Korean J Physiol Pharmacol
Vol 6: 261–267, October, 2002

Interactions between Estradiol-17 β-BSA and Calcitropic Hormones in Ca^{2+} Uptake in Renal Proximal Tubule Cells

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The aim of the present study was to investigate the interaction of estradiol-17 β-bovine serum albumin (E_2-BSA) and calcitropic hormones, such as parathyroid hormone, calcitonin, and vitamin D, in regulation of Ca^{2+} uptake in primary cultured renal proximal tubule cells. Statistically significant increase in Ca^{2+} uptake was found from 2 hours after E_2-BSA (10^{-9} M) treatment, while estradiol-17 β (10^{-9} M) did not affect. Treatment of the cells with E_2-BSA (10^{-9} M) together with parathyroid hormone (PTH) (10^{-8} M), vitamin D (10^{-8} M), or calcitonin (10^{-8} M) significantly stimulated Ca^{2+} uptake by 32.50%, 29.30%, or 27.75%, respectively, compared with the control. However, calcitropic hormones did not exhibit any synergistic effect on the E_2-BSA-induced stimulation. E_2-BSA significantly increased cAMP generation and PKC activity. The stimulatory effect of cotreatment of E_2-BSA and PTH or vitamin D was blocked by SQ22536 (an adenylate cyclase inhibitor) and staurosporine (a PKC inhibitor), but the effect of cotreatment of E_2-BSA and calcitonin was not blocked. Furthermore, 8-Br-cAMP and TPA (an artificial PKC promoter) increased Ca^{2+} uptake by 25.51% and 16.47%, respectively, compared with the control. In conclusion, E_2-BSA combined with calcitropic hormones regulated Ca^{2+} uptake partially via cAMP and PKC-dependent mechanisms in renal proximal tubule cells.

Key Words: E_2-BSA, Ca^{2+} uptake, PTH, vitamin D, calcitonin, cAMP, PKC

INTRODUCTION

Besides its effects on the reproductive system, estradiol-17 β has been shown to have multiple physiological functions, such as growth, bone mineralization, cardiovascular functions, and brain masculinization by acting on different target tissues (Sharpe et al, 1998). Estrogen may play a role in calcium homeostasis, especially in women. The kidney is an important organ for the maintenance of calcium homeostasis, mediated by the principal physiological regulators, including parathyroid hormone (PTH), calcitonin, and 1,25-dihydroxyvitamin D_3 (1,25$[OH]_2D_3$), through an influence of renal calcium reabsorption in human (Friedman & Gesek, 1993). Although the estrogen effect may be mediated by other hormones or factors, estrogen receptors have been found in renal proximal tubule cells (Hagenfeldt & Eriksson, 1988; Han et al, 1999). Recently, several studies report that rapid and presumably non-genomic effects of estrogens include a rapid release of cAMP (Picotto et al, 1996), activation of phospholipase C (Graber et al, 1993), and rapid elevation of intracellular calcium concentration (Audy et al, 1996). Therefore, it is possible that estrogen may have a direct effect on the renal calcium handling. Furthermore, estrogen deficiency affects renal calcium transport, resulting in the impairment of extracellular calcium balance (Prince & Dick, 1997) and perhaps in the pathogenesis of osteoporosis. However, there are little data on the regulatory effects of estrogen on calcium handling of renal proximal tubule cells.

Effects of estrogen on PTH levels must be interpreted in the light of the circulating plasma ionized calcium concentration. Durate et al (1988) suggested that estradiol-17 β could directly stimulate the PTH secretion from human parathyroid adenomas in a short-term in vitro culture. This short-term effect of estrogen could be due to its membrane effect (Morley et al, 1992). Estrogen may also have effects on the circulating calcitriol concentration in addition to effects on ionized calcium. Serum 1,25 $[OH]_2D_3$ levels increase after estrogen treatment (Brumbaugh et al, 1975; Van Hoof et al, 1999). To date, in vitro studies have not shown any direct effects of estrogen on the calcitriol action, although many studies in animals have shown marked stimulatory effects of parenteral estrogen on calcitriol production (Criddle et al, 1997). The pathogenetic importance of systemic hormones such as calcitonin, PTH, and metabolites of vitamin D in postmenopausal osteoporosis is still open to debate, because of conflicting the available data.

