Guanosine 3',5'-monophosphate is not a marker of the proepileptic activity of valproic acid in hippocampal brain tissues

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ABSTRACT

Objective: Guanosine 3',5' monophosphate (cGMP) can be used as a marker of the epileptogenicity of proconvulsant drugs. As valproic acid (VPA), at certain concentrations, acts as a proconvulsant agent in hippocampal pyramidal neurons when tested in the veratridine model, this investigation was conducted to study the effect of proconvulsant concentrations of VPA on the basal level of cGMP in hippocampal tissue.

Methods: Experiments were performed using standard radioimmunoassay techniques in hippocampal tissues from rats. This study was carried out at the Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, Texas, United States of America between 1996-1997.

Results: We found that veratridine (0.3, 1 and 2 µM, n=3) increased the level of cGMP in hippocampal tissue in a concentration dependent manner. However, VPA at proepileptic concentrations (0.1, 2, 5 µM, n=3), did not significantly affect the basal level of cGMP when added alone or with veratridine (0.3 µM)

Conclusion: Guanosine 3',5' monophosphate is not a marker of the proepileptic activity of VPA in brain tissues.

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It is well documented that convulsant agents, which cause cellular depolarization such as veratridine, can cause an elevation in guanosine 3',5' monophosphate (cGMP). This elevated level of cGMP produced by veratridine is a consequence of a cellular depolarization and not as a direct effect of the depolarizing agents on cyclic nucleotide synthesis or degradation. This increase in cGMP represents a marker of epileptic activity. On the other hand, agents which produce antiepileptic activity inhibited cGMP accumulation in the brain. Previously, we have found that valproic acid (VPA) enhanced veratridine induced seizure-like activity in hippocampal pyramidal neurons. The enhancement of veratridine bursting by VPA was accompanied by depolarization of the resting membrane potential. The proepileptic activity of VPA was attributed to its effect on sodium channels.

The specific aim of this study is to answer the following question: Does the depolarization induced by VPA in our veratridine model lead to an increase in cGMP accumulation in hippocampal tissue? In other words, is cGMP a marker for the proepileptic activity of VPA in veratridine model?
**Methods.** The current experiments were performed on male Sprague-Dawley rats [Harlan Sprague-Dawley Inc, Indianapolis, United States of America (USA)] weighing 150-300 g. The rat was quickly decapitated by means of a small animal guillotine. To reach the animal's brain, the skull bone was removed by small bone rongeurs and the dura was carefully cut with small scissors. A stainless steel spatula was used to lift the brain out of the skull. The brain was immediately and gently transferred to a petri dish filled with cold (0°C) oxygenated (95% O₂, 5% CO₂) artificial cerebrospinal fluid (ACSF). The brain was divided midsagittally into 2 hemispheres. From each hemisphere, a transverse block containing the hippocampal tissue was dissected. This block was fixed on a slicer stage using cyanoacrylate glue. Transverse slices of 500 µm thickness were cut using a vibroslice (Campden Instruments Limited). The hippocampi were dissected and suspended in a beaker filled with fresh ACSF solution gently gassed with 95% O₂ and 5% CO₂. Hippocampal tissue was incubated for one hour at 37°C. At the end of the incubation period, the samples were divided into 10 aliquots (25-40 mg tissue) and placed in 3 ml of fresh ACSF. The test agent was added to each aliquot to achieve the desired concentration and then incubated for 20 minutes at 37°C. Control aliquots were treated in identical fashion but no agent was added. The sample was extracted by homogenization in one ml of 4 mM ethylenediamine tetraacetic acid (EDTA) solution to prevent enzymatic degradation of cGMP. Coagulation of protein was achieved by heating the samples in a boiling water bath for 5 minutes. Following centrifugation (2000 rpm), the supernatant was collected for measurement of cGMP level using Amersham's cGMP [3H] assay system. Guanosine 3',5' monophosphate assay is based on the competition between unlabeled cGMP and a fixed quantity of tritium labeled cGMP for binding to antiserum. The kit assay tubes and the tubes that have the unknowns were placed in ice/water bath throughout the assay. Following the procedure described in the booklet provided from Amersham, the tubes were incubated at 2-8°C for one and a half hours. The antibody bound cGMP was separated from the unbound nucleotide by the addition of one ml of ice cold ammonium sulphate followed by centrifugation. The supernatant was decanted and the precipitate which contains the antibody bound complex was dissolved in one ml of distilled water. The activity of the precipitate was determined by liquid beta scintillation counting. The concentration of the unlabeled cGMP was calculated from a linear standard curve. The EDTA treated brain tissue precipitate was solubilized by the addition of 20 µL of 1 N (normality) sodium hydroxide (NaOH) and assayed for protein using bicinchoninic acid (BCA) protein assay available from Pierce Biotechnology incorporation, Rockford, Illinois, USA. In this assay bovine serum albumin was used as a standard. Cyclic guanosine monophosphate was expressed as p moles/mg tissue protein.

