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Endogenous Regucalcin Suppresses the Enhancement of Protein Phosphatase Activity in the Cytosol and Nucleus of Kidney Cortex in Calcium-Administered Rats

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Abstract The suppressive role of endogenous regucalcin (RC), which is a regulatory protein of calcium signaling, in the enhancement of protein phosphatase activity (PPA) in the cytosol and nucleus of kidney cortex in calcium-administered rats was investigated. Calcium content in the kidney cortex was significantly increased at 0.5–5 h after a single intraperitoneal administration of calcium chloride solution (10 mg Ca/100 g body weight) to rats. The analysis with Western blotting of RC protein showed that RC levels in the cytosol and nucleus were significantly increased 0.5–5 h after the administration of calcium (10 mg/100 g). PPA toward phosphotyrosine, phosphoserine, and phosphothreonine was found in the cytosol and nucleus of kidney cortex. PPA toward three phosphoamino acids in the cytosol and nucleus was significantly increased by the administration of calcium (10 mg/100 g). The presence of anti-RC monoclonal antibody (25 ng/ml) in the enzyme reaction caused a significant increase in PPA toward phosphotyrosine, phosphoserine, and phosphothreonine in the cytosol and nucleus of kidney cortex in normal rats. The effect of anti-RC monoclonal antibody (25 ng/ml) in increasing PPA toward three phosphoamino acids in the cytosol and nucleus was significantly enhanced in calcium-administered rats. The effect of anti-RC monoclonal antibody (25 ng/ml) in increasing PPA in the cytosol and nucleus of normal rats and calcium-administered rats was completely abolished by the addition of RC (10^{-6} M) in the enzyme reaction mixture. The present study suggests that endogenous RC suppresses the enhancement of PPA in the cytosol and nucleus of kidney cortex in calcium-administered rats. J. Cell. Biochem. 85: 553–560, 2002.

Key words: regucalcin; calcium signaling; protein phosphatase; cytosol; nucleus; rat kidney

Calcium ion (Ca^{2+}) plays a pivotal role in the regulation of many cell functions. The Ca^{2+} effect in cells is mediated through Ca^{2+}-dependent protein kinase, which are related to a signal transduction due to hormonal stimulation [Cheung, 1984; Heizman and Hunziker, 1991; Kraus-Friedman and Feng, 1996]. Regucalcin (RC) was found as a kind of Ca^{2+}-binding protein in which the EF-hand motif of Ca^{2+}-binding site is not present in its molecule [Yamaguchi and Yamamoto, 1978; Shimokawa and Yamaguchi, 1993]. In recent years, it has been shown that RC plays the multifunctional role as a regulatory protein for Ca^{2+} signaling in cells [Yamaguchi, 2000a,b; Reviews].

RC is localized in liver and kidney cortex, but not in the medulla of rats [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993; Yamaguchi and Kurota, 1995]. The role of RC in liver cells is well known. However, the regulatory role of RC in kidney cells is not fully clarified. It has been shown recently that RC can regulate intracellular Ca^{2+} homeostasis [Kurota and Yamaguchi, 1997a,b; Xue et al., 2000] and that this protein has an inhibitory effect on Ca^{2+}-dependent protein kinases [Kurota and Yamaguchi, 1997c, 1998] and protein phosphatases [Morooka and Yamaguchi, 2001a,b] in rat renal cortex. RC may have a role in the regulation of cell function in kidney cortex.

Kidney RC mRNA expression has been shown to be stimulated by calcium administration to rats [Yamaguchi and Kurota, 1995]. The increase in RC protein in the kidney cortex of

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calcium-administered rats is unknown, however. The present study was undertaken to determine whether RC level is increased in the kidney cortex of calcium-administered rats and the endogenous RC can regulate protein phosphatase activity (PPA) in the kidney cortex. Dephosphorylation of many phosphorylated proteins is regulated by protein phosphatase in many cells [Hunter, 1995], and the enzyme may play an important role in the regulation of kidney function. We found that the calcium administration-induced increase in endogenous RC in the kidney cortex suppresses the enhancement of PPA in the renal cytosol and nucleus of calcium-administered rats.

MATERIALS AND METHODS

Chemicals

o-Phospho-L-tyrosine, *o*-phospho-L-serine, and *o*-phospho-L-threonine were obtained from Sigma Chemical Co. (St Louis, MO). Streptavidin-peroxidase conjugate was obtained from Tago, Inc. (Burlingame, CA). NHS-LC-biotin was obtained from Pierce (Rockford, IL). Calcium chloride and other chemicals were purchased from Sigma Chemical Co. and Wako Pure Chemical Company (Osaka, Japan). The reagents were dissolved in water. Some reagents were passed through ion-exchange resin to remove metal ions.

Animals

Male Wistar rats (80–100 g, Japan SLC, Hamamatsu, Japan) were fed with commercial laboratory chow (solid, Oriental Yeast Co. Ltd., Tokyo) containing 57.5% carbohydrate, 1.1% calcium, and 1.1% phosphorus at room temperature of 25°C, and distilled water, ad libitum.

