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Expression of plasma membrane calcium pump mRNA in rat intestine: effect of age and 1,25-dihydroxyvitamin D

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Abstract

The capacity of the small intestine to actively transport Ca declines markedly with increasing age in the rat. The basal-lateral plasma membrane Ca pump is thought to be an important component of the active transport mechanism. Therefore, the purpose of this study was to determine if there are changes in the expression of the intestinal Ca pump with age. mRNA levels were quantitated by Northern and dot blot analysis using a cDNA probe based on the sequence of the plasma membrane Ca pump expressed in the rat intestine (PMCA1). In the duodenum, Ca pump mRNA levels were 3–4-times higher in young (2 months) rats compared to adult (12 months) and old (27 months) rats. In the ileum, Ca pump mRNA levels were one third those of the duodenum, and ileal levels were higher in young rats compared to adult rats. These changes in mRNA levels with age and segment were significantly correlated with Ca pump activity as measured in basal-lateral membrane vesicles in vitro. To determine intestinal responsiveness to $1,25(OH)_2D$, rats were fed a strontium diet to induce vitamin D deficiency. In young animals, $1,25(OH)_2D$ significantly increased Ca pump mRNA levels 4-fold in the duodenum. $1,25(OH)_2D$ had a similar effect in the adult duodenum. These studies demonstrate that there are changes in Ca pump mRNA levels with age and intestinal segment. Since there was no change in the capacity of $1,25(OH)_2D$ to increase Ca pump mRNA levels, the decline in Ca pump expression may be due to the age-related decrease in serum $1,25(OH)_2D$ rather than to decreased responsiveness to $1,25(OH)_2D$.

Keywords: Calcium pump; Intestine; Age; 1,25-Dihydroxyvitamin D; (Rat)

1. Introduction

Intestinal absorption of calcium (Ca) declines with age in humans [1] and rats [2–4]. In addition, the capacity of 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the biologically active metabolite of vitamin D, to stimulate active Ca transport also declines with age [4,5]. An important component of the Ca transport mechanism is the ATP-dependent Ca pump found on the basal-lateral surfaces of the intestinal absorptive cell. There are strong correlations between active Ca transport and basal-lateral membrane Ca uptake with regard to intestinal segment [7], villus-crypt axis [8], and stimulation by $1,25(OH)_2D$ [7,8]. Using basal-lateral membrane vesicles, we have shown a decline in Ca pump capacity in rat duodenum with age [6].

The purpose of the present study was to determine if the expression of the intestinal Ca pump varied with age and intestinal segment. This has been made possible by the cloning of rat plasma membrane Ca pumps [9,10], and the identification of the isoform (PMCA1) found in the rat intestine [10]. The mRNA for this isoform has been found to be increased by $1,25(OH)_2D$ in the intestine [11]. In addition, the Ca pump in the chick intestine has been cloned and sequenced, and the expression of this pump is modulated by $1,25(OH)_2D$ and dietary Ca [12]. Using the cDNA probe for PMCA1, we have studied the expression of the intestinal Ca pump with age and intestinal segment. In addition, we also determined whether the capacity of $1,25(OH)_2D$ to increase expression of the intestinal Ca pump changed with age.

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2. Materials and methods

Experiments were performed using young (2 months), adult (12 months), and old (27 months) male virgin Fischer 344 rats (F344/NNIA). The mean lifespan of these animals is 29 months of age [13]. Animals were obtained from the National Institute on Aging colony maintained by Harlan Industries (Indianapolis, IN). Rats were maintained under microbial barrier conditions and were fed a semisynthetic diet containing 1.2% calcium, 0.8% phosphorus, and 3.3 IU/g vitamin D-3 (Purina rodent chow, Ralston-Purina, St. Louis, MO).

To make rats deficient in $1,25(OH)_2D$, they were fed a high strontium diet [5,14]. The diet consisted of a low calcium diet (Teklad Test Diet #170120, Madison, WI) containing 0.8% strontium, and it was fed for 6 days [5,14]. To determine the effect of $1,25(OH)_2D$, rats were given a single intraperitoneal injection of $1,25(OH)_2D$ (300 ng/100 g body weight) dissolved in ethanol. Control rats were given vehicle only. Rats were killed 16 h after dosing with $1,25(OH)_2D$.

To collect tissue, rats were killed, and blood was drained into heparinized tubes. The heparinized blood was spun, and the plasma was frozen for later analysis of plasma $1,25(OH)_2D$. The abdominal cavity was exposed by midline incision. The proximal duodenum (0–5 cm distal to the pylorus) and distal ileum (0–10 cm proximal to the caecum) were removed, slit lengthwise, and scraped with glass microscope slides to remove mucosa. Mucosa was quick-frozen for later analysis of Ca pump mRNA.

