Glucocorticoids differentially regulate expression of duodenal and renal calbindin-D9k through glucocorticoid receptor-mediated pathway in mouse model

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Lee, Geun-Shik, Kyung-Chul Choi, and Eui-Bae Jeung. Glucocorticoids differentially regulate expression of duodenal and renal calbindin-D9k through glucocorticoid receptor-mediated pathway in mouse model. Am J Physiol Endocrinol Metab 290: E299–E307, 2006. First published October 11, 2005; doi:10.1152/ajpendo.00232.2005.—Dexamethasone (Dex) is a member of the glucocorticoids (GCs), and is broadly used as an anti-inflammatory medication. Continuous administration with GCs induces adverse effects and suffering in humans (i.e., osteoporosis) due to negative calcium balance derived from low re- and absorption in the duodenum and kidney. A cytosolic calcium-binding protein, calbindin-D9k (CaBP-9k), is dominantly expressed in the renal and intestinal tissues involved in calcium re- and absorption and plays an active role in calcium transport. In the present study, we employed adrenalectomized (ADX) and sham-treated (Sham) male mice to examine the effect of Dex on CaBP-9k gene expression in the duodenum and kidney. Dex significantly reduced the levels of duodenal CaBP-9k mRNA and protein, and it restored ADX-induced decrease in renal CaBP-9k protein compared with the level of Sham control. Dex treatment increased calcium and phosphate levels in the sera of both Sham and ADX mice. In a time course experiment, Dex significantly decreased duodenal CaBP-9k at the transcriptional and translational levels at 3 days, whereas it temporarily increased CaBP-9k mRNA and protein levels at 12 and 24 h. Altered CaBP-9k expression by Dex was completely reversed by mifepristone, an antagonist for the GC receptor (GR). In addition, duodenal CaBP-9k and GR were colocalized on the enterocyte (duodenocyte), supporting a role for GR in regulating CaBP-9k. In ovariectomized (OVX) and ADX female mice daily treated with Dex for 3 days, duodenal CaBP-9k was expressed at the same level as in male mice. Also, no cross-activity of progesterone and Dex on their receptors was observed. Taken together, these results indicate that mouse CaBP-9k gene may be regulated by Dex in a tissue-specific manner, and reduced duodenal CaBP-9k via the GR pathway may take part in negative calcium absorption of GC-induced osteoporosis, whereas renal CaBP-9k may not be involved in the regulation of calcium homeostasis.

duodenum; kidney; uterus; osteoporosis; dexamethasone

GLUCOCORTICOIDS (GCs) have been used to treat diseases that have an inflammatory component, such as asthma, rheumatoid arthritis, and atopic dermatitis. The effectiveness of GCs as an anti-inflammatory agent is offset by the occurrence of side effects, including osteoporosis, hypothyroid-pituitary-adrenal axis suppression, growth retardation, cataract formation, skin thinning, and bruising (37). Of these, potentially the most serious and debilitating disease is GC-induced osteoporosis (GIO) (40). Potential mechanisms of GCs to induce GIO have been suggested (46). GCs inhibit bone formation by suppressing the proliferation and activity of osteoblasts and osteocytes and reduce bone cells by increasing apoptosis (50). The decreased absorption of gastrointestinal calcium ions and increased excretion of urinary calcium ions are caused by GCs. In addition, a number of critical mediators for bone homeostasis, such as sex hormones, growth hormone, insulin-like growth factor 1, and transforming growth factor-β are also suppressed by GCs. Taken together, GIO is a complicated disease resulting from alterations in a number of molecular mechanisms.

Even though studies to investigate the effect of GCs on bone cells have been carried out in the past several decades, its effect on intestinal calcium transport and renal calcium elimination are not clear. In humans and rats, intestinal calcium absorption decreases in the presence of long-term and high doses of GCs (34). Decrease in active transcellular transport and in normal brush-border vesicle uptake of calcium, and reduced calcium-binding proteins in the intestine, are considered cellular mechanisms resulting in diminished intestinal calcium absorption (18, 26, 49). In parallel, it has been widely accepted that GCs increase renal calcium elimination, and it increased the epithelial calcium transporters and calbindin-D28k (39). Long-term treatment with GCs in patients resulted in marked hypercalcuria and fasting urine calcium excretion (11, 43). This imbalance of calcium induced by GCs promotes secondary hyperparathyroidism, and the increased parathyroid hormones cause bone reabsorption due to the increase in osteoclast activity (59), which eventually result in osteoporosis.