Isolation of RC

RC is markedly expressed in rat liver cytosol [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993] from which it was isolated. RC in the cytosol fraction (the supernatant of 105,000*g*) of rat liver homogenate was purified to electrophoretic homogeneity by gel filtration on Sephadex G-75 and G-50 followed by ion-exchange chromatography on diethylaminoethyl (DEAE)-cellulose, as reported previously [Yamaguchi and Yamamoto, 1978].

Anti-RC Antibody

Anti-RC antibody was a monoclonal antibody raised against RC prepared by using standard methods [Omura and Yamaguchi, 1999]. Mouse (BALB/C, Japan SLC) were subcutaneously injected with 0.1 mg/animal of antigen (rat liver RC) emulsified with Freund's complete adjuvant, and 19 days later antigen (0.25 mg/animal) was intraperitoneally injected with Freund's incomplete adjuvant. Animals were sacrificed by bleeding 3 days after the last injection. Spleen cells were prepared from immuned mouse, and fused into myeloma cells. Anti-RC monoclonal antibody-producing cells (hybridoma cells) were obtained by screening. The IgG from hybridoma cells was isolated through protein A-agarose column (Sigma).

Calcium Administration

Calcium chloride was dissolved in sterile distilled water as the concentrations of 5, 10, and 20 mg Ca/ml. The solution (0.5 ml/100 g body weight of rats) was intraperitoneally administered to rats, and the animals were killed by bleeding at 0.5, 1, 2, and 5 h after calcium administration.

Preparation of Kidney Cortex Cytosol

Kidney cortex was removed, frozen immediately, cut into small pieces, suspended (1:4) in 0.25 M sucrose solution and homogenized in Potter–Elvehjem homogenizer with a Teflon pestle. The homogenates were spun at 5,500*g* in a refrigerated centrifuge for 10 min to remove mitochondria. The 5,500*g* supernatant was spun at 105,000*g* for 60 min, and the supernatant fraction (cytosol) was pooled to assay PPA and to analysis PPA and to analysis Western blotting for RC. Protein concentration was determined by the method of Lowry et al. [1951] using bovine serum albumin as the standard.

Isolation of Nuclei

The nuclei from kidney cortex were isolated by the procedure of Jones et al. [1989] with a minor modification. Kidney cortex was homogenized in a Potter–Elvehjem homogenizer with a Teflon pestle in 40 ml of TKM solution (50mM Tris-HCl, pH 4.5, 25 mM KCl, 5 mM $MgCl_2$) containing 0.25 M sucrose and 1.0 mM EGTA. The homogenate was filtered through three layers of cheesecloth. The homogenate was centrifugated at 700*g* for 10 min. The pellets were homogenized (five strokes) in 40 ml of the same solution and centrifuged again at 700*g* for 10 min. The pellet was resuspended in 24 ml of

the same solution by homogenization (five strokes), and 6 ml was added to each of four tubes containing 12 ml of TKM including 2.3 M sucrose. The upper layer and the sucrose cushion were removed with an aspirator. Then it was resuspended in ice-cold 0.25 M sucrose solution by hand homogenization to assay PPA and to analysis Western blotting for RC. Assay of marker enzymes showed that there was less than 5% contamination by microsomes, plasma membranes, or mitochondria. DNA content in the nuclei was determined using the diphenylamine reaction [Burton, 1956]. Protein concentration was determined by the method of Lowry et al. [1951].

Determination of Calcium in Kidney Cortex

Kidney cortex tissues were rinsed with a cold 0.25-M sucrose solution. The amount of calcium in the kidney cortex was determined by atomic absorption spectrophotometry after digestion with nitric acid [Yamaguchi and Kurota, 1995]. Calcium content was expressed as microgram (μg) per gram (g) wet tissue.

Western Blot Analysis

The cytosol and nuclei from renal cortex homogenate were used for Western blot analysis [Wessendorf et al., 1993; Tsurusaki et al., 2000]. Aliquots of cytosol or nucleus containing 25 or 100 μg of protein, respectively, were mixed with 5 × sample buffer, boiled for 5 min, and SDS–PAGE was performed by the method of Laemmli [1970] using 12% polyacrylamide resolving gel. After SDS–PAGE, the proteins were then transferred onto a polyvinylidene difluoride membrane at 100 mA for 4 h. The membranes were incubated with a polyclonal rabbit anti-RC antibody [Yamaguchi and Isogai, 1993], which was diluted 1:2,000 in 10 mM Tris-HCl, pH 8, containing 150 mM NaCl, 0.1% (w/v) skim milk for 1 h. The membranes incubated with antibody were washed four times with washing buffer. Then the membranes were incubated for 1 h with horseradish peroxidase linked anti-rabbit IgG, which was diluted 1:5,000 with washing buffer containing 5% (w/v) skim milk, and washed again. Detection of the protein bands was performed using an enhanced chemiluminescent kit following the manufacturer's instructions. The molecular size of the detecting protein was determined by running the standard protein molecules of known sizes in parallel. The density of protein bands was quantified by densitometer scanning (Dual wavelength Flying-spot Scanner. CS-9000, Shimadzu Company, Japan).

