Regulatory Mechanism of Polarized Membrane Transport by Glucocorticoid in Renal Proximal Tubule Cells: Involvement of $[Ca^{2+}]i$

Ho Jae HAN, Dae Hoon KIM, Soo Hyun PARK, Young Soon LEE[1)], Jang Hern LEE[1)] and Il Suk YANG[1)]

Department of Veterinary Physiology, College of Veterinary Medicine, Hormone Research Center, Chonnam National University, Kwangju, 500–757 and [1)]College of Veterinary Medicine, Seoul National University, Suwon, 440–744 Korea

(Received 17 February 1999/Accepted 24 June 1999)

ABSTRACT. We examined the effect of glucocorticoids on brush border membrane transporters and, furthermore, the involvement of Ca^{2+} in its action in the primary cultured rabbit renal proximal tubule cells (PTCs). Dexamethasone (DEX, 10^{-9} M) decreased Pi uptake by 17%; whereas DEX affected neither α-methyl-glucopyranoside (α-MG) uptake nor Na^+ uptake. The DEX-induced inhibition of Pi uptake was due to a decrease of V_{max}. In contrast, other steroid hormones such as progesterone, testosterone, and 17β-estradiol (10^{-9} M) did not induce inhibition of Pi uptake. In order to examine the involvement of Ca^{2+} in DEX-induced inhibition of Pi uptake, PTCs were treated with A 23187 (10^{-6} M, Ca^{2+} ionophore). A 23187 also inhibited Pi uptake, mimicking DEX action in Pi uptake. Treatments with W-7 (10^{-4} M, calmodulin dependent kinase inhibitor), KN-62 (10^{-6} M, Ca^{2+}/calmodulin-dependent protein kinase II inhibitor), and BAPTA/AM (10^{-6} M) or TMB-8 (10^{-4} M) (intracellular Ca^{2+} mobilization blockers) blocked the DEX-induced inhibition of Pi uptake. However, nifedifine, methoxyverapamil (10^{-6} M, L-type Ca^{2+} channel blockers), and EGTA (1 mM, extracellular Ca^{2+} chelator) did not block it. In conclusion, DEX inhibited Pi uptake via, in part, Ca^{2+}/calmodulin pathway mediated by intracellular Ca^{2+} mobilization in the PTCs. —KEY WORDS: Ca^{2+}/calmodulin, dexamethasone, kidney, Na-Pi cotransporter.

J. Vet. Med. Sci. 61(11): 1197–1202, 1999

Glucocorticoids have been reported to have diverse effects on glucose metabolism [20], blood pressure [12], and renal function [18]. Glucocorticoids are also important regulators of renal phosphate transporter. The primary site of action of glucocorticoids is the proximal tubular apical brush border membrane and proximal tubular inorganic phosphate reabsorption plays a key role in overall Pi homeostasis [4, 18]. However, the action of glucocorticoids on Pi transport is controversial. In rat proximal tubule and chicken kidney cells glucocorticoids decrease tubular reabsorption of phosphate [22, 30]. In contrast, dexamethasone increased Pi uptake in opossum kidney (OK) cells [17]. In addition, the mechanisms responsible for the action of glucocorticoids on Pi uptake remain to be clarified. Among signal pathways, calcium is reported to be involved in the action of glucocorticoids in several tissues. Raising serum Ca^{2+} levels stimulates phosphate absorption in the proximal tubule of parathyroidectomized rat and Ca^{2+} stimulates the sodium-dependent phosphate absorption [1, 26]. Ning and Sanchez showed that calmodulin can interact with glucocorticoid receptor complex [21]. Therefore, intracellular Ca^{2+} may also regulate Pi transport in the kidney. However, the roles of Ca^{2+} in the action of glucocorticoids on Pi uptake of the proximal tubules are unclear. To test the effects of glucocorticoids, dexamethasone (DEX), a synthetic glucocorticoid which is poor substrate for 11β-hydroxysteroid dehydrogenase type-2, was used [7].

A convenient means to evaluate the effects of hormones on renal tubule epithelial cells is to use *in vitro* cell culture systems. A primary cultured rabbit renal proximal tubule cells (PTCs) culture system has been developed that forms confluent monolayers of polarized epithelial cells when grown in a serum-free medium supplemented with insulin and transferrin. The primary cells have been observed to retain a number of differentiated functions typical of the renal proximal tubule, including Na^+-dependent sugar transport, Na^+-dependent phosphate transport, and probenecid sensitive *p*-aminohippuric acid transport [9, 16, 33]. Therefore, the membrane transport studies, such as those establishing Pi uptake, conducted with such PTCs in hormonally defined, serum-free culture conditions have the particular advantage that the results can be directly compared with the original renal tissue [32]. Thus, we investigated the effect of DEX on the renal proximal brush border membrane transporters function and the involvement of Ca^{2+} in its action in the PTCs. The results demonstrated that DEX inhibited Pi uptake in the PTCs, which is, in part, mediated by cytosolic Ca^{2+} mobilized from the intracellular Ca^{2+} storage pool.