A convenient means to evaluate the effects of estradiol-17 β on calcium handling in renal proximal tubule cells is to use differentiated cell cultures. In this study, we used primary rabbit kidney proximal tubule cell cultures. This primary culture system could be a compared with previous transport

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ABBREVIATIONS: E_2-BSA, estradiol-17 β-bovine serum albumin; PTH, parathyroid hormone; PTCs, primary rabbit renal proximal tubule cells.

studies conducted with renal tissue. The primary rabbit renal proximal tubule cells (PTCs) retain a number of differentiated functions distinctive of the renal proximal tubule (Chung et al, 1982; Han et al; 1999). The observation on the responses of the primary PTCs to hormones also indicates that this cell culture system consists of a population of cells, which is highly enriched with cells originating from the renal proximal tubule. Among typical responses of the renal proximal tubule is the stimulation of cAMP production by PTH, unlike arginine vasopressin and calcitonin (Taub et al, 1989; Dunlay & Hruska, 1990).

The present study was undertaken to examine the nongenomic effect of estradiol-17β on Ca^{2+} uptake in the PTCs using covalently linked estradiol-17β to bovine serum albumin (E_2-BSA) via a carboxymethyl oxime bridge in order to restrict the entry of estradiol-17β through the cell membrane. Also, it was to examine interactions of E_2-BSA and calcitropic hormones on Ca^{2+} uptake in the PTCs.

METHODS

Materials

Dulbecco's Modified Eagle's Medium (D-MEM), Ham's nutrient mixture F-12 (F-12), and Class IV collagenase were purchased from Life Technologies (Grand Island, NY). Estradiol-6-*O*-carboxymethyl oxime-BSA (E_2-BSA) (estradiol: BSA, 32:1), PTH, calcitonin, vitamin D, staurosporine, TPA (phorbol 12-myristrate 13-acetate), 8-bromo adenosine-3', 5'-cyclic Monophosphate (8-Br-cAMP), nifedipine, and methoxyvera pamil (D600) were obtained from Sigma Chemical Company (St. Luis, MO). SQ 22536 was purchased from Biomol (Plymouth Meeting, PA). $^{45}Ca^{2+}$ was purchased from Dupont/NEN (NEN, Boston, MA). [^{3}H]-cyclic AMP assay system (code TRK 432) and protein kinase C enzyme assay system (code RPN 77) were purchased from Amersham International (UK).

Methods

Primary cell culture: Male New Zealand white rabbits (1.5~2.0 kg) were used for the experiments. Primary rabbit kidney proximal tubule cell cultures were prepared by the modified method of Chung et al (1982). Kidneys were perfused via the renal artery, first with PBS and subsequently with D-MEM/F-12 containing 0.5% iron oxide (wt/vol), until the kidney turned gray-black in color. Renal cortex were sliced and then homogenized with 4 strokes of a sterile glass homogenizer. The homogenate was filtered first through a 253 μm and then an 83 μm mesh filter. Tubules and glomeruli on top of the 83 μm filter were transferred into sterile D-MEM/F-12 medium containing a magnetic stirring bar. Glomeruli (containing iron oxide) were removed with a magnetic stirring bar. The remaining proximal tubules were briefly incubated in D-MEM/F-12 containing 60 μg/ml collagenase (Class IV) and 0.025% soybean trypsin inhibitor. The dissociated tubules were then washed by centrifugation, resuspended in D-MEM/F-12 containing the two supplements, and transferred into tissue culture dishes. The PTCs were grown in 50 : 50 mixture of Dulbecco's Modified Eagle's Medium (D-MEM) and Ham's nutrient mixture F-12 with 15 mM HEPES and 20 mM sodium bicarbonate, but lacking phenol red (pH 7.4). Immediately prior to the use of the medium, two growth supplements (5 μg/ml insulin, 5 μg/ml transferrin) were added. PTCs were maintained in a humidified 5% CO_2-air environment in phenol red-free D-MEM/F-12 medium at 37°C. Medium was changed one day after plating and every three days thereafter.

$^{45}Ca^{2+}$ uptake experiments: Ca^{2+} uptake was determined by a modification of the method described by Chirayath et al (1998). To study the effect of E_2-BSA on $^{45}Ca^{2+}$ uptake, completely confluent monolayers were incubated with and without 10^{-9} M E_2-BSA for various times (0~24 hours) before the experiments. After culture medium was removed by aspiration, the monolayers were gently washed twice with the uptake buffer (140 mM NaCl, 2 mM KCl, 1 mM KH_2PO_4, 10 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM glucose, 5 mM L-alanine, 5 M indomethacin, and 10 mM HEPES/Tris, pH 7.4). The monolayers were then incubated at 37°C for 30 min in an uptake buffer containing 1 μCi/ml $^{45}Ca^{2+}$. At the end of the incubation period, the monolayers were again washed three times with ice-cold uptake buffer, and the cells were solubilized in 1 ml of 0.1% SDS. To determine the intracellularly $^{45}Ca^{2+}$ incorporated, 900 μl of each sample was transferred into scintillation vials and counted in a liquid scintillation counter (LS 6500, Beckman Instruments, Inc., Fullerton, CA). The remainder of each sample was used for protein determination by Bradford method (1976). The radioactivity counts in each sample were then normalized with protein content and corrected for zero-time uptake per mg protein. All uptake measurements were carried out in triplicate.