Assay of PPA

Phosphatase activity towards phosphotyrosine, phosphoserine, and phosphothreonine was assayed at 30°C in 20 mM Tris-HCl, pH 8.0, 1.0 mM NaCl, 6 mM $MgCl_2$, 0.5 mM dithiothreitol, 9 mM phosphoaminoacids, and cytosol (0.6–0.9 mg protein/ml) or nuclei (0.9–1.4 mg protein/ml; 0.7–0.9 mg DNA/ml) as reported elsewhere [Pallen and Wang, 1983; Fruman et al., 1992]. In the separate experiments, the above reaction mixture contained either vehicle or anti-RC antibody (25 ng/ml) with an effective concentration. The enzyme reaction was terminated after 15 min by the addition of 1.0 ml of ice-cold 10% trichloroacetic acid and centrifuged to precipitate protein. Inorganic phosphate released in the supernatant was quantified by the method of Nakamura and Mori [1958]. Results were expressed as nanomoles of inorganic phosphate liberated per minute per milligram of cytosol or nuclear protein.

Statistical Analysis

Data were expressed as the mean ± SEM. The significance of the difference between the values was estimated by Student's *t*-test. We also used a multiway ANOVA comparison test to compare the treatment groups. A P value of < 0.05 was considered to indicate a statistically significant difference.

RESULTS

Increase in Calcium Content in the Kidney Cortex of Calcium-Administered Rats

The change in calcium content in the kidney cortex of calcium-administered rats is shown in Figure 1. Rats received a single intraperitoneal (i.p.) administration of calcium chloride solution (10 mg Ca/100 g body weight), and they were killed 0.5, 1, 2, and 5 h after the administration (Fig. 1A). Calcium content in the kidney cortex was significantly increased by the administration of calcium to rats (Fig. 1A). The increase was remarkable at 5 h after calcium administration. The effect of increasing doses of calcium (2.5, 5, and 10 mg/100 g body weight) on calcium content in the kidney cortex of rats is shown in Figure 1B. Rats were killed 1 h after a single i.p. administration of calcium (2.5, 5.0,

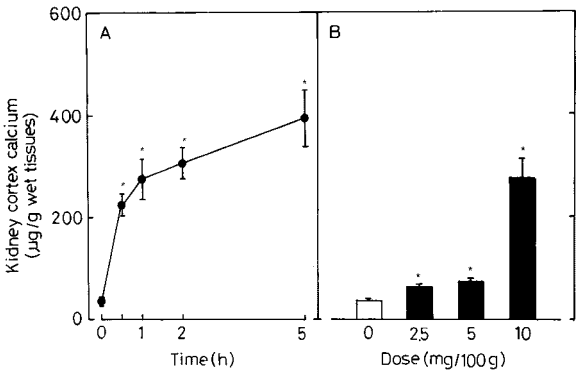

Fig. 1. Increase in calcium content in the kidney cortex of calcium-administered rats. **A**: Rats received a single i.p. administration of calcium chloride (10 mg Ca/100 g body weight) and they were killed 0.5, 1, 2, and 5 h after the administration. **B**: Rats received a single i.p. administration with increasing doses (2.5, 5, and 10 mg Ca/100 g) of calcium chloride, and 1 h later they were killed. Each value is the mean ± SEM of five rats. $*P < 0.01$, compared with the control value.

and 10 mg/100 g). Calcium content in the kidney cortex was significantly increased by the dose of calcium (2.5 and 5 mg/100 g) (Fig. 1B). The increase was remarkable with the dose of 10 mg Ca/100 g.

Change in RC in the Kidney Cortex of Calcium-Administered Rats

RC mRNA expression in the kidney cortex of rats is significantly increased by the administration of calcium [Yamaguchi and Kurota, 1995], suggesting that endogenous RC is increased in the kidney cortex of calcium-administered rats. The analysis with Western blotting of RC protein in the cytosol and nucleus of renal cortex from normal rats and calcium-administered rats is shown in Figures 2 and 3. RC levels were significantly ($P < 0.01$) increased in the cytosol and nucleus of kidney cortex obtained at 0.5, 1, 2, and 5 h after the administration of calcium (10 mg/100 g body weight) (Figs. 2A and 3A). When rats were killed 1 h after the administration of calcium (2.5, 5, and 10 mg/100 g), RC levels in the cytosol and nucleus were significantly ($P < 0.01$) increased by the lowest dose (2.5 mg Ca/100 g) of calcium (Figs. 2B and 3B).

Change in PPA in the Kidney Cortex of Calcium-Administered Rats

The change in PPA toward phosphotyrosine in the cytosol and nucleus of kidney cortex of calcium-administered rats is shown in Figure 4.

