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Suppressive Role of Endogenous Regucalcin in the Regulation of Protein Phosphatase Activity in Rat Renal Cortex Cytosol

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Abstract The role of endogenous regucalcin, which is a regulatory protein of calcium signaling, in the regulation of protein phosphatase activity in the cytosol of rat renal cortex was investigated. Protein phosphatase activity toward phosphotyrosine, phosphoserine, and phosphothreonine was found in the cytosol of kidney cortex. The addition of regucalcin (50–250 nM) in the enzyme reaction mixture caused a significant decrease in protein phosphatase activity toward three phosphoamino acids. The effect of calcium (25 μM) and calmodulin (2.5 μg/ml) in increasing protein phosphatase activity toward three phosphoamino acids was significantly decreased by the addition of regucalcin (100 nM). Protein phosphatase activity toward three phosphoamino acids was significantly increased in the presence of anti-regucalcin monoclonal antibody (10–50 ng/ml) in the enzyme reaction mixture. The effect of antibody (25 ng/ml) in increasing the enzyme activity was significantly inhibited by cyclosporin A (10^{-5} M) or vanadate (10^{-5} M). Regucalcin in the kidney cortex cytosol was clearly decreased by the administration of saline (0.9% NaCl) for seven days in rats. Protein phosphatase activity toward three phosphoamino acids was significantly decreased by saline administration. The effect of anti-regucalcin antibody (25 ng/ml) in increasing protein phosphatase activity toward three phosphoamino acids was not seen in the renal cortex cytocol of saline-administered rats. The present study demonstrates that endogenous regucalcin plays a suppressive role in the regulation of protein phosphatase activity in the cytoplasm of rat kidney cortex. J. Cell. Biochem. 81:639–646, 2001. © 2001 Wiley-Liss, Inc.

Key words: regucalcin; calcium-binding protein; protein phosphatase; kidney cortex

Calcium ion (Ca^{2+}) plays an important role in the regulation of many cell functions. The Ca^{2+} effect in cells is amplified by Ca^{2+} -dependent protein kinases, which are related to a signal transduction due to hormonal stimulation [Heizman and Hunziker, 1991; Kraus-Friedman and Feng, 1996; Malviya and Rogue, 1998]. Recently, regucalcin, a calcium-binding protein, has been demonstrated to have a regulatory role for Ca^{2+} signaling in cells [Yamaguchi, 2000a, 2000b].

Regucalcin is mainly localized in liver, although its relatively higher levels are also found in kidney cortex but not the medulla of rats [Shimokawa and Yamaguchi, 1992, 1993a; Yamaguchi and Isogai, 1993]. Regucalcin mRNA expression in the liver and renal cortex of rats is markedly stimulated by the administration of calcium [Shimokawa and Yamaguchi, 1993b; Yamaguchi and Kurota, 1995], indicating that the expression is mediated through Ca^{2+} signaling in the cells [Murata and Yamaguchi, 1999].

The role of regucalcin in kidney cortex cells is not yet fully known. In recent years, it has been shown that regucalcin may play a cellular role as an activator in the ATP-dependent Ca^{2+} pump in the basolateral membranes [Kurota and Yamaguchi, 1997a], the microsomes [Kurota and Yamaguchi, 1997b], and the mitochondria [Xue et al., 2001] from rat kidney cortex cells. Also, this protein has an inhibitory effect on Ca^{2+}/calmodulin-dependent protein kinase [Kurota and Yamaguchi, 1997c] and protein kinase C [Kurota and Yamaguchi, 1998] activities in rat renal cortex cytosol. Thus, regucalcin

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may have a role in the regulation of Ca^{2+}-related cellular function in kidney cortex.

The present study, furthermore, was undertaken to clarify the effect of regucalcin on protein phosphatase activity in rat renal cortex cytosol. Dephosphorylation of many phosphorylated proteins is regulated by protein phosphatase in cells [Hunter, 1995]. Protein phosphatase may play an important role in the regulation of kidney cells. Investigation of whether regucalcin has the regulatory effect on protein phosphatase activity in rat renal cortex cytosol found that endogenous regucalcin can inhibit this activity.

MATERIALS AND METHODS

Chemicals

Calmodulin [5,2000 U/mg protein from bovine brain], *o*-phospho-L-tyrosine, *o*-phospho-L-serine, *o*-phospho-L-threonine, cyclosporin A, and okadaic acid 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, vanadate (neutralized with KOH), and other chemicals were purchased from Sigma Chemical Co. and Wako Pure Chemical Company (Osaka, Japan). The reagents were dissolved in distilled water. Some reagents were passed through ion-exchange resin to remove metal ions.

