilyaev,⁴ Alexander N. Moskvina,⁴ Alexander I. Krivchenko,⁴
Claude A. Piantadosi,^{1,2,3} Barry W. Allen^{1,2 *}

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ABBREVIATIONS:

HBO₂ (Hyperbaric Oxygen), **ATA** (Atmospheres Absolute), **AED** (Antiepileptic Drug), **CBZ** (Carbamazepine), **GBP** (Gabapentin), **LTG** (Lamotrigine), **OXC** (Oxcarbazepine), **PRM** (Primidone), **TGB** (Tiagabine), **VGB** (Vigabatrin), **VPA** (Valproic Acid), **ZNS** (Zonisamide)

ABSTRACT [250 words]

Breathing oxygen at sufficiently elevated pressures can trigger epileptiform seizures. Therefore, we tested the hypothesis that pre-treatment with FDA-approved antiepileptic drugs could prevent seizure onset in hyperoxia at 5 atmospheres absolute. We selected drugs from two putative functional categories, Na⁺-channel antagonists and GABA enhancers, each administered intraperitoneally at four doses in separate groups of C57BL/6 mice. The drugs varied in efficacy at the doses used. Of the five tested Na⁺-channel antagonists, carbamazepine and lamotrigine more than tripled seizure latency compared to values seen in vehicle controls. Primidone, zonisamide and oxcarbazepine were less effective. Of the four GABA reuptake inhibitors, tiagabine and vigabatrin also increased seizure latency by more than three times control values; valproic acid was less effective, and the GABA synthesis promoter gabapentin was intermediate in effectiveness. We infer that Na⁺-channel function and GABA neurotransmission may be critical targets in the pathophysiology of CNS O₂ toxicity. Because these essential components of neuronal excitation and inhibition are also implicated in the pathogenesis of other seizure disorders, including generalized epilepsy, we propose that, at some level, common pathways are involved in these pathologies, although the initiating insults differ. Furthermore, hyperoxic exposures are not known to cause the spontaneously-recurring seizures that characterize true clinical epilepsy. Nonetheless, experimental studies of hyperbaric oxygen toxicity could provide new insights into molecular mechanisms of seizure disorders of various etiologies. In addition, the neuropathology of hyperbaric oxygen is particularly relevant to the hypothesis held by some investigators that oxidative stress is an etiological factor in clinical epilepsies.

INTRODUCTION

Oxygen is a convulsant when breathed for a sufficient time at partial pressures of 2.5 atmospheres absolute (ATA) or above, and latency to onset of epileptiform patterns on the EEG and tonic-clonic neuromotor responses is inversely proportional to inspired Po_2 . This has been well established in human and animal studies that have been conducted for many years, primarily to devise procedures for protecting personnel exposed to hyperbaric oxygen (HBO_2) in certain occupational and therapeutic environments (Balentine, 1982; Behnke et al., 1935; Clark and Thom, 2003; Donald, 1947a; Donald, 1947b; Paton, 1967). Vulnerable groups include military and civilian divers, crew attempting escape from a disabled submarine and patients undergoing hyperbaric oxygen therapy.

Although many drugs have been developed to prevent seizures of various etiologies, particularly those of broad clinical relevance, none have been specifically formulated to protect against seizures in HBO_2 , and it is unlikely that pharmaceutical companies would undertake the research needed to do so, since the human population at risk is small. Up to now, the only reliable methods for avoiding neurotoxicity in HBO_2 have been to limit the dose and duration of hyperbaric oxygenation.

Although the events or conditions that initiate seizures in hyperbaric oxygen and in other seizure disorders may differ, the final common pathways and ultimate molecular targets may be similar. Indeed, some investigators have proposed that oxidative stress plays a contributory role in epilepsy (Patel, 2004; Pearson et al., 2015; Zsurka and Kunz, 2015). We reasoned, therefore, that antiepileptic drugs (AEDs) could prevent or delay seizures of CNS O_2 toxicity and as a corollary, AEDs with known mechanisms-of-action might be used as investigative probes to further elucidate events that evoke hyperoxic seizures, although the true mechanisms of action of some of these drugs are not completely understood and may not fit neatly into their nominal functional classes. Furthermore, if a broad range of AEDs are found to prevent or delay seizures

in HBO₂, and if there are parallels between the ictal events in HBO₂ and those seen in other seizure disorders, the hyperbaric model could elucidate mechanisms of various seizure-related disorders, including those due to the acquired and idiopathic epilepsies, traumatic brain injury and other causes. However, it must be understood that most animal models used in epilepsy research only simulate the seizures of epilepsy rather than epilepsy itself (Loscher, 2011), and this would apply to HBO₂ as well.