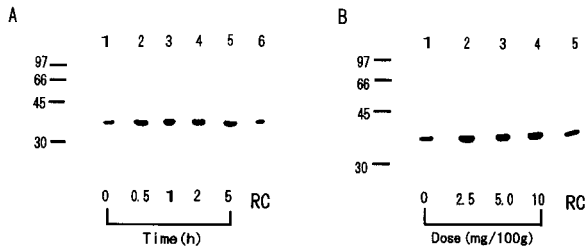

Fig. 2. Analysis of RC protein in the cytosol of kidney cortex in calcium-administered rats. **A**: Rats received a single i.p. administration of calcium (10 mg/100 g body weight), and they were killed 0.5, 1, 2, and 5 h after the administration. **B**: Rats received a single i.p. administration of calcium (2.5, 5, and 10 mg/100 g), and 1 h later they were killed. Western blot analysis was carried out on the extracts (25 μg of cytosolic proteins) obtained from the kidney cortex of normal or calcium-administered rats. RC (0.1 μg) as the marker was used in **lane 6** (A) and **lane 5** (B). The figure shows one of four experiments with separate rats. The densitometric data showed: (A), 205 ± 15.6, 200 ± 18.9, 197 ± 19.1, and 191 ± 20.2 (percent of control; mean ± SEM of four rats) at 0.5, 1, 2, and 5 h after calcium administration, respectively; (B), 177 ± 10.7, 153 ± 9.6, and 163 ± 11.3 with the dose of 2.5, 5, and 10 mg Ca/ml, respectively.

Rats were killed 0.5, 1, 2, and 5 h after a single i.p. administration of calcium (10 mg/100 g body weight). The enzyme activity was measured in a medium containing either vehicle or anti-RC monoclonal antibody (25 ng/ml). PPA in the cytosol was significantly increased 0.5–5 h after calcium administration. The presence of anti-RC monoclonal antibody (25 ng/ml) caused a

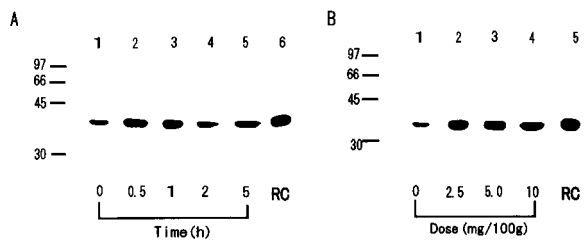

Fig. 3. Analysis of RC protein in the nucleus of kidney cortex in calcium-administered rats. **A**: Rats received a single i.p. administration of calcium (10 mg/100 g body weight), and they were killed 0.5, 1, 2, and 5 h after the administration. **B**: Rats received a single i.p. administration of calcium (2.5, 5, and 10 mg/100 g), and 1 h later they were killed. Western blot analysis was carried out on the extracts (100 μg of nuclear proteins) obtained from the kidney cortex of normal or calcium-administered rats. RC (0.1 μg) as the marker was used in **lane 6** (A) and **lane 5** (B). The figure shows one of four experiments with separate rats. The densitometric data showed: (A), 211 ± 15.3, 201 ± 16.9, 158 ± 9.8, and 179 ± 12.3 (percent of control; mean ± SEM of four rats) at 0.5, 1, 2, and 5 h after calcium administration, respectively; (B), 262 ± 20.9, 278 ± 25.1, and 225 ± 32.0 with the dose of 2.5, 5, and 10 mg Ca/100 g, respectively.

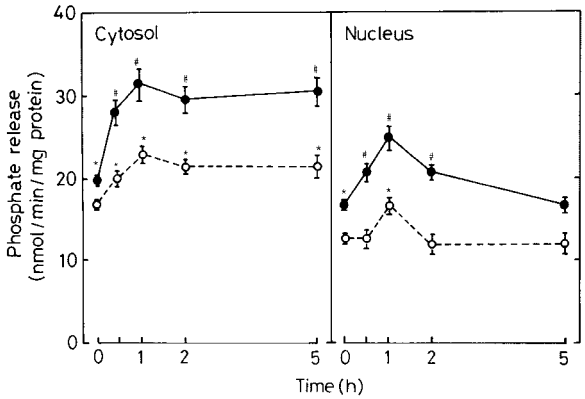

Fig. 4. Change in PPA toward phosphotyrosine in the cytosol and nucleus of kidney cortex in calcium-administered rats. Rats received a single i.p. administration of calcium (10 mg/100 g body weight), and they were killed 0.5, 1, 2, and 5 h after the administration. The enzyme activity was measured in a reaction mixture containing the cytosol or nucleus in the absence or presence of anti-RC monoclonal antibody (25 ng/ml). Each value is the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value obtained at zero time without antibody addition. $\#P < 0.01$, compared with the value obtained by antibody addition at zero time. ○, without antibody; ●, with antibody.