Animals

Male Wister 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 Regucalcin

Regucalcin is markedly expressed in rat liver cytosol [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993], from which it was isolated. Regucalcin in the cytosol fraction (the supernatant of 10,5000*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-Regucalcin Antibody

Anti-regucalcin antibody was a monoclonal antibody raised against regucalcin prepared using standard methods [Omura and Yamaguchi, 1999]. Mouse (BALB/C, Japan SLC) were subcutaneously injected with 0.1 mg per animal of antigen (rat liver regucalcin) 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 three days after the last injection. Spleen cells were prepared from immuned mouse, and fused into myeloma cells. Anti-regucalcin monoclonal antibody-producing cells (hybridoma cells) were obtained by screening. The IgG from hybridoma cells was isolated through protein A-agarose column (Sigma).

Saline Administration

Sodium Chloride (0.9%, saline), which was dissolved in distilled water, was freely ingested daily as drinking water for 2 or 7 days in normal rats (4 weeks old). Control animals were given distilled water. Rats were sacrificed by cardiac puncture under light anesthesia with ether. Kidneys were immediately removed.

Preparation of Kidney Cortex Cytosol

Rats were sacrificed by cardiac puncture, and the 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 protein phosphatase activity and to analysis Western blotting for regucalcin.

Protein concentration was determined by the method of Lowry et al. [1951] using bovine serum albumin as the standard.

Assay of Protein Phosphatase Activity

Protein phosphatase activity toward various phosphoamino acids was assayed at 30°C in 1.0 ml of reaction mixture containing 20 mM Tris-HCI (pH 8.0), 100 mM NaCl, 6 mM $MgCl_2$, 0.5 mM dithiothreitol, 9 mM phosphoamino acid (neutrazied with NaOH solution) in kidney cortex cytosol (0.7–0.9 mg protein/ml) as reported elsewhere [Pallen and Wang, 1983;

Fruman et al., 1992]. In separate experiments, the reaction mixture contained either vehicle, anti-regucalcin antibody (10–50 ng/ml), regucalcin (50–250 nM), cyclosporin A(10^{-5} M), okadaic acid (10^{-5} M), or vanadate (10^{-5} M). The enzyme reaction was terminated after 15 min by the addition of 1.0 ml of ice-cold 10% trichloacetic acid and centrifuged to precipitate protein. Released inorganic phosphate in the supernatant was quantified by the method of Nakamura and Mori [1958]. Results were expressed as nanomoles of inorganic phosphate liberated per min per mg of cytosolic protein.

Western Blot Analysis

The homoganate from renal cortex tissues was centrifuged for 1 h at 105,000*g* at 4°C, and the supernatant (cytosol) was used for Western blot analysis [Wessendorf et al., 1993; Yamaguchi et al., 2000]. Aliquots of cytosol were mixed with 5 × Laemmli 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 polyvinrylidene difluoride membrane at 100 mA for 4 h. The membranes were incubated with a polyclonal rabbit anti-regucalcin antibody [Yamaguchi and Isogai, 1993], which was diluted 1:2000 in 10 mM Tris-HCl, pH 8, containing 150 mM NaCl, 0.1% (w/v) Tween 20 (washing buffer), and 5% (w/v) skim milk for one hour. The membranes incubated with antibody were washed four times with washing buffer. Then membranes were incubated for 1 h with horseradish peroxidase linked anti-rabbit IgG which was diluted 1:5000 with washing buffer containing 5% (w/v) skim milk, and again they were washed. Detection of the protein bands was performed using a enhanced chemiluminescent kit following the manufacture instructions. The molecular size of the detecting protein was determined by running the standard protein molecules of known sizes in parallel.

Statistical Analysis

Data were expressed as the mean ± S.E.M. The significance of the difference between the values was estimated by Student's *t*-test. Also, we used a multiway ANOVA and Turky-Kramer multiple comparison test to compare the treatment groups. A P value of < 0.05 was considered to indicate a statistically significant difference.

