SODIUM VALPROATE AND BRAINSTEM ENERGETICS

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The effect of the anticonvulsant sodium valproate on cerebral brainstem energy metabolism has been investigated. Stupor and coma were produced in mice by the intraperitoneal injection of sodium valproate at a dose of 600 mg/kg. Glucose, glycogen, ATP, and phosphocreatine were measured in small tissue samples from the ascending reticular activating system. Levels of all metabolites were either normal or elevated in precoma and comatose mice as compared to controls. These data are consistent with the concept that sodium valproate does not have a primary action through depletion of high energy phosphates.

INTRODUCTION

Sodium valproate, an effective anticonvulsant, is used to control a variety of seizure disorders. However, sodium valproate has side effects associated with its use. One side effect, drowsiness, is commonly found with normal usage; the second, coma may follow an overdose of the drug (1, 2). Interestingly, sodium valproate is an isomer of octanoic acid, a medium chain fatty acid which is elevated in hepatic encephalopathy and causes symptoms similar to the side effects of sodium valproate. Octanoic acid, when injected into experimental animals, produces an encephalopathic state characterized by drowsiness, stupor and coma (17). Octanoic acid has recently been shown to have a selective effect on energy metabolism in cells from the reticular activating system in mice rendered comatose by the intraperitoneal injection of the fatty acid (12). The selective effect includes a decrease in high energy phosphate reserves during the precoma
stage as well as during early coma. Such a diminution of energy stores correlates with decreased electrical output, a condition conducive to the development of coma. In fact a similar set of metabolic responses has been shown in the reticular formation in animals made comatose by either hyperammonemia (10) or hypoglycemia (11). The concept has been proposed that a selective decrease in reticular formation energy metabolism may be a “triggering” feature of all metabolic encephalopathies which have coma as a prevalent feature.

Sodium valproate has been shown to alter cerebral GABA metabolism, and the mode of action relates to its probable effect on both GABA transaminase and GAD (glutamic acid decarboxylase) activities (13). The net result is that GABA levels are elevated in the brain of sodium valproate treated animals (3, 15). Previous studies on the effects of sodium valproate and cerebral energy metabolism have centered on whole brain of newborn animals (16), or on cortical and cerebellar tissues (8). Results from these studies have shown that sodium valproate administration results in either no change or in an increase in energy reserves in these regions. Nevertheless, the uniqueness of metabolic response in the reticular formation in other types of metabolic coma prompted us to investigate the effects of valproic acid in reticular formation energy metabolism, and the results are the basis for this report.

EXPERIMENTAL PROCEDURE

Female Swiss-Albino mice weighing 20–22 grams were obtained commercially, and housed in standard laboratory cages with food and water ad lib. Experimental animals were injected intraperitoneally with sodium valproate at a dose of 600 mg/kg. Control mice received an equivolume injection of saline. Mice were sacrificed by rapid submersion in liquid N₂ 1 minute after the onset of coma. A second group was sacrificed after injection at a time when they were drowsy but still able to right themselves (precoma). Control animals were sacrificed and their brains processed simultaneously with the experimental groups.

Following sacrifice, the mouse brains were removed in a Wedeen cryostat at −20°C, mounted in brain paste and sectioned into sections 20 microns thick. Coronal sections were taken through the brainstem at the level of the inferior (posterior) colliculus. These sections were placed in specially constructed aluminum holders and vacuum desiccated at −40°C for 18–24 hours. Following this treatment, the samples can be removed and manipulated at room temperature without loss of labile metabolites. Freeze-dried sections were viewed through a stereo dissecting microscope and small 100–400 nanogram samples were dissected from the reticular formation and the inferior colliculus. These samples were weighed on a quartz fiber fishpole balance and placed into oil well racks for metabolite analysis. Glucose, glucogen, GABA, ATP and P-creatine were all measured using NADP-NADPH coupled reactions, and enzymatic cycling to enhance fluorescence. A more detailed description of these methodologies has been previously published (4, 7). Data was statistically analyzed using the Mann-Whitney U test (14).