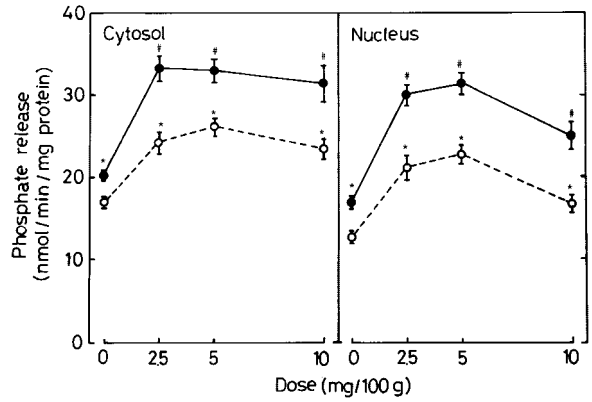

Fig. 5. Effect of increasing doses of calcium chloride on PPA toward phosphotyrosine in the cytosol and nucleus of kidney cortex in rats. Rats received a single i.p. administration of calcium (2.5, 5, and 10 mg/100 g body weight), and 1 h later they were killed. The enzyme activity was measured in a reaction mixture containing the cytosol or nucleus in the absence or presence of anti-RC monoclonal antibody (25 ng/ml). Each value is the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value obtained at zero time without antibody addition. $\#P < 0.01$, compared with the value obtained by antibody addition at zero time. ○, without antibody; ●, with antibody.

significant increase in PPA in the kidney cortex cytosol obtained at zero time (control). The increase in PPA of the kidney cortex cytosol obtained at 0.5–5 h after calcium administration was significantly enhanced in the presence of anti-RC monoclonal antibody (25 ng/ml) in the enzyme reaction mixture. Meanwhile, the nuclear PPA was significantly increased 1 h after calcium administration. The presence of anti-RC monoclonal antibody (25 ng/ml) caused a significant increase in the enzyme activity of normal rat kidney nucleus. The increase in PPA in the nucleus obtained at 0.5–2 h after calcium administration was significantly enhanced in the presence of anti-RC monoclonal antibody (25 ng/ml) in the enzyme reaction mixture.

The effect of increasing doses of calcium (2.5, 5, and 10 mg/100 g body weight) on PPA toward phosphotyrosine in the kidney cortex of rats is shown in Figure 5. A significant increase in PPA was seen with the dose of 2.5 mg Ca/100 g. The effect of anti-RC monoclonal antibody (25 ng/ml) in increasing PPA was significantly enhanced in the cytosol and nucleus of kidney cortex in calcium (2.5–10 mg/100 g)-administered rats as compared with that of normal rats.

The effect of RC addition on the anti-RC monoclonal antibody-increased PPA toward phosphotyrosine in the cytosol and nucleus of rat kidney cortex is shown in Figure 6. Kidney cortex was obtained 1 h after the administration of calcium (10 mg/100 g). The effect of anti-RC monoclonal antibody in increasing PPA in the cytosol and nucleus of kidney cortex obtained

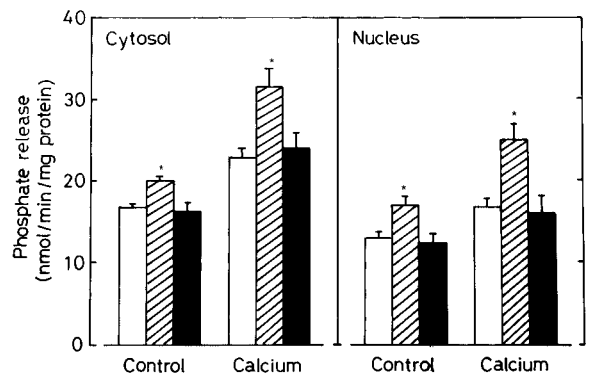

Fig. 6. Effect of RC addition on anti-RC monoclonal antibody-increased PPA toward phosphotyrosine in the cytosol and nucleus of normal and calcium-administered rats. Rats received a single i.p. administration of calcium (10 mg/100 g body weight), and 1 h later they were killed. The enzyme activity was measured in a reaction mixture containing either vehicle, anti-RC monoclonal antibody (25 ng/ml) or RC (10^{-6} M) plus anti-RC monoclonal antibody (25 ng/ml) in the presence of the cytosol or nucleus. Each value is the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value. White bars, control (none); hatched bars, antibody; black bars, antibody plus RC.

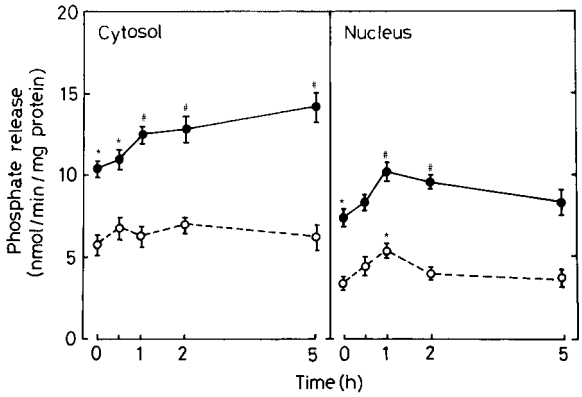

Fig. 7. Change in PPA toward phosphoserine in the cytosol and nucleus of kidney cortex in calcium-administered rats. Rats received a single i.p. administration of calcium (10 mg/100 g body weight), and they were killed 0.5, 1, 2, and 5 h after the administration. The enzyme activity was measured in a reaction mixture containing the cytosol or nucleus in the absence or presence of anti-RC monoclonal antibody (25 ng/ml). Each value is the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value obtained at zero time without antibody addition. $\#P < 0.05$, compared with the value obtained by antibody addition at zero time. ○, without antibody; ●, with antibody.

