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Phosphorus intake regulates intestinal function and polyamine metabolism in uremia

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Phosphorus intake regulates intestinal function and polyamine metabolism in uremia. This study found that 5/6-nephrectomized uremic rats showed secondary hyperparathyroidism as reflected by an increase in their serum parathyroid hormone (PTH) level in association with a decrease in serum 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D]. These changes recovered partially upon phosphorus restriction. Calcium absorption and gene expression of calbindin-D_{9k} were decreased in uremia and were also improved by phosphorus restriction. In uremia, intestinal spermidine/spermine N^1-acetyltransferase activity was decreased, while ornithine decarboxylase (ODC) activity and its gene expression were potentiated. Enhancement of c-*fos* and c-*jun* gene expressions was also observed in uremia. These phenomena suggest that the intestinal villus may proliferate in uremia. Phosphorus restriction prevented increases in the expression of ODC, c-*fos* and c-*jun* observed in uremia. Since phosphorus restriction caused a rise in the serum 1,25-$(OH)_2$D level, the role of 1,25-$(OH)_2$D in uremia-induced intestinal dysfunction was examined. A single injection of 1,25-$(OH)_2D_3$ to uremic rats caused an increase in the steady-state calbindin-D_{9k} mRNA level, and decreases in steady state c-*fos* and ODC mRNA levels, suggesting that the deficiency of 1,25-$(OH)_2D_3$ is responsible for intestinal dysfunction in uremia. In conclusion, altered polyamine metabolism caused by 1,25-$(OH)_2$D deficiency is intimately involved in intestinal dysfunction and the development of the proliferative state of the intestinal villus in uremia.

Renal insufficiency is characterized by several alterations in mineral homeostasis. Uremic intoxication *per se* may lead to reduced intestinal calcium absorption in the proximal segments of the small intestine of patients [1–3] and rats [4]. Reduced renal function results in phosphate retention [5], and the high level of PTH with its severe effects on several organ systems are believed to be an inevitable homeostatic response, which increases the fractional excretion of phosphate [6]. Renal dysfunction also leads to low serum 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], probably due to phosphorus restriction and to the decreased activity of 1α-hydroxylase in the kidney [7], which is essential for the production of 1,25-$(OH)_2$D. Low levels of 1,25-$(OH)_2$D lead to decreased serum calcium [3, 8] and enhanced synthesis and secretion of parathyroid hormone (PTH) [9–14]. Secondary hyperparathyroidism occurs even in the early stage of renal insufficiency, and is followed by the development of renal osteodystrophy [15]. Even at an early stage, chronic renal failure increases PTH mRNA levels without altering serum 1,25-$(OH)_2$D and calcium levels [16]. The development of 1,25-$(OH)_2$D resistance in chronic renal failure is thought to reduce the action of 1,25-$(OH)_2$D [17, 18].

In support of the major role of phosphorus retention [15, 19] in the pathogenesis of secondary hyperparathyroidism, previous reports have shown that the decreased phosphorus filtration rate prevents [20] or ameliorates [21–23] secondary hyperparathyroidism. Hyperphosphatemia results in hypocalcemia by several possible mechanisms, including inhibition of the activity of the renal enzyme 1α-hydroxylase [7, 24], which is already limited by the decrease in renal mass. In normal humans [25] and in patients with moderate chronic renal failure [22, 23], restriction of dietary phosphorus increases the production of 1,25-$(OH)_2$D, thus decreasing the levels of immunoreactive PTH. However, phosphorus restriction may reverse hyperparathyroidism in uremia by itself, independently of any change in calcium or 1,25-$(OH)_2$D [26].

Intracellular concentrations of polyamines are highly regulated and they play essential roles in regulating cell growth and differentiation. Ornithine decarboxylase (ODC) catalyzes the formation of putrescine from ornithine, a rate-limiting step in polyamine biosynthesis. Another rate-limiting enzyme, spermidine/spermine N^1-acetyltransferase (SAT), also participates in the formation of putrescine from spermidine or spermine [27]. The importance of polyamines in modulating cell growth and differentiation is well established [28–30]. Administration of vitamin D to vitamin D-deficient chicks [31] or rats [32] resulted in a considerable increase in the duodenal villus length. The duodenal ODC and SAT activities increased markedly with a concomitant increase in the duodenal content of putrescine after a single injection of 1,25-$(OH)_2D_3$ into vitamin D-deficient chicks [33–35]. Putrescine is therefore intimately involved in vitamin D action in modulating the morphological and functional development of the intestinal villus [36]. However, the intestinal polyamine metabolism in chronic renal failure is not well understood.