In this study we assessed the ability of nine FDA-approved AEDs, administered individually, to increase seizure latency in mice exposed to 100% oxygen at 5 ATA for 60 min. This level and duration of hyperoxia reliably elicits seizures in this species in a reasonable period of time (60 min or less) without producing a significant degree of direct cardiopulmonary injury (Demchenko et al., 2007).

Since abnormal propagation of excitatory neurotransmission and attenuation of inhibitory neurotransmission are established pathogenic factors in clinical epilepsies (Jefferys, 2010) and are also assumed to be factors in CNS O₂ toxicity (Colton and Colton, 1982; Dean et al., 2003; Demchenko and Piantadosi, 2006), we chose to evaluate the protective efficacy in HBO₂ of AEDs from two functional classes relevant to excitatory and inhibitory functions: sodium-channel antagonists and GABA transmission enhancers. We compared our findings with published values for the efficacy of the same AEDs in a well-established animal model of epileptic seizures, maximal electroshock (MES).

RESULTS

Seizure Activity in Vehicle-Treated Mice. The 24 mice treated with vehicle (0.9% NaCl or DMSO) and exposed to HBO₂ at 5 ATA exhibited neuromotor responses that progressed in 2 or 3 stages, comparable to those of the Racine Scale (Racine, 1972). In stage I, restlessness, intensive grooming, slight tremors, twitching vibrissae and transient muscular spasms were observed. In stage II, persistent, rhythmic spasms appeared in the face and body along with bilateral forelimb clonus and escape behaviors. Stage III, if it occurred, was characterized by generalized tonic-clonic convulsions. Stages I and II were seen in 23 (>95%) of the vehicle-treated mice; one mouse was unresponsive. All three stages occurred in 17 (>70%) of these animals. In the remaining 7 (29%) vehicle-treated mice, stage III was not seen; instead, the signs of stage II suddenly disappeared and behavioral arrest was observed for the remainder of the 60-min exposure. For the purpose of evaluating the efficacy of AEDs, we defined the termination of seizure latency to coincide with either the first signs of stage II or with behavioral arrest, either of which would incapacitate human subjects and therefore mark the limit of safe exposure to HBO₂.

Seizure Activity in AED-Treated Mice. Each of the tested AEDs delayed the onset of CNS O₂ toxicity in a dose-dependent fashion (Table 1, Figures 1 and 2). However, almost all of these animals exhibited either signs of stage II or behavioral arrest at some point during a 60-min exposure. Stage III was observed in 47% of these animals, mostly those treated with lower doses. Of the Na-channel antagonists, carbamazepine (CBZ) and lamotrigine (LTG) were the most effective, increasing seizure latency by more than three times that observed in animals treated with vehicle; whereas primidone (PRM), zonisamide (ZNS) and oxcarbazepine (OXC) were least effective, and at the lowest doses used they failed to increase seizure latency significantly ($P \leq 0.05$), compared to vehicle controls.

Of the GABA transmission enhancers, the reuptake inhibitors tiagabine (TGB) and vigabatrin (VGB) were the most effective, increasing seizure latency by more than three times compared to vehicle controls, when administered at the highest doses. Valproic acid (VPA) was the least effective. The GABA-synthesis promoter gabapentin (GBP) was intermediate in effectiveness. Only VGB provided significant increases in seizure latency at the lowest dose.

Equally-effective Doses. To provide a uniform standard for evaluating efficacy, we used linear regression analysis ($R^2 > 0.95$ in all cases) to predict an equally-effective dose (EQD) for each AED that would increase seizure latency by a fixed multiple of the mean latency observed in the corresponding vehicle controls (Figures 3A and 3C). Because the Na^+ -channel antagonists LTG and CBZ and the GABA enhancers VGB and TGB each increased latency by more than a factor of 3, we selected EQD_{3x} as a convenient standard for comparison. For the other 5 drugs, this parameter was estimated by extrapolation along the calculated lines of best fit. This procedure was validated by comparison with ED₅₀'s obtained using log Probit Analysis (Litchfield and Wilcoxon, 1949). Since the latter method assumes all-or-none responses, latency data from the first 40 minutes of each hyperbaric exposure were used, an interval in which some animals exhibited seizure activity and some did not. For each drug, we found good qualitative agreement between the EQD_{3x} and the ED₅₀.