**Drugs and Chemicals.** All the chemicals were obtained from Sigma Chemical Company. Stock solutions of valproic acid were prepared by dissolving the drugs in distilled water. Veratridine was dissolved in 0.1 mM of hydrogen chloride. The composition of the ACSF was: sodium chloride 127 mM, calcium chloride 2.5 mM, potassium chloride 4.7 mM, magnesium chloride 1.2 mM, sodium hydrogen carbonate 22 mM, sodium dihydrogen phosphate 1.2 mM and glucose 11 mM. The pH of this solution is kept at 7.4.

**Statistics.** Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using the 2-way analysis of variance. P value of <0.05 was considered statistically significant.

**Results.** Veratridine (0.3, 1 and 2 µM, n=3) increased the level of cGMP in hippocampal tissue in a concentration dependent manner (Figure 1). These concentrations of veratridine were used to induce seizure like activity in hippocampal pyramidal neurons.1 Basal level of cGMP was 4.73 ± 1.2 p mole/mg (mean ± SEM). However, VPA at proepileptic concentrations (0.1, 2, 5 mM, n=3) did not significantly affect the basal level of cGMP when added alone (Figure 2) or with veratridine (0.3 µM) at the concentration used in our electrophysiological experiments to induce bursting (Figure 3).

**Discussion.** We found that veratridine increased the level of cGMP in hippocampal tissue in a concentration dependent manner. Proepileptic concentrations of VPA did not significantly affect the level of cGMP when added alone or immediately before veratridine. Our conclusion is that the depolarization induced by VPA in hippocampal pyramidal neurons did not induce or enhance veratridine increase in cGMP.

Numerous investigations have studied the role of cyclic nucleotides in the regulation of neuronal activity. It has been found that the concentration of cGMP undergoes significant changes in certain areas of the central nervous system with the development of epileptic seizures.1 Most of the convulsant drugs that induce seizures in vivo elevate the level of cGMP in the brain.2 Focal penicillin epilepsy in cat cerebral cortex was associated with an increase in cGMP level during the ictal phase of the seizure.4 The seizure induced by pentylenetetrazol was accompanied by an increase

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in the level of cGMP. Cyclic GMP increased after injection of bicuculline gama-aminobutyric acid receptor blocker, N-methyl-D-aspartate agonist and strychnine. Moreover, an increase in the level of cGMP was documented during amygdaloid carbachol kindling in the rat. Some anticonvulsants are known to decrease the level of cGMP. Phenytoin inhibited electroshock seizures-induced elevation in cGMP in mice at a dose which suppresses epileptic activity. The administration of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate / kainate receptor antagonist 6-nitro-7-sulfamoylbenzo (f) quinoxaline-2,3-dione decreases cGMP in rat hippocampus.

We found that veratridine increased the level of cGMP in a concentration dependent manner. There are 2 theories that explain the mechanism by which veratridine induces an elevation in cGMP. The first theory states that veratridine prolongs inactivation of sodium channels and so increases sodium permeability into the cell, thus producing depolarization. This cellular depolarization leads to influx of calcium which presumably activates guanylate cyclase and increases the level of cGMP. The second theory linked the formation of cGMP in the brain tissue to the activation of voltage-dependent calcium channels. In neuroblastoma cell lines number 115 cells, study showed that the increase in cGMP produced by veratridine is completely dependent on extracellular calcium. Study found that the increase in cGMP is attenuated by a calcium channel blocker but not by a sodium channel blocker.

We have shown that the proepileptic phase of VPA is accompanied by a cellular depolarization that is attributed to the opening of sodium channels. Similar to veratridine, we were expecting the cellular depolarization induced by VPA to increase the level of cGMP. Our results fail to demonstrate this expectation. Valproic acid did not induce any change in the level of cGMP. The explanation of these results is not fully clear. Based on this, cGMP is not a marker for the proepileptogenisity of VPA.

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References

2. Fedele E, Varnier G, Raiteri M. In vivo microdialysis study of GABA(A) and GABA(B) receptors modulating the glutamate receptor/NO/cyclic GMP pathway in the rat hippocampus. Neuropsychopharmacology 1997; 36: 1405-1415.