MATERIALS AND METHODS

Materials: Male New Zealand White rabbits (1.5–2.0 kg) were used for these experiments. Dulbecco's Modified Eagle's medium/Nutrient Mixture F-12 (Ham) (D-MEM/F-12, 1:1), Class IV collagenase and soybean trypsin inhibitor were purchased from Life Technologies (Grand Island, NY). Dexamethasone (DEX), testosterone, progesterone, 17β-estradiol, α-methyl-D-glucopyranoside (α-MG), ethylene glycol-bis (β-amino ethyl ether)-N,N,N',N'-tetra acetic acid (EGTA), 8-(N,N-diethylamino)-octyl-3, 4,5-trimethoyoxy-benzoate (TMB-8), 1,2-bis(aminophenoxy)ethane-N,N,N',N'-tetra acetic acid tetrakis (acetomethyl ester) (BAPTA/AM), n-6(aminohexyl)-5-chloro-1-naphthalene-

sulfonamide (W-7), 1-[N,O-bis(5-isoquinolinesulfonyl) -N-methyl-l-tyrosyl]-4-phenyl-piperazine (KN-62), nifedifine, methoxyverapamil, and ouabain were obtained from the Sigma Chemical Company (St. Louis, MO). $^{22}Na^+$, ^{14}C-α-methyl-D-glucopyranoside (^{14}C-α-MG), and ^{32}Phosphate (^{32}Pi) were purchased from Dupont/NEN. All other reagents were of the highest purity commercially available. Liquiscint was obtained from National Diagnostics (Parsippany, NY).

Isolation of rabbit renal proximal tubules and culture conditions: Primary rabbit renal proximal tubule cell cultures were prepared by the method of Chung *et al.* [9]. The PTCs were grown in D-MEM/F-12 medium with 15 mM HEPES and 20 mM sodium bicarbonate (pH 7.4). Immediately prior to the use of the medium, two growth supplements (5 μg/m*l* insulin and 5 μg/m*l* transferrin) were added.

Cell growth study: To determine the effects of DEX, PTCs in culture were initiated in 35 mm dishes for cell growth studies. Briefly, tubules were inoculated at one-fourth the normal inoculum (the normal inoculum being 0.3 mg protein/dish). During this time, cell counts were determined on days 5, 9, and 13 from triplicate culture plates using a Coulter Model ZF particle counter.

Marker enzymes assay: The confluent monolayers were incubated with 10^{-9} M DEX for 4 hr before marker enzymes assay. Alkaline phosphatase activity was assayed by the method of Bessy *et al.* using *p*-nitrophenylphosphate as a substrate [5]. Leucine aminopeptidase activity was assayed by the method of Green *et al.* using L-leucine-*p*-nitroanilide as a substrate [15]. γ-glutamyl transpeptidase activity was determined by the method of Tate and Meister using γ-glutamyl-*p*-nitroanilide as substrate and glycylglycine as the amino acid acceptor [29]. Each determination was made using triplicate dishes and was standardized with respect to protein. Protein determination was performed by the methods of Bradford using bovine serum albumin as a standard [6].

Uptake studies: The confluent monolayers were incubated with 10^{-9} M DEX for 4 hr before the uptake experiments. The Pi uptake experiment was conducted as described by Rabito [24]. After the culture medium was removed by aspiration, the monolayers were gently washed twice with the uptake buffer (150 mM NaCl, 1.2 mM $MgSO_4$, 0.1 mM $CaCl_2$, and 10 mM MES/Tris, pH 7.4). After the washing procedure, the monolayers were incubated at 37°C for 30 min in an uptake buffer containing 1.5 μCi/m*l* ^{32}Pi and 1 mM unlabeled phosphate. 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 m*l* of 0.1% SDS. To determine the ^{32}Pi incorporated intracellulary, 900 μ*l* of each sample was removed and counted in a liquid scintillation counter (Beckmann Instruments, Inc., Fullerton, CA). The remainder of each sample was used for protein determination. The radioactivity counts in each sample were then normalized with respect to protein and were corrected for zero-time uptake per mg protein. All uptake measurements were made in triplicate.

Na^+ uptake experiment was conducted by the method of Rindler *et al.* [25], and α-MG uptake experiment by the method of Sakhrani *et al.* [27]. Next steps were conducted as described in Pi uptake.

Statistical analysis: Results were expressed as means ± standard errors (S.E.). The difference between two mean values was analyzed with the ANOVA test. The difference was considered statistically significant when $p<0.05$.