cAMP assay: The confluent monolayers were preincubated with 100 μM IBMX for 30 min at 37°C to inhibit degradation of cAMP and were then incubated with E_2-BSA (10^{-9} M) for 30 min. The sample was extracted by homogenization in buffer containing 4 mM EDTA to prevent enzymatic degradation of cAMP, followed by heating for 5 min in boiling water bath to coagulate protein. After centrifugation at 3,000 rpm for 5 min, the cAMP fraction in the supernatants was transferred into a new tube and stored at 4°C. The samples were assayed for cAMP by the use of [^{3}H]-cAMP assay system.

PKC assay: For PKC assay, PTCs grown in 35 mm plates were incubated with E_2-BSA (10^{-9} M) for 30 min and washed with 2 ml ice-cold buffer [10 mM Tris-HCl (pH 7.5), 0.25 M sucrose, 0.2 mM $CaCl_2$, 1 mM phenylmethylsufonyl fluoride (PMSF), 10 μg/ml leupeptin and 10 mM benzamidine] and were scraped off into the tube, and homogenized by POLYTRON® PT 1200 in the same ice-cold buffer (1 ml). After centrifugation at 12,000 rpm for 5 min, the PKC fraction in the supernatant was transferred into a new tube and stored at 4°C for measurement of PKC activity. PKC activity was measured as the rate of phosphorylation of peptide substrate using the PKC assay system kit and expressed as pmol of phosphate/mg protein/min.

Statistical analysis: All results are presented as means ±standard errors (SEM). Statistical differences between two mean values were analyzed by Students t-test. Statistical significance was defined as $P<0.05$.

RESULTS

Effect of estradiol-17β and E_2-BSA on Ca^{2+} uptake

To determine the time-response for the effect of E_2-BSA

on Ca^{2+} uptake, PTCs were incubated with estradiol-17β or E_2-BSA for various time intervals. As shown in Fig. 1, a statistically significant increase in Ca^{2+} uptake was found after 2 hours of the incubation with E_2-BSA. In contrast, estradiol-17β did not affect Ca^{2+} uptake.

Interaction of E_2-BSA and PTH, vitamin D, or calcitonin on Ca^{2+} uptake

To determine the effective concentrations and time for Ca^{2+} uptake to PTH, PTCs were incubated with different concentrations of PTH (10^{-12}, 10^{-10}, 10^{-8}, or 10^{-7} M) and for various time intervals. PTH ($>10^{-8}$ M) significantly stimulated Ca^{2+} uptake from 2 hours after the incubation (Fig. 2A). As shown in Fig. 2B, 8-hour incubation with 10^{-8} M PTH significantly stimulated Ca^{2+} uptake (383.83$\pm$27.43 pmol/mg protein/min vs. 319.75$\pm$6.04 pmol/mg protein/min in the control; $P<0.05$). We examined the interaction of E_2-BSA and PTH on Ca^{2+} uptake. Thus, PTCs were incubated with E_2-BSA alone (10^{-9} M) or together with PTH (10^{-8} M) for 8 hours. As shown in Fig. 2C, E_2-BSA alone or together with PTH stimulated Ca^{2+} uptake significantly (407.21$\pm$12.93 or 425.25$\pm$22.98 pmol/mg protein/min, respectively) compared with the control (320.93$\pm$16.43 pmol/mg protein/min; $P<0.05$). However, the Ca^{2+} uptake in response to E_2-BSA combined PTH was not significantly different from that with E_2-BSA alone.

To determine the effective concentration and treatment time for the Ca^{2+} uptake to vitamin D, PTCs were incubated with different concentrations of vitamin D (10^{-12}, 10^{-10}, 10^{-8}, or 10^{-7} M) and for various time intervals. As shown in Fig. 3A, the incubation with 10^{-8} M vitamin D for 8 hours significantly stimulated Ca^{2+} uptake (383.08$\pm$5.03 pmol/mg protein/min for 10^{-8} M vitamin D vs. 308.49 $\pm$11.43 pmol/mg protein/min for the control; $p<0.05$). Vitamin D ($>10^{-8}$ M) significantly stimulated Ca^{2+} uptake from 2 to 16 hour-incubation (Fig. 3B). PTCs were incubated with E_2-BSA alone (10^{-9} M) or together with vitamin D (10^{-8} M) for 8 hours. Fig. 3C showed that E_2-BSA alone or E_2-BSA combined with vitamin D stimulated Ca^{2+} uptake by 407.21$\pm$12.93 or 414.97$\pm$20.45 pmol/mg protein/min, respectively, compared with the control (320.93$\pm$16.43 pmol/mg protein/min; $P<0.05$). However, there was no significant difference in the effect between E_2-BSA alone and E_2-BSA combined with vitamin D.