Plasma membrane Ca pump mRNA levels were measured by Northern and dot blotting. Total RNA was isolated using RNAzol (Tel-Test, Friendswood, TX). For Northern blotting, RNA was fractionated on a 1.2% glyoxyl/DMSO gel and transferred to a nylon membrane (Micro Separations, Westborough, MA). For dot blots, 4–6 dilutions of each sample were spotted onto the nylon membranes. Membranes were hybridized using a cDNA probe containing 2500 base pairs from the 3′ end of the mRNA for the rat brain plasma membrane calcium pump PMCA1 [9]. This probe was kindly supplied by Dr. Gary Shull, University of Cincinnati College of Medicine, Cincinnati, OH. The PMCA1 isoform of the plasma membrane Ca pump is the only one expressed to any degree in the intestine [10]. Membranes were hybridized with the random-primed probe, washed, and exposed to X-ray film. For quantitation, the film dots were quantified by densitometry and normalized to total RNA applied, using the linear portion of the dilution curve. In some experiments, blots were stripped and rehybridized with a probe to beta-actin (Oncor, Gaithersburg, MD).

Serum $1,25(OH)_2D$ was measured using a commercial kit (ImmunoNuclear, Stillwater, MN). 1,25-Dihydroxyvitamin D was partially purified by C-18 and silica Sep-Pak cartridges. 1,25-Dihydroxyvitamin D was quantitated by a receptor binding assay, using calf thymus cytosol as the source of binding protein.

The data from these experiments are reported as the mean ± S.E. of the number of animals indicated. Statistical analyses were performed using two-tailed Student's *t*-test. A confidence level of 95% or greater was considered significant.

3. Results

The expression of plasma membrane Ca pump mRNA in the duodenum with age was studied by Northern blotting (Fig. 1). The duodenum was studied initially since it shows the greatest decline in Ca absorption with age [2–4]. Northern blots revealed a major band with a size of approx. 5.3 kb and two minor bands of 8.3 and 4.5 kb in each age group. This pattern is similar to what has been reported previously in the rat intestine using probes based on the PMCA1 sequence [10,11]. There was no detectable change in the mRNA sizes with age, but the intensity of the hybridization was markedly decreased in the 12 and 27-month-old animals compared to the 2 month rats. Individual bands were quantitated by transmittance densitometry. The major 5.3 kb band decreased from 33.1 OD/mg RNA in 2 month rats to 9.2 and 9.4 OD/mg in 12 and 27 month rats, respectively. The minor 8.3 kb band decreased from 11.3 OD/mg in 2

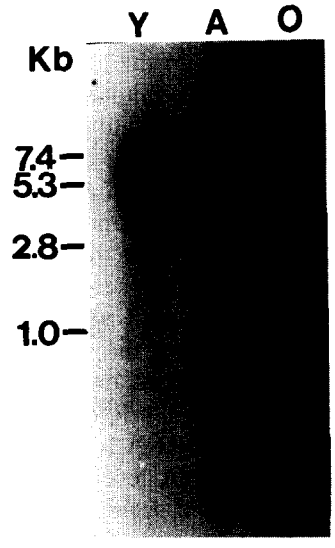

Fig. 1. Expression of plasma membrane Ca pump mRNA in the duodenum with age. Total RNA from the duodena of rats aged 2, 12 and 27 months of age (four rats per age group) was separated by electrophoresis, transferred to nylon and probed with radiolabeled cDNA probe for the plasma membrane Ca pump (PMCA1). Numbers represent molecular size (in kb) determined from size markers.

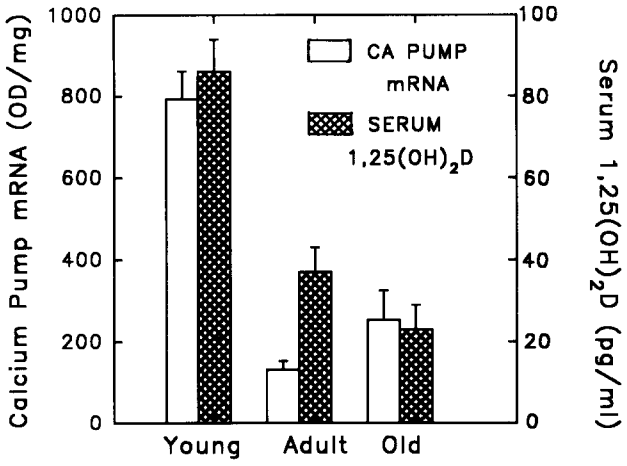

Fig. 2. Effect of age on calcium pump mRNA and serum $1,25(OH)_2D$. Calcium pump mRNA and serum $1,25(OH)_2D$ levels were determined in young (2 months), adult (12 months) and old (27 months) rats. Calcium pump mRNA was determined by dot blot of total RNA from individual rat duodena. Serum $1,25(OH)_2D$ was determined by receptor binding assay. Bars are the mean ± S.E. of four rats in each age group.

month rats to 3.7 and 5.5 OD/mg in 12 and 27 month rats, respectively. The 4.5 kb band was too diffuse to quantitate. Thus, the 5.3 kb and 8.3 kb mRNAs showed a parallel decline with age.