DISCUSSION

We have demonstrated that a range of FDA-approved AEDs, of two functional classes, can significantly delay seizure onset in extreme hyperoxia. Although other investigators have tested the protective efficacy of two such drugs in HBO₂, CBZ and VGB (Bitterman and Halpern, 1995; Hall et al., 2013; Harel et al., 1978; Reshef et al., 1991; Tzuk-Shina et al., 1991), we know of no study in which the anticonvulsant efficacies of multiple AEDs were compared in hyperbaric hyperoxia.

Among the Na⁺-channel antagonists we tested, CBZ and LTG were the most effective in increasing seizure latency and exhibited the highest potencies, as shown in Figure 3A. Clinically, CBZ is considered “first line anticonvulsant therapy for generalized tonic-clonic and partial seizures” (Reinikainen et al., 1987), whereas LTG is used as initial therapy for focal epilepsy, idiopathic generalized epilepsy and absence seizures in children (Biton et al., 2005; Coppola et al., 2004). Both of these drugs impose use-dependent blocks on neuronal sodium channels during rapid, repetitive, sustained firing (Clemens et al., 2007; Goldenberg, 2010; Vreugdenhil and Wadman, 1999). LTG may also inhibit neuronal synthesis of glutamate and aspartate, diminishing the release of these excitatory neurotransmitters (Lee et al., 2008).

The remaining Na⁺-channel antagonists, OXC, ZNS, and PRM, were less effective in HBO₂ but still doubled seizure latency when administered in high doses. OXC and ZNS are used clinically to treat both focal and generalized seizures in adults and children, but not generalized tonic-clonic seizures (Goldenberg, 2010; Kothare et al., 2004). The mode of action of OXC is comparable to that of CBZ, imposing a use-dependent blockade of sodium channels (Reinikainen et al., 1987). ZNS, however, is chemically and structurally unrelated to the other AEDs, and its primary mechanism of action is to block both voltage-dependent sodium channels and T-type calcium channels (Goldenberg, 2010; Leppik, 2004). As for PRM, its mode of action is difficult to study as such, since it is converted to phenobarbital, phenylethylmalonamide (PEMA) and

several other metabolites; it may act by imposing a use-dependent blockade of voltage-gated sodium channels and by increasing GABA-mediated chloride influx (Lasoń et al., 2011).

Of the GABA-enhancing AEDs we tested, TGB and VGB were the most effective and had the highest potencies. TGB increases GABA concentration in the synaptic cleft by inhibiting its reuptake, and it is used clinically as an adjunctive treatment for focal seizures (Ängehagen et al., 2003; Lasoń et al., 2013). VGB irreversibly inhibits the degradation of GABA by GABA-transaminase and is used to treat refractory focal seizures (Thomas et al., 1996) and as a monotherapy (Aicardi et al., 1996). The other GABA-enhancers, GBP and VPA, significantly increased seizure latency in HBO₂ only at high doses. GBP elevates GABA levels in the brain by an unknown mechanism and although structurally similar to GABA, it is not known to act directly on GABA receptors. VPA may increase GABA synthesis by stimulating glutamate decarboxylase (GAD) activity. It also modulates voltage-gated sodium currents during sustained, rapid, repetitive neuronal firing (Taverna et al., 1998).