The calcium-binding protein calbindin-D9k (CaBP-9k) is a cytosolic protein with a high affinity for calcium ions (10, 27). A number of tissues, such as intestine, uterus, placenta, kidney, and bone tissues of mammals express the CaBP-9k gene (3, 15, 23, 24, 35, 36, 44, 47). Intestinal CaBP-9k is involved in intestinal calcium absorption and is regulated at the transcriptional and posttranscriptional levels by 1,25-dihydroxyvitamin D3, the hormonal form of vitamin D in rodents (13, 45, 56). In addition, it has been formerly demonstrated that the expression...
of duodenal CaBP-9k may be involved in 1,25-dihydroxycholecalciferol in humans (54). Furthermore, the vitamin D regulation of this gene may also be important in reproductive tissues (28, 52). Although the intestinal level of CaBP-9k is clearly vitamin D controlled, its placental and uterine levels do not respond to vitamin D depletion. Therefore, CaBP-9k does not seem to be under the control of vitamin D despite the presence of vitamin D receptors in the uterus. Rather, CaBP-9k appears to be controlled by sex hormones (12, 31). Renal CaBP-9k is expressed at the distal convoluted tubules and is suspected as facilitating the calcium reabsorption (41).

The regulation of CaBP-9k in gastrointestinal tissues is not completely understood. The vitamin D-responsive DNA element is found in the CaBP-9k gene, which has been shown to regulate the expression of CaBP-9k in the intestine (14). CaBP-9k is actively expressed in the enterocytes, which are dominant epithelial cells in the duodenal mucosa (54). Its expression level decreases downstream from the duodenum where the levels are barely detectable in the distal ileum and large intestine. CaBP-9k has been thought to be one of the important factors for calcium absorption and metabolism in the intestine. Thus, in the present study, the regulation of duodenal and renal CaBP-9k by GCs was explored in a mouse model. Duodenal and renal expressions of CaBP-9k mRNA and protein levels were analyzed by Northern blot and immunoblot analysis. Alternation of serum calcium and phosphate levels was monitored after dexamethasone (Dex) treatment, and mifepristone, a GC receptor (GR) antagonist, was employed to examine the role of GR. In addition, the spatial expression between CaBP-9k and GR in the duodenum and kidney was determined whether the Dex-induced effect is derived through a GR-mediated pathway. Finally, female mice were also treated with Dex or progesterone (P4) to determine whether the regulations of duodenal, renal, and uterine CaBP-9k were identical compared with male mice and to examine whether GCs and P4 would share GR.

MATERIALS AND METHODS

Experimental animals and treatments. In the present study, mature male and female mice (11- to 130 g old) were obtained from Dae Han Biolink (Eumsung, Chungbuk, Korea). All animals were housed in polycarbonate cages and used after acclimatization to an environmentally controlled room (temperature: 23 ± 2°C, relative humidity: 50 ± 10%, frequent ventilation and 12:12-h light-dark cycle). We performed bilateral adrenalectomy (ADX) in 15 male mice under ether anesthesia. Only skin incision (Sham, n = 10 mice) was performed as a control. After 1 wk, Sham or ADX male mice were euthanized to examine the effects of ADX on CaBP-9k expression. Two groups of Sham and ADX mice (n = 20 per group) were daily treated subcutaneously with saline as a vehicle or 10 mg/kg body wt Dex (Sigma-Aldrich, St. Louis, MO) for 7 days (6, 22, 42). The mice were euthanized at 24 h after final injection. In addition, serum samples were collected for measuring the blood calcium and phosphate levels. In a time-dependent experiment, four groups of male mice (n = 5 per group) were given subcutaneous injection with saline or 10 mg/kg body wt Dex and euthanized at 12 or 24 h after injection. In addition, two groups were daily given subcutaneous injection with saline or Dex for 3 days and euthanized at 24 h after final injection. For the GR antagonist treatment, 30 mice were divided into three groups (n = 10 per group) and treated one time or given 3-day treatment with saline, Dex, or Dex plus mifepristone (50 mg/kg body wt, Sigma-Aldrich) and euthanized at 24 h after final injection. ADX and ovariectomized (OVX) female mice were divided into three groups (total n = 15) and daily treated subcutaneously with saline, P4 (Sigma-Aldrich), or Dex (10 mg/kg body wt) for 3 days and euthanized 24 h after final injection. All experimental procedures and animal use were approved by the Ethics Committee of the Chungbuk National University.

