Calcium Metabolism of Brain in Acute Renal Failure

EFFECTS OF UREMIA, HEMODIALYSIS, AND PARATHYROID HORMONE

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ABSTRACT Studies were carried out to evaluate the changes in content of calcium and magnesium in brain during acute uremia in dogs. Ca content in gray and white matter of brain increased significantly after 3 days of acute uremia and this increment was prevented by thyroparathyroidectomy (TPTX). The administration of parathyroid extract (PTE) to normal dogs and TPTX uremic animals produced a significant rise in brain Ca. These changes were not related to alteration in the concentration of Ca in plasma or cerebrospinal fluid, to changes in calcium-phosphorus product, or to changes in blood pH. Furthermore, the infusion of large amounts of phosphate to vitamin D₃-treated animals with suppressed parathyroid gland activity produced marked elevation in calcium-phosphorus product but no significant change in brain Ca. Also, uremia in vitamin D₃-treated TPTX dogs failed to increase calcium content in brain despite marked elevation in calcium-phosphorus product. Hemodialysis significantly reduced Ca content of brain but the values were still significantly higher than normal. Mg content increased modestly only in the white matter of uremic dogs with intact parathyroid glands and in normal dogs and TPTX uremic dogs receiving PTE. The results indicate that (a) acute uremia of 3 days is associated with a marked rise of Ca content of brain and modest increment of Mg in certain parts of the brain, and (b) these alterations are not related to uremia, per

se, but are dependent on the presence of excess parathyroid hormone. It is suggested that the neurological abnormalities noted in acute uremia may be related in part to the rise in the Ca content of brain.

INTRODUCTION

In studies carried out in our laboratory on the effects of acute renal failure on water and electrolyte metabolism of the central nervous system, we found significant elevations in the calcium content of brain. This finding was surprising since Ca content of brain usually remains stable even with acute changes in serum Ca (1, 2).

Many studies indicate that parathyroid hormone (PTH)¹ may enhance entry of Ca into different types of cells, such as skin (3), cornea (4), and blood vessels (5). In acute renal failure in man, the serum levels of PTH are elevated (6), and there is evidence that hyperactivity of the parathyroid glands occurs rapidly following acute renal failure in animals (7). It is possible that uremia, per se, or the elevation in the blood levels of PTH in renal failure are the factors promoting the entry of Ca into the brain. The present study was undertaken to test this hypothesis.

METHODS

Studies were made in nine groups of mongrel dogs of both sexes, weighing 15–22 kg, as follows: (a) nine normal dogs; (b) eight dogs with uremia of 3 days duration; (c) 12 dogs with uremia of 3 days duration which were treated either with rapid hemodialysis (blood flow 12 ml/

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¹Abbreviations used in this paper: CSF; cerebrospinal fluid; TPTX, thyroparathyroidectomy; PTE, parathyroid extract; PTH, parathyroid hormone.

kg/min for 100 min, six dogs), or slow hemodialysis (blood flow 5 ml/kg/min for 210 min, six dogs); (d) six normal dogs which received parathyroid extract (PTE) injection (Eli Lilly and Company, Indianapolis, Ind.), 100 U twice a day for 3 days; (e) four normal dogs which were subjected to thyroparathyroidectomy (TPTX) and studied 3 days later; (f) five dogs which underwent thyroparathyroidectomy (TPTX) and were then rendered uremic for 3 days; (g) six dogs which were subjected to TPTX and then made uremic. With the induction of uremia, PTE, 100 U four times daily, was administered for 3 days. (h) four dogs received vitamin D₃ in adequate doses to produce and maintain an elevation in serum calcium to levels of 12.0–13.5 mg/100 ml for a period of a month. These animals then received on 3 successive days infusions of saline containing buffered sodium phosphate (pH 7.4) to deliver 1.5 g of elemental phosphorus over a period of 3 h. These infusions resulted in a rise in serum phosphorus to 12.8–18.9 mg/100 ml without hypocalcemia. Hyperphosphatemia was usually present for an additional 6–8 h after each infusion. This procedure produced a marked elevation in calcium-phosphorus product in animals with suppressed parathyroid gland function. The animals were sacrificed immediately after the end of the last phosphate infusion. (i) Four dogs were subjected to thyroparathyroidectomy (TPTX). After the appearance of hypocalcemia, treatment with vitamin D₃ in adequate doses was initiated to produce and maintain an elevation in serum Ca to levels of 12.0–13.5 mg/100 ml for a period of 3–4 wk. Uremia was then induced

and the animals were sacrificed 3 days later for studies of brain Ca.