The Ca pump mRNA levels in the duodena of individual rats were quantitated by dot blot (Fig. 2). The mRNA levels in adult and old animals were significantly decreased to 17% and 32% of the young animals, respectively. There was no significant difference between the mRNA levels of the adult and old animals. These changes in Ca pump mRNA levels paralleled the changes in serum $1,25(OH)_2D$ with age. The serum $1,25(OH)_2D$ levels in the adult and old animals were significantly decresed compared to young animals (Fig. 2). There was no significant difference in serum $1,25(OH)_2D$ levels between adult and old animals.

Next, the expression of Ca pump mRNA was determined in both the duodenum and ileum in young (2 months) and adult (12 months) rats and compared to Ca pump activity (Table 1). As seen previously, duodenal mRNA levels in the adult were significantly decreased compared to those of the young. mRNA levels in the ileum were only about one third to one half those of the duodenum. Ileal mRNA also declined with increased age. The ileal mRNA levels in adult animals were 58% of young mRNA levels. These changes in mRNA levels with age and segment are similar to the changes previously observed in Ca uptake by isolated basal-lateral membrane vesicles (Table 1). To examine this relationship quantitatively, Ca pump activity was plotted as a function of Ca pump mRNA levels (Fig. 3). There was a significant correlation between the two parameters ($P < 0.01$), suggesting that changes in Ca pump mRNA levels could account for the changes in basal-lateral Ca pump activity with age and segment.

Table 1
Calcium pump mRNA and calcium pump activity

Age	Intestinal segment	Ca pump mRNA (OD/mg RNA)	Ca pump activity (nmol/mg)
Young	Duodenum	968 ± 57	1.64 ± 0.16
Young	Ileum	313 ± 35	0.37 ± 0.05
Adult	Duodenum	370 ± 20	0.24 ± 0.02
Adult	Ileum	184 ± 23	0.15 ± 0.02

Table entries are the mean ± S.E. of triplicate determinations. Measurements were made in young (2 months) and adult (12 months) F344 rats. Calcium pump mRNA was quantitated by dot blot. Calcium pump activity was taken as ATP-dependent calcium uptake by basal-lateral membrane vesicles. Calcium uptake was measured by rapid filtration after 10 min in the presence of 5 μM calcium [6].

To determine if the adult intestine demonstrated decreased responsiveness to $1,25(OH)_2D$, the effect of $1,25(OH)_2D$ on Ca pump mRNA levels was studied in strontium-fed rats (Table 2). Feeding strontium reduced Ca pump mRNA to low levels in both young and adult duodena. Injection of $1,25(OH)_2D$ markedly increased Ca pump mRNA to the same levels 16 h later in both young and adult rats. Actin mRNA levels in the same experiment did not change significantly with age and $1,25(OH)_2D$ administration (Table 2). Finally, serum $1,25(OH)_2D$ was measured in both control and $1,25(OH)_2D$-treated groups. In the young, serum $1,25(OH)_2D$ was increased from 33 ± 3 to 201 ± 54 pg/ml, and in the adult it was increased from 42 ± 4 to 206 ± 12 pg/ml. There was no significant difference between the young and adult animals with regard to serum $1,25(OH)_2D$ levels.

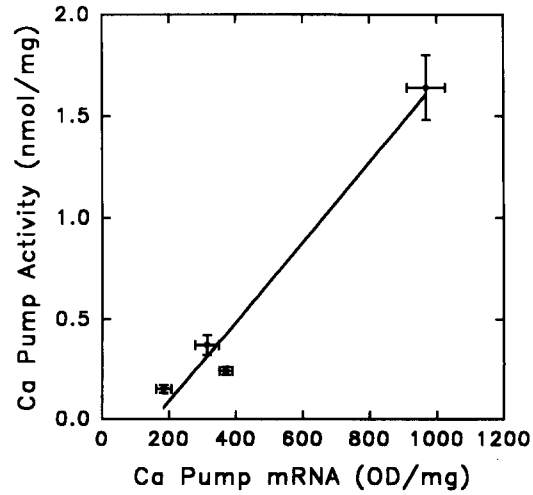

Fig. 3. Correlation of calcium pump mRNA levels and Ca pump activity. Since there was a parallel between calcium pump mRNA levels and activity (Table 1), pump activity was plotted as a function of mRNA levels. The data was fitted by a linear regression line with the equation $y = 0.0020x - 0.29$ ($r = 0.989$).

