Regional Cerebral Na⁺K⁺ ATPase Activity following Octanoate Administration

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Summary

Sodium octanoate in an 0.2 M solution was administered to rabbits by continuous slow IV infusion over 4 hr. Controls were given identical infusions of normal saline. The animals were then sacrificed, brains were removed, and specific areas were isolated and assayed for Na⁺K⁺ ATPase activity. Significant inhibition of regional Na⁺K⁺ ATPase activity was detected in cortex, thalamus, hypothalamus, pons, and medulla of rabbits given octanoate when compared to controls.

Speculation

Elevated serum concentrations of short-chain fatty acids may produce coma in patients with Reye's syndrome and hepatic encephalopathy by inhibition of cerebral Na⁺K⁺ ATPase activity with resultant disruption of normal transport across neuronal and glial membranes.

Continuous IV infusion of the short-chain fatty acid sodium octanoate into unanesthetized rabbits produces significant hyperventilation, coma, seizures, hypotonia, and electroencephalographic changes (9). The mechanisms underlying the central nervous system alterations are unknown. One previous report (1) suggested that octanoate inhibited Na⁺K⁺ ATPase activity in rat brain slices *in vitro*. However, no effort was made to identify possible regional differences, nor have *in vivo* studies been performed. The present study was designed to examine regional Na⁺K⁺ ATPase activity in rabbit brain *in vivo* following a continuous IV infusion of octanoate.

MATERIALS AND METHODS

Two-kg albino rabbits were prepared according to the experimental protocol described in detail in a previous report (9). An 0.2 M solution of sodium octanoate was infused at a rate of 0.19 ml/min over a 4-hr period through the ear vein. Control animals were given continuous infusions of normal saline. At the end of the four-hr period, the animals received a rapid IV injection of 100 mg sodium pentobarbital, and the brains were immediately exposed, removed, and placed on ice. Under a dissecting microscope, sections of approximately 50 to 100 mg were isolated rapidly, frozen on dry ice, and weighed. Each sample was mixed with distilled water in a 20:1 ratio of water to brain by volume. (In preliminary studies in this laboratory, no difference in ATPase activity was found using water or deoxycholate; it was thus decided to use water). This mixture was then homogenized in a glass homogenizer with a Teflon pestle for 50 strokes at 4°C and centrifuged for 10 min at 400 × g and 4°C, and the supernatant was frozen and used for ATPase determination. ATPase was measured by a modification of the method of Samson and Quinn (6). Total ATPase activity was measured by taking 0.2 ml of the supernatant and adding 0.2 ml of 50 mM Tris buffer, pH 7.8. This

mixture was added to a reaction mixture containing 100 mM sodium chloride, 30 mM potassium chloride, 3 mM magnesium chloride, and 3 mM Tris-ATP to make a total volume of 2 ml. Mg²⁺ ATPase was measured in a reaction mixture similar but omitting sodium chloride and potassium chloride. The mixtures were incubated at 45°C for 10 min, and the reaction was stopped by addition of 1 ml 5% trichloroacetic acid. Inorganic phosphorus was measured using 1 ml of the supernatant by the method of Fiske and Subbarow (2). Na⁺K⁺ ATPase activity was calculated by subtracting Mg²⁺ ATPase from total ATPase activity. Protein concentration in the brain homogenate samples was determined by the method of Lowry *et al.* (4), and the results were expressed as mM inorganic phosphorus released per min per mg protein.

Serum octanoate concentrations were determined as described previously (9).

RESULTS

No significant alterations in blood pressure or partial pressure of oxygen were observed during the 4-hr period of octanoate infusion. Marked hyperventilation and hypotonia were observed in rabbits treated with octanoate and were similar to findings reported previously (9). Pupillary dilatation was also observed in all experimental animals. Mean concentration of octanoate in blood at the end of 4 hr was 380 μmoles/liter.

Na⁺K⁺ ATPase activity was assayed in cortex, hypothalamus, thalamus, basal pons, tegmental pons, and medulla. In every area of brain examined, ATPase activity was significantly reduced compared to activity measured in control brains (Table 1).

DISCUSSION

The clinical effects of short-chain fatty acid administration on experimental animals are striking. Hyperventilation to a respiratory rate of 240 (from a baseline of 90) is a consistent finding. Hypotonia, unresponsiveness, seizures, and pupillary dilation also occur. Recent studies in this laboratory have suggested that octanoate may alter blood-brain barrier permeability to injected 1-DOPA in rats (8). The present observation of inhibition of regional cerebral Na⁺K⁺ ATPase activity in rabbits *in vivo* is significant because it provides one mechanism by which octanoate produces its toxic effects on the central nervous system. Na⁺K⁺ ATPase is thought to regulate ion transport across cell membranes (7). Interference with normal ionic transport across neuronal and glial membranes could certainly result in coma and seizures. Such interference could also result in a loss of functional integrity of the blood-brain barrier.

This observation has implications for several human metabolic and toxic encephalopathies in which short-chain fatty acid elevations have been reported. Reye's syndrome is an acute encephalopathy in children which presents with coma, seizures, and hyperventilation. Serum short-chain fatty acid elevations (including octanoic acid) are observed in these patients (5, 10) and appear to

Table 1. Na⁺K⁺ ATPase activity ($\mu\text{M PO}_4^-$ per min per mg protein)

	Control	n ¹	Experimental	n	P ²
Cortex	0.1672 $\pm$ 0.05 ³	7	0.0638 $\pm$ 0.02	7	<0.001
Thalamus	0.2139 $\pm$ 0.13	6	0.0809 $\pm$ 0.02	5	<0.05
Hypothalamus	0.1523 $\pm$ 0.05	3	0.0911 $\pm$ 0.03	6	<0.05
Tegmental pons	0.2404 $\pm$ 0.14	5	0.0873 $\pm$ 0.05	5	<0.05
Basal pons	0.2730 $\pm$ 0.15	7	0.0950 $\pm$ 0.02	5	<0.05
Medulla	0.1779 $\pm$ 0.05	5	0.0818 $\pm$ 0.05	7	<0.02

¹ n, number of samples in each group.² One-tailed Student's *t* test.³ $\bar{x} \pm$ S.D.

correlate with the severity of the illness (11). Patients with hepatic encephalopathy from a variety of causes also have elevated serum and cerebrospinal fluid concentrations of short-chain fatty acids (3). After a 4-hr infusion of octanoate, serum concentrations of this substance are of the same order of magnitude as are those in patients with Reye's syndrome (5), suggesting that the concentrations of fatty acids in these patients may be high enough to account for the clinical symptomatology. The fact that the short-chain fatty acid sodium octanoate inhibits Na⁺K⁺ ATPase activity in experimental animals suggests that this may be a possible mechanism for these human encephalopathies. Further studies are required to identify potential therapeutic agents to counteract the toxic effects of excess short-chain fatty acids on the central nervous system.

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ERRATUM

In the article by Lee *et al.* "Genetic Influence on Serum IgD Levels" in *Pediatric Research*, **14**: 60 (1980), the value of the variance of monozygote pairs in the IgD level was incorrectly printed. The correct value is 0.03.

We regret the error.