In the present investigation, intestinal functions related to calcium absorption and polyamine metabolism were examined in rats with chronic renal failure. The effects of a phosphorus restricted diet and a single injection of 1,25-$(OH)_2D_3$ in uremic rats were also examined to clarify the interaction between polyamine metabolism and 1,25-$(OH)_2D_3$ in the intestine of rats with chronic renal failure.

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Methods

Materials

[α^{32}P]-dCTP (3000 Ci/mmol) was purchased from Dupont/New England Nuclear (Boston, MA, USA). Complementary DNA (cDNA) for c-*fos* [37], c-*jun* [38], mouse ODC [39], calbindin-D_{9k} [40] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) [41] were labeled with [α^{32}P]-dCTP using hexadeoxynucleotide random primers. L-[1-^{14}C] ornithine (2.96 mCi/mmol) and [1-^{14}C] acetyl coenzyme A (55 mCi/mmol) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA) and ICN Biochemicals Inc. (Costa Mesa, CA, USA), respectively.

Animals and study protocol

The protocol of the experiment was approved by the Animal Research Committee of Osaka City University, and care of the animals was in accordance with the standards of this institution (*Guide for Animal Experimentation, Osaka City University*).

Uremic rats were produced by 5/6 nephrectomy as described previously [18]. Both sham operated (Sham) and uremic rats were maintained on a diet (Type B modified; Oriental Yeast Co., LTD., Tokyo, Japan) containing 1.09% calcium and 0.536% phosphate for seven days after nephrectomy. Then the uremic rats were divided into two groups; normal phosphorus diet (Uremic-N) and phosphorus-restricted diet (Uremic-P). The diets contained 1.09% calcium and 2000 IU vitamin D_3 per kg diet, and the phosphorus concentrations of normal phosphorus diet and phosphorus-restricted diet were 0.536 and 0.136%, respectively. Sham operated rats were maintained on a normal phosphorus diet during the experiment. They were each fed a diet for seven weeks before the study. The rats were fasted for 18 hours before the experiments but were allowed to drink water freely. The rats were bled by aortic puncture and sacrificed under ether anesthesia. Serum was kept for biochemical analysis. The lumen and outer surface of the excised duodenum were thoroughly rinsed with cold phosphate buffered saline (PBS), and kept in cold PBS until used for the determination of calcium transport. Some duodena were frozen in liquid nitrogen, and stored at −80°C until used for Northern blot and enzymatic analyses. To analyze the effect of a single injection of 1,25-$(OH)_2D_3$, 2.0 μg/kg body wt of 1,25-$(OH)_2D_3$ was administered intraperitoneally to some Uremic-N rats. Duodena were collected and stored as described above until used for Northern blot analysis.

Calcium absorption in rat intestine

In the intestines, intestinal ^{45}Ca absorption was measured by the everted gut-sac technique [42]. Data are expressed as the ratio (S/M) of the tracer concentration in the serosal medium (inside the sac) to the concentration of the tracer in the mucosal medium (outside). In addition, the final tracer concentration in each compartment is expressed as the percentage of the average tracer concentration in the Sham group.

Measurement of enzyme activity

ODC activity was determined by the release of $^{14}CO_2$ formed from L-[1-^{14}C] ornithine by the method described previously [43]. The frozen tissues were excised and homogenized with 4 vol of ice-cold 10 mM Tris-HCl (pH 7.5) containing 200 μM pyridoxal phosphate, 0.1 mM EDTA, and 2.5 mM dithiothreitol. The homogenate was centrifuged at 30,000 × g for 30 minutes at 4°C. The supernatant (90 μl) was added to glass tubes (12 × 100 mM) containing 0.5 μCi of L-[1-^{14}C] ornithine (10 μl) and 80 nmol of L-ornithine (10 μl) each. The glass tubes were sealed with a rubber stopper through which a syringe needle was placed. A paper disk carrying 50 μl of Soluene-350 (Packard Instrument Company, Downers Grove, IL, USA) was impaled on the syringe needle. The incubation time was 60 minutes at 37°C. The reaction was terminated by adding 0.2 ml of 50% trichloroacetic acid and the tube was incubated for an additional 60 minutes to trap $^{14}CO_2$ in Soluene-350 absorbed on the paper disk. The paper disk was then transferred to a vial containing 5 ml of toluene-based scintillation fluid (0.4% PPO, 0.01% POPOP) and counted for its radioactivity by a Beckman liquid scintillation counter (LC 5801).

