TITLE: Vigabatrin Protects Against Hypoxia in Rats.

AUTHORS: Cristobal Garcia, M.D., Leonid Bunegin, B.S., Maurice S. Albin, M.D., M.Sc.(Anes.)

AFFILIATION: Neuroanesthesia Laboratory, Department of Anesthesiology, University of Texas Health Science Center at San Antonio.

Vigabatrin (8-vinyl GABA) is an irreversible selective, enzyme activated inhibitor of GABAtransaminase and appears to be effective as an anticonvulsant in both animal models and man.[1] The purpose of this study is to determine whether the ability of vigabatrin to increase GABA levels might be protective against CNS damage suffered during hypoxia. The protocol used in this study was approved by the Animal Care Committee, University of Texas Health Science Center at San Antonio. Twenty one Sprague-Dawley rats, mean weight 400 gm were trained daily for two weeks and conditioned to turn to the left in a "T" maze at zero indecision level. The rats were divided into three groups; Groups I (n=7) received 1500 mg/kg^{-1} of intraperitoneal vigabatrin; Group 2 (n=7) received 750 mg/kg^{-1} of intraperitoneal vigabarin and Group 3 (n=7) received 1 cc of intraperitoneal saline solution. Three hours after injection one animal of each group was placed on an airtight flow through box with a flow of 15 lts/min consisting of 3.7% O_2 in nitrogen. The percent O_2 was monitored via mass spectrometry at the inflow site and by a polarographic device at the outflow side. The chamber temperature was maintained between 31-33°C. Rats breathed this hypoxic mixture for 8 minutes and body temperature was supported with a heating pad after the drug was injected until the end of the experiment. The duration of hypoxia was 8 min for each set of animals. After hypoxic insult, rats were removed from the chamber and given cardiopulmonary support if necessary, temperature was measured rectally and the animals were permitted to recover in a warmed cage. Twenty four hours and one week after the hypoxic insult they were given 10 trial runs each in the maze, with the average run time and number of errors recorded. Data was compared between control, vigabatrin 1500 mg/kg^{-1} and vigabatrin 750 mg/kg^{-1} using paired t test with $p<0.05$ considered significant. There was no significance (NS) between control prehypoxia and 750 mg/kg^{-1} vigabatrin pre-posthypoxia and vigabatrin 1500 mg/kg^{-1} pre-posthypoxia; and between vigabatrin 750 mg/kg^{-1} pre-posthypoxia and vigabatrin 1500mg/kg pre-posthypoxia. Three animals of control group died within 24 hours after the experiment and the four remaining rats died within the next 48 hours. No animal in this group was able to run the maze at any moment after the hypoxic insult; the animals in this group were inactive, somnolent and sometimes seizing after hypoxia. Two animals died in the 1500 mg/kg^{-1} vigabatrin group (24 hours and 72 hours after hypoxia). No animal died in the 700 mg/kg^{-1} vigabatrin group. These animals were behaving normally 24 hours after the experiment. The deaths of the two rats in the high dose vigabatrin group might be explained in the basis that the 1500 mg/kg^{-1} used is near the LD50. Our data indicates that vigabatrin 750 mg/kg^{-1} not only protected the rats from death caused by hypoxia, but also protected the animals from neurobehavioral deficits that are usually produced with hypoxic insult.

Maze Run Times, Temperature and Deaths

	Control	Vigabatrin 1500	Vigabatrin 750
Maze Run Time Prehypoxia	7.2 sec ±0.22	7.2 sec ±0.37	7.3 sec ±0.32
Maze Run Time 24 hrs after Hypoxia	*	8.3 sec ±0.38	8.14 sec ±0.39
1 week after Hypoxia	*	8.6 sec ±0.42	8.28 sec ±0.4
2 weeks after Hypoxia	*	8.7 ±0.41	8.0 ±0.39
Deaths	7	2	0
Temp.	36.5 °C ±0.1	36.3 °C ±0.1	37.1 °C ±0.2

*p<0.05 Data presented as mean ± SEM.

Title: OLIGODENDROGLIAL CELL DEATH OCCURS IN THE ABSENCE OF CALCIUM IN A DEENERGIZATION MODEL OF HYPOXIA AND PERIVENTRICULAR LEUKOMALACIA

Authors: MS Jurkowitz-Alexander, Ph.D., LA Horrocks, Ph.D., TD Simmons, B.A., CM Hohl, Ph.D., RA Altschuld, Ph.D., and JS McDonald, M.D.
Affiliation: Departments of Anesthesiology and Physiological Chemistry, The Ohio State University, Columbus, OH 43210

Hypoxic injury to oligodendroglial cells is a key event in development of periventricular leukomalacia, the major hypoxic/ischemic disease affecting premature infants (1). The mechanism(s) of cell injury are not known. The role of calcium (Ca) in mediating hypoxic cell injury in an oligodendroglia glioma cell line (ROC-1) was examined. Amobarbital (amytal) was used to block mitochondrial ATP synthesis and mimick the state of oxygen deprivation. Cell morphology, viability, and nucleotide and nucleoside contents were examined.

Methods. ROC-1 cells were incubated in Krebs-Henseleit media containing glucose at pH 7.0 and 37°C. Medium (+Ca) was 1.2 mM with respect to Ca and medium (-Ca) contained 100 μM EGTA and no Ca. Deenergization (DE) was achieved by inhibiting mitochondrial electron transport with amytal and glycolysis with iodoacetate. Viability was assessed by LDH release and trypan blue exclusion analyses. Intracellular contents of adenine and purine nucleotides and nucleosides were measured.

Results. In glucose-containing media cells maintained viability for >4 H in media (+Ca) and (-Ca). Within 10 min after DE, blebs formed and cells began to lose viability; by 70 min 100% of cells were nonviable. Rates of bleb formation and viability loss were the same in cells incubated in presence or absence of Ca. During the first 6 min of deenergization, ATP levels declined to less than 1.5% of control values of 40 nmol/min/mg while AMP, IMP, and ADP contents rose in (-Ca) and in (+Ca) media.

Blockade of the voltage dependent calcium channels with verapamil or inhibition of Na+,K+-ATPase with ouabain (1.5 mM) did not change the DE-induced viability loss. In (+Ca) medium, the ionophore A23187 induced rapid cell death with or without DE. In (-Ca) medium, A23187 reduced intracellular free Ca concentration but had no effect on viability loss. Buffering of intracellular free Ca with Quin 2 did not attenuate DE-induced viability loss in either (-Ca) or (+Ca) media.

Conclusion. The cell death is dependent on depletion of ATP. Viability loss in the Ca-free medium seems to occur without an increase in intracellular free Ca. The formation of blebs on membrane surfaces may indicate disruption of cytoskeleton and subsequent plasma membrane dysfunction (2). Cell death could also be due to increased lipolysis with accumulation of end products.

References. 1. Developmental Pathology of the Neonate. Elsevier/North Holland Biomedical Press, Amsterdam. 399-408, 1977. 2. Nature, Lond. 325, 78-81, 1987.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/73/3A/NA/666763/0000542-199009001-00742.pdf by guest on 19 April 2024