Uremia was produced by bilateral ureteral ligation done through a suprapubic incision. During the subsequent 3 days these animals ate small amounts of food and had free access to water. The success of TPTX was ascertained by a fall in plasma Ca of at least 2 mg/100 ml within 48 h. In groups e–g, plasma Ca was maintained at 6–8 mg/100 ml by intravenous injection of 10 ml of 0.9 M CaCl₂ twice a day.

Measurements of Ca and magnesium were made in brain cortical gray matter, subcortical white matter, arterial plasma, and cisternal cerebrospinal fluid (CSF), and water content was determined in brain white and gray matter. The urea, creatinine, inorganic phosphorus, and osmolality were determined in plasma and CSF, while pH, Pco₂, and bicarbonate were measured in arterial blood. The techniques of the dialytic procedures and the various analytical methods have been detailed elsewhere (8, 9).

Specimens were obtained in the following manner: Animals were anesthetized with sodium pentobarbital and intubated with an endotracheal tube. Ventilation was carried out with a Harvard Respirator (Harvard Apparatus Co., Inc., Millis, Mass.) at a rate of 25 strokes/min and initial tidal volume of 8.5 cm³/kg. The tidal volume was then adjusted to maintain a Pco₂ of about 35 mm Hg. After obtaining samples of arterial blood and cisternal CSF, the top of the skull was removed with a trephine and rongeur, leaving the dura mater intact. The entire operative field

TABLE I
Effects of Uremia, Parathyroid Hormone, and Hemodialysis on the Concentration of Calcium, Magnesium, and Phosphorus in Plasma and Cerebrospinal Fluid

	Plasma								Cerebrospinal fluid		
	Ca	Mg	P	Cr	Urea	HCO ₃	pH	Osmolality	Ca	Mg	P
	mg/100 ml	mg/100 ml	mg/100 ml	mM	mM	mM		mosmol/kg H ₂ O	mg/100 ml		
Normal (9)	10.3 ±0.2	1.9 ±0.1	4.0 ±0.3	0.8 ±0.1	5.0 ±0.2	20.0 ±0.9	7.36 —	304 ±1	4.8 ±0.2	2.4 ±0.1	0.6 ±0.1
Normal + TPTX (4)	6.5 ±0.3	1.9 ±0.2	5.0 ±0.4	0.8 ±0.1	5.2 ±0.3	20.8 ±1.0	7.38 —	291 ±2	4.6 ±0.2	2.3 ±0.1	1.2 ±0.1
Normal + PTE (6)	12.8 ±0.5	1.7 ±0.1	5.3 ±0.2	1.1 ±0.2	6.4 ±0.8	21.1 ±0.5	7.36 —	305 ±1	5.7 ±0.3	2.3 ±0.1	1.1 ±0.2
Uremic (8)	9.8 ±0.2	2.4 ±0.1	11.4 ±1.2	11.7 ±0.8	72.0 ±5.0	13.8 ±0.8	7.22 —	349 ±7	3.8 ±0.4	2.3 ±0.1	1.5 ±0.1
Uremic + TPTX (5)	6.8 ±0.5	2.6 ±0.2	11.1 ±2.3	10.5 ±0.6	56.6 ±2.0	13.6 ±0.7	7.24 —	347 ±9	4.3 ±0.3	2.3 ±0.2	2.7 ±0.7
Uremic + TPTX + PTE (6)	9.8 ±0.4	2.8 ±0.2	11.5 ±1.7	12.6 ±0.3	62.0 ±5.0	13.6 ±0.6	7.23 —	341 ±5	6.1 ±0.3	2.7 ±0.1	1.7 ±0.3
Uremic, after HD (12)	8.5 ±0.2	2.1 ±0.1	6.1 ±0.6	5.2 ±0.1	24.6 ±1.6	18.8 ±0.4	7.33 —	305 ±1	4.0 ±0.2	2.0 ±0.1	1.9 ±0.1
Normal + vitamin D ₃ + Phosphate infusion (4)	11.0 ±0.2	1.4 ±0.1	16.0 ±1.4	— —	— —	— —	— —	— —	— —	— —	— —
TPTX + vitamin D ₃ + Uremia (4)	12.2 ±0.5	2.2 ±0.2	9.5 ±0.3	9.8 ±0.8	51.1 ±8.2	11.8 ±1.2	7.18 —	344 ±10	6.2 ±0.3	2.6 ±0.1	2.6 ±0.5

Data are presented as mean ±1 SE. The mean pH was calculated from the mean of the hydrogen ion concentration. The arterial Pco₂ for all animals was maintained at 33–37 mm Hg. The numbers in parentheses are the numbers of animals studied. Cr, creatinine; HD = hemodialysis; P = phosphate.