RNA extraction and Northern blot analysis. Mice were euthanized, and the duodenum, kidney, and uterus were rapidly excised and washed in cold sterile saline (0.9% NaCl). Total RNA was prepared from the uterus with Trizol reagent (Invitrogen, Carlsbad, CA), and the concentration of RNA was determined by the absorbance at 260 nm. Total RNA was denatured by heating at 85°C for 10 min. Ten micrograms of total RNA were electrophoresed on 1% formaldehyde denaturing agarose gels for 1 h at 110 V. The 18S rRNA served as an indicator of the quantity of total RNA. The RNA was then transferred from the agarose gel to a nylon membrane with a vacuum blotter (Bio-Rad, Hercules, CA) according to the manufacturer’s suggested procedure. RNA was UV cross-linked to the membrane with a Gene Cross-Linker (Bio-Rad). The membranes were prehybridized in 50% formamide, 5× SSPE, 5× Denhardt’s, 0.1% SDS, and 0.1 mg/ml salmon sperm DNA for 2 h at 42°C. The radiolabeled CaBP-9k probe (1) was prepared from the Random Primer DNA Labeling Kit (Takara Bio, Otsu, Shiga, Japan) according to the manufacturer’s suggested procedure. The [32P]dCTP-labeled CaBP-9k probe was added to the hybridization solution and incubated overnight at 42°C. The membranes were washed three times at 42°C in 2× SSC, 0.1% SDS, at 54°C in 1× SSC, 0.1% SDS, and at 68°C in 0.1× SSC, 0.1% SDS. The membranes were then exposed to X-ray film (Eastman Kodak, Rochester, NY). The films were scanned and analyzed by Quantity One program (Gel Doc EQ, Bio-Rad).

Serum collection and biochemical analysis. Blood was collected from each mouse, transferred to serum separator tubes, and centrifuged at 2,000 rpm for 15 min and aliquoted. A colorimetric assay, using Biomek Robotics (Beckman-Coulter, Fullerton, CA) and reagents from Sigma-Aldrich were used to measure serum calcium and phosphate levels.

Western blot analysis. Protein was extracted with Proteprep (iNtRON Bio, Sungnam, Kyungki-Do, Korea) according to the supplier’s instructions. Thirty micrograms of cytosolic protein per lane were resolved by SDS-PAGE (12% acrylamide) and transferred to nitrocellulose membrane with a Trans-Blot Cell (Bio-Rad) according to the manufacturer’s protocol. The membranes were then blocked overnight with phosphate-buffered saline containing 0.05% Tween-20 (PBS-T) and 5% skim milk. The membranes were then incubated in primary antibodies diluted in 1% bovine serum albumin (BSA) for 1 h at room temperature. Primary antibodies to CaBP-9k (diluted 1:3,000; Swant, Bellinzona, Switzerland), and β-actin (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA) were employed. β-Actin was not altered by GC as an internal control (25). The horseradish peroxidase-conjugated anti-rabbit IgG (diluted 1:3,000 in 1% BSA, Santa Cruz Biotechnology) was used as the secondary antibody. Membranes were incubated with Western Lighting Chemiluminescence reagent (PerkinElmer Life Sciences, Boston, MA) according to the manufacturer’s protocol and exposed to X-ray film (Eastman Kodak). The films were scanned and analyzed by Quantity One program (Gel Doc EQ, Bio-Rad).