RESULTS

Characterization of Protein Phosphatase Activity in Renal Cortex Cytosol

The effect of calcium chloride addition on protein phosphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex is shown in Figure 1. The addition of calcium (25 or 50 μM) in the enzyme reaction mixture caused a significant increase in protein phosphatase activity toward phosphotyrosine,and phosphothreonine. The effect of calcium in increasing the enzyme activity was weakened by increasing the concentrations of calcium (100 μM).

The effect of calmodulin addition on protein phosphatase activity in rat renal cortex cytosol is shown in Figure 2. The presence of calmodulin (2.5 or 50 μg/ml) in the enzyme reaction mixture caused a significant increase in protein phosphatase activity toward phosphoserine and phosphothreonine. Such an increase was not seen in the case of phosphotyrosine.

The effect of various inhibitors on protein phosphatase activity without calcium or calmodulin addition is shown in Figure 3. Protein phosphatase activity toward phosphotyrosine in kidney cortex was significantly decreased by the addition of okadaic acid (10^{-5} M) or vanadate (10^{-5} M), although cyclosporin A (10^{-5} M) did not have an effect. The enzyme activity toward phosphoserine was significantly decreased in the presence of cyclosporin A (10^{-5} M). Such an effect was not seen by the addition of

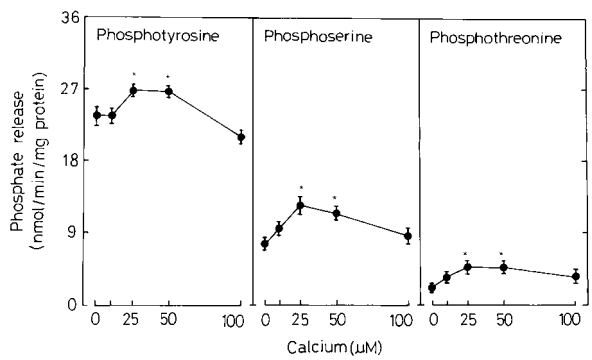

Fig. 1. Effect of calcium addition on protein phosphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex. The enzyme reaction mixture contained either vehicle or calcium chloride (25, 50, or 100 μM) without calmodulin addition. Each value represents the mean ± S.E.M. of five experiments with different rats. *$P < 0.01$, compared with the control (none) value.

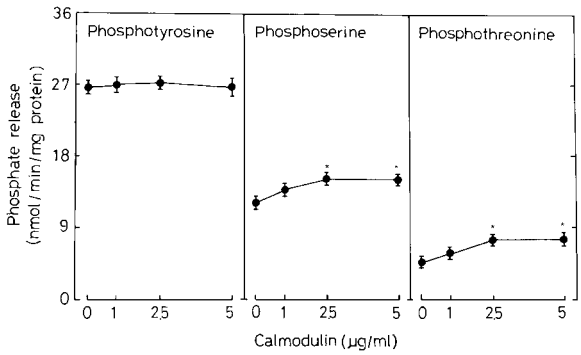

Fig. 2. Effect of calmodulin addition on protein phosphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex. The enzyme reaction mixture contained either vehicle or calmodulin (1, 2.5, or 5 μg/ml) with calcium (25 μM) addition. Each value represents the mean ± S.E.M. of five experiments with different rats. $*P < 0.01$, compared with the control (none) value.

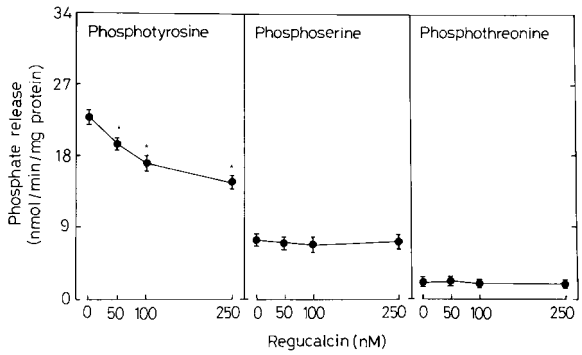

Fig. 4. Effect of regucalcin addition on protein phosphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex. The enzyme reaction mixture contained either vehicle or regucalcin (50, 100, or 250 nM) without calcium and calmodulin addition. Each value represents the mean ± SEM of five experiments with different rats. $*P < 0.01$, compared with the control (non) value.

okadaic acid (10^{-5} M). Phosphatase activity toward phosphothreonine was not significantly altered by cyclosporin A (10^{-5} M), okadaic acid (10^{-5} M) or vanatate (10^{-5} M).