RESULTS

Effects of dexamethasone on growth and brush border membrane enzyme activities: DEX has been implicated in modulating PTCs growth and function *in vivo*. In the first set of experiments, we examined the growth responsiveness of PTCs to DEX. Figure 1 depicts the time course growth of PTCs. PTCs were treated with DEX (10^{-12}–10^{-6} M) during medium exchange every 3 days. Cell counts were determined on days 5, 9, and 13. Cell numbers increased logarithmically from day 5 to 13. On day 9, DEX did not affect the growth of PTCs (Fig. 1). In the experiments to measure the brush border membrane marker enzyme activity, alkaline phosphatase and γ-glutamyl transpeptidase activity were not significantly different from the control. However, leucine aminopeptidase exhibited significantly reduced activity (Table 1).

Effects of dexamethasone on transporters activities: Cells were incubated with DEX prior to Pi uptake. As shown in Fig. 2A, DEX inhibited Pi uptake by 17% after 4 hr, and by 21% after 8 hr. This effect was less pronounced with a more prolonged preincubation time (3% after 24 hr, p=NS). Following the treatment of DEX for 4 hr, the relationship between DEX concentration and inhibition of Pi uptake was

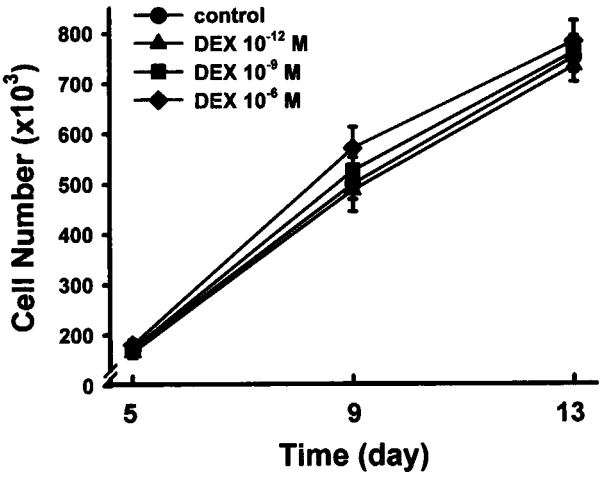

Fig. 1. The effect of dexamethasone on cell growth. PTCs were treated with dexamethasone (10^{-12}–10^{-6} M) during medium exchange every 3 day. Cell counts were determined on days 5, 9, and 13 using a Coulter Model ZF particle counter. Value are means ± S.E. of 9 separate experiments performed on 3 different cultures.

Table 1. Effect of dexamethasone on the brush border membrane enzyme activity of the primary cultured renal proximal tubular cells

	Control	Dexamethasone
Alkaline phosphatase (nmoles *p*-nitrophenyl phosphate released/mg protein/min)	16.8 ± 1.8	20.1 ± 2.9
Leucine aminopeptidase (nmoles *p*-nitroanilide released/mg protein/min)	164.9 ± 5.2	133.0 ± 5.1*
γ-Glutamyl transpeptidase (nmoles *p*-nitroanilide released/mg protein/min)	23.4 ± 1.3	22.1 ± 1.6

Values are means ± S.E. of four to five independent experiments with triplicate dishes. * $p<0.05$ vs. control.

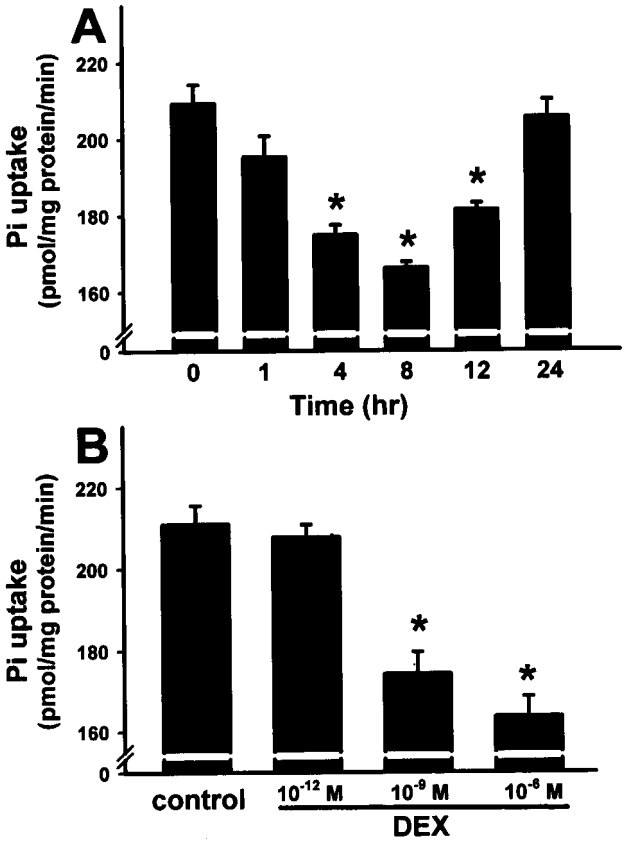

Fig. 2. Time course (A) and dose response curve (B) of dexamethasone on the inhibition of Pi uptake. PTCs were incubated with 10^{-9} M dexamethasone for various periods of time (0–24 hr) or with different doses of dexamethasone (0–10^{-12} M) for 4 hr before Pi uptake was performed. Values are means ± S.E. of 12 separate experiments with triplicate dishes. * $p<0.05$ vs. control.