The extract for assay of SAT activity was prepared as described previously [27]. The frozen tissues were excised and homogenized with 4 vol of ice-cold 50 mM Tris-HCl (pH 7.8) containing 0.25 M sucrose. The homogenate was centrifuged at 105,000 × g for 60 minutes, and the resultant supernatant was used for the assay of SAT activity. The supernatant was diluted with 4 vol of distilled water, and 50 μl aliquots of the supernatant were incubated with 0.3 μmol of spermidine, 10 μmol Tris-HCl (pH 7.8), and 40 nCi of [1-^{14}C]acetyl coenzyme A (CoA) in the final volume of 100 μl at 37°C for 10 minutes. The reaction was terminated by chilling followed by the addition of 20 μl of 1 M NH_2OH·HCl; the reaction mixture was placed in a boiling water bath for three minutes. Aliquots of 50 μl of the reaction mixture were spotted onto a Whatman P81 paper disk (2.4 cm in diameter). The paper disk was washed with tap water and then five times with 1 ml aliquots of water followed by three washes with 1 ml aliquots of ethanol on a filter. The paper disk was dried and transferred to a vial containing 5 ml of toluene-based scintillation fluid, and the incorporation of radioactivity into monoacetylspermidine was measured with a Beckman liquid scintillation counter.

RNA isolation and hybridization

Total RNA was isolated from pooled duodena by acid guanidinium thiocyanate-phenol-chloroform extraction [44]. Poly(A)$^+$-RNA was obtained by using oligo(dT)-latex (Takara Shuzo Co., Ltd., Kyoto, Japan). The RNA was electrophoresed in a 1% agarose gel containing formaldehyde, transferred to a nylon filter (Hybond N, Amersham International, plc, Buckinghamshire, UK). The nylon filter was prehybridized and hybridized as described [18]. The density of each band was determined by a laser densitometer and expressed as an arbitrary unit (AU).

Biochemical parameters

Serum calcium, phosphate, urea nitrogen and creatinine were measured by an autoanalyzer. Serum PTH was measured by radioimmunoassay using antibody raised against N-terminal rat PTH [45]. The serum 1,25-$(OH)_2D$ level was determined as described [46].

Statistical analysis

Value were expressed as the mean ± SD. Statistical analysis was performed by Student's *t*-test or one-way ANOVA and multiple comparison (Scheffe type).

Table 1. Serum parameters of rats used in the experiment

	Sham	Uremic-N	Uremic-P
N	16	20	21
Body weight *g*	472.1 ± 37.3	309.8 ± 91.4[a]	356.6 ± 54.4[a]
Urea nitrogen *mg/dl*	18.9 ± 5.7	232.7 ± 126.2[a]	183.9 ± 117.5[a]
Calcium *mEq/liter*	4.99 ± 0.55	3.28 ± 0.59[a]	5.71 ± 0.63[a,b]
Phosphate *mg/dl*	5.68 ± 0.76	15.91 ± 7.93[a]	8.21 ± 1.58[a]
PTH *pg/ml*	73.1 ± 40.4	1165.5 ± 415.7[a]	215.2 ± 171.2[a]

Values are expressed as the mean ± SD of 16 to 21 rats.

Abbreviations are: *N*, number of animals; PTH, parathyroid hormone; Sham, sham operated rats; Uremic-N, 5/6 nephrectomized rats maintained on a normal phosphorus diet; Uremic-P, 5/6 nephrectomized rats maintained on a phosphorus-restricted diet.

