J.Sci.Soc.Thailand, 18(1992)41-51

CALCIUM FLUXES ACROSS *IN SITU* INTESTINAL LOOPS IN OVARIECTOMIZED RATS

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(Received 31 January 1992)

ABSTRACT

This study aimed to evaluate effects of ovariectomy and estradiol replacement on calcium fluxes across different intestinal segments. Sham-operated and ovariectomized (OV) rats had either in situ *duodenal and colonic loops filled with 0.5 mM $CaCl_2$ + ^{45}Ca or jejunal and ileal loops filled with 2.5 mM $CaCl_2$ + ^{45}Ca. Five to six weeks of ovariectomy was found to induce hypocalcemia which could be prevented by daily injection of 3 ug estradiol/100 g rat. In sham rats, both duodenal and colonic loops exhibited net secretion while jejunal and ileal loops showed net absorption of calcium. Ovariectomy significantly enhanced the plasma to lumen flux of calcium in duodenal and colonic loops while reducing the lumen to plasma flux of calcium in jejunal and ileal loops. These changes in calcium fluxes were prevented by estradiol administration. From this data, it could be concluded that reduction in passive absorption of calcium in the jejunum and ileum together with enhanced calcium secretion in the duodenum and colon constituted calcium loss from the gastrointestinal tract which may partly account for ovariectomy induced hypocalcemia.*

INTRODUCTION

The positive effect of estrogens on intestinal calcium absorption has been reported.[1-4] It was also well documented in avian species that sex hormones exert control on the 1,25 $(OH)_2D_3$-1αhydroxylase.[5-7] Moreover, since the serum levels of 1,25 $(OH)_2D_3$ which were markedly elevated in pregnant women[8-10] and reduced in post-menopausal women,[11] positively correlated with the level of sex hormone in these two groups of women, it was proposed that estrogens may induce increase in intestinal calcium transport via enhancing production of 1,25 $(OH)_2D_3$.

However, later evidence showed that estrogen-induced rise in 1,25 $(OH)_2D_3$ levels was due to an increase of serum vitamin D binding protein, so that the level of free 1,25 $(OH)_2D_3$ was unchanged both in the case of estrogen deficiency as in postmenopausal osteoporosis[12] and in the case of physiologic hyperestrogenism as in pregnancy.[8, 13, 14] A recent investigation further demonstrated that the impairment of 1, 25 $(OH)_2D_3$ stimulation of calcium absorption in postmenopausal women was probably due to an end organ resistance in a hypoestrogenic condition and not to the level of 1,25 $(OH)_2D_3$[15]. Moreover, ovariectomy did not significantly alter the metabolism of 25 $(OH)_2D_3$ and 1,25 $(OH)_2D_3$,[16-19] or the amount of calcium binding

protein in duodenal mucosa of rats.[17, 18] Thus, a reduction in calcium absorption in estrogen deficiency may not be directly related to vitamin D metabolites.

Regardless whether estrogen influenced calcium absorption directly of indirectly via 1,25 $(OH)_2D_3$, it was interesting to see how calcium absorption in various intestinal segments was affected by the absence of estrogen. We used the in situ intestinal loop method to study calcium fluxes in duodenum and colon (active transport predominance) and jejunum and ileum (passive transport predominance).

MATERIALS AND METHODS

Animals

Femal Wistar rats weighing 180-220 g were obtained from the Animal Centre, Salaya, Mahidol University. The animals were maintained on a 12 hr light/dark cycle and were fed a pelleted rat diet from F.F. Zuellig Ltd., Thailand.

The animals were randomly devided into 3 groups, two of which were ovariectomized while the remaining group was sham-operated. Ovariectomy, performed under light anesthesia, consisted of excision of the ovaries and the surrounding fat. One group of overiectomized rats received daily intramuscular injection of 3 μg estradiol/100 g body weight dissolved in corn oil starting on the day after ovariectomy. The other group of ovariectomized rats received only the vehicle corn oil. Both ovariectomized and sham operated rats were raised for 5-6 weeks until the experiment.