examined. Significant inhibition was achieved by DEX concentrations equal to or higher than 10^{-9} M (Fig. 2B). This DEX action was selective for Pi uptake since DEX did not cause changes in the activities of α-MG uptake, Na^+ uptake, and $^{86}Rb^+$ uptake (Table 2). In order to investigate the effects of DEX or Pi uptake kinetics, the affinity (Km) of Pi for Na/Pi cotransporter and the maximum velocity (Vmax) of Pi uptake were calculated using a Lineweaver-Burk plot. Phosphate concentrations varied from 0.0625 to 1 mM. Figure 3 presents the results of the kinetic analysis of Pi uptake in the presence and the absence of 10^{-9} M DEX. Kinetic studies showed a decrease in V_{max} values of Pi uptake from 234.7 ± 17.2 to 178.3 ± 14.1 nmol/mg protein/min, while apparent K_m values were unchanged.

To evaluate whether the effect of DEX on Pi uptake was mimicked by other steroids, PTCs were treated with either 17 β-estradiol, testosterone, or progesterone at a concentration of 10^{-9} M for 4 hr, respectively. None of these hormones affected Pi uptake (Fig. 4).

Involvement of Ca^{2+} on dexamethasone-induced inhibition of Pi uptake: To examine the role of Ca^{2+} on Pi uptake, the PTCs were treated with A 23187 (10^{-6} M, Ca^{2+} ionophore). As shown in Fig. 5, A 23187 significantly inhibited Pi uptake, mimicking the DEX-induced inhibition of Pi uptake. Therefore we examined the role of Ca^{2+} or calmodulin on DEX-induced inhibition of Pi uptake. PTCs were treated with W-7 (10^{-4} M, calmodulin dependent kinase inhibitor) or KN-62 (10^{-6} M, Ca^{2+}/calmodulin dependent protein kinase II inhibitor) prior to the treatment with DEX. W-7 and KN-62 completely blocked DEX response (Fig. 5), and these results suggest that Ca^{2+}/calmodulin pathway is involved in the DEX-induced inhibition of Pi uptake.

Role of intracellular Ca^{2+} mobilization on dexamethasone-induced inhibition of Pi uptake: To know the source of cytosolic free Ca^{2+}, PTCs were treated with

Table 2. Effects of dexamethasone on ^{32}Pi, ^{14}C-α-MG, ^{22}Na, and ^{86}Rb uptake (pmol/mg protein/min)

	^{32}Pi	^{14}C-α-MG	^{22}Na	^{86}Rb
Control	209.8 ± 9.7	576.2 ± 24.3	1382.3 ± 60.0	274.2 ± 9.6
Dexamethasone	173.3 ± 9.3*	530.4 ± 24.7	1449.9 ± 70.6	293.9 ± 18.5

Values are means ± S.E. of 12 separate experiments with triplicate dishes. * $p<0.05$ vs. each control.

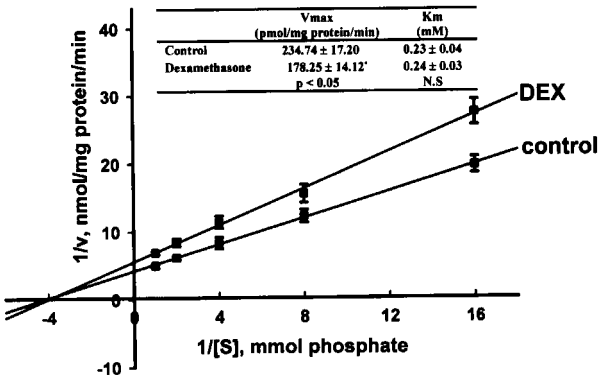

Fig. 3. Effect of dexamethasone on the kinetic parameters of Pi uptake. PTCs were treated with vehicle or dexamethasone (10^{-9} M) for 4 hr before Pi uptake. Phosphate uptake was measured in a buffer containing ^{32}Pi (1.5 μCi/m*l*) in the presence of unlabeled Pi (0.0625–1 mM). Values are means ± S.E. of 9 separate experiments with triplicate dishes. * $p<0.05$ vs. control.