Effect of Endogenous Regucalcin of Protein Phosphatase Activity in Renal Cortex Cytosol

The effect of regucalcin addition on protein phosphatase activity in the cytosol of rat kidney cortex is shown in Figure 4. The addition of regucalcin (50, 100, or 250 nM) in the enzyme reaction mixture caused a significant decrease in protein phosphatase activity toward phosphotyrosine. The enzyme activity toward phosphoserine or phosphothreonine was not significantly altered by the addition of regucalcin (50, 100, or 250 nM). The effect of calcium (25 μM) and calmodulin (2.5 μg/ml) in increasing protein phosphatase activity toward phosphotyrosine, phosphoserine, or phosphothreonine was significantly decreased by the addition of regucalcin (100 nM) in the enzyme reaction mixture (Fig. 5).

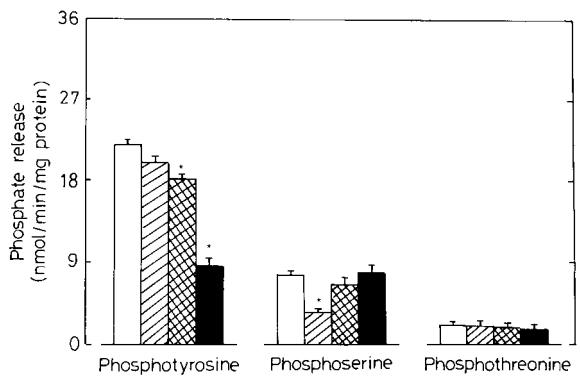

Fig. 3. Effect of various inhibitors on protein phosphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex. The enzyme reaction mixture contained either vehicle, cyclosporin A (10^{-5} M), okadaic acid (10^{-5} M) or vanadate (10^{-5} M) without calcium and calmodulin addition. Each value represents the mean ± S.E.M. of five experiments with different rats. $*P < 0.01$, compared with the control (none) value. White bars, control; hatched bars, cyclosporin A; double hatched bars, okadaic acid; black bars, vanadate.

The effect of anti-regucalcin monoclonal antibody on protein phosphatase activity in the cytosol of rat renal cortex is shown in Figure 6. The presence of anti-regucalcin monoclonal antibody (10, 25, and 50 ng/ml) in the enzyme reaction mixture caused a significant increase in protein phosphatase activity toward phosphotyrosine, phosphoserine, and phosphothreonine. The effect was completely abolished by the addition of regucalcin (100 nM) in the enzyme reaction mixture (data not shown). Moreover, protein phosphatase activity toward three phosphoamino acids in the absence of anti-regucalcin monoclonal antibody was not significantly altered in the presence of nonimmune IgG (100 ng/ml) in the enzyme reaction mixture (data not shown).

The effect of anti-regucalcin monoclonal antibody (25 ng/ml)in increasing protein phosphatase activity toward phosphotyrosine was significantly decreased by the addition of vanadate (10^{-5} M) (Fig. 7). The effect of antibody was not significantly altered by cyclosporin A(10^{-5} M) or okadaic acid (10^{-5} M). Meanwhile,

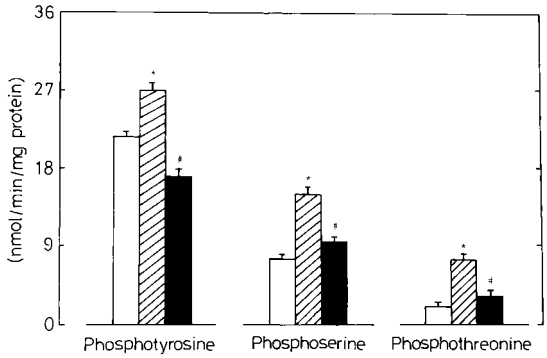

Fig. 5. Effect of regucalcin on the calcium and calmodulin addition-increased protein phosphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex. The enzyme reaction mixture contained either vehicle or regucalcin (100 nM) with the addition of calcium (25 μM) plus calmodulin (2.5 μg/ml). Each value represents the mean ± S.E.M. of five experiments with different rats. $*P < 0.01$, compared with the control (none) value. $\#P < 0.01$, compared with the value for calcium and calmodulin addition. White bars, control; hatched bars, calcium plus calmodulin; black bars, calcium and calmodulin plus regucalcin.