Variance analysis: [a] $P < 0.05$ vs. Sham; [b] $P < 0.05$ vs. Uremic-N

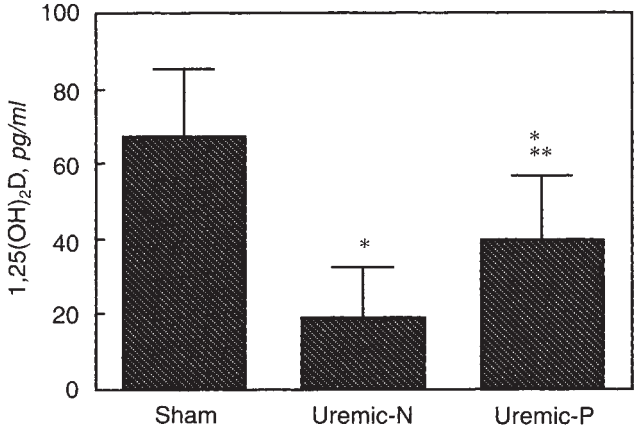

Fig. 1. *Serum 1,25-$(OH)_2D$ levels of rats used in the experiment.* Uremic and sham-operated rats (Sham) were produced as described in the **Methods** section. Uremic rats were maintained on normal phosphorus diet (Uremic-N) or phosphorus-restricted diet (Uremic-P) for seven weeks. The serum 1,25-$(OH)_2D$ level was determined as described [46]. Values are expressed as the mean ± SD of 16 to 21 rats. $^{*}P < 0.05$ vs. Sham; $^{**}P < 0.05$ vs. Uremic-N.

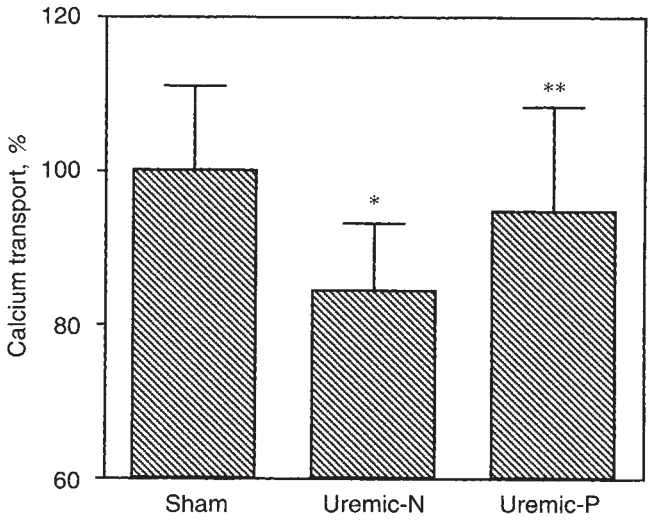

Fig. 2. *Effects of uremia and phosphorus-restricted diet on rat intestinal calcium absorption.* Intestinal calcium absorption was studied as described in the **Methods** section. Values are expressed as the mean ± SD of 16 to 21 rats. $^{*}P < 0.05$ vs. Sham; $^{**}P < 0.05$ vs. Uremic-N.

Results

Biochemical profiles of the uremic rats

The 5/6-nephrectomy resulted in characteristic changes in chronic renal failure: characteristic body wt, serum urea nitrogen, serum phosphate, serum calcium, serum PTH (Table 1) and serum 1,25-$(OH)_2D$ levels (Fig. 1).

The uremic rats fed a normal phosphorus diet (Uremic-N) and those fed a phosphorus restricted diet (Uremic-P) had reduced body wts ($P < 0.05$) and significantly increased serum urea nitrogen ($P < 0.05$) compared to the sham-operated rats (Sham). Distinctive differences in calcium and phosphate metabolism between the Sham group and the Uremic-N group were evident as serum phosphate was significantly increased while serum calcium was reduced in the Uremic-N group ($P < 0.05$). The Uremic-N group also showed decreased serum 1,25-$(OH)_2D$ levels ($P < 0.05$) and increased serum PTH ($P < 0.05$), thus it appears to be a relevant animal model for secondary hyperparathyroidism. The phosphorus-restricted diet reversed the hypocalcemia, hyperphosphatemia, high concentration of serum PTH and low concentration of serum 1,25-$(OH)_2D$ levels observed in the Uremic-N group ($P < 0.05$).

Calcium absorption and calbindin-D_{9k} gene expression in the intestine of uremic rats

Uremic-N group experienced a significant (84%) decrease in intestinal calcium absorption compared to the Sham group, which was restored to the level in the Sham group by phosphorus restriction (Fig. 2). The changes in calcium absorption were partly accompanied by the changes in calbindin-D_{9k} gene expression (Fig. 3). Steady-state levels of calbindin-D_{9k} mRNA in the intestine of Uremic-N group were significantly decreased (0.32 AU) compared to the Sham group (1.0 AU). The gene expression was recovered partially in the Uremic-P group (0.62 AU) compared to the Uremic-N group.