As shown in Fig. 4A and B, a significant increase in Ca^{2+} uptake of PTCs was observed when incubated with 10^{-8} M calcitonin for 8 hours, compared with the control (379.33 $\pm$10.06 pmol/mg protein/min vs. 320.93$\pm$16.43 pmol/mg

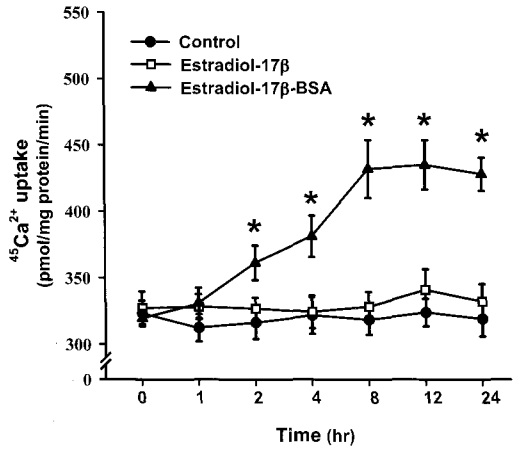

Fig. 1. Time course of the effects of estradiol-17β and E_2-BSA on Ca^{2+} uptake. PTCs were incubated in the presence and absence of estradiol-17β (E_2) and E_2-BSA (10^{-9} M), respectively. Values are means$\pm$SEM of four independent experiments with triplicate dishes. * $P<0.05$ vs. control.

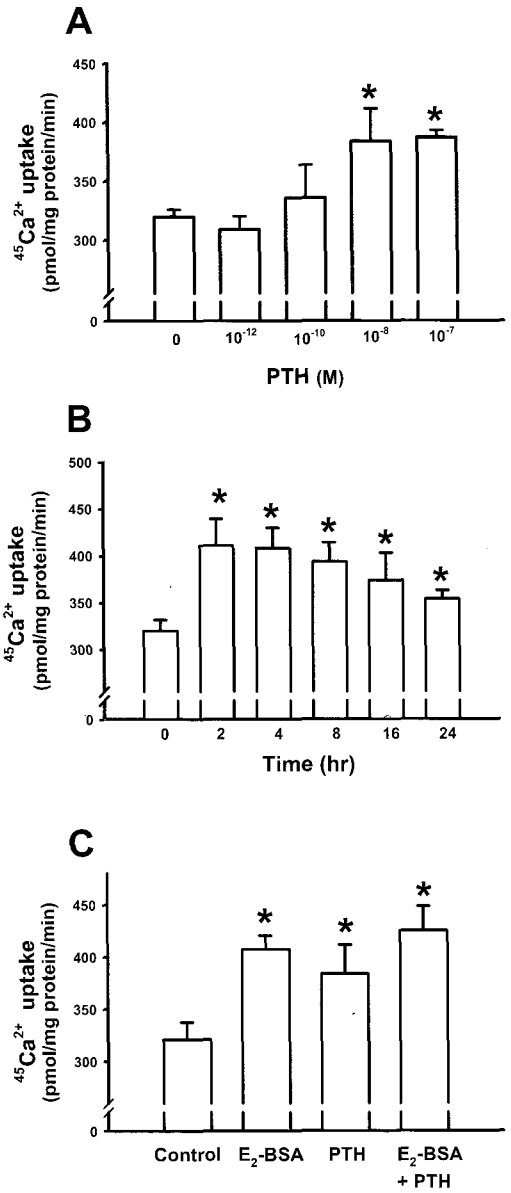

Fig. 2. (A) Dose response of PTH on Ca^{2+} uptake. PTCs were treated with 0, 10^{-12}, 10^{-10}, 10^{-8}, or 10^{-7} M PTH for 8 hours before uptake experiment. (B) Time course of the effects of PTH on Ca^{2+} uptake. PTCs were incubated with PTH (10^{-8} M) for up to 24 hours before uptake experiment. (C) Effects of E_2-BSA combined with and without PTH on Ca^{2+} uptake. PTCs were treated with E_2-BSA (10^{-9} M) or/and PTH (10^{-8} M) for 8 hours. Values are means$\pm$SEM of three independent experiments with triplicate dishes. * $P<0.05$ vs. control.