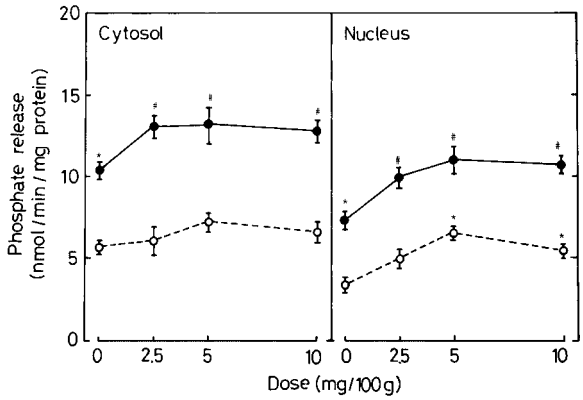

Fig. 8. Effect of increasing doses of calcium chloride on PPA toward phosphoserine in the cytosol and nucleus of kidney cortex in rats. Rats received a single i.p. administration of calcium (2.5, 5, and 10 mg/100 g body weight), and 1 h later they were killed. The enzyme activity was measured in a reaction mixture containing the cytosol or nucleus in the absence or presence of anti-RC monoclonal antibody (25 ng/ml). Each value is the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value obtained at zero time without antibody addition. $\#P < 0.01$, compared with the value obtained by antibody addition at zero time. ○, without antibody; ●, with antibody.

from control or calcium-administered rats was completely abolished by the addition of RC (10^{-6} M).

The change in PPA toward phosphoserine in the cytosol and nucleus of kidney cortex in calcium-administered rats is shown in Figure 7. The enzyme activity in the cytosol and nucleus of kidney cortex in control (normal) rats was significantly increased in the presence of anti-RC monoclonal antibody (25 ng/ml) in the reaction mixture. The effect of anti-RC monoclonal antibody (25 ng/ml) in increasing PPA was significantly enhanced in the renal cortex cytosol obtained at 2–5 h after calcium administration. Such an enhancement was also seen in the nucleus of kidney cortex obtained at 1 and 2 h after calcium administration. Moreover, the enhancing effect of anti-RC monoclonal antibody (25 ng/ml) on PPA in the cytosol and nucleus of kidney cortex obtained at 1 h with the lowest dose (2.5 mg/100 g) of calcium (Fig. 8).

The change in PPA toward phosphothreonine in the cytosol and nucleus of kidney cortex in calcium-administered rats is shown in Figure 9. The administration of calcium (10 mg/100 g body weight) caused a significant increase in PPA in the cytosol and nucleus of rat kidney cortex. The effect of anti-RC monoclonal antibody (25 ng/ml) in increasing the enzyme activity was significantly enhanced in the cytosol and nucleus of kidney cortex obtained from calcium-administered rats. The effect of increasing doses of calcium (2.5, 5, and 10 mg/100 g) on PPA toward phosphothreonine in the

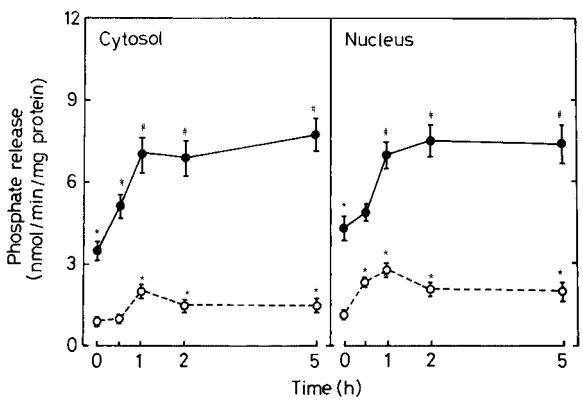

Fig. 9. Change in PPA toward phosphothreonine in the cytosol and nucleus of kidney cortex in calcium-administered rats. Rats received a single i.p. administration of calcium (10 mg/100 g body weight), and they were killed 0.5, 1, 2, and 5 h after the administration. The enzyme activity was measured in a reaction mixture containing the cytosol or nucleus in the absence or presence of anti-RC monoclonal antibody (25 ng/ml). Each value is the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value obtained at zero time without antibody addition. $\#P < 0.01$, compared with the value obtained by antibody addition at zero time. ○, without antibody; ●, with antibody.

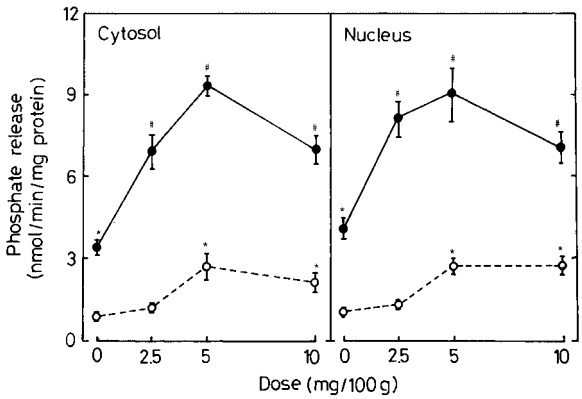

Fig. 10. Effect of increasing doses of calcium chloride on PPA toward phosphothreonine in the cytosol and nucleus of kidney cortex in rats. Rats received a single i.p. administration of calcium (2.5, 5, and 10 mg/100 g body weight), and 1 h later they were killed. The enzyme activity was measured in a reaction mixture containing the cytosol or nucleus in the absence or presence of anti-RC monoclonal antibody (25 ng/ml). Each value is the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value obtained at zero time without antibody addition. $\#P < 0.01$, compared with the value obtained by antibody addition at zero time. ○, without antibody; ●, with antibody.