Polyamine metabolism and expression of proto-oncogenes in uremic rats

Since polyamine metabolism is involved in cell proliferation and function, we examined the metabolism of polyamine in the intestine of uremic rats. The Uremic-N group experienced a significant decrease (49.9%) in SAT activity compared to the Sham group, which was reversed significantly to the level of Sham group by phosphorus restriction in the Uremic-P group (89.6%) (Fig. 4). Steady state levels of ODC mRNAs in the Uremic-N group were significantly increased (1.7 AU) as compared to the Sham group (1.0 AU), while the increment in such gene expression was partially normalized in the Uremic-P group (1.3 AU; Fig. 3). ODC activity was also significantly increased in the Uremic-N group (463%), and the increase was partially normalized in the Uremic-P group (200%) (Fig. 6). Steady state levels of c-*fos* and c-*jun* mRNAs in the Uremic-N group were increased (2.6 and 1.9 AU) as compared to the Sham group (1.0 AU), while the increment in such gene expression was ameliorated in the Uremic-P group (Fig. 5).

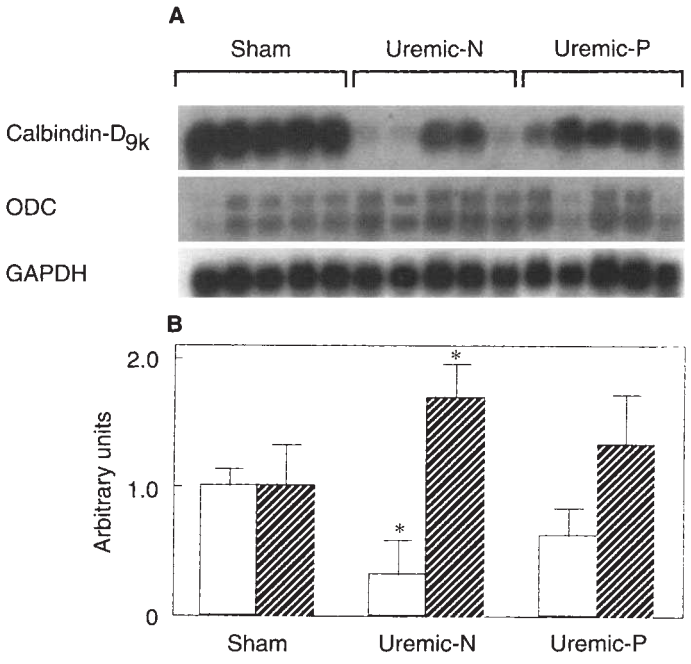

Fig. 3. *Effects of uremia and phosphorus-restricted diet on steady state mRNA levels of calbindin-D_{9k} and ODC.* The steady-state mRNA level of calbindin-D_{9k} and ODC were examined by Northern blot analysis (10 μg/lane). **A.** Results of Northern blot analysis. **B.** The density of their mRNA bands normalized to GAPDH mRNA levels. Sham mRNA levels normalized to GAPDH mRNA level were expressed as 1.0 AU. The calbindin-D_{9k} mRNA is shown in the open bars and the ODC mRNA is shown in the hatched bars. The blot shown is reproducible for two independent experiments. $^{*}P < 0.05$ vs. Sham.

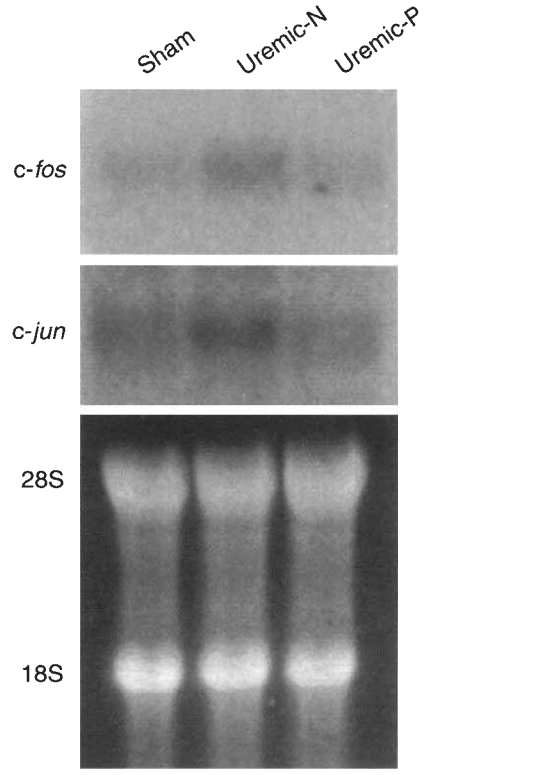

Fig. 5. *Effects of uremia and phosphorus-restricted diet on steady state mRNA levels of c-fos and c-jun.* The steady-state mRNA level of c-*fos* and c-*jun* was examined by Northern blot analysis (20 μg/lane). Equal loading of the gel was confirmed by ethidium bromide staining of the ribosomal RNA. The blot shown is reproducible for two independent experiments.