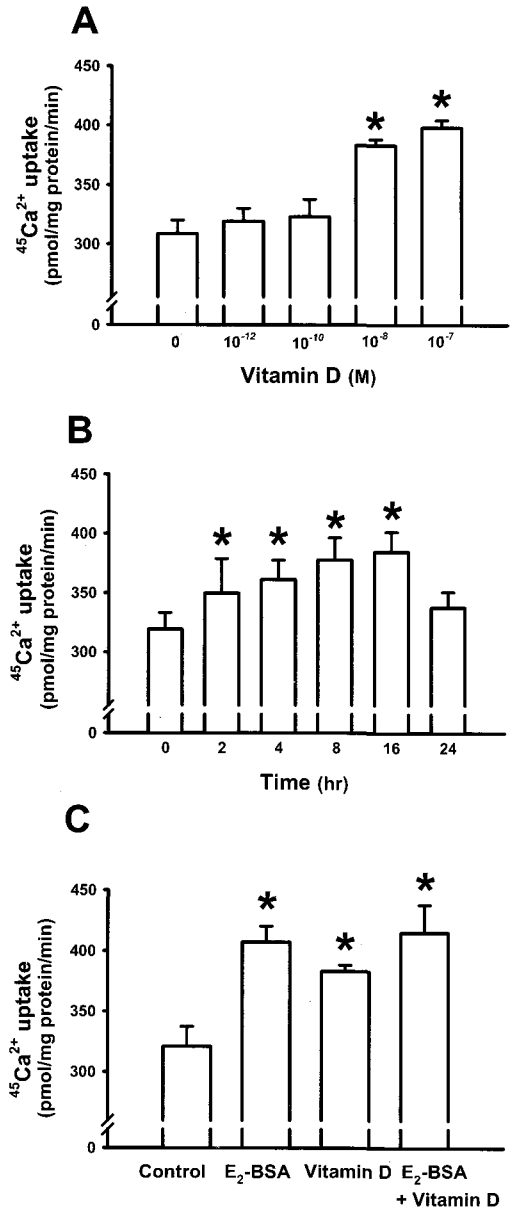

Fig. 3. (A) Dose response of vitamin D on Ca^{2+} uptake. PTCs were treated with 0, 10^{-12}, 10^{-10}, 10^{-8}, or 10^{-7} M vitamin D for 8 hours before uptake experiment. (B) Time course of the effects of vitamin D on Ca^{2+} uptake. PTCs were incubated with vitamin D (10^{-8} M) for up to 24 hours before uptake experiment. (C) Effect of E_2-BSA combined with and without vitamin D on Ca^{2+} uptake. PTCs were treated with E_2-BSA (10^{-9} M) or/and vitamin D (10^{-8} M) for 8 hours. Values are means±SEM of three independent experiments with triplicate dishes. * $P<0.05$ vs. control.