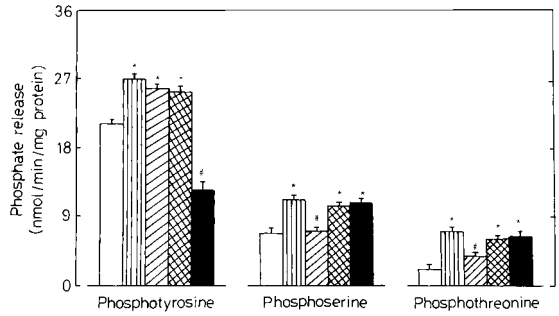

Fig. 7. Effect of various inhibitors on the anti-regucalcin monoclonal antibody-increased protein phosphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex. The enzyme reaction mixture contained either vehicle, cyclosporin A (10^{-5} M), okadaic acid (10^{-5} M) or vanadate (10^{-5} M) in the presence or absence of anti-regucalcin monoclonal antibody (25 ng/ml). Each value represents the mean ± S.E.M. of five experiments with different rats. White bars, control; lined bars, antibody; hatched bars, antibody plus cyclosporin A; double hatched bars, antibody plus okadaic acid, black bars, antibody plus vanadate.

the anti-regucalcin monoclonal antibody (25 ng/ml)-increased protein phosphatase activity toward phosphoserine or phosphothreonine was completely prevented by the addition of cyclosporin A(10^{-5} M) (Fig. 7). Such an effect was not seen in the presence of okadaic acid (10^{-5} M) or vanadate (10^{-5} M).

Saline Administration-Induced Decrease in Protein Phosphatase Activity in Renal Cortex Cyrosol

Regucalcin mRNA expression in the renal cortex of rats is markedly suppressed by the intake of saline for 7 days [Shinya et al., 1996],

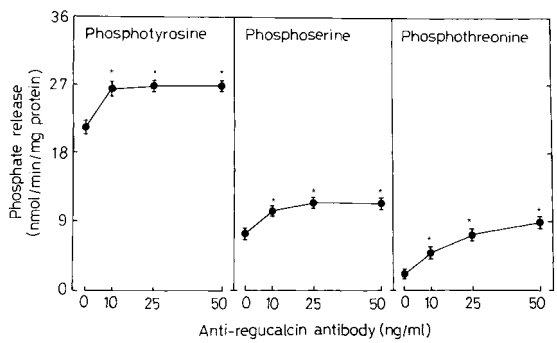

Fig. 6. Effect of anti-regucalcin monoclonal antibody on protein phoaphatase activity toward various phosphoamino acids in the cytosol of rat kidney cortex. The enzyme reaction mixture contained either vehicle or anti-regucalcin monoclonal antibody (10, 25, or 50 ng/ml) without calclium and calmodulin addition. Each value represents the mean ± S.E.M. of five experiments with different rats.

suggesting that endogenous regucalcin is decreased in the kidney cortex cytosol of saline-administered rats. The analysis with Western blotting of regucalcin protein in the cytosol of renal cortex from normal rats and saline-administered rats is shown in Figure 8. Regucalcin level was significantly ($P < 0.01$) decreased in the renal cortex cytosol of rats administered saline for 7 days, as compared with that of control (normal) rats; the densitometric data showed 92 ± 1 or 65 ± 3 (% of control; mean ± S.E.M of four rats) at 2 or 7 days with saline administration, respectively.

The effect of saline administration on protein phosphatase activity in the renal cortex cytosol of rats administered for 2 or 7 days as a drinking water is shown in Figure 9. Protein phosphatase activity toward phosphotyrosine, phosphoserine, or phosphothreonine was significantly decreased by saline administration for 7 days. The effect of anti-regucalcin monoclonal antibody (25 ng/ml) in increasing protein phosphatase activity toward phosphotyrosine, phosphoserine, or phosphothreonine was not seen in the presence of the antibody in enzyme reaction mixture containing the renal cortex cytosol of rats administratered saline for 7 days (Fig. 10).