Immunohistochemical staining. The localization of CaBP-9k protein was examined by immunohistochemistry. Duodenum and kidney were embedded in paraffin. Sections (5-μm thick) were rehydrated in descending grades of ethanol. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in PBS-T for 20 min. Incubation of the sections in 10% normal goat serum (NGS) for 2 h at room temperature blocked nonspecific reaction. Sections were subsequently incubated with a polyclonal rabbit antibody specific to CaBP-9k (diluted 1:1,000, Swant) or GR (1:500, Santa Cruz Biotechnology) dissolved in 10% NGS at room temperature for 1 h. After a washing with PBS-T, the sections were incubated with the biotinylated secondary antibody (rabbit IgG; Vector Laboratories, Burlingame, CA) for 30 min at 37°C and further incubated with
ABC-Elite for 30 min at 37°C. Diaminobenzidine (Sigma-Aldrich) was used as a chromagen, and the sections were counterstained with hematoxylin followed by mounting with a coverslip.

Data analysis. Data were analyzed by nonparametric one-way analysis of variance using the Kruskal-Wallis test, followed by Dunnett’s test for multiple comparisons to vehicle. Values of data were converted to ranks for these tests. All statistical analyses were performed with SPSS for Windows Edition (SPSS, Chicago, IL). \( P < 0.05 \) was considered statistically significant.

RESULTS

Effects of Dex and endogenous adrenal hormones on duodenal and renal CaBP-9k expression in male mice. To understand the role of endogenous adrenal hormones in duodenal and renal CaBP-9k regulation, 11-wk-old male mice were operated on by ADX or Sham as a control. Expression levels of duodenal and renal CaBP-9k mRNA and protein were observed by Northern and Western blot assays, respectively. No significant change in the expression levels of duodenal CaBP-9k mRNA and protein was observed in ADX mice compared with those of Sham control (Fig. 1). Although renal CaBP-9k mRNA was not altered, its protein level significantly decreased in ADX mice (Fig. 1), implying that certain factors from the adrenal gland may take part in the translation or degradation of renal CaBP-9k.

In addition, the effect of Dex on duodenal and renal CaBP-9k expression was evaluated in ADX and Sham mice after daily treatment with Dex (10 mg/kg) for 7 days. Dex treatment resulted in a significant decrease in duodenal CaBP-9k mRNA and protein, and its altered expression level did not differ between ADX and Sham mice (Fig. 2A), suggesting that repressed duodenal CaBP-9k expression by Dex may have contributed to the decrease in intestinal calcium absorption. In the kidney, the level of CaBP-9k mRNA was not altered by Dex, whereas CaBP-9k protein level was significantly decreased in ADX mice compared with Sham control, as shown in Fig. 2B. In addition, ADX-induced decrease in renal CaBP-9k protein was restored to the level of Sham control by replacement with Dex (Fig. 2B), suggesting that endogenous GCs may be an important factor for supporting renal CaBP-9k homeostasis.

In addition, the blood from these mice was collected to analyze the serum levels of calcium and phosphate ions. Long-term treatment of mice with Dex significantly increased serum calcium (Fig. 3A) and phosphate (Fig. 3B) levels in both Sham and ADX mice, respectively, implying that Dex-treated mice appear to show calcium imbalance condition.

Time-dependent effects of Dex on duodenal and renal CaBP-9k expression in male mice. To determine the regulation of duodenal and renal CaBP-9k expression by Dex in a time-dependent manner, male mice were treated with a pharmacological dose of Dex (10 mg/kg) for one time or were daily injected for 3 days. As shown in Fig. 4, duodenal CaBP-9k mRNA (A) and CaBP-9k protein (B) levels were not altered by Dex at 12 h and 1 day after a single injection compared with controls. However, treatment of mice with Dex significantly decreased the duodenal expressions of CaBP-9k mRNA and protein for 3 days (Fig. 4, A and B). It is of interest that a single treatment with Dex significantly induced renal CaBP-9k mRNA expression at 12 and 24 h. However, this altered expression was not observed at 3 days (Fig. 4C). In parallel with its mRNA level, renal CaBP-9k protein was also increased 1 day after a single treatment with Dex and was returning to control level in the 3-day-treated group, as shown in Fig. 4D. These results indicate that Dex has a distinct mechanism in modulating renal CaBP-9k expression compared with its regulation by Dex in the duodenum.