Our finding that certain Na⁺-channel antagonists and GABA-enhancers provide a sustained anticonvulsant effect in extreme hyperoxia supports our hypothesis that, at some level, similar molecular targets are involved in HBO₂-neurotoxicity and in other seizure disorders for which the efficacies of these drugs have been established clinically. Indeed, a correlation between Na⁺-channel activation and increased neuronal firing has been documented in HBO₂ (Garcia et al., 2003; Mulkey et al., 2003). Likewise, the role of diminished inhibitory neurotransmission in HBO₂ seizures has been well described (Colton and Colton, 1982; Colton and Colton, 1986; Colton and Colton, 1978). Clearly, certain drugs that beneficially modulate neuronal function in other seizure disorders also protect against hyperoxic seizures. It is thought-provoking that the pattern of relative efficacy of most of the drugs we tested¹, when

¹ VGB is excluded from this comparison because it is ineffective in the MES model (Loscher et al., 1994; Waszkielewicz et al., 2011).

plotted in order of increasing ED_{50} , closely resembles that seen for the same drugs administered to mice subjected to maximal electroshock, MES (Bialer et al., 2004), when doses are expressed as molar equivalents (Chmielewska and Lamparczyk, 2008) (Table 2 and Figure 4). The difference in magnitudes of the ED_{50} 's for the two experimental models is likely due to the fact that the intensities of the two kinds of stimuli are unequal. Perhaps a lower PO_2 , for example 3 ATA, would result in a closer correspondence in doses, something to be determined by investigators who may wish to pursue the parallels between these two experimental models.

From our results, we infer that AEDs with specific mechanisms-of-action can be used as tools to investigate mechanisms of CNS O_2 toxicity. This inference, however, is limited by the fact that the true mechanisms of action of some of these drugs may not fit neatly into their nominal classes. For example, some ostensible Na^+ -channel antagonists may enhance GABA neurotransmission and inhibit that of glutamate, for example PRM. Other Na^+ -channel antagonists may also block L-type or T-type Ca^{2++} channels. The polyspecificity of certain AEDs has been extensively reviewed (Czapinski et al., 2005; Fariello, 1993; Lasoń et al., 2011; Sills and Brodie, 2001).

Our findings also support the idea that HBO_2 could be added to the list of established techniques (Grone and Baraban, 2015; Raol and Brooks-Kayal, 2012; Rogawski, 2006) used to elicit epileptiform seizures in animal models. It must be said, however, that the epileptiform events seen in CNS O_2 toxicity are not strictly speaking “epilepsy”, since that term implies the spontaneous recurrence of seizures. Yet if a comparison is to be made, which form of clinical epilepsy most closely parallels CNS O_2 toxicity? The idea that reactive oxygen and nitrogen species (RONS) are produced uniformly throughout the brain would support the view that CNS oxygen toxicity begins in both hemispheres and, therefore, most closely resembles primary generalized epilepsy. However, cerebral blood flow, oxidative metabolism and the concentration of antioxidant enzymes exhibit significant differences in various brain regions (Allen et al.,

2009; Demchenko and Piantadosi, 2006; Demchenko et al., 2012). This is consistent with a multifocal initiation of seizure activity in HBO₂ and the concept that hyperoxic seizures are partial seizures with secondary generalization.

Hyperbaric oxygen, therefore, has interesting properties as a convulsant. For example, administration by inhalation does not involve the stresses induced in conscious animals by capture and injection of a drug or placement of stimulating electrodes. Also, oxygen is a lipophilic gas that diffuses readily to target tissues, assuring rapid kinetics of distribution (Micarelli et al., 2013). Finally, the stages of seizure progression—including prodromal phases and recruitment of neurons beyond an initial focus—can be studied in isolation by a careful titration of the inspired Po₂ which can be achieved by adjusting the pressure in the hyperbaric chamber, without having to alter the concentration of O₂ in the inspired gas. In our previous work, we have exploited these characteristics to delineate several pathophysiological states that precede the onset of hyperoxic seizures, by exposing rodents to levels of HBO₂ ranging from 3 to 6 ATA. These preliminary phases include sequential changes in the afferent and efferent limbs of the autonomic nervous system, cardiovascular function, EEG, and neuromotor responses (Allen et al., 2009; Demchenko and Piantadosi, 2006; Demchenko et al., 2012).