DISCUSSION

Protein phosphorylation–dephosphorylation is a universal mechanism by which numerous

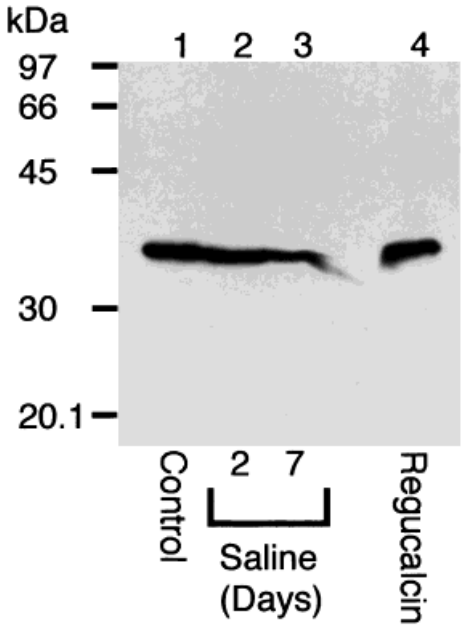

Fig. 8. Quantitative analysis of regucalcin protein in the cytosol of rat renal cortex. Rats were fed with daily intake of saline as a drinking water for 2 or 7 days. Western blot analysis was carried out on the extracts (25 μg of cytosolic proteins) obtained from the kidney cortex of normal or saline-administered rats. **Lane 1**, normal rat kidney cortex; **lane 2**, saline-administered rats (2 days); **lane 3**, saline-administered rats (7 days); **lane 4**, regucalcin (0.1 μg) as the marker. The figure shows 1 of 4 experiments with separate samples.

cellular events are regulated. It has become apparent that there may exist as many as 1,000 phosphatases which, like the kinases, are just as elaborately and rigorously controlled [Hunter, 1995; Wang et al., 1995]. The precise physiologic

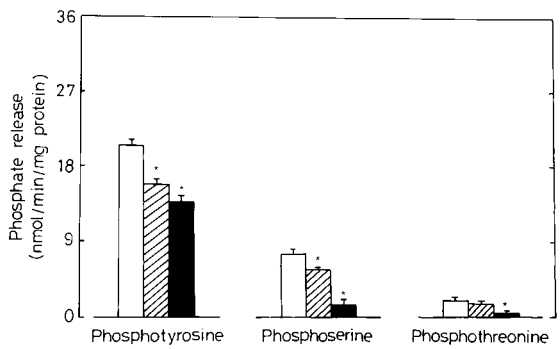

Fig. 9. Alteration in protein phosphatase activity toward various phosphoamino acids in the renal cortex cytosol of rats administered saline.Rats were sacrificed by bleeding at 2 and 7 days with daily intake of saline as drinking water. The enzyme activity was measured in a reaction mixture without calcium and calmodulin. Each value represents the mean ± S.E.M. of five experiments with different rats. $*P < 0.01$, compared with the control (none) value. White bars, control; hatched bars, 2 days with saline intake; black bars, 7 days with saline intake.

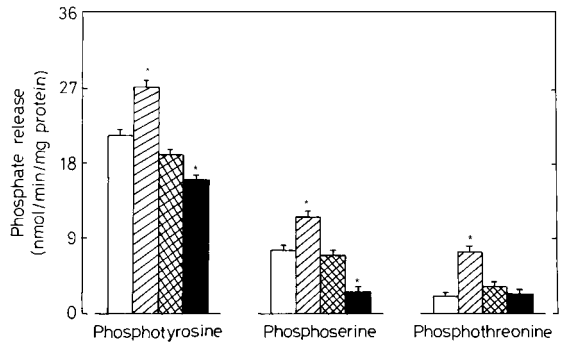

Fig. 10. Effect of anti-regucalcin monoclonal antibody on protein phosphatase activity toward various phosphoamino acids in the renal cortex cytosol of salin-administered rats. Rats were sacrificed by bleeding at 2 and 7 days with daily intake of saline as drinking water. The enzyme reaction mixture contained either vehicle or anti-regucalcin monoclonal antibody (25 ng/ml). Each value represents the mean ± S.E.M. of five experiments with different rats. $*P < 0.01$, compared with the control (none) value. $^{\#}P < 0.01$, compared with the value for calcium and calmodulin addition. White bars, control for normal rats; hatched bars, antibody addition for normal rats; double hatched bars, antibody addition for 2 days-saline intake; black bars, antibody addition for 7 days-saline intake.

role of phosphatases is to dephosphorylate a wide variety of phosphoproteins, and it may serve an important cellular function.

Protein phosphatyase activity toward phosphotyrosine, phosphoserine, and phosphothreonine was seen in the cytosol of rat renal cortex, indicating that protein tyrosine phosphatases and protein serine/threonine phosphatases are present in renal cortex cytosol. Protein phosphatase activity toward phosphoserine and phosphothreonine in renal cortex cytosol was enhanced by the addition of calcium and calmodulin in the enzyme reaction mixture, indicating the existence of Ca^{2+}/calmodulin-dependent protein phosphatases (calcineurin).