cytosol and nucleus of kidney cortex of rats is shown in Figure 10. Kidney cortex was obtained 1 h after calcium administration. A significant increase in the enzyme activity in the cytosol and nucleus of normal rat kidney cortex was induced by the administration of calcium (5 and 10 mg/100 g). The enhancing effect of anti-RC monoclonal antibody (25 ng/ml) was seen in the dose of 2.5–10 mg Ca/100 g.

DISCUSSION

The present study demonstrates that calcium administration caused a significant increase in calcium content of rat kidney cortex and a corresponding elevation of RC levels in the cytosol and nucleus. Kidney RC mRNA expression has been shown to be stimulated by calcium administration to rats [Yamaguchi and Kurota, 1995], and the expression may be partly mediated through the Ca^{2+}/calmodulin-dependent signaling [Murata and Yamaguchi, 1999]. Calcium administration may increase RC protein by stimulating RC mRNA expression in the kidney cortex of rats. RC has been shown to translocate from the cytoplasm to the nucleus of rat liver [Tsurusaki et al., 2000]. Presumably, calcium administration increases RC protein in the cytoplasm of rat kidney cortex, and the protein is translocated to the nucleus.

Calcium administration caused a significant increase in PPA toward phosphotyrosine and phosphothreonine in the cytosol and nucleus of rat kidney cortex, suggesting that the increase in enzyme activity is partly mediated through Ca^{2+} signaling, which result from the augmentation of renal calcium content. It has been shown that the addition of calcium in the enzyme reaction mixture increases protein phosphatase activity toward phosphotyrosine and phosphothreonine in the cytosol and nucleus of rat kidney cortex in vitro [Morooka and Yamaguchi, 2001a,b].

The presence of anti-RC monoclonal antibody in the enzyme reaction mixture caused a significant increase in PPA toward three phosphoamino acids in the cytosol and nucleus of normal rat kidney cortex, indicating that the enzyme activity is regulated by endogenous RC. The effect of anti-RC monoclonal antibody in increasing PPA toward three phosphoamino acids was significantly enhanced in the cytosol and nucleus of kidney cortex in calcium-administered rats. This finding demonstrates that the calcium administration-induced increase in endogenous RC suppresses the enhancement of PPA toward phosphotyrosine, phosphoserine, and phosphothreonine in the cytosol and nucleus of kidney cortex in calcium-administered rats.

PPA toward phosphoserine in the cytosol and nucleus of kidney cortex of rats was not appreciably increased by calcium administration. However, the effect of anti-RC monoclonal antibody in increasing PPA toward phosphoserine was significantly enhanced in the cytosol and nucleus of calcium-administered rats. This result suggests that the calcium administration-increased RC has a potential effect in the suppression for protein serine phosphatase activity in the cytosol and nucleus of kidney cortex. Protein serine phosphatase activity has been shown to be increased by calcium addition into the enzyme reaction mixture containing the cytosol or nucleus of normal rat kidney cortex in vitro [Morooka and Yamaguchi, 2001a,b]. This increase is completely inhibited by RC addition.

Kidney cortex possesses nephrons including glomeruli and tubules. The re-absorption of urinary calcium in kidney is promoted by transcellular Ca^{2+} transport in the epithelial cells of renal tubules [Ng et al., 1982; Agus et al., 1997]. The regulation of intracellular Ca^{2+} homeostasis is important in the promotion of

transcellular Ca^{2+} transport. The augmentation of Ca^{2+} in the kidney cortex cells may affect the cellular functions. RC, which is a regulatory protein for Ca^{2+} signaling, may play a physiologic role in the regulation of Ca^{2+}-dependent cellular functions for the increase of intracellular Ca^{2+} in renal cortex cells. The present findings that endogenous RC can suppress the enhancement of PPA in the cytosol and nucleus of kidney cortex in calcium-administered rats in vitro, may support the view that RC can regulate cell function related to Ca^{2+}.

In conclusion, it has been demonstrated that calcium administration causes an increase in regucalcin protein in the cytosol and nucleus of kidney cortex in rats, and that the endogenous regucalcin suppresses the enhancement of PPA in the renal cortex and nucleus of calcium-administered rats.

REFERENCES

Agus ZS, Chiu PJS, Goldberg M. 1997. Regulation of urinary calcium excretion in the rats. Am J Physiol 232:F545–F549.

Burton A. 1956. Study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J 62:315–323.

Cheung WY. 1984. Calmodulin plays a pivotal role in cellular regulation. Science 202:19–27.