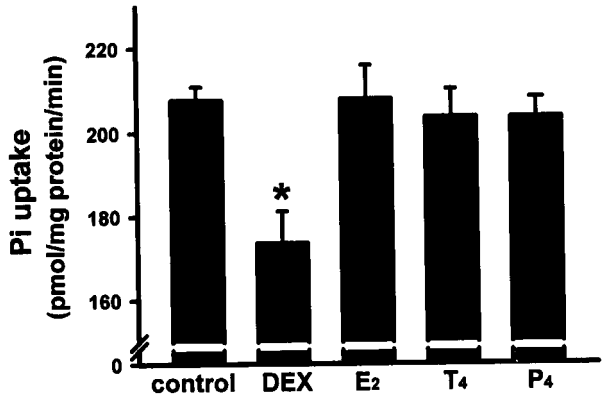

Fig. 4. Effect of several steroid hormones on Pi uptake. PTCs were treated with dexamethasone, 17β-estradiol (E_2), testosterone (T_4), and progesterone (P_4) (10^{-9} M) for 4 hr before Pi uptake. Values are means ± S.E. of 12 separate experiments with triplicate dishes. * $p<0.05$. vs. control.

BAPTA/AM (10^{-6} M) and TMB-8 (10^{-4} M) (intracellular Ca^{2+} mobilization blockers) alone prior to the treatment with DEX. BAPTA/AM and TMB-8 alone had no significant effect on Pi uptake. However, they completely blocked DEX-induced inhibition of Pi uptake (Fig. 6A). Therefore, as a next step, we examined the role of extracellular Ca^{2+}. PTCs were reported to have a L type Ca^{2+} channel. Thus PTCs were treated with nifedifine and methoxyverapamil (10^{-6} M, L type Ca^{2+} channel blockers) prior to the treatment with DEX. They did not block the DEX-induced inhibition of Pi uptake, and EGTA (1 mM, extracellular Ca^{2+} chelator) also did not affect the DEX-induced inhibition of Pi uptake (Fig. 6B). These results suggest that cytosolic free Ca^{2+} originating not from the extracellular Ca^{2+} influx but from the intracellular Ca^{2+} storage pool may be involved in the DEX-induced inhibition of Pi uptake.

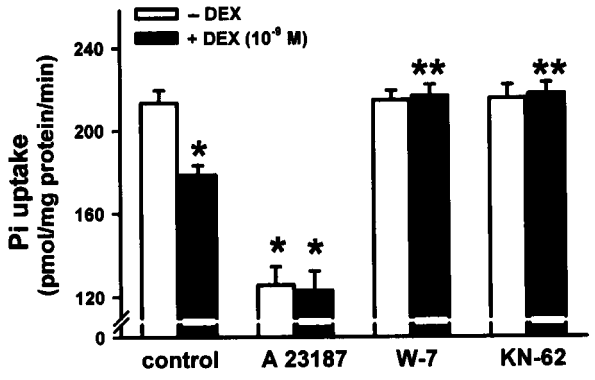

Fig. 5. Effect of Ca^{2+} on dexamethasone-induced inhibition of Pi uptake. PTCs were treated dexamethasone (10^{-9} M) alone or together with A 23187 (10^{-6} M) for 4 hr. In the same experiments, PTCs were incubated with W-7 (10^{-4} M) or KN-62 (10^{-6} M) for 30 min prior to the treatment of dexamethasone and then were treated with dexamethasone (10^{-9} M) for 4 hr. Values are means ± S.E. of 9 separate experiments with triplicate dishes. * $p<0.05$ vs. control, ** $p<0.05$ vs. dexamethasone.