Involvement of GR in regulation of duodenal and renal CaBP-9k. To clarify the mechanism underlying Dex-induced decrease in duodenal CaBP-9k at 3 days and increase in renal CaBP-9k at 24 h by a single treatment with Dex, mifepristone (RU-486), a GR antagonist, was employed in the present study. As shown in Fig. 5, duodenal CaBP-9k protein was suppressed by Dex, and pretreatment with RU-486 completely reversed the Dex-induced decrease in duodenal CaBP-9k protein. On the other hand, pretreatment with RU-486 prior to Dex reversed the Dex-induced increase in renal CaBP-9k protein, as seen in Fig. 5, implying that the effect of Dex on the expression of duodenal and renal CaBP-9k may be derived through a GR-dependent pathway.

\( \text{Fig. 1. Effect of adrenalec} \text{tomy (ADX) on duodenal and renal calbindin-D9k (CaBP-9k) expression. One week after sham operation (Sham) or ADX, expression levels of CaBP-9k mRNA or CaBP-9k protein in duodenum and kidney were analyzed by Northern and Western blot assays as described in MATERIALS AND METHODS. Renal expression of CaBP-9k protein was significantly decreased in ADX mice compared with Sham control (represented on right as bar graphs; means ± SE of duplicated values from all samples expressed as %CaBP-9k/3S rRNA or β-actin). All experiments were more than duplicated, and exemplary samples are shown.} \, ^*P < 0.05 \text{ vs. each vehicle.} \)
The duodenal and renal CaBP-9k proteins were immunohistochemically localized in the duodenum and kidney related to the expression of GR protein to elucidate their spatial regulation. Duodenal CaBP-9k protein was moderately stained on the cytoplasm of the enterocyte (duodenocyte; Fig. 6, A and B), and GR was also observed in the nuclei of these duodenocytes (Fig. 6C), supporting a role for GR in regulating CaBP-9k. In the kidney, intense staining for CaBP-9k protein was observed in the distal convoluted tubules (Fig. 6, D and E), whereas GR protein was localized in the glomerular cells (Fig. 6F).

Effects of P₄ and Dex on uterine, duodenal, and renal CaBP-9k expression in ADX and OVX female mice. Dex and P₄ have cross-binding capability to GR or progesterone receptor (PR), and female mice were exposed to P₄ in normal physiological conditions. In this study, to elucidate the cross-effect of PR and GR and the role of Dex on female mice, ADX and OVX were performed to avoid interferences by their endogenous hormones in mice. The levels of duodenal CaBP-9k mRNA and protein were significantly repressed when the mice were treated daily with 10 mg/kg Dex for 3 days (Fig. 7A). However, P₄ did not show any effect on duodenal CaBP-9k expression. In the kidney, Dex did not alter the expression levels of renal CaBP-9k mRNA and protein, nor did P₄ have any effect in this tissue (Fig. 7B). As expected, P₄ induced uterine CaBP-9k protein expression (Fig. 7C). Taken together, these results indicate that no cross-activity between P₄ and Dex on their receptors was observed, and the effect of Dex on the regulation of duodenal, renal, and uterine CaBP-9k was identical in these tissues in both male and female mice.

DISCUSSION

It has been widely accepted that GCs can reduce intestinal calcium absorption by decreasing active transport and normal brush-border vesicle uptake of calcium (26, 49). GCs have been demonstrated to reduce the synthesis of calcium-binding proteins in chicken (18). In mammalian duodenum and kidney, calcium ions actively enter through the epithelial calcium...
Fig. 4. Time-dependent effect of Dex on duodenal and renal CaBP-9k expression. Mature male mice (11 wk) were injected daily with Dex (10 mg/kg body wt) for 1–3 days and euthanized at 12 or 24 h after final injection as described in MATERIALS AND METHODS. Top: duodenal CaBP-9k mRNA (A) and CaBP-9k protein (B). Bottom: renal CaBP-9k mRNA (C) and CaBP-9k protein (D). Bar graphs summarize data from densitometric analysis (means ± SE of duplicated values from all samples expressed as %CaBP-9k/18S rRNA or %CaBP-9k/β-actin). ∗P < 0.05 vs. each vehicle.