EXPERIMENTAL PROCEDURE

After an overnight fast, the animal was anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal injection). Tracheostomy and cannulation ot the femoral artery and vein were performed for blood collection and fluid replacement. Body temperature was maintained at 37°C by an overhead heating lamp connected to the temperature regulator (YSI Model 73A, Ohio, USA)

In situ intestinal loop method was performed as follows. Through the midline abdominal incision, the distal end of the bile duct and pyloric sphincter were ligated. Each rat had two 5 cm loops prepared, duodenal and colonic loop or jejunal and ileal loops. The duodenal and colonic loops were prepared by making a ligation 5 cm distal to the pyloric ligation or the ceco-colonic junction, respectively, the jejunal loop was prepared by making ligations at 10-15 cm distal to the pyloric ligation and the ileal loop was prepared from a segment 1-6 cm proximal to the ileocecal junction. After the loop was washed with 0.9% NaCl, it was filled with one of the following test solutions.

Duodenal and colonic loops : 0.4 ml of 0.5 mM $CaCl_2$ + 1 uCi ^{45}Ca, pH 7.0
Jejunal and ileal loops : 0.4 ml of 2.5 mM $CaCl_2$ + 1 uCi ^{45}Ca, pH 7.0

The concentration of 0.5 mM $CaCl_2$ was lower than the plasma ionized calcium (1.25 mM) and therefore active transport of calcium presumably predominated over passive transport in

this condition. The duodenum and colon were selected for the use of 0.5 mM $CaCl_2$ because they wsere two segments of intestine known to be capable of active transport. As for the jejunum and ileum, the concentration of calcium in the test solution was 2.5 mM, therefore passive transport of calcium predominated.

$^{45}CaCl_2$ was purchased from Radiochemical Centre, Amersham, UK with specific activity of 2.1 mCi/ml and 78 MBq/ml.

During the 30 minute incubation period, blood samples were collected at 5, 15 and 30 minutes. At the end of the experiment, the intestinal loops were removed and their contents were determined for volume, total calcium, and ^{45}Ca concentrations. Calcium fluxes were calculated according to the following equations.[20]

$$\text{Net absorption} = (V_i)\,(^{40}Ca_i) - (V_f)(^{40}Ca_f)/L$$

$$Ca_{P\text{-}L} = \frac{(V_i)(^{45}Ca_1) - (V_f)(^{45}Ca_i)}{(^{45}Ca_i/^{40}Ca_i + ^{45}Ca_f/^{40}Ca_f)/2L}$$

$$Ca_{P\text{-}L} = Ca_{L\text{-}P} - \text{net absorption}$$

where V is the volume of test solution; ^{40}Ca, ^{40}Ca concentration, ^{45}Ca, ^{45}Ca concentration; L, the length of intestinal loop. $Ca_{L\text{-}P}$, calcium movement from lumen; $Ca_{P\text{-}L}$, calcium movement into the lumen; and subscripts i and f are initial and final, respectively. It was assumed that the recycle of ^{45}Ca into the lumen solution was negligible.[20, 21]

Analyses

Blood samples were centrifuged immediately and the plasma was kept at 4°C until analysis on the same day. The plasma calcium concentration was determined by atomic absorption spectrophotometry (Varian AA 575). The radioactivity of ^{45}Ca was estimated by the standard liquid scintillation technique using an LKB-Wallac liquid scintillation counter (Model 1219) with quench corrected by the external standard ratio method.

All data were presented as mean ±SEM. Student's t-test was used for comparing two sets of data. Multiple comparisons were made using analysis of variance (ANOVA) and the significance of difference between groups was determined by Duncan's test. A value of $P < 0.05$ was considered significant.

RESULTS

All three groups of rats ie., sham, ovariectomized and ovariectomized plus estradiol received daily injection of either corn oil or 3 ug estradiol/100 g in corn oil. At the end of 5-6 week treatment, the body weights of sham, ovariectomized and ovariectomized rats with estradiol were 214±3, 225±6 and and 194±2 g, respectively. The body weight was significantly increased ($P < 0.05$) after ovariectomy. The plasma calcium concentration of ovariectomized rats was significantly lower than that of sham ie., 2.15±0.04 vs 2.50±0.02 mM, $P < 0.05$. The