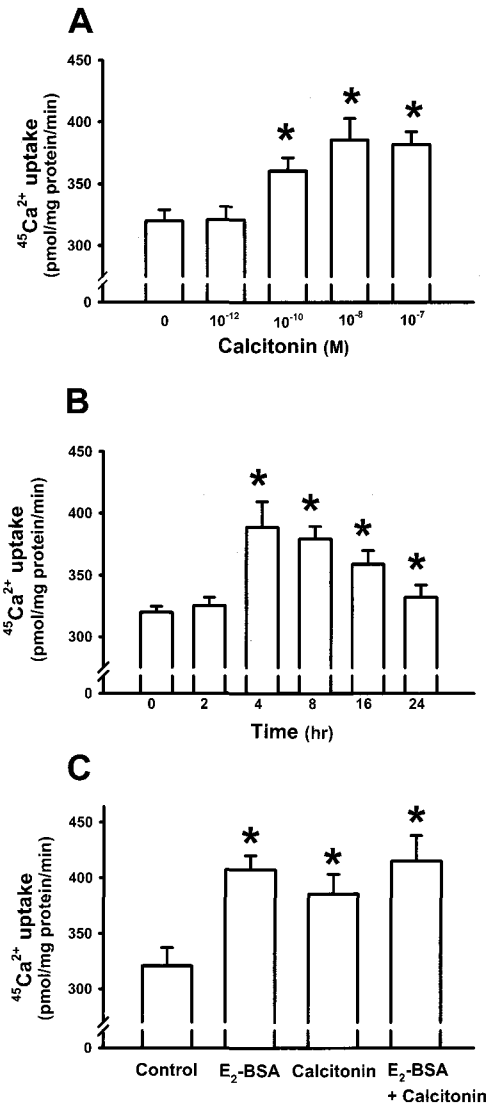

Fig. 4. (A) Dose response of calcitonin on Ca^{2+} uptake. PTCs were treated with 0, 10^{-12}, 10^{-10}, 10^{-8}, or 10^{-7} M calcitonin for 8 hours before uptake experiment. (B) Time courses of the effects of calcitonin on Ca^{2+} uptake. PTCs were incubated with calcitonin (10^{-8} M) for up to 24 hours before uptake experiment. (C) Effect of E_2-BSA combined with and without calcitonin on Ca^{2+} uptake. PTCs were treated with E_2-BSA (10^{-9} M) or/and calcitonin (10^{-8} M) for 8 hours. Values are means±SEM of three independent experiments with triplicate dishes. * $P<0.05$ vs. control.

protein/min). E_2-BSA combined with calcitonin (409.97± 12.68 pmol/mg protein/min) also stimulated Ca^{2+} uptake by 127.75% compared to the control (Fig. 4C), however, there was no significant difference, when compared with E_2-BSA alone.

Effects of PKA and PKC promoters and inhibitors on E_2-BSA combined with and without calcitropic hormone-induced Ca^{2+} uptake

To further identify the second messengers that mediate the stimulatory action of E_2-BSA combined with calcitropic hormones on Ca^{2+} uptake in proximal tubule cells, the effects of PKA and PKC activators as well as inhibitors were examined. Fig. 5A shows that 8-Br-cAMP and TPA (an artificial PKC activator) increased Ca^{2+} uptake by 25.51% and 16.47%, respectively, compared with the con-

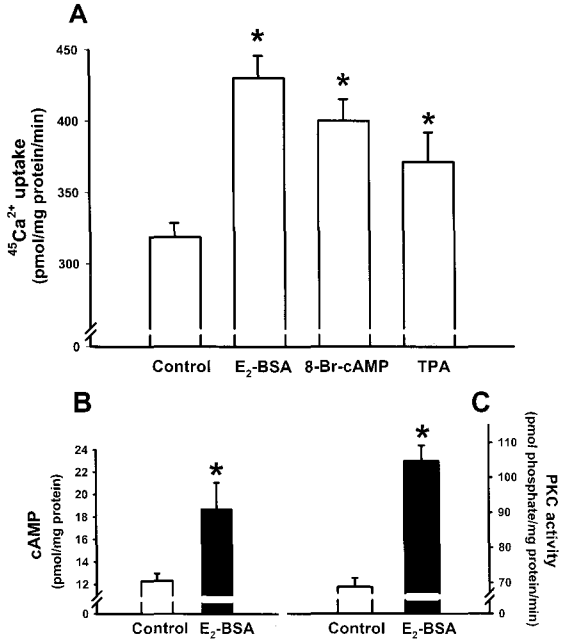

Fig. 5. (A) Effects of 8-bromoadenosine 3', 5'-cyclic monophosphate (8-Br-cAMP) and 12-O-tetradecanoylphorbol-13-acetate (TPA) on Ca^{2+} uptake. After PTCs were incubated with E_2-BSA (10^{-9} M), 8-Br-cAMP (10^{-6} M), or TPA (0.02 ng/ml) for 8 hours, Ca^{2+} uptake was measured. (B, C) Effects of E_2-BSA on cAMP and total PKC activity. With PTCs incubated with E_2-BSA (10^{-9} M) or vehicle for 30 min, cAMP and PKC activity were measured. * $P<0.05$ vs. untreated E_2-BSA.

trol. Determination of cAMP/PKC showed that 10^{-9} M E_2-BSA significantly stimulated cAMP production (control: 12.31 ± 0.64 vs. E_2-BSA: 18.74 ± 2.30 pmol/mg protein; $P<0.05$) and the total PKC activity (control: 68.74 ± 2.54 vs. E_2-BSA: 104.71 ± 4.51 pmol phosphate/mg protein/min; $P<0.05$) (Fig. 5B, C).

Fig. 6A, B show that the addition of SQ 22536 (an adenylate cyclase inhibitor) blocked the Ca^{2+} uptake stimulated by E_2-BSA together with PTH or vitamin D. Staurosporine (a PKC inhibitor) also blocked Ca^{2+} uptake stimulated by combination of E_2-BSA with PTH or vitamin D. Furthermore, SQ 22536 and staurosporine blocked Ca^{2+} uptake stimulated by calcitropic hormone alone. However, SQ 22536 or staurosporine did not block the Ca^{2+} uptake stimulated by the cotreatment of E_2-BSA and calcitonin (Fig. 6C).