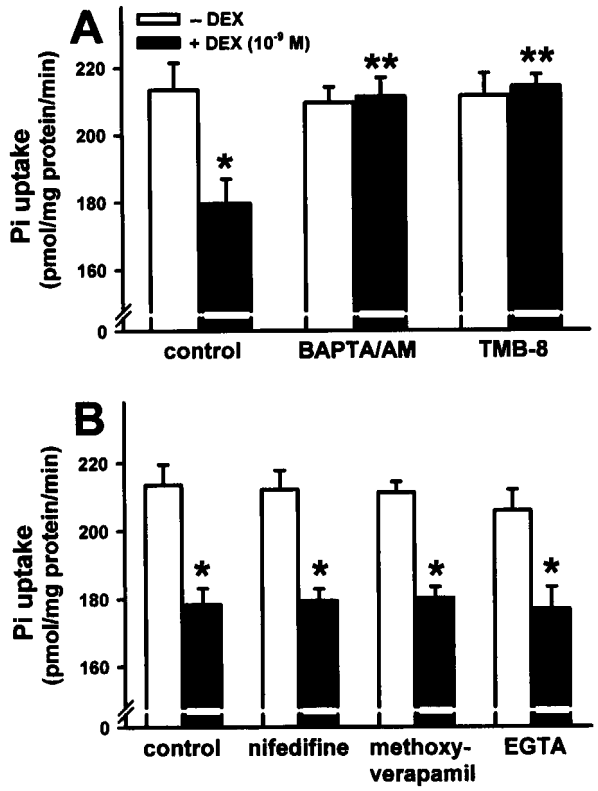

Fig. 6. Effect of intracellular Ca^{2+} mobilization (A) and extracellular Ca^{2+} influx (B) on dexamethasone-induced inhibition of Pi uptake. PTCs were treated with BAPTA/AM (10^{-6} M), TMB-8 (10^{-4} M), methoxyverapamil (10^{-6} M), nifedifine (10^{-6} M), or EGTA (1 mM) for 30 min prior to treatment with dexamethasone (10^{-9} M) and then were treated with dexamethasone (10^{-9} M) for 4 hr. Values are means ± S.E. of 9 separate experiments with triplicate dishes. * $p<0.05$ vs. control, ** $p<0.05$ vs. dexamethasone.

DISCUSSION

In this study, we document the first evidence that DEX inhibited Pi uptake in primary cultured rabbit renal proximal tubule cells, which may be mediated by Ca^{2+} mobilized from the intracellular Ca^{2+} storage pool. Recently, there was a report that DEX inhibited proliferation of human renal cell carcinomas [28]. In addition DEX decreased alkaline phosphatase in neonatal murine calvarial cells [8] and increased γ-glutamyltransferase activity in the plasma membrane of a highly differentiated hepatoma cell line [3]. In the present study, DEX did not affect the cell growth, or alkaline phosphatase and γ-glutamyl transpeptidase activity; however, it did decrease leucine aminopeptidase activity. Based upon these results, we hypothesized that glucocorticoids regulate various properties of PTCs. Thus, we examined the regulatory mechanism of glucocorticoids on brush border membrane transporters in PTCs.

It is widely accepted that glucocorticoids are important regulators of phosphate transport in the kidney [17]. Glucocorticoids injected *in vivo* into rats also decreased Na^+-phosphate cotransport in renal brush-border membrane vesicles prepared from the animals [14]. In the present study, DEX inhibited Pi uptake in the PTCs. Its effect was due to a decrease in the V_{max}. It is unclear whether the change in V_{max} reflects a change in the number of active transport proteins in the membrane or a change in the transport properties of existing proteins. In addition to the inhibition of Na^+/Pi cotransporter in the renal cell plasma membrane, glucocorticoid increased Na^+/H^+ antiporter activity [14], Na^+/glucose cotransporter activity, Na^+/proline cotransporter activity [2], and Na^+/K^+-ATPase [19]. However, in the PTCs DEX did not change Na^+ uptake and α-MG uptake. These results suggest that DEX is selective for Pi uptake in the PTCs. Inhibition of Pi uptake is specific to DEX, since treatment of the cells with other steroid hormones such as 17β-estradiol, progesterone, and testosterone did not inhibit Pi uptake. The inhibition of Pi uptake by DEX may be mediated by a glucocorticoid receptor. This assumption was based upon the report that rabbit proximal tubule had DEX-specific binding sites [13], and that the inhibition of Na^+/Pi transport by DEX in OK cells was receptor-mediated [31]. Further study will be required to characterize the receptor in PTCs.

Several lines of evidence suggest that Ca^{2+} signal is involved in glucocorticoid action. In PC12 cells, glucocorticoids inhibited nicotine-induced calcium influx via protein kinase C [23]. In our present study, treatment with Ca^{2+} ionophore mimicked the DEX-induced inhibition of Pi uptake, and calmodulin dependent kinase inhibitor and Ca^{2+} calmodulin dependent protein kinase II inhibitor blocked the DEX-induced inhibition of Pi uptake. These results suggest that the Ca^{2+}/calmodulin pathway may be an important signal in the DEX-induced inhibition of Pi uptake in PTCs. Several reports are in accordance with our results. In lymphocytes, glucocorticoids induced a sustained elevation of the cytosolic Ca^{2+}, and the increase of calmodulin mRNA and calmodulin inhibitor blocked glucocorticoid-induced apoptosis [11]. In mouse L929 cells, there is a functional interaction between calmodulin and the glucocorticoid receptor [21]. However, these results are not consistent with many reports, in which glucocorticoid has no effect on $[Ca^{2+}]i$ [10]. These differences may be due to the different experimental model systems or species.