TABLE II
Changes in Brain Content of Water, Calcium, and Magnesium

	Gray matter			White matter		
	H ₂ O	Ca	Mg	H ₂ O	Ca	Mg
	%	mg/kg dry wt		%	mg/kg dry wt	
Normal (9)	80.20 ±0.36	263 ±9	732 ±4	69.09 ±0.57	161 ±11	434 ±3
Normal + TPTX (4)	80.30 ±0.28	267 ±4	742 ±17	67.58 ±0.26	162 ±2	453 ±14
Normal + PTE (6)	80.16 ±0.44	317* ±8	737 ±12	68.62 ±0.66	190† ±3	464* ±4
Uremic (8)	80.57 ±0.64	389* ±22	768 ±29	69.53 ±0.77	267* ±19	519* ±27
Uremic + TPTX (5)	79.85 ±0.23	269 ±9	742 ±14	67.93 ±0.25	144 ±10	452 ±10
Uremic + TPTX + PTE (6)	80.54 ±0.38	360* ±20	738 ±14	68.97 ±0.36	194† ±5	476* ±8
Uremic + HD (12)	81.28 ±0.28	305† ±15	694† ±15	70.66 ±0.12	188† ±7	414 ±13
Normal + vitamin D ₃ + Phosphate infusion (4)	79.57 ±0.34	285 ±6	672† ±31	— —	— —	— —
T-PTX + vitamin D ₃ + Uremia (4)	81.89 ±0.18	258 ±11	792 ±16	66.98 ±0.28	136 ±9	406 ±26

Data are presented as mean ±1 SE. The numbers in parentheses are the numbers of animals studied.

HD, hemodialysis.

* $P < 0.01$ versus normal values.

† $P < 0.05$ versus normal values.

was thoroughly lavaged with 0.9% NaCl, in order to remove all bone fragments. The dura mater was then removed and the operative field was lavaged with artificial CSF. The whole brain was then scooped out with a spatula. Cortical gray matter and subcortical white matter were separated by blunt dissection and triplicate samples of both gray and white matter, each about 0.5 g, were placed in tared flasks and weighed to 0.1 mg. All samples were dried at 105°C for 48 h and then reweighed to determine water content. The tissue samples were then extracted in 0.75 N HNO₃ for 24 h and measurements of Ca and Mg were made in the supernates (9).

In four normal animals, the concentration of citrate in serum was measured before and after 3 days of acute uremia produced as described above. These measurements were made by Bio-Science Laboratories, Van Nuys, Calif., according to the method of Taussky (10).

RESULTS

The results are presented in Tables I and II, and Fig. 1. In dogs with acute uremia, the levels of Ca in blood and CSF were lower than those of normal animals whereas the concentration of phosphorus was markedly elevated in the uremic animals (Table I). Also, the plasma pH and the concentration of bicarbonate were significantly lower in the uremic dogs than in the normal animals.

In uremic animals the brain content of Ca increased significantly: It was 389 ± 22 (SE) mg/kg dry wt (normal 263 ± 9 , $P < .01$) in gray matter and 267 ± 19 mg/kg dry wt (normal 161 ± 12 , $P < 0.01$) in white matter (Table II and Fig. 1).

Both slow and rapid hemodialysis reduced the blood

FIGURE 1 The effects of uremia, TPTX, and administration of PTE on the Ca content of gray matter of brain. Each bar represents the mean value and the brackets are ±1 SE.

concentrations of urea and creatinine and the plasma osmolality by similar amounts, while the plasma concentrations of phosphorus and bicarbonate and arterial pH returned towards normal. The concentrations of Ca in plasma and CSF after dialysis were not different from those in uremic animals, and the values were similar at the end of both slow and rapid hemodialysis. Therefore, the data from all 12 animals were pooled in one group. At the end of hemodialysis, the brain content (gray matter) of Ca was 305 ± 15 (SE) mg/kg dry wt, a value significantly ($P < 0.01$) lower than that seen in the uremic animals. However, the Ca content of brain remained higher than in normal dogs ($P < 0.05$).

The administration of PTE for 3 days to normal dogs elevated the plasma levels of Ca to 12.8 ± 0.5 mg/100 ml; the brain content of Ca was 317 ± 8 mg/kg dry wt in gray matter and 190 ± 3 mg/kg dry wt in the white matter; both values are significantly higher than normal.