The activity of phosphatase toward phosphotyrosine in renal cortex cytosol was significantly decreased by regucalcin addition in the enzyme reaction mixture containing the cytosolic protein. Regucalcin may have an inhibitory effect on protein tyrosine phosphatases in renal cortex cytosol. Now, regucalcin addition did not cause a significant inhibitory effect on phosphatase activity toward phosphoserine and phosphothreonine in renal cortex cytosol. However, regucalcin addition had a significant inhibitory effect on Ca^{2+}/calmodulin-increased protein phosphatase activity toward three phosphoamino acids. The presence of anti-regucalcin

monoclonal antibody in the enzyme reaction mixture causes a significant increase in protein phosphatase activity toward three phosphoamino acids in renal cortex cytosol. This finding suggests that endogenous regucalcin in renal cortex cytosol reveals an inhibitory effect on protein phosphatase activity toward three phosphoamino acids without Ca^{2+}/calmodulin addition. From these results, it is assumed that endogenous regucalcin, which is present in low amount in renal cortex cytosol, may have an inhibitory effect on protein serine/threonine phosphatase activities. Presumably, the effect of regucalcin addition in decreasing protein serine/threonine phosphatase activity in the cortex cytosol is saturated for regucalcin addition with greater amount than that of the endogenous protein. Alternatively, regucalcin has an inhibitory effect on the activities of protein tyrosine phosphatase and protein serine/threonine phosphatises in rat renal cortex cytosol. The identification of specific molecules of protein phosphatases, which is inhibited by regucalcin, remains to be elucidated.

The effect of anti-regucalcin antibody in increasing protein phosphatase activity in renal cortex cytosol was significantly inhibited in the presence of cyclosporin A, an inhibitor of calcineurin [Fruman et al., 1992], or vanadate, an inhibitor of protein tyrosine phosphatases [Hunter, 1995]. These results support the view that endogenous regucalcin inhibits protein tyrosine phosphatases and protein serine/threonine phosphatases in renal cortex cytosol. At present, a physiological significance of regucalcin, which inhibits the activity of various protein phosphatases in renal cortex cells, is unknown. However, regucalcin can inhibit the activity of Ca^{2+} -dependent protein kinases in renal cortex cytosol [Kurota and Yamaguchi, 1997c, 1998]. Presumably, regucalcin plays a regulatory role in the cellular signaling system which is involved in phosphoprotein due to inhibiting the activity of protein kinases and protein phosphatases in renal cortex cytosol. Further studies are needed to determine a physiologic role of regucalcin in renal cells.

A pathophysiologic role of regucalcin in kidney is unknown. Saline administration may induce a hypertensive state which is related to kidney function. Regucalcin mRNA expression in the renal cortex has been shown to be suppressed by the administration of saline as a drinking water for 7 days in rats [Shinya et al., 1996; Shinya and Yamaguchi, 1997]. The present study demonstrates that regucalcin level in the renal cortex cytosol was significantly decreased by saline administration for 7 days in rats. The expression of regucalcin in rat renal cortex cells was suppressed in saline-administered rats. Saline administration may be a tool to study a physiologic and pathophysiologic in kidney.

Protein phohphatase activity toward three phosphoamino acids was found to be decreased in the renal cortex cytosol of saline-administratered rats for 7 days. The mechanism by which saline administration causes a reduction of protein phosphatase activity is unknown. This, however, may be involved in an impairment of renal function of saline-administered rats [Shinya and Yamaguchi, 1997]. Interestingly, the effect of anti-regucalcin monoclonal antibody in increasing protein phosphatase activity toward three phosphoamino acids was not seen in the renal cortex cytosol of rats administered saline for 7 days. This result may be partly due to a decrease in endogenous regucalcin in the renal cortex of saline-administered rats, supporting a role of endogenous regucalcin in the regulation of protein phosphatase activity. Thus, saline administration could induce a reduction of regucalcin expression and protein phosphatase activity in rat renal cortex cytosol. Presumably, regucalcin plays a pathophysiologic role in the attenuation of renal cell function in saline-administered rats.

In conclusion, it has been demonstrated that endogenous regucalcin plays a suppressive role in the regulation of various protein phosphatases in rat renal cortex cytosol.

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