A relatively new hypothesis of epilepsy pathogenesis is that oxidative stress is a factor in epileptic seizure initiation (Martinc et al., 2012; Patel, 2004; Waldbaum and Patel, 2010). Therefore, the seizures of CNS O₂ toxicity could provide an appropriate model for direct investigation of redox-dependent mechanisms by which neuronal hyperexcitability and hypersynchrony might develop. A large body of work in the hyperbaric literature demonstrates that reactive oxygen and nitrogen species (ROS and RNS) interact with the machinery of neuronal excitation and synaptic transmission. Thus, the activation of diatomic oxygen by an initial one-electron reduction to the superoxide anion ($\cdot\text{O}_2^-$) that occurs in mitochondria and at other enzymatic sites, as well as the endogenous production of nitric oxide (NO $\cdot$) are both

increased in HBO₂. In the thermodynamically-favored events that follow, other reactive oxygen and nitrogen species are generated, including hydrogen peroxide (H₂O₂), the hydroxyl radical (OH•) and peroxynitrite (ONOO⁻) (D'Agostino et al., 2007; Demchenko et al., 2003). The brief half-life of these reactive species limits their scope of action, but where their sources of production are intimately co-located with redox-sensitive neuronal targets, significant alterations in membrane excitability and neurotransmission can occur. For example, ion-sensitive and voltage-sensitive channels that regulate permeability to Na⁺, K⁺ and Ca²⁺ all present potential targets (Matalon et al., 2003; Mulkey et al., 2003), as do the mechanisms involved in the biosynthesis, re-uptake and metabolism of neurotransmitters. In this regard, GABA is particularly important, as there are multiple redox-sensitive targets in its pathways of synthesis, release, uptake and catabolism. Alterations in these processes can perturb the equilibrium between excitation and inhibition in specific brain regions, resulting in increased neuronal firing that propagates across brain structures (Demchenko and Piantadosi, 2006). Ultimately, increased oxidative and nitrosative stresses that occur in HBO₂ drive excessive neuronal excitation that results in epileptiform patterns on the EEG and tonic-clonic neuromotor responses (Allen et al., 2009; D'Agostino et al., 2007; Demchenko et al., 2001; Demchenko et al., 2005).

The amino acid cysteine is a critical constituent at many functional sites in the CNS and is particularly vulnerable to both ROS and RNS through S-oxidation and S-nitrosylation, and both of these processes are intensified in HBO₂. Thus, cysteine residues in the beta-1 subunit of voltage-gated Na⁺-channels form a stabilizing disulfide bridge, and its disruption can lead to certain idiopathic epilepsies (Wallace et al., 1998). Likewise, calcium-activated BK-type K⁺ channels contain cysteine string proteins, consisting of multiple linked cysteines, which have critical regulatory functions (Kyle et al., 2013). Also, the so-called Cys-loop super-family of receptors includes acetylcholine, 5-HT₃, glycine and GABA_a receptors (Connolly and Wafford,

2004; Pan et al., 2000). Furthermore, signals mediated through cysteine oxidation regulate key mitochondrial functions, in the CNS and elsewhere (Mailloux et al., 2014). The cysteines in these examples, as well as those in other critical locations in the CNS, are all potentially subject to oxidative or nitrosative attack.

Conclusions. The findings presented here support our hypothesis and its corollary: a range of FDA-approved AEDs can prevent or delay seizures in extreme hyperoxia, and AEDs with known mechanisms-of-action can serve as investigative probes to elucidate seizure mechanisms in HBO₂. Thus, some of the AEDs we tested increased seizure latency to more than triple that observed in vehicle controls. Furthermore, the significant protective efficacy in HBO₂ of AEDs known to block Na⁺-channel function or to enhance GABA-mediated inhibition clearly demonstrate that Na⁺-channel function and GABA neurotransmission are critical targets of hyperbaric oxygen and, by implication, are significant nodes on pathways to CNS toxicity. By extension, since disturbances of Na⁺-channel function and GABA-mediated inhibition are also implicated in other seizure disorders; the body of information that has been accrued by the many studies of CNS toxicity in HBO₂ may be useful well beyond the traditional fields of hyperbaric medicine and physiology. In addition, hyperbaric oxygen is particularly well-suited to pursue the hypothesis that oxidative stress is a contributive factor in some forms of epilepsy.

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METHODS and MATERIALS

Experiments were performed on conscious C57BL/6 mice (weighing 19-25g and approximately 2.5 months in age) at the Center for Hyperbaric Medicine and Environmental Physiology, Duke University Medical Center (Durham, NC, USA), and at the Institute of Evolutionary Physiology and Biochemistry, Russian Academy Sciences (St. Petersburg, Russia). Animal use protocols were approved independently by the Institutional Animal Care and Use Committee of Duke University and the Ethical Review Board of the Institute of Evolutionary Physiology and Biochemistry. The drugs, hyperbaric exposures, assessments of animal responses and methods for analyzing results were the same at both experimental sites.