The source of Ca^{2+} in the various glucocorticoid-induced cell responses including PTCs has not yet been clarified. The Ca^{2+} source may be the intracellular Ca^{2+} storage pool or extracellular Ca^{2+}. BAPTA/AM and TMB-8 have been used to block mobilization of intracellular Ca^{2+} in many studies, and in the present study, BAPTA/AM and TMB-8 (intracellular Ca^{2+} mobilization blockers) also blocked it. On the other hand, PTCs were reported to have a L type Ca^{2+} channel, which contributes to the Ca^{2+} influx during cell response [34], and the treatment of nifedifine and methoxyverapamil did not block the DEX-induced inhibition of Pi uptake. In addition, EGTA (extracellular Ca^{2+} chelator) did not block it either. These results suggest that DEX-induced inhibition of Pi uptake may be mediated by cytosolic Ca^{2+} mobilized from intracellular Ca^{2+} storage pools. Further studies on additional signaling pathways remain to elucidate the precise uptake mechanisms.

In conclusion, DEX selectively inhibited Pi uptake via, in part, Ca^{2+}/calmodulin pathway mediated by intracellular Ca^{2+} mobilization in the PTCs.

ACKNOWLEDGEMENT. This study was supported by grants awarded to Dr. H. J. Han from Korea Science and Engineering Foundation (KOSEF 971–65–36–2, HRC 1998G0301).

REFERENCES

1. Amiel, C., Kuntziger H., Couette, S., Coureau, C. and Bergounioux, N. 1976. Evidence for a parathyroid hormone-independent calcium modulation of phosphate transport along the nephron. *J. Clin. Invest.* 57: 256–263.
2. Arar, M., Levi, M. and Baum, M. 1994. Maturational effects of glucocorticoids on neonatal brush-border membrane phosphate transport. *Pediatr. Res.* 35: 474–478.
3. Barouki, R., Chobert, M-N., Billon, M-C., Finidori, J., Tsapis, R. and Hanoune, J. 1982. Glucocorticoid hormones increase the activity of gamma-glutamyltransferase in a highly differentiated hepatoma cell line. *Biochim. Biophys. Acta* 721: 11–21.
4. Berndt, T. J. and Knox, F. G. 1992. Renal regulation of phosphate excretion, pp. 2511–2532. *In*: The Kidney: Physiology and Pathophysiology (Seldin, D.W. and Giebisch, G. eds.), Raven Press, New York.
5. Bessy, O. A., Lowly, O. H. and Brock, M. J. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J. Biol. Chem.* 164: 321–329.
6. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254.
7. Brown, R. W., Chapman, K. E., Kotolevtsev, Y., Yau, J. L.,

Lindsay, R. S., Brett, L., Leckie, C., Murad, P., Lyons, V. Mullins, J. J., Edwards, C. R. and Seckl, J. R. 1996. Cloning and production of antisera to human placental 11 beta-hydroxysteroid dehydrogenase type 2. *Biochem. J.* 313: 1007–1017.