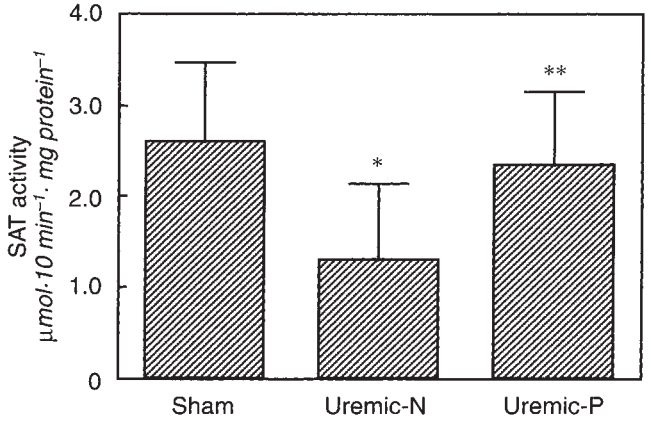

Fig. 4. *Effects of uremia and phosphorus-restricted diet on rat intestinal SAT activity.* SAT activity was studied as described in the **Methods** section. Values are expressed as the mean ± SD of 12 rats. $^{*}P < 0.05$ vs. Sham $^{**}P < 0.05$ vs. Uremic-N.

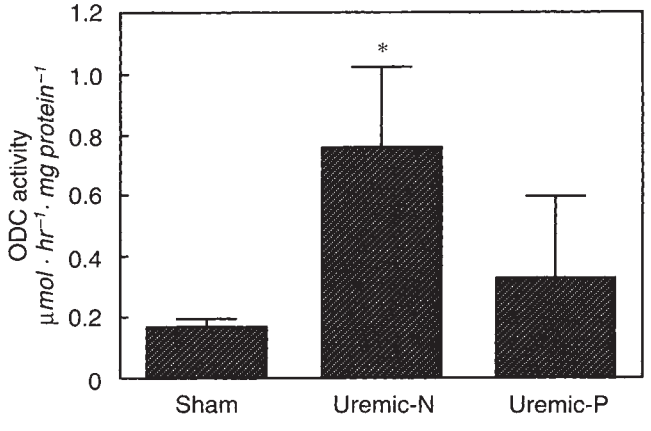

Fig. 6. *Effects of uremia and phosphorus-restricted diet on rat intestinal ODC activity.* ODC activity was studied as described in the **Methods** section. Values are expressed as the mean ± SD of five rats. $^{*}P < 0.05$ vs. Sham.

Effect of a single injection of 1,25-$(OH)_2D_3$ on gene expressions in uremic rats

To clarify whether 1,25-$(OH)_2D_3$ was involved in the beneficial effect of phosphorus restriction in uremic rats, gene expressions of calbindin-D_{9k}, ODC, and c-*fos* after a single injection of 1,25-$(OH)_2D_3$ (2 μg/kg body wt) were determined in uremic rats. 1,25-$(OH)_2D_3$ treatment caused an increase of steady state calbindin-D_{9k} mRNA at 2 to 24 hours (1.0, 1.186, 2.580, 2.687 and 2.993 AU for 0, 2, 6, 12 and 24 hr, respectively), while it caused decreases in steady state levels of c-*fos* mRNA (1.0, 0.489, 0.389. 0.264 and 0.257 AU for 0, 2, 6, 12 and 24 hr, respectively) and of ODC mRNA (1.0, 0.852, 0.516, 0.481 and 0.881 AU for 0, 2, 6, 12 and 24 hr, respectively; Fig. 7).