Hyperbaric Exposures. Groups of 4 to 6 freely-moving mice, selected at random, were placed in a hyperbaric chamber in separate cages. Compression to 5 ATA was accomplished at $0.7 \text{ ATA} \cdot \text{min}^{-1}$, and exposures to 100% O_2 lasted for up to 60 min. Chamber temperature and relative humidity were maintained at $23 \pm 0.5^\circ \text{C}$ and $60 \pm 2\%$. Animals were continuously observed for signs of CNS HBO_2 toxicity, and in some cases video recordings were made for later review.

Experimental Design. AEDs included 5 Na^+ -channel antagonists and 4 GABA enhancers, each of which was administered in four different doses in separate groups of animals. Two additional groups were used as vehicle controls. Each treatment or control group comprised 12 animals. A total of 456 mice were used in the 36 treatment groups and the NaCl and DMSO vehicle controls. AEDs were obtained in clinical formulations, crushed to powder, if necessary, and dissolved in 0.9% NaCl or DMSO, according to their solubilities. Prepared stock solutions were diluted so that the intended dose ($\text{mg} \cdot \text{kg}^{-1}$) could be administered IP in a volume of $0.005 \text{ ml} \cdot \text{g}^{-1}$ body weight. The range of doses and the time allowed for onset of action for each drug were based on known pharmacokinetics, clinical practice, animal models of epilepsy (Bialer et al., 2004) and our preliminary testing of some of the drugs.

Estimation of Efficacy. For each dose of a drug, we evaluated mean seizure latency, the time interval that began when chamber pressure reached the target pressure of 5 ATA and ended when persistent, rhythmic spasms, bilateral forelimb clonus or escape behaviors first appeared. To estimate relative efficacies, we used linear regression analysis of mean seizure latencies (expressed as multiples of latencies observed in vehicle controls) plotted against $\log_{10}$ doses to estimate an equally-effective dose (EQD) for each drug.

Statistical Analysis. Mean seizure latencies observed after pretreatment with each drug-dose combination were compared with those observed in the corresponding vehicle controls using 2-way ANOVA. Significance was accepted at $P \leq 0.05$.

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FIGURE LEGENDS

Figures 1 and 2: Seizure Latencies for Conscious Mice exposed to HBO₂ at 5 ATA, after pretreatment with one of 2 functional classes of AEDs administered individually at 4 different doses, are shown as multiples of latencies seen in mice treated with vehicle controls. **Na⁺-Channel Antagonists:** *PRM* (Primidone), *ZNS* (Zonisamide), *OXC* (Oxcarbazepine), *LTG* (Lamotrigine), and *CBZ* (Carbamazepine). **GABA Enhancers:** *VPA* (Valproic Acid), *GBP* (Gabapentin), *VGB* (Vigabatrin), and *TGB* (Tiagabine). Statistically significant differences ($p \leq 0.05$) among maximally-effective doses are indicated with linked tick marks.

Figure 3: Equally-effective doses (EQD's) and ED₅₀'s in HBO₂ at 5 ATA.

A and C. Regression lines ($R^2 > 0.95$) are plotted as mean seizure latencies (expressed as multiples of latencies in vehicle controls) against log₁₀ AED doses. Broken lines intersect regression curves at each point where the corresponding dose is predicted to produce seizure latency 3x that observed in vehicle controls.

B and D. Estimated ED₅₀'s are obtained by plotting mean seizure latencies (expressed as probability units, *probits*) against log₁₀ AED doses. Broken lines intersect dose-response curves at each point where the corresponding dose results in the probability that half of the treated animals will experience seizures and half will not.

Na⁺ Channel Antagonists: ○ *LTG* (Lamotrigine), □ *CBZ* (Carbamazepine), △ *OXC* (Oxcarbazepine), ■ *ZNS* (Zonisamide) and ● *PRM* (Primidone).