Effect of Ca^{2+} channel inhibitor on E_2-BSA-induced Ca^{2+} uptake

In order to determine whether L-type Ca^{2+} channel blocker, such as nifedipine and methoxyverapamil, affects the E_2-BSA-induced stimulation of Ca^{2+} uptake, they were applied to the PTCs 30 min before the E_2-BSA treatment. As shown in Fig. 7, nifedipine did not inhibit the E_2-BSA-stimulated Ca^{2+} uptake, but methoxyverapamil slightly blocked it. These results suggest that the E_2-BSA-stimulated Ca^{2+} uptake in PTCs was mediated by the methoxyverapamil-sensitive L-type Ca^{2+} channel. They also suggest that the E_2-BSA-induced stimulation of Ca^{2+} uptake may be mediated not only by L-type Ca^{2+} channel, but also by some other pathways.

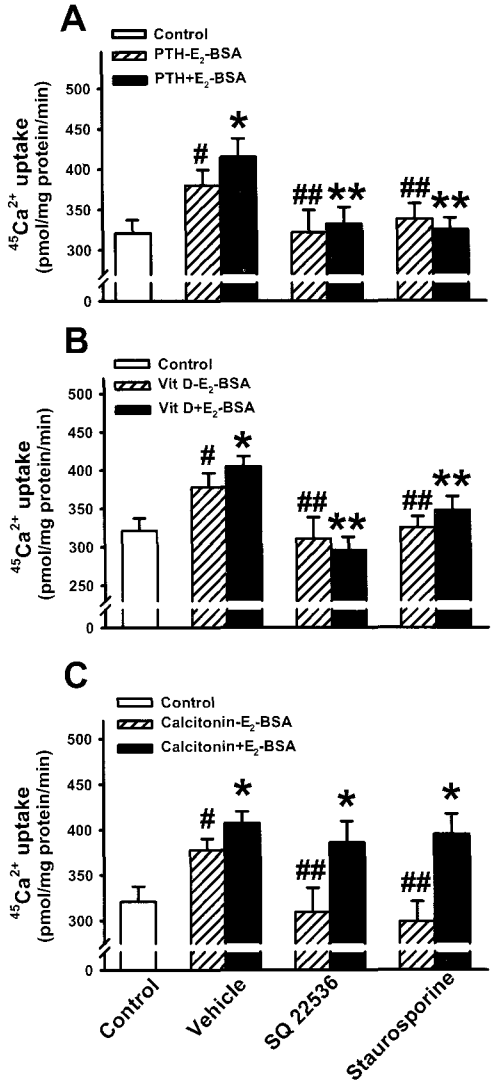

Fig. 6. Effects of SQ 22536 and staurosporine on the E_2-BSA combined with and without calcitropic hormone-induced stimulation of Ca^{2+} uptake. PTCs were incubated with SQ 22536 (10^{-6} M) or staurosporine (10^{-7} M) for 30 min prior to the treatment of E_2-BSA and PTH (A), vitamin D (B), or calcitonin (C). And PTCs were then treated with E_2-BSA (10^{-9} M) and PTH (10^{-8} M), vitamin D (10^{-8} M), or calcitonin (10^{-8} M) for 8 hours. Values are means $\pm$ SEM of three independent experiments with triplicate dishes. * $P<0.05$ vs. control, ** $P<0.05$ vs. E_2-BSA and PTH or vitamin D, # $P<0.05$ vs. control, ## $P<0.05$ vs calcitropic hormone alone.

DISCUSSION

The present work was undertaken to elucidate interactions of E_2-BSA and calcitropic hormones on Ca^{2+} uptake in renal proximal tubule cells. In our study we used primary cultured rabbit renal proximal tubule cells incubated in serum-free D-MEM/F-12 medium lacking phenol red. Our results showed that E_2-BSA, PTH, calcitonin, and

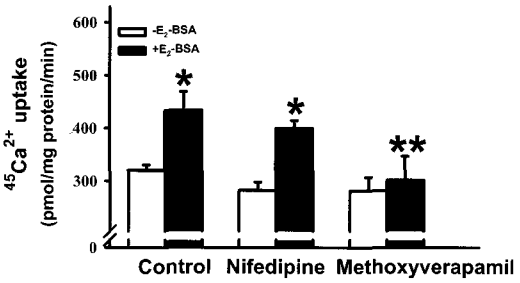

Fig. 7. Effects of nifedipine and methoxyverapamil on E_2-BSA-induced Ca^{2+} uptake. PTCs were incubated with nifedipine (10^{-5} M) and methoxyverapamil (10^{-6} M) for 30 min prior to the treatment of 10^{-9} M E_2-BSA. Ca^{2+} uptake was measured after 8 hours. Values are means ± SEM of three independent experiments with triplicate dishes. * $P<0.05$ vs. control, ** $P<0.05$ vs. E_2-BSA.