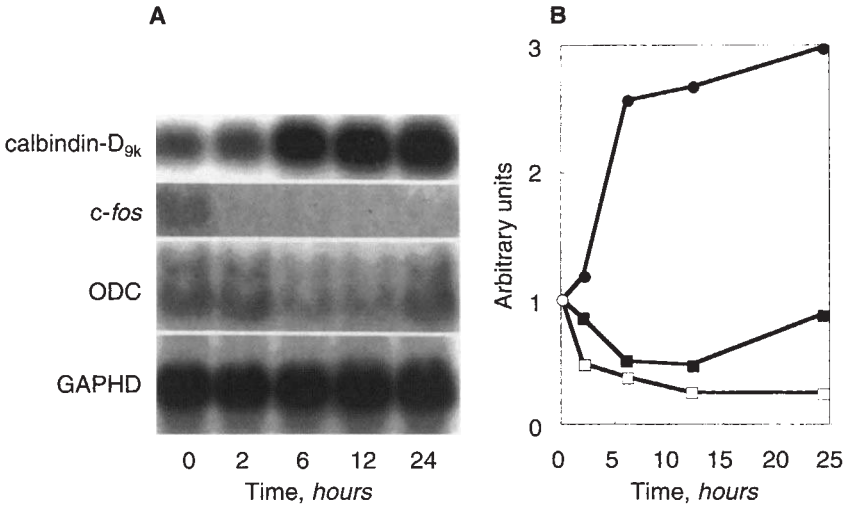

Fig. 7. *Effect of a single injection of 1,25-$(OH)_2D_3$ on steady state mRNA levels of calbindin-D_{9k}, c-fos, and ODC.* One microgram of poly(A)$^+$RNA was used for Northern blot analysis. **A.** Results of Northern blot analysis. **B.** The density of their mRNA bands normalized to GAPDH mRNA levels. Basal mRNA levels normalized to GAPDH mRNA level at time 0 were expressed as 1.0 AU. The blot shown was reproducible in two independent experiments. Symbols are: (●) calbindin-D_{9k}; (■) ODC; (□) c-*fos*.

Discussion

This study shows that polyamine metabolism is altered in the intestine of uremic rats. This alteration may be associated with intestinal dysfunction. Moreover, polyamine metabolism seems to be regulated by phosphate metabolism in chronic renal failure.

Our present data confirmed the previous finding that intestinal calcium absorption was decreased in uremic rats [1, 2, 4]. This impairment of function was completely restored by phosphorus restriction, suggesting that phosphate metabolism plays an important role in intestinal function in uremia. Our results could be affected by differences in intake of other nutrients in addition to phosphorus. We showed for the first time that polyamine metabolism was also altered in rat intestine with chronic renal failure. ODC activity, which has been reported to have crucial roles in the regulation of cell proliferation and differentiation, was increased in the uremic intestine. This increase was also suppressed by a phosphorus-restricted diet, indicating that intestinal ODC activity as well as intestinal function was regulated by phosphate metabolism. Northern blot hybridization analysis showed that changes in ODC activities correlated well with the ODC mRNA level, suggesting that an increase in ODC activity in uremia occurs by stimulation of transcription or stabilization of mRNA. Further, the expression of c-*fos* and c-*jun*, possible cell growth associated proto-oncogenes, was also enhanced in uremic intestine, which was partially reversed by phosphorus restriction. Taken together, it may be hypothesized that villi in the uremic intestine are proliferate more than in control intestine, resulting in decreased calcium absorption. Supporting this finding is that uremic rats were reported to have consistently longer villi and deeper crypts [47]. Since enhancement of growth regulating molecule expression as well as suppression of intestinal function were possibly the results of impaired phosphate metabolism, it may be hypothesized that phosphate metabolism is an important determinant of proliferation and differentiation of intestinal epithelial cells.

It seems likely that the effect of phosphorus restriction on intestinal function and polyamine metabolism in uremia was mediated in part by the increase of 1,25-$(OH)_2D$, as suggested by the following findings. First, a decrease in serum 1,25-$(OH)_2D$ levels in uremic rats was restored partially by phosphorus restriction as reported previously [22, 23]. Second, changes of expression of the calbindin-D_{9k} gene, one of the 1,25-$(OH)_2D$-dependent cytosolic calcium-binding proteins, were in good agreement with those of intestinal calcium absorption. This protein was shown to be involved in intracellular calcium transport and homeostasis [48]. Finally, a single injection of 1,25-$(OH)_2D_3$ to uremic rats caused decreases in steady state levels of ODC and c-*fos* mRNAs, and a dramatic rise in the calbindin-D_{9k} mRNA level. These changes were similar to those induced by a phosphorus-restricted diet in uremic rats. 1,25-$(OH)_2D_3$ treatment also caused the serum PTH level to decrease at 6 to 24 hours (data not shown). Since changes in levels of ODC and c-*fos* mRNA after the 1,25-$(OH)_2D_3$ treatment preceded that of serum PTH level, it seems unlikely that PTH mediated the effect of 1,25-$(OH)_2D_3$ in suppressing the gene expression of these genes.