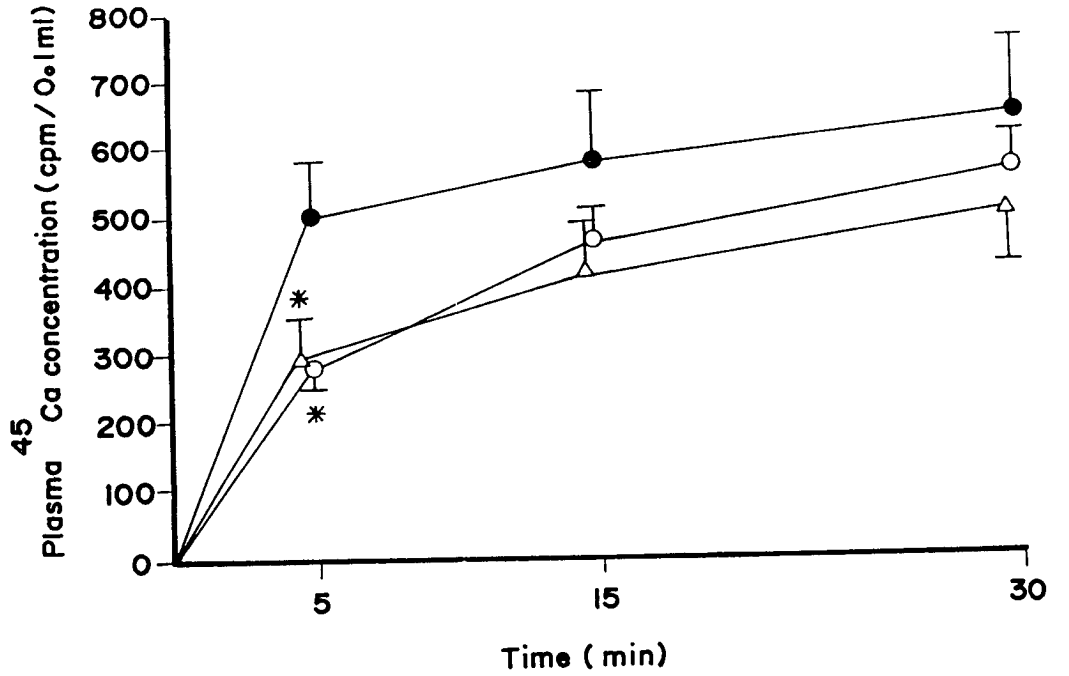

Fig. 1 The plasma ^{45}Ca concentrations (cpm/0.1 ml plasma) during the 30 minute in situ intestinal loop incubation in sham (●——●, n = 7) and ovariectomized rats given daily intramuscular injection of either corn oil (○——○, n = 7) or 3 ug estradiol/100 g body weight (△——△, n = 7). Each rat had the duodenal and colonic loops filled with 0.4 ml of 0.5 mM $CaCl_2$ + ^{45}Ca solution.
*$P < 0.05$, compared with sham.