Fig. 5. Effect of RU-486 (RU) on Dex-induced duodenal and renal CaBP-9k protein. Two groups of mature (11-wk) male mice were injected with RU-486, a glucocorticoid receptor (GR) antagonist, at 30 min before being treated with Dex. Bar graphs summarize data from densitometric analysis (means ± SE of duplicated values from all samples expressed as %CaBP-9k/β-actin). ∗P < 0.05 vs. vehicle; ∗∗P < 0.05 vs. Dex treated.
transporter-1 or -2 from the lumen and bind to the calcium-binding proteins CaBP-9k or CaBP-28k and then are discharged to the blood by the plasma membrane Ca\(^{2+}\)-ATPase 1 or Na/Ca exchanger (7). However, it is unknown which protein is affected by GCs to the malfunction of renal and duodenal calcium re- and absorption. In the present study, we primarily focused on the effects of GCs on the regulation of CaBP-9k, the most abundant protein, in duodenum and kidney to understand calcium imbalance during GIO status.

To determine whether endogenous factors from adrenal glands are involved in regulating CaBP-9k expression, the duodenal and renal expressions of CaBP-9k were analyzed in ADX mice by Northern blot analysis and immunoblot assays. In the duodenum, no difference in CaBP-9k mRNA and protein levels was observed in ADX mice compared with Sham controls, whereas the renal CaBP-9k protein level significantly decreased in ADX mice compared with Sham controls. These results imply that certain factors from the adrenal gland may take part in the regulation of renal CaBP-9k protein, and endogenous factors involved in modulating the renal CaBP-9k protein need to be investigated. Reduced renal CaBP-9k by the removal of adrenal glands (ADX) was partially explained by the fact that renal tubular cells, which have GR in the primary cell culture condition, may need an exogenous supplement with GCs to maintain their normal phenotype (17, 38).

Male mature mice were daily administered Dex, a synthetic GC, for 7 days to examine the role of Dex on the regulation of CaBP-9k, as previously described (42). Both duodenal CaBP-9k mRNA and CaBP-9k protein were progressively diminished after Dex treatment in both ADX and Sham mice. Reduced CaBP-9k expression was observed after 3-day Dex treatment in a time-dependent manner. Intestinal CaBP-9k has been reported to be regulated by 1,25-dihydroxyvitamin D\(_3\) at the transcriptional and posttranscriptional levels (13, 45, 56). Furthermore, recent studies have indicated that duodenal CaBP-9k expression involves 1,25-dihydroxycholecalciferol and sex steroid hormones (53, 54). Vitamin D- and sex steroid hormone-responsive DNA elements are located in the promoter region of CaBP-9k gene, and receptor binding to the promoter region that can regulate the transcription of this gene (14). However, GC-responsive DNA elements have not been demonstrated on the promoter region of the CaBP-9k gene. Suppressed gene expression by GCs may be controlled by the transcriptional factor AP1 (21). The serum level of
1,25-dihydroxyvitamin D₃ was lower in some series and normal in others, and serum calcitriol level was also variable across studies (9, 51, 58). In addition, Braun et al. (8) and Hahn et al. (20) found that prednisone had little effect on serum dihydroxylated vitamin D levels in patients, whereas intestinal calcium absorption fell, suggesting that the GC-related impairment in calcium absorption may be independent of vitamin D. Therefore, reduced duodenal CaBP-9k is not due to suppression of vitamin D by Dex but is derived from the activation of GR by Dex attested with GR antagonist.

