PTH INCREASES CA++ TRANSPORT IN RAT BRAIN SYNAPTOSOMES IN UREMIA

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INTRODUCTION

Central nervous system (CNS) dysfunction is a major complication of patients with endstage renal failure. The clinical manifestations of this disorder are well described in several recent reviews (1). The biochemical basis for the CNS dysfunction of uremia is not well understood and is not completely corrected by dialysis. Studies of the CNS in humans and animals with renal failure have revealed no consistent pathologic changes (2) and biochemical studies in the brain of animal models of renal failure have also been generally unrevealing. The brain content of several ions (Na, K, Mg, Cl, bicarbonate) and water are normal (2,3), and so is brain intracellular pH (pHi) (3). Brain content of high-energy phosphate compounds (ATP, phosphocreatine) is also normal, although their turnover rate appears to be decreased (4). It appears that there may be altered permeability of the uremic brain to certain molecules, such as sodium, potassium, and inulin (5).

Among the few pathological findings in uremic brain which have been described is increased brain content of calcium, which appears to be parathyroid hormone (PTH) dependent (6). There are also associated abnormalities of the electroencephalogram, which are also felt to be dependent on PTH (7,8). Additionally, it is also felt that increased levels of aluminum in the brain may be responsible for some forms of uremic encephalopathy (9). However, removal of some of the potential "uremic neurotoxins", such as aluminum or PTH, does not necessarily reverse the encephalopathy, although parathyroidectomy in uremic subjects does result in improvement of both physiologic testing and the electroencephalographic abnormalities (10). Thus, it appears that other biochemical alterations are most likely present in uremia, and these alterations may play a significant role in the development of uremic encephalopathy. Further elucidation of these abnormalities in \textit{in vivo} whole animal models appears to be rather difficult.

With studies in \textit{in vitro} systems, other investigators have found abnormalities in both enzyme activity and certain transport phenomena. In erythrocytes from uremic subjects, it has been shown (11-13) that there may be abnormalities of sodium transport. In extracts from uremic rat brain, the Na-K ATPase enzyme activity was reported to be normal to low (14,15).

Because of the difficulty of directly measuring calcium transport in the brain of mammalian species, recent workers have performed calcium transport studies in an \textit{in vivo} system called synaptosomes. These are membrane vesicles that are formed from presynaptic nerve terminals in the brain (16) by homogenization and differential centrifugation of the
cerebral cortex. Under these conditions the presynaptic nerve terminals are sheared off and reseal to form intact vesicles called synaptosomes (17-19). Many enzymatic and metabolic properties which are identical to the intact nerve cell have been demonstrated in synaptosomes, and transport studies with sodium and neurotransmitter substances have also been evaluated (16,20-22). Under these conditions, the synaptosomes have proven to be a reliable and highly reproducible model with which to study alterations in CNS function.

There are five major pathways by which calcium can either enter or leave synaptosomes. These include voltage dependent Na+ channel, voltage dependent Ca++ channel, Na-Ca exchanger and Ca-ATPase pump. The Na-Ca exchanger and the Ca-ATPase pump are the two major calcium efflux pathways, while the Na+-Ca++ exchange participates in both calcium influx and calcium efflux (23-25). In nonexcitable cells, these transport mechanisms serve to actively extrude calcium against a high calcium concentration gradient of 10,000:1 (outside to inside cell). Calcium transport by the Na+-Ca++ exchanger is dependent on the sodium concentration gradient such that calcium is transported in a direction opposite to that of the sodium movement (26). The larger the sodium gradient, the greater the amount of calcium which will be exchanged for sodium (27). The stoichiometry for this process is such that three sodiums are exchanged for each calcium transported (28).

In the present studies, experiments were carried out to determine the effect of uremia on calcium transport in synaptosomes. However, based on the results of the studies, additional experiments were performed to determine if PTH was responsible for the calcium transport abnormalities that were observed.

METHODS

Experiments were performed on 200 gm male Sprague Dawley rats (29) and were carried out in normal (BUN = 20 ± 3 mg/dl), uremic (BUN = 250 ± 25 mg/dl), uremic parathyroidectomized (PTX-U) (BUN = 230 ± 18 mg/dl) and PTX-U rats which received 2.8 μg/day PTH x 7 days. Rats were made acutely uremic by performing bilateral ureteral ligation under general anesthesia (16). Depending on the experimental protocol, at between 30-45 hours of uremia, they were decapitated and their forebrains removed and placed in ice cold isolation media (320 mM sucrose, 0.2 mM K-EDTA, 5 mM TRIS-HCI, pH 7.4 at 0-4°C). In the PTX-U rats that were treated with parathyroid extract, decapitation was performed at approximately 30 hours of uremia (BUN = 180 mg/dl) vs. 45 hours in the uremic group. This modification was necessary because PTX-U rats which received PTH did not generally survive beyond 35 hours of acute uremia. In these instances all groups that were simultaneously studied were sacrificed at the same time.

Isolation of Synaptosomes

After the forebrain was removed and placed in ice cold isolation media, it was minced finely with scissors and washed thoroughly with the same media to remove all trace of blood. The brain extract was homogenized slowly, and centrifuged with a Beckman centrifuge at graded spins of 1300 G and 18000 G to obtain the crude synaptosomal-mitochondrial pellet (16). The purified synaptosomal fraction was then obtained by differential centrifugation on a discontinuous Ficoll gradient. At the end of the spin, the synaptosomal fraction was resuspended in isolation media and kept on ice until transport studies were performed.

Assessment of Metabolic Properties of Synaptosomes

Protein concentration of the synaptosomal preparations were determined as described by Lowry et al. (30). Internal synaptosomal volume prior to performing uptake studies was measured using 14C-methoxy inulin and tritiated water as described by Padan and associates (31). During experimental conditions, internal volumes were followed using tritiated mannitol as a marker for intracellular water (16). Tritiated mannitol was used to follow vesicular volume during the experiments instead of 14C-methoxy inulin because of the