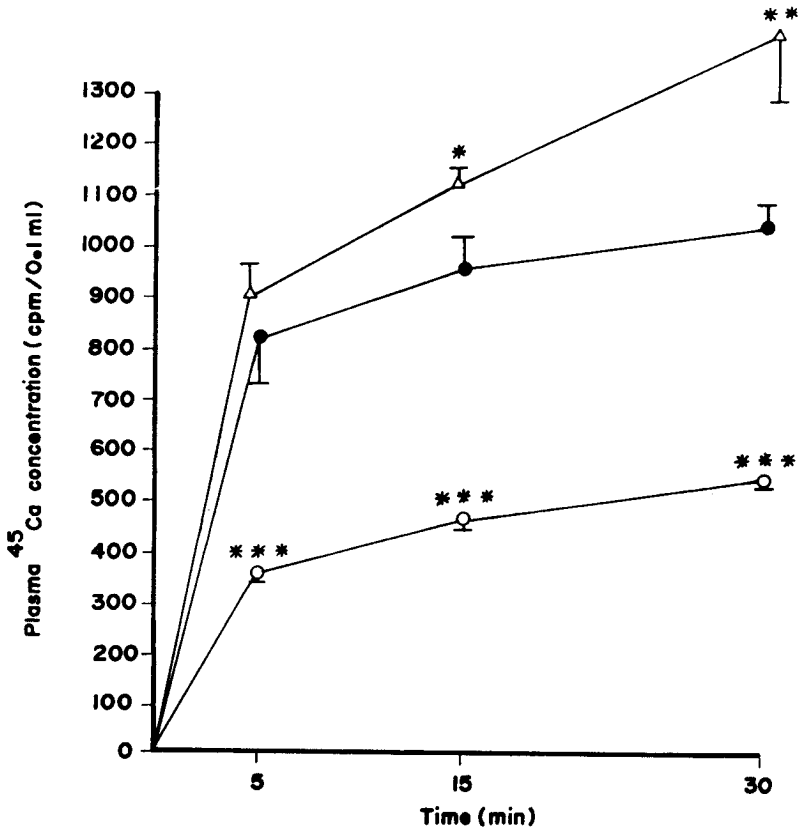

Fig. 2 The plasma ^{45}Ca concentration (cpm/0.1 ml plasma) during the 30 minute in situ intestinal loop incubation in sham (●——●, n = 6) and ovariectomized rats given daily intramuscular injection of either corn oil (○——○, n = 7) or 3 ug estradiol/100 g body weight (△ ————△, n = 7). Each rat had the jejunal and ileal loops filled with 0.4 ml of 2.5 mM $CaCl_2$ + ^{45}Ca solution.
*P < 0.05, **P < 0.01, and ***P < 0.001, compared with sham.

by daily administration of estradiol. Since estrogen is known to enhance hepatic production of plasma albumin, the decrease in plasma total calcium following ovariectomy and the return to normal level after estrogen treatment may be secondary to changes in serum albumin which binds about 43% of circulating plasma calcium.[28] However, there was also a possibility that ovariectomy-induced hypocalcemia may partly be accounted for by a reduction in the intestinal net calcium absorption.

As seen in Figure 1, when the test solution contained 0.5 mM calcium which was lower than the plasma ionized calcium, and active calcium transport presumably predominated,[29] ovariectomy and estrogen replacement had no effect on calcium absorption as represented by the plasma ^{45}Ca. The slight reduction in plasma ^{45}Ca in ovariectomized group at 5 min may be due to dilution effect since the heavier ovariectomized rats and control rats received the same dose of ^{45}Ca in the intestinal loops. In contrast, when passive calcium absorption predominated at luminal calcium of 2.5 mM (Figure 2), the plasma ^{45}Ca was markedly reduced after ovariectomy, calcium absorption recovered after estradiol replacement. It seemed unlikely that the 45% reduction in plasma ^{45}Ca was due to diluton rather than to effects on intestinal transport.

Our data implied that the effects of estrogens and 1,25 $(OH)_2$ D_3 on calcium absorption were not directly related. If the effects of estrogens were expressed via stimulation of 1,25 $(OH)_2D_3$ production which has been demonstrated only in birds[5-7] and not in mammals, then ovariectomized rats should have exhibited a reduction in the active transport of calcium in the duodenum which is the target of 1,25 $(OH)_2D_3$.[30] Thomas and Ibarra[18]using an in vitro everted gut sac technique, have recently demonstrated that duodenal active transport of calcium observed over 90 minute period in nonfasted ovariectomized rats was related to the increased growth rate and hyperphagia. Our data, On the other hand, showed no change in the lumen to plasma flux of calcium. We have no explanation for the difference in these results except that in our experiments, in situ intestinal loop technique was used and the fasted animals were studied for 30 minutes.

In situ intestinal loop experiments further showed that with the luminal calcium of 0.5 mM, estrogen deficiency resulted in an increase in the plasma to lumen flux of calcium which led to increased net secretion in the duodenal and colonic loops. How ovariectomy induced this increase in calcium secretion was not clear. However, from the data, it was probable that ovariectomy led to an increase in the permeability of the epithelium in such a way that calcium secretion which occurred passively via the paracellular pathway,[31] down the concentration gradient from plasma to lumen was enhanced. Since the colon transported calcium by active absorption[32] rather than by paracellular absorption,[33] this distal intestinal segment probably played a significant role in conserving calcium. Moreover, an increase in calcium secretion by the colon may constitute a route for substantial loss of calcium in ovariectomized rats.

With the luminal calcium concentration of 2.5 mM, it was interesting to find that both jejunal and ileal loops of ovariectomized rats exhibited a significant reduction in both directional fluxes of calcium. Since the reduction in the lumen to plasma flux was greater than the reduction in the plasma to lumen flux, net absorption of calcium was significantly reduced

in the jejunum. In intact animals with the luminal calcium concentration of 2.5 mM which was higher than that of the plasma ionized calcium, calcium secretion normally occurred against the concentration gradient, but down the electrical gradient towards the negative lumen. Ovariectomy-induced hypocalcemia further elevated the concentration gradient against which calcium secretion occurred. This would explain a reduction in the plasma to lumen flux of calcium in the jejunal and ileal loops of ovariectomized rats. Of more importance was the reduction in the lumen to plasma flux of calcium in these distal small intestinal segments of ovariectomized rats. Since these segments were known to be regions for absorption of large loads of calcium,[33] a marked reduction would undoubtedly result in a loss of calcium from the intestine.