8. Chen, T. L. and Fry, D. 1999. Hormonal regulation of the osteoblastic phenotype expression in neonatal murine calvarial cells. *Calcif. Tissue Int.* 64: 304-309.
9. Chung, S. D., Alavi, N., Livingston, D., Hiller, S. and Taub, M. 1982. Characterization of primary rabbit kidney cultures that expresses proximal tubule functions in a hormonally defined medium. *J. Cell Biol.* 95: 118–126.
10. Doolan, C. M., O'Sullivan G. C. and Harvey, B. J. 1998. Rapid effects of corticosteroids on cytosolic protein kinase C and intracellular calcium concentration in human distal colon. *Mol. Cell. Endocrinol.* 138: 71–79.
11. Dowd, D. R., MacDonald, P. N., Komm, B. S., Haussler, M. R. and Miesfeld, R. 1991. Evidence for early induction of calmodulin gene expression in lymphocytes undergoing glucocorticoid-mediated apoptosis. *J. Biol. Chem.* 266: 18423–18426.
12. Edwards, C. R., Ebenediktsson, R., Lindsay, R. S. and Seckl, J. R. 1993. Dysfunction of placental glucocorticoid barrier: link between the fetal environment and adult hypertension? *Lancet* 341: 355–357.
13. Farman, N., Vandewalle, A. and Bonvalet, J. P. 1983. Autoradiographic determination of dexamethasone binding sites along the rabbit nephron. *Am. J. Physiol.* 244: F325-F334.
14. Freiberg, J. M., Kinsella, J. and Sacktor, B. 1982. Glucocorticoids increase the Na^+-H^+ exchange and decrease the Na^+ gradient-dependent phosphate-uptake systems in renal brush border membrane vesicles. *Proc. Natl. Acad. Sci. (U.S.A.)* 79: 4932–4936.
15. Green, M. N., Tsou, K. C., Bressler, R. and Seligman A. M. 1955. The colorimetric determination of leucine aminopeptidase activity with L-leucil-β-naphthylamide hydrochlroride. *Arch. Biochem. Biophys.* 57: 457–474.
16. Han, H. J., Kang, J. W., Park, K. M., Lee, J. H. and Yang, I. S. 1996. Functional characterization of primary culture cells grown in hormonally defined, serum-free and serum-supplemented medium. *Korean J. Vet. Res.* 36: 551–563.
17. Kafmann, M., Muff, R. and Fischer, J. A. 1991. Effect of dexamethasone on parathyroid hormone (PTH) and PTH-related protein regulated phosphate uptake in opossum kidney cells. *Endocrinology* 128: 1819–1824.
18. Kinsella, J. L. 1990. Action of glucocorticoids on proximal tubule transport systems. *Semin. Nephrol.* 10: 330–338.
19. Lee, Y. C., Lin, H. H. and Tang, M. J. 1995. Glucocorticoid upregulated Na-K-ATPase alpha- and beta-mRNA via an indirect mechanism in proximal tubule cell primary cultures. *Am. J. Physiol.* 268: F862–F867.
20. Lindsay, R. S., Lindsay, R. M., Waddell, B. J. and Seckl, J. R. 1996. Prenatal glucocorticoid exposure leads to offspring hyperglycemia in the rat: studies with 11β-hydroxysteroid dehydrogenase inhibitor carbenoxolone. *Diabetologia* 39: 1299–1305.
21. Ning, Y-M. and Sanchez E. R. 1995. Evidence for a functional interaction between calmodulin and the glucocorticoid receptor. *Biochem. Biophysic. Res. Com.* 208: 48–54.
22. Noronha-Blob, L. and Sacktor, B. 1986. Inhibition by glucocorticoids of phosphate transport in primary cultured renal cells. *J. Biol. Chem.* 261: 2164–2169.
23. Qui, J., Lou, L-G., Huang X-Y., Lou S-H., Pei, G. and Chen, Y-Z. 1996. Nongenomic mechanisms of glucocorticoid inhibition of nicotine-induced calcium influx in PC12 cells: involvement of protein kinase C. *Endocrinology* 139: 5103–5108.
24. Rabito, C. A. 1983. Phosphate uptake by a kidney cell line (LLC-PK). *Am. J. Physiol.* 245: F22-F31.
25. Rindler, M. J., Taub, M. and Saier, M. H. Jr. 1979. Uptake of $^{22}Na^+$ by cultured Dog Kidney Cell (MDCK). *J. Biol. Chem.* 254: 11431–11439.
26. Rouse, D. and Suki, W. N. 1985. Modulation of phosphate absorption by calcium in the rabbit proximal convoluted tubule. *J. Clin. Invest.* 76: 630–636.
27. Sakharani, L. M., Badie-Dezfooly, B., Trizna, W., Mikhail, N., Loew, A. G., Taub, M. and Fine, L. G. 1984. Transport and metabolism of glucose by renal proximal tubular cells in primary culture. *Am. J. Physiol.* 246: F757–F764.
28. Takenawa, J., Kaneko, Y., Okumara, K., Yoshida, O., Nakayama, H. and Fujita, J. 1995. Inhibitory effect of dexamethasone and progesterone *in vitro* on proliferation of human renal cell carcinomas and effects on expression of interleukin-6 or interleukin-6 receptor. *J. Urol.* 153: 858–862.
29. Tate, S. S. and Meister, A. 1974. Interaction of γ-glutamyl transpeptidase with amino acids, dipeptides, and derivatives and analogs of glutathione. *J. Biol. Chem.* 249: 7593–7602.
30. Turner, S. T., Kiebzak, G. M. and Dousa, T. P. 1982. Mechanism of glucocorticoid effect on renal transprot of phosphate. *Am. J. Physiol.* 243: C227–C236.
31. Vrtovsnik, F., Jourdain, M., Cherqui, G., Lefebvre, J. and Friedlander, G. 1993. Glucocorticoid inhibition of Na-Pi cotransport in renal epithelial cells in mediated by protein kinase C. *J. Biol. Chem.* 269: 8872–8877.
32. Waqar, M. A., Seto, J., Chung, S. D., Hiller-Grohol, S. and Taub, M. 1985. Phosphate uptake by primary renal proximal tubule cell culture grown in hormonally defined medium. *J. Cell. Physiol.* 124: 411–423.
33. Yang, I. S., Goldinger, J. M., Hong, S. K. and Taub, M. 1988. Preparation of basolateral membranes that transprot p-aminohippurate from primary cultures of rabbit kidney proximal tubule cells. *J. Cell. Physiol.* 135: 481–487.
34. Zhang, M. I. N. and O'Neil, R. G. 1996. A regulated calcium channel in apical membranes of renal proximal tubule cells. *Am. J. Phsyiol.* 271: C1757–1764.