Thyroparathyroidectomy in normal dogs resulted in a fall in plasma Ca concentration to 6.5 ± 0.3 mg/100 ml. Despite the hypocalcemia, the Ca content of the gray (267 ± 4 mg/kg dry wt) and the white (162 ± 2 mg/kg dry wt) were not different from that of normal dogs. Uremia for 3 days in dogs previously subjected to TPTX was not associated with an increase in Ca content of brain. The Ca content in gray matter was 269 ± 9 mg/kg dry wt and it was 144 ± 10 mg/kg dry wt in white matter. The administration of PTE to TPTX uremic dogs elevated the concentration of Ca in plasma to 9.8 ± 0.4 mg/100 ml, a value not different from both normal and uremic animals with intact parathyroid glands. In these dogs, the Ca content of both the gray (360 ± 20 mg/kg dry wt) and the white (194 ± 5 mg/kg dry wt) matter were significantly higher than the value in normal dogs and approached values seen in the uremic dogs with intact parathyroid glands.

The infusion of phosphate in vitamin D₃-treated animals produced marked elevation in serum phosphorus and in calcium-phosphorus product but only a slight and insignificant increment in brain calcium (286 ± 6 vs. 263 ± 9 [normal] mg/kg dry wt). Moreover, the values of calcium in brain in these animals were markedly and significantly lower ($P < 0.01$) than those observed in uremic animals with intact parathyroid gland function despite higher calcium-phosphorus product in the former group. Uremia in vitamin D₃-treated TPTX dogs did not cause an elevation in the calcium content of brain (258 ± 11 vs. 263 ± 9 [normal] mg/kg dry wt), and the values were significantly lower than those observed in uremic animals with intact parathyroid glands ($P < 0.01$). The failure of calcium content of brain to increase in this group of uremic animals occurred despite marked elevation in plasma calcium-phosphorus product.

The concentration of citrate in serum rose from $0.24 \pm$

0.02 to 0.62 ± 0.11 mM ($P < 0.02$) after 3 days of uremia. A significant elevation in the concentration of Mg in plasma occurred in the uremic animals and the values returned to normal after hemodialysis. TPTX or the administration of PTE did not alter the plasma levels of Mg and the concentrations of Mg in CSF were not affected by various experimental maneuvers (Table I). There were small and inconsistent changes in the Mg content of the gray matter of brain. However, the Mg content in white matter was modestly but significantly higher than normal in uremic dogs with intact parathyroid glands and after administration of PTE to normal or to TPTX uremic animals (Table II).

DISCUSSION

The data of the present study demonstrate that acute uremia of 3 days duration is associated with a 47% increment in the Ca content of the gray matter and with a 66% increase in that of the white matter. These changes occurred despite a small fall in the concentration of Ca in plasma and CSF. The results indicate that either uremia, per se, and/or some other metabolic consequences of the uremic state, such as metabolic acidosis or secondary hyperparathyroidism, are responsible for this phenomenon. The latter is known to occur rapidly after acute renal failure (6).

The demonstration in the present study that TPTX before the induction of uremia completely prevented the rise in Ca content of both gray and white matter of the brain indicates that uremia, per se, cannot be the only factor underlying the increment in brain Ca. This observation rather points to the role of the parathyroid glands in this phenomenon. The findings that PTE administration to normal or to uremic TPTX dogs increased significantly the Ca content of the brain further support the latter possibility. It could be argued, however, that the lack of rise in Ca content of brain in TPTX uremic dogs is due to the hypocalcemia, and that the increment in brain Ca in the normal dogs receiving PTE is related to the hypercalcemia. These possibilities seem remote. First, TPTX in normal dogs was associated with hypocalcemia but brain Ca remained normal. Second, data from our laboratory² indicate that despite the elevation and maintenance of serum Ca at levels of 13.0–15.0 mg/100 ml for a period of 2–3 mo by the oral administration of vitamin D₃, the content of Ca in gray matter was 260 ± 14 mg/kg dry wt, a value not different from normal. Thirdly, other investigators have shown that variations in the level of plasma calcium are not associated with alterations in brain Ca (1, 2).

Theoretically, extracellular metabolic acidosis, either directly or by increasing the ionization of Ca in plasma,

² S. G. Massry and J. W. Coburn. Unpublished data.

may promote entry of Ca into brain. However, our data do not support such a contention. The Ca content of brain was markedly and significantly higher in intact uremic dogs than in TPTX uremic animals, despite similar plasma pH and bicarbonate concentration in the two groups.