From these data, estrogens appeared to have influence over calcium movements partly by altering calcium fluxes possibly by changing epithelial permeability. Estradiol administration returned calcium fluxes to the pattern observed in sham rats. It seemed that the lack of estrogens increased calcium loss by secretion from the duodenum and colon, and decreased the passive absorption of calcium in the jejunum and ileum. Although the present study didnot directly investigate the effect of estrogens or lack of them on the permeability of the intestinal epithelia, we would like to speculate that estrogens normally helped to regulate the epithelial permeability in such a way that passive or paracellular transport of calcium especially in the jejunum and ileum was enhanced, and calcium secretion in the duodenum and colon was restricted.

The effect of estrogens on intestinal calcium absorption in part may be attributed to the reported action of estrogens in increasing total body calcium.[34] Although the present study was done in rats, the findings that estrogens increased passive absorption of calcium may have important implications for therapy of postmenopausal osteoporosis. Estrogen treatment together with sufficient calcium supplements to elevate the luminal calcium concentration to a level at which passive absorption could readily take place, may be effective in enhancing the intestinal calcium absorption.

ACKNOWLEDGEMENTS

The authors are pleased to thank Wasana Saeng-umnart for her skilled technical assistance and Kosoom Vongthai for typing the manuscript.

This study was supported by the National Research Council of Thailand and the Mahidol University.

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บทคัดย่อ

จุดประสงค์ของการวิจัยนี้เพื่อศึกษาถึงอิทธิพลของฮอร์โมนเพศหญิงต่อการเคลื่อนแคลเซี่ยมระหว่างเลือดและโพรงลำไส้ส่วนต่าง ๆ ซึ่งการเปลี่ยนแปลงที่ลำไส้นี้อาจเป็นสาเหตุหนึ่งของการเปลี่ยนแปลงระดับแคลเซี่ยมในเลือด

สัตว์ทดลองที่ใช้คือหนูขาวกลุ่มควบคุม กลุ่มตัดรังไข่ และกลุ่มตัดรังไข่แต่ได้ฮอร์โมนอีสโตรเจนชดเชย หลังจาก 5-6 อาทิตย์ หนูขาวแต่ละตัวจะถูกผูกลำไส้ส่วน duodenum และ colon เป็นลักษณะถุงบรรจุสารละลาย 0.5 mM $CaCl_2$ + ^{45}Ca หรือ ถุงลำไส้ส่วน jejunum และ ileum บรรจุสารละลาย 2.5 mM $CaCl_2$ + ^{45}Ca จากการทดลองพบว่า 5-6 อาทิตย์หลังตัดรังไข่แคลเซี่ยมในเลือดมีระดับลดลง และการฉีดอีสโตรเจนมีผลป้องกันไม่ให้เกิดการลดของระดับแคลเซี่ยม หนูกลุ่มควบคุมมี net secretion ของแคลเซี่ยมในลำไส้ส่วน duodenum และ colon และมี net absorption ของแคลเซี่ยมที่ jejunum และ ileum หนูที่ตัดรังไข่มีการหลั่งแคลเซี่ยมจากเลือดสู่โพรงลำไส้ส่วน duodenum และ colon มากขึ้น ส่วน jejunum และ ileum มีการดูดซึมแคลเซี่ยมลดลง การเปลี่ยนแปลงเหล่านี้จะไม่เกิดขึ้นหากฉีดอีสโตรเจนชดเชย

ดังนั้นจึงพอสรุปได้ว่า หนูที่ไม่มีรังไข่จะดูดซึมแคลเซี่ยมแบบพาสซีพลดลงใน jejunum และ ileum และจะหลั่งแคลเซี่ยมมากขึ้นใน duodenum และ colon ซึ่งอาจเป็นสาเหตุหนึ่งของการสูญเสียแคลเซี่ยมและภาวะแคลเซี่ยมต่ำในเลือด