4. Discussion

These studies demonstrate a decrease in the expression of the intestinal Ca pump in both the duodenum and the ileum with age. These changes correlate with decreased Ca pump activity as measured in basal-lateral membrane vesicles. This suggests that decreased expression of the Ca pump mRNA may be responsible for the observed decrease in pump activity.

The decline in Ca pump activity, along with the reported age-related decline in intestinal calbindin [15], could account for the decline in duodenal Ca transport with age [2,4,17]. Calbindin is thought to play a role in the movement of Ca across the intestinal cell to the basal-lateral membrane [16]. There is a correlation between the decline in active Ca transport and the age-related decline in calbindin and Ca pump mRNA. The active transport of Ca (S/M), as measured by everted duodenal sacs in these animals, is 4.49 ± 0.46, 0.71 ± 0.03, and 0.65 ± 0.02 at 1, 12, and 18 months, respectively [2,17]. Thus, the greatest decline in Ca transport is between 1 and 12 months of age, which is also the period of the greatest decline in Ca pump (Fig. 2) and calbindin expression [15]. After 12 months, there is no further decrease in Ca transport or in Ca pump mRNA or calbindin mRNA levels.

These studies also suggest that the decline in Ca pump expression is related to the age-related decline in serum $1,25(OH)_2D$. $1,25(OH)_2D$ stimulates both Ca pump activity [7,8] and Ca pump mRNA levels [11,12]. Serum $1,25(OH)_2D$ decreases the most between 2 and 12 months of age, and Ca pump expression (Fig. 2) and calbindin expression [15] decline during this time as well. Thus, the data are consistent with the age-related decline in serum $1,25(OH)_2D$ being the primary event. This, in turn, leads to secondary decreases in Ca pump and calbindin expression which result in a decline in intestinal Ca absorption.

On the other hand, there is no evidence for an age-related decrease in intestinal responsiveness to $1,25(OH)_2D$ at the mRNA level. In the present studies, $1,25(OH)_2D$ increased Ca pump mRNA to the same level in both young and adult rats (Table 2). However, the capacity of $1,25(OH)_2D$ to increase active Ca transport by the duodenum does decline with age [4,5]. $1,25(OH)_2D$ increases active Ca transport (S/M) to 2.10 ± 0.11 in 1.5 month old strontium-fed rats after 16 h. However, it increases Ca transport to only 1.15 ± 0.09 in 12 month old rats [5]. These findings suggest that the step in the action of $1,25(OH)_2D$ which is altered with age is distal to the increase in mRNA levels.

These studies also provide additional evidence that the intestinal basal-lateral Ca pump is the PMCA1 isoform of the plasma membrane pump. Previously, it was shown that mRNA for this isoform is increased by $1,25(OH)_2D$ in the intestine [11]. The greatest increase is seen in the villus tip cells, which contain the most Ca pump activity [8]. In the present study, we have shown that the greatest expression of the PMCA1 isoform is in the duodenum, which has the greatest Ca pump activity [7]. We have also shown that the expression of this isoform declines with age in both the duodenum and ileum, as does Ca pump activity [6].

In addition to the age-related decline in the intestinal basal-lateral Ca pump, age-related decreases in other Ca pumps have been reported. These include the Ca pumps in heart sarcoplasmic reticulum [18], skeletal muscle sarcoplasmic reticulum [19], synaptosomal plasma membranes [20], and parotid basolateral membranes [21]. Thus, altered Ca pump activity may be a common feature of the aging process with a variety of physiological consequences.

Table 2
Effect of $1,25(OH)_2D$ on Ca pump and actin mRNA levels

Age	Control	$1,25(OH)_2D$-treated
	Ca Pump mRNA (OD/mg)	
Young	28± 8	102± 1*
Adult	10± 3	107±10*
	Actin mRNA (OD/mg)	
Young	249±79	208±53
Adult	250±70	304±88

Table entries are the mean±S.E. of three animals. Asterisk (*) indicates significantly different from control ($P < 0.05$, t-test). Young (2 months) and adult (12 months) rats were fed a strontium diet and then given a single intraperitoneal injection of $1,25(OH)_2D$ (300 ng/100 g body weight) or vehicle only (control). RNA was isolated from the duodenum 16 h later and calcium pump mRNA was quantitated by dot blot. Blots were then stripped and reprobed for actin.

Acknowledgements

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