GABA Enhancers: ◇ *TGB* (Tiagabine), □ *GBP*, △ *VGB* (Vigabatrin), ○ *VPA* (Valproic Acid),

Figure 4: The pattern of ED₅₀'s for 8 AEDs in mice exposed to HBO₂ at 5 ATA closely tracks with that of the same drugs tested in mice using the maximal electroshock (MES) model, when normalized ED₅₀ doses are expressed in molar values.

FIGURES

Figure 1

Figure 2

Figure 3

Figure 4

TABLES

Table 1: Effects of AED/dose Combinations vs Vehicle in HBO₂ at 5 ATA

AEDs [Vehicle]	AEDs Dose (mg · kg ⁻¹)	Numbers of Mice	Time Allowed for Onset of Action (Min)	Mean Seizure Latency (Min)	Standard Error of the Mean	Significance vs Vehicle
VEHICLE CONTROLS						
0.9% NaCl		12	60	12.8	1.7	—
DMSO		12	60	13.5	1.6	—
Na ⁺ -CHANNEL ANTAGONISTS						
Carbamazepine	5	12	60	22.4	2.7	* $P \leq 0.03$
(CBZ)	10	12		34.8	3.2	* $P \leq 0.01$
Tegetrol®	15	12		45.6	2.4	* $P \leq 0.01$
[DMSO]	25	12		49.6	3.6	* $P \leq 0.01$
Lamotrigine	2	12	60	28.2	3.3	* $P \leq 0.01$
(LTG)	5	12		35.7	4.2	* $P \leq 0.01$
Lamictal®	10	12		41.0	3.4	* $P \leq 0.01$
[DMSO]	20	12		44.0	4.1	* $P \leq 0.01$
Primidone	5	12	60	15.0	2.0	—
(PRM)	20	12		21.5	2.4	* $P \leq 0.03$
Mysoline®	80	12		28.7	2.9	* $P \leq 0.01$
[DMSO]	160	12		30.3	5.0	* $P \leq 0.01$
Zonisamide	10	12	60	16.5	2.0	—
(ZNS)	20	12		21.0	3.5	* $P \leq 0.03$
Zonegran®	40	12		27.6	3.5	* $P \leq 0.03$
[0.9 %NaCl]	80	12		28.9	3.9	* $P \leq 0.01$
Oxcarbazepine	5	12	60	16.6	1.8	—
(OXC)	15	12		24.5	4.0	* $P \leq 0.03$
Trilepta®	45	12		33.6	4.1	* $P \leq 0.01$
[DMSO]	135	12		37.3	3.9	* $P \leq 0.01$
GABA ENHANCERS						
Gabapentin	10	12	120	16.8	2.5	—
(GBP)	30	12		25.3	4.7	* $P \leq 0.01$
Neurontin®	100	12		32.0	3.2	* $P \leq 0.01$
[0.9 %NaCl]	300	12		37.3	4.2	* $P \leq 0.01$
Tiagabine	1	12	60	22.1	3.9	—
(TGB)	2	12		31.8	4.6	* $P \leq 0.02$
Gabatrik®	6	12		43.6	3.8	* $P \leq 0.01$
[0.9 %NaCl]	9	12		46.9	3.3	* $P \leq 0.01$
Valproic Acid	150	12	60	23.8	4.2	—
(VPA)	300	12		25.7	4.4	* $P \leq 0.03$
Depakene®	500	12		29.8	3.4	* $P \leq 0.01$
[0.9 %NaCl]	600	12		35.8	3.7	* $P \leq 0.01$
Vigabatrin	50	12	240	18.2	3.3	* $P \leq 0.01$
(VGB)	150	12		35.3	3.6	* $P \leq 0.01$
Sabril®	300	12		43.6	4.3	* $P \leq 0.01$
[0.9 %NaCl]	500	12		45.4	4.2	* $P \leq 0.01$

Table 2: ED₅₀ for AEDs in 5 ATA HBO₂ vs MES

AED	ED ₅₀ HBO ₂ 5 ATA		ED ₅₀ Max Electro Shock	
	mg · kg ⁻¹	μM · kg ⁻¹	mg · kg ⁻¹	μM · kg ⁻¹
LTG	7	27	8	29
TGB	3	8	5	12
CBZ	13	54	8	33
OXC	82	325	14	57
PRM	110	504	12	55
ZNS	204	961	41	191
GBP	136	794	78	456
VPA	648	4,500	263	1,826
VGB	190	1,471	—	—