vitamin D stimulated Ca^{2+} uptake in PTCs. Such E_2-BSA effects have been demonstrated to be initiated at the plasma membrane level, however, the nature and characteristics of the receptor mediating these effects have not yet been clearified. Indeed, a novel G-protein coupled membrane estrogen receptor has been characterized in murine macrophage cell lines (Benten et al, 2001). Calcium influx stimulated by both estradiol-17β and E_2-BSA is inhibited by pertussis toxin but not by classic estrogen receptor blockers such as tamoxifen and raloxifen (Lieberherr et al, 1993). There are several different opinions on the effect of estrogen replacement therapy on the level of the circulating calcitropic hormones. If estrogen causes a fall in ionized calcium and a rise in PTH, one would expect decreased excretion in urinary calcium. Studies of oral estrogen replacement have usually, but not always, found a rises in total or/and free calcitriol levels (Cheema et al, 1989; Dick et al, 1995), especially in osteoporotic women (Gallagher et al, 1980), whereas studies of transdermal estrogen have not observed any effects of estrogen on the calcitriol level (Selby & Peacock, 1986; Dick et al, 1995). The stimulating effect of estrogen on the total 1,25 $(OH)_2D_3$ level has been documented by some studies (Brumbaugh et al, 1975), but not in other studies (Zofkova & Kancheva, 1996).

In contrast to the well-known genomic actions of estrogen, which are mediated by the classical nuclear receptors of estrogen (α and β), rapid effects of estradiol-17β occurring in seconds to few minutes after stimulation have recently been described in several different cell models (Lagrange et al, 1997; Collins et al, 1999; Falkenstein et al, 2000). Estrogen receptors have been found in renal proximal tubule cells, and there may be estrogen effects on renal Ca^{2+} transport that are independent of it's effects on the bone and calcitropic hormones (Prince, 1994). In the present study, we investigated direct interactions of E_2-BSA with calcitropic hormones in the regulation of Ca^{2+} uptake by renal proximal tubule cells. E_2-BSA and calcitropic hormones alone or together markedly stimulated Ca^{2+} uptake, but there was no significant difference. Furthermore, the E_2-BSA combined with and without calcitropic hormone-induced stimulation of Ca^{2+} uptake was blocked by SQ22536 (an adenylate cyclase inhibitor) and staurosporine (a PKC inhibitor). These results indicate that the interaction of E_2-BSA and calcitropic hormones regulated Ca^{2+} uptake mediated by nongenomic pathway. Indeed, our earlier in vitro studies indicated a direct effect of estrogen on the function of PTCs on cAMP accumulation and PKC activity.

Several lines of evidence have suggested that the activity of renal calcium channels is modulated by phosphorylation (Bkaily et al, 1984; Hosey et al 1988). Our results provided an evidence for the participation of the cAMP/PKC pathway in E_2-BSA-mediated stimulation of Ca^{2+} uptake. A temporal correlation between the changes in Ca^{2+} uptake and cAMP/PKC levels in response to E_2-BSA was observed. Consistent with these observations, fast increase in cAMP level after either in vitro or in vivo treatments with estradiol-17β have been observed in rat intestinal, bone, and uterine cells, and in human intact uterus, and in breast cancer cells (Fujimoto et al, 1994; Fiorelli et al, 1996). Moreover, we found that the effect of E_2-BSA was suppressed by a cAMP/PKC antagonist, which suggested that the PKA/PKC-dependent phosphorylation was involved in estradiol-17β-dependent Ca^{2+} uptake in proximal tubule cells. It has been proposed that the cAMP/PKC generated by the nongenomic pathway synergises with the steroid hormone receptor-mediated activation of gene transcription by phosphorylation of the receptor or associated transcription factors (Parikh et al, 1980; Le Goff et al, 1994). Therefore, it is possible that estradiol-17β participates in the hormonal regulation of calcium metabolism in renal proximal tubule cells through genomic and nongenomic pathways. In conclusion, the present study demonstrated that E_2-BSA and calcitropic hormone-induced stimulation of Ca^{2+} uptake in PTCs was partly mediated by PKA and PKC signal pathways.

ACKNOWLEDGEMENTS

This study was financially supported by Stem Cell Research Center (MK102KL010001-02k1201-02410) of Ministry of Science and Technology.

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