Fruman DA, Klee CB, Bierer BE, Burakoff SJ. 1992. Calcineurin phosphatase activity in T lymphocytes is inhibited by FK 506 and cyclosporin A. Proc Natl Acad Sci USA 89:3686–3690.

Heizman CW, Hunziker W. 1991. Intracellular calcium-binding proteins: more sites than insights. Trends Biochem Sci 16:98–103.

Hunter T. 1995. Protein kinases and phosphatase: the Yin and Yang of protein phosphorylation and signaling. Cell 80:225–236.

Jones DP, McConkey DJ, Nicotera P, Orrenius S. 1989. Calcium activated DNA fragmentation in rat liver nuclei. J Biol Chem 264:6398–6403.

Kraus-Friedman N, Feng L. 1996. The role of intracellular Ca^{2+} in the regulation of gluconeogenesis. Metabolism 42:389–403.

Kurota H, Yamaguchi M. 1997a. Activatory effect of calcium-binding protein regucalcin on ATP-dependent calcium transport in the basolateral membranes of rat kidney cortex. Mol Cell Biochem 169:149–156.

Kurota H, Yamaguchi M. 1997b. Regucalcin increases Ca^{2+}-ATPase activity and ATP-dependent calcium uptake in the microsomes of rat kidney cortex. Mol Cell Biochem 177:201–207.

Kurota H, Yamaguchi M. 1997c. Inhibitory effect of regucalcin on Ca^{2+}/calmodulin-dependent protein kinase activity in rat renal cortex cytosol. Mol Cell Biochem 177:239–243.

Kurota H, Yamaguchi M. 1998. Inhibitory effect of calcium-binding protein regucalcin on protein kinase C activity in rat renal cortex cytosol. Biol Pharm Bull 21:315–318.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 224:680–685.

Lowry OH, Rosebrogh NH, Farr AL, Randdal RJ. 1951. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–273.

Morooka Y, Yamaguchi M. 2001a. Suppressive role of endogenous regucalcin in the regulation of protein phosphatase activity in rat renal cortex cytosol. J Cell Biochem 81:639–646.

Morooka Y, Yamaguchi M. 2001b. Inhibitory effect of regucalcin on protein phosphatase activity in the nuclei of rat kidney cortex. J Cell Biochem 83:111–120.

Murata T, Yamaguchi M. 1999. Binding of kidney nuclear proteins to the 5′-flanking region of the rat gene for Ca^{2+}-binding protein regucalcin: involvement of Ca^{2+}/calmodulin signaling. Mol Cell Biochem 199:35–40.

Nakamura M, Mori K. 1958. Colorimetric determination of inorganic phosphorus in the presence of glucose-1-phosphate and adenosine triphosphate. Nature 18:1441–1442.

Ng RCK, Peraino RA, Suki WN. 1982. Divalent cation transport in isolated tubules. Kidney Int 22:492–497.

Omura M, Yamaguchi M. 1999. Regucalcin of protein phosphatase activity by regucalcin localization in rat liver nuclei. J Cell Biochem 75:437–445.

Pallen CJ, Wang JH. 1983. Calmodulin-stimulated dephosphorylation of *p*-nitrophenylphosphate and free phosphotyrosine by calcineurin. J Biol Chem 258:850–855.

Shimokawa N, Yamaguchi M. 1992. Calcium administration stimulates the expression of calcium-binding protein regucalcin mRNA in rat liver. FEBS Lett 305:151–154.

Shimokawa N, Yamaguchi M. 1993. Molecular cloning and sequencing of the cDNA coding for a calcium-binding protein regucalcin from rat liver. FEBS Lett 327:251–255.

Tsurusaki Y, Misawa H, Yamaguchi M. 2000. Translocation of regucalcin to rat liver nucleus: involvement of nuclear protein kinase and protein phosphatase regulation. Int J Mol Med 6:655–660.

Wessendorf JHM, Garfinkel S, Zhan X, Brown S, Maciag T. 1993. Identification of a nuclear localization sequence within the structure of the human interleukin-1α precursor. J Biol Chem 268:22100–22104.

Xue JH, Takahashi H, Yamaguchi M. 2000. Stimulatory effect of regucalcin on mitochondrial ATP-dependent calcium uptake activity in rat kidney cortex. J Cell Biochem 80:285–292.

Yamaguchi M. 2000a. Role of regucalcin in calcium signaling. Life Sci 66:1769–1780.

Yamaguchi M. 2000b. The role of regucalcin in nuclear regulation of regenerating liver. Biochem Biophys Res Commun 276:1–6.

Yamaguchi M, Isogai M. 1993. Tissue concentration of calcium-binding protein regucalcin in rats by enzyme-linked immunoabsorbent assay. Mol Cell Biochem 122:66–68.

Yamaguchi M, Kurota H. 1995. Expression of calcium-binding protein regucalcin mRNA in the kidney cortex of rats: the stimulation by calcium administration. Mol Cell Biochem 146:71–77.

Yamaguchi M, Yamamoto T. 1978. Purification of calcium binding substance from soluble fraction of normal rat liver. Chem Pharm Bull 26:1915–1918.