It was reported that administration of vitamin D to vitamin D-deficient chicks and rats resulted in considerable growth in the villi [31, 32], which was associated with increases in duodenal ODC and SAT activities and in the tissue content of putrescine. As known, intestinal mucosal cells originate in progenitor cells present in the crypt region of the villi and differentiate and migrate toward the villus tips. Shinki et al [35] suggested that SAT activity was intimately involved in calcium absorption because it is at high levels in the villus tips. In contrast, the activity of ODC was reported to be located predominantly in the crypt region and in the lower part of villi [35], suggesting an essential role for ODC and putrescine in the regulation of epithelial cell proliferation. As speculated from these findings, an increase in ODC activity in uremia may be a feature of intestinal epithelial cell proliferation, while a decrease in SAT activity could be related to impaired intestinal calcium absorption. So putrescine, the product of ODC and SAT, is somehow involved in the 1,25-$(OH)_2D$ action in maintaining the morphological and functional development of the intestinal villus mucosa. A single injection of 1,25-$(OH)_2D_3$ into vitamin D-deficient chicks produces a marked increase of putrescine accumulation in the duodenum [33, 36]. Administering specific inhibitors of putrescine synthesis to newborn chicks significantly decreased the duodenal content of putrescine in association with a reduction in calcium transport activity. The putrescine depletion also induced shortening of the duodenal villus length. The inhibition of calcium absorption and villus

length in the putrescine-depleted chicks was almost completely restored by administering putrescine to the birds. The effect of the putrescine depletion and its supplementation on the duodenal villus length and the calcium absorption was reproduced in 5-week-old vitamin D-deficient chicks given vitamin D_3 or 1,25-$(OH)_2D_3$. Therefore, taking these findings into consideration together with our results, it seems possible to conclude that the decrease in intestinal calcium transport activity and the expression of calbindin D_{9k} mRNA in uremic rat intestine may arise from the impairment of polyamine biosynthesis. The locations of ODC and SAT activities in uremic intestine and the difference between uremic and normal intestine need to be elucidated to prove this theory.

Of note is that ODC activity and its gene expression were down-regulated by 1,25-$(OH)_2D_3$ in uremia, in contrast to the up-regulation in vitamin D-deficient animal models [33–35]. It is somewhat difficult to understand the mechanism of this phenomenon from our current results. However, the response of uremic intestine to 1,25-$(OH)_2D_3$ seems to be complicated compared to vitamin D-deficient intestine, that is, attenuated up-regulation of vitamin D receptor and potentiated induction of 24-hydroxylase gene expression as described previously [18]. We have recently shown that maturation of intestinal epithelial cells was impaired in uremic state as evidenced by the distribution of 24-hydroxylase [49] and by increased expressions of PKC α, PKC δ, and PKC ζ [50]. In this study, we have further demonstrated the impairment of the differentiation of intestinal epithelial cells as evidenced by decreases of differention markers such as calcium transport activity, calbindin D_{9k} expression and SAT activity. As a result of the impairment of the differentiation, the proliferation of intestinal epithelial cells seemed enhanced in uremic state, which is in sharp contrast to the vitamin D-deficient state. The enhanced proliferation of intestinal epithelial cells was reflected by: (i) the morphological study indicating a considerable increase in the villus length and (ii) increases of proliferation markers such as ODC activity and its gene expression, and oncogene expressions. 1,25-$(OH)_2D$ is known to stimulate both proliferation and differentiation of intestinal epithelial cells. In vitamin D deficient state, both processes are impaired. Administration of 1,25-$(OH)_2D$ to vitamin D deficient animal normalized both impairment in such state. However, since only differentiation is impaired in uremic state in association with the resultant enhancement of proliferation, when differentiation impairment is normalized by 1,25-$(OH)_2D_3$ administration, the enhanced proliferation should be reduced to a normal level. Therefore, 1,25-$(OH)_2D$ seems to have an effect on the proliferation of intestinal epithelial cells in a uremic state totally different from that in a vitamin D-deficient state.

In summary, altered polyamine metabolism existed and was probably associated with impaired intestinal function in rats with chronic renal failure. Phosphate metabolism was an important regulator of intestinal polyamine metabolism and calcium absorption, which is mediated, at least in part, by 1,25-$(OH)_2D$.

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