A sustained GC injection resulted in marked hypercalciuria, mediated in the distal tubule, even in the presence of GC’s excess (19), implying that GCs regulate renal calcium reabsorption directly or indirectly. Although most calcium in the kidney is resorbed in the proximal tubule and cortical thick ascending limb by a paracellular pathway involving diffusion of calcium down its electrochemical gradient or via solvent drag phenomenon, calcium reabsorption in the distal convoluted tubule and connecting segment occurring via a transcellular route plays an important role in the fine tuning of whole body calcium homeostasis (41). Renal CaBP-9k is present in the distal convoluted tubule and connecting segment occurring via a transcellular route plays an important role in the fine tuning of whole body calcium homeostasis (41). Renal CaBP-9k is present in the distal convoluted tubule involving active transport (32). It is hypothesized that renal CaBP-9k is expressed at the site of calcium reabsorption. However, mice treated with a pharmacological dose of Dex did not display altered renal expression of CaBP-9k mRNA or protein. Interestingly, ADX-induced suppression of renal CaBP-9k protein was reversed by long-term supplementation with Dex, supporting the hypothesis that endogenous GCs from adrenal gland sustain renal CaBP-9k homeostasis. In a time-dependent experiment, Dex induced renal transcription of CaBP-9k gene at 12 and 24 h after a single Dex injection and retreated to the vehicle level at 3 days. In parallel with its mRNA level, its translation increased at 24 h and decreased to the control level at 3 days. This short-term induction of CaBP-9k suggested that exogenous GCs might affect renal CaBP-9k homeostasis, as renal CaBP-9k expression was reduced by ADX. By use of a GR antagonist, increased expression of renal CaBP-9k by Dex was completely reversed by RU-486, indicating that this renal expression of CaBP-9k may be dependent on GR mediation. To monitor the GIO status, Ferrari et al. (19) demonstrated that an increased calcium excretion resulted from inhibiting 11β-hydroxysteroid, which converted cortisol to receptor-inactive cortisone and was mediated by the mineralocorticoid receptor rather than by GR. In the 7-day Dex-treated mice, serum calcium and phosphate levels increased, indicating the secondary hyperparathyroidism due to a negative calcium balance by GCs (40). In addition, sustained GCs stimulated bone reabsorption resulting from the suppressed activity of osteoblast cells and enhanced capacity of osteoclasts (29, 40).

Uterine CaBP-9k mRNA and protein in mice are exclusively controlled by P₄ and expressed during the progestin-dominant cycle because of mice carrying a progesterone-responsive element on its promoter region (1, 2, 30). GCs and progestins bind to their receptors, which share many structural and functional similarities, including virtually identical DNA recognition specificity (55). In the present study, using ADX and OVX female mice, we demonstrated the cross-activity of GCs and progestins on duodenal, renal, and uterine CaBP-9k mRNA and protein expression and compared CaBP-9k expression pattern with a male GIO mouse model. P₄ had no effect on the duodenal and renal CaBP-9k expression, but P₄ is a major
factor in regulating uterine CaBP-9k. Although GCs and progestins share receptors, uterine CaBP-9k was regulated by only progestins, suggesting that CaBP-9k may have a distinct biological mechanism in an organ-specific manner. The same dose of Dex in the female mouse model diminished duodenal CaBP-9k and induced the renal CaBP-9k expression. However, increased expression of renal CaBP-9k was emphasized because renal CaBP-9k was suppressed by ADX and restored by the exogenous Dex supplement. Taken together, these results suggest that Dex has an identical effect in male and female mice.

CaBP-9k is actively expressed in enterocytes, the major epithelial cells of the duodenal mucosa (54). Its expression level decreases downstream from the duodenum, with levels barely detectable in the distal ileum and large intestine (54). CaBP-9k is thought to protect the cells against the potential cytotoxicity of free calcium ions and participates in transport-ations, and molecular biology.

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In this study, we demonstrated that the effect of GCs on the regulation of CaBP-9k in calcium re- or absorbent organs, i.e., duodenum and kidney, and elucidated the potential role of the CaBP-9k in these organs. Sustained Dex suppressed duodenal CaBP-9k expression, whereas its renal level was not altered. In addition, Dex has been shown to regulate mouse CaBP-9k expression in a tissue-specific manner. Dex inhibited CaBP-9k expression in the duodenum, which temporally induced its expression in the kidney or did not affect its levels in the uterus. Reduced duodenal CaBP-9k via the GR pathway may take part in negative calcium absorption of GIO, but the renal CaBP-9k may not be involved.

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