Another factor that should be considered is the role of the elevation in plasma calcium-phosphorus product in the genesis of the increment in brain calcium in uremia. Two lines of evidence indicate that this factor did not play a paramount role in this phenomenon. First, there is no significant correlation between the calcium content of brain and the plasma calcium-phosphorus product observed in all 35 animals with uremia ($r = 0.28$, $P > 0.1$). Second, the elevation of calcium-phosphorus product in animals with suppressed parathyroid gland activity (vitamin D₃-treated dogs) receiving phosphate infusion and in the uremic vitamin D₃-treated TPTX dogs did not produce a significant increment in brain Ca. It is of interest that the Ca content of other tissues in the latter two groups (lung: 842 ± 121 and 1329 ± 179 mg/kg dry wt and renal cortex: $2,164 \pm 388$ and $1,170 \pm 144$ mg/kg dry wt, respectively) were significantly greater than those observed in normal dogs (lung 539 ± 10 and renal cortex 368 ± 8 mg/kg dry wt). These observations suggest that the mechanism of Ca accumulation in brain is different than in other tissues. It is apparent that Ca can accumulate in tissues other than the brain in the absence of excess PTH when calcium-phosphorus product in blood is elevated. It also seems unlikely that the modest elevation in the concentration of citrate in serum caused the observed rise in brain calcium; citrate forms nonionizable complexes with calcium, and there is no evidence to indicate that such complexes enter the brain easier than calcium ion.

Although there were no consistent changes in the Mg content of the gray matter, the content of this ion was modestly but significantly higher than normal in white matter only in uremic dogs with intact parathyroid glands, and in normal dogs and in TPTX uremic animals receiving PTE; the increments were 19.5%, 6.9% and 9.6%, respectively. These observations suggest that PTH may also enhance the entry of Mg in certain parts of the brain.

The significant fall in the content of Ca in brain after hemodialysis was surprising. Theoretically, this phenomenon may be due to amelioration of the uremic state, correction of acidosis, creation of a gradient for Ca which favors its movement from brain into CSF or plasma, or removal of excess PTH from blood. We have already presented reasons which militate against the role of uremia and extracellular acidosis in the alterations of Ca content of brain. The creation of a gradient favoring movement of Ca from brain into CSF or plasma demands

that the concentration of Ca in the latter two compartments is significantly reduced by hemodialysis. Our data indicate that hemodialysis did not cause significant changes in the concentration of Ca in CSF or plasma. Removal of PTH from the circulation could occur either by its actual dialysis or by its adsorption onto the dialyzing membranes. There are no data available on the clearances of PTH by dialysis in patients with uremia. However, recent work indicates that several immunoreactive fragments of PTH exist in the circulation of patients with renal failure and that the fragment with the lowest molecular weight possesses biological activity (11). If the metabolism of PTH in the uremic dogs is similar to that of patients with renal failure, one can postulate that the partial removal from the circulation of the biologically active low molecular weight fragment of PTH may cause a fall in brain Ca after hemodialysis. It is interesting that despite the significant fall in brain Ca by dialysis, the values were still higher than normal ($P < 0.05$); this observation is consistent with partial removal of biologically active fragments of PTH by dialysis.

Although the present data provides the first demonstration that excess PTH promotes entry of Ca into brain, other investigators have shown similar effects in other tissues. Bernstein, Pletka, Hattner, Hampers, and Merrill (5) reported that Ca content of aorta is significantly higher in rats with uremia than in aorta of normal animals, and that such a change was prevented by parathyroidectomy. In addition, Massry et al. (3) demonstrated a significantly elevated content of Ca in skin of uremic patients with clinically overt secondary hyperparathyroidism, and that subtotal parathyroidectomy in these patients was followed by a fall in Ca content of skin. Furthermore, Berkow, Fine, and Zimmerman (4) have shown that PTH can increase the content of Ca in the cornea.

It is of interest to speculate on the possible clinical implications of the findings of the present study. Some of the neurological and behavioral abnormalities found in acute uremia, such as lethargy, slow mentation, and confusion are similar to those seen in patients with hyperparathyroidism. These symptoms are usually corrected by removal of the adenoma in patients with hyperparathyroidism and by dialysis in patients with acute uremia. It is conceivable that the increased levels of Ca which could be present in the brain of such patients may be at least partly responsible for the observed mental aberrations.

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