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Inhibitory Effect of Regucalcin on Protein Phosphatase Activity in the Nuclei of Rat Kidney Cortex

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Abstract The role of regucalcin, which is a regulatory protein of calcium signaling, in the regulation of protein phosphatase activity in the nuclei of rat kidney cortex was investigated. Protein phosphatase activity towards phosphotyrosine, phosphoserine, and phosphothreonine was found in the nuclei. The enzyme activity towards three phosphoamino acids was significantly increased by the addition of calcium chloride (10–50 μM) in the enzyme reaction mixture. This increase was significantly inhibited by trifluoperazine (25 or 50 μM), an antagonist of calmodulin. The presence of regucalcin (50 or 100 nM) in the enzyme reaction mixture caused a significant decrease in protein phosphatase activity towards three phosphoamino acids. This effect was also seen in the presence of calcium (25 μM) and/or calmodulin (5 μg/ml). Protein phosphatase activity towards three phosphoamino acids was significantly increased in the presence of anti-regucalcin monoclonal antibody (25 or 50 ng/ml) in the enzyme reaction mixture. This effect was completely blocked by the addition of regucalcin (100 nM). The effect of antibody (25 ng/ml) in increasing protein phosphatase activity towards phosphotyrosine was significantly inhibited by vanadate (10^{-4} M). Also, the antibody's effect towards phosphoserine and phosphothreonine was significantly inhibited by cyclosporin A (10^{-5} M). Endogenous regucalcin was found in the nuclei of rat kidney cortex using Western blot analysis. Nuclear regucalcin level was significantly reduced by the administration of saline (0.9% NaCl) for seven days in rats. Protein phosphatase activity towards three phosphoamino acids was significantly decreased by saline administration. The effect of anti-regucalcin monoclonal antibody (25 ng/ml) in increasing protein phosphatase activity towards three phosphoamino acids was weakened in the renal cortex nuclei of saline-administrated rats. The present study demonstrates that endogenous regucalcin plays a suppressive role in the regulation of protein phosphatase activity in the nuclei of rat kidney cortex cells. J. Cell. Biochem. 83: 111–120, 2001.

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

Calcium ion (Ca^{2+}) plays an important role in the regulation of many cell functions. The Ca^{2+} effect in cells is partly modulated by Ca^{2+}-binding protein [Cheung, 1984; Heizman and Hunziker, 1991; Kraus-Friedman and Feng, 1996]. Regucalcin 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 demonstrated that regucalcin plays the multifunctional role as a regulatory protein for Ca^{2+} signaling in cells [Yamaguchi, 2000a,b; in review].

Regucalcin is mainly localized in liver, although its relatively higher levels are also found in kidney cortex but not in the medulla of rats [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993]. There is a growing evidence that regucalcin can regulate intracellular Ca^{2+} homeostasis [Takahashi and Yamaguchi, 2000], activation of various Ca^{2+}-dependent protein kinases [Katsumata and Yamaguchi, 1998], protein phosphatases [Omura and Yamaguchi, 1999], and nuclear functions with proliferative cells [Yamaguchi and Kanayama, 1996; Inagaki and Yamaguchi, 2000] in liver cells. However, the role of regucalcin in kidney cells is not fully known.

It has been shown recently that regucalcin may play a cellular role as an activator in the ATP-dependent Ca^{2+} pump in the basolateral

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membranes [Kurota and Yamaguchi, 1997a], the microsomes [Kurota and Yamaguchi, 1997b], and the mitochondria [Xue et al., 2000] from rat kidney cortex cells. Also, this protein has an inhibitory effect on Ca^{2+}-dependent protein kinases [Kurota and Yamaguchi, 1997c, 1998] and protein phosphatases [Morooka and Yamaguchi, 2001] in rat renal cortex cytosol. Regucalcin may play a regulatory role in kidney function. Whether this protein plays a role in the regulation of the nuclear function in rat renal cortex cells, however, is unknown.

The present study, therefore, was undertaken to clarify the effect of regucalcin on protein phosphatase activity in the nuclei of rat kidney cortex. Dephosphorylation of many phosphorylated proteins is regulated by protein phosphatase in cells [Hunter, 1995]. This enzyme may play an important role in the regulation of the nuclear function in kidney cortex cells. We found that regucalcin can inhibit protein phosphatase activity in the nuclei of rat kidney cortex.

MATERIALS AND METHODS

Chemicals

Calmodulin (52,000 U/mg protein from bovine brain), *o*-phospho-L-tyrosine, *o*-phospho-L-serine, *o*-phospho-L-threonine, trifluoperazine, 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 (80–100g, 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 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-Regucalcin Antibody

Anti-regucalcin antibody was a monoclonal antibody raised against regucalcin prepared by 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.

Isolation of Nuclei

The nuclei from kidney cortex were isolated by the procedure of Jones et al. [1989] with a minor modification. Rats were killed by cardiac puncture, and the kidney cortex were then removed, cut into small pieces, and homogenized in a Potter-Elvehjem homogenizer with a Teflon pestle in 40 ml of the same solution containing 0.25 M sucrose and 1.0 mM EGTA. The homogenate was filtered through three layers of cheesecloth. The homogenate was filtered by centrifugation 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 tubes were centrifuged at 37,000*g* for 30 min. The upper layer and the sucrose cushion were removed with an aspirator. Then it was resuspended in the incubation medium (125 mM KCl, 2 mM potassium phosphate, 25 mM Hepes, 4 mM $MgCl_2$, pH 7.0) by hand homogenization. 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].

Assay of Protein Phosphatase Activity

Phosphatase activity towards phosphotyrosine, phosphoserine, and phosphothreonine was assayed at 30°C in 1.0 mM NaCl, 6 mM $MgCl_2$, 0.5 mM dithiothreiol, 9 mM phosphoaminoacid, and nuclei (0.7 mg protein/ml; 0.2–0.3 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, calcium chloride (5–50 μM), anti-regucalcin (RC) antibody (10–50 ng/ml), trifluoperazine (10–50 μM), cyclosporin A (10^{-6} M), okadaic acid (10^{-6} 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% trichloroacetic acid and centrifuged to precipiate 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 minutes (min) per milligram (mg) of nuclear protein. Protein concentration was determined by the method of Lowry et al. [1951] using bovine serum albumin as a standard.

Western Blot Analysis

The nuclei from renal cortex homogenate were used for Western blot analysis [Wessendorf et al., 1993; Tsurusaki et al., 2000]. Aliquots of nucleus (containing 100 μg of protein) 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 polyvinylidene 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 1:2,000 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 manufacture'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).

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 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 Nuclei

The effect of calcium chloride addition on protein phosphatase activity towards various phosphoamino acids in the nuclei of rat kidney cortex is shown in Figure 1. The addition of calcium (10, 25, or 50 μM) in the enzyme reaction mixture caused a significant increase in protein phosphatase activity towards phosphotyrosine and phosphoserine. Protein phosphatase activity towards phosphothreonine was significantly raised by the concentration of 25 and 50 μM calcium.

The effect of trifluoperazine, an antagonist of calmodulin [Vincenzi, 1982], on the calcium-increased protein phosphatase activity in the nuclei of rat kidney cortex is shown in Figure 2. The presence of trifluoperazine (25 or 50 μM) in the enzyme reaction mixture caused a significant decrease in protein phosphatase activity towards phosphotyrosine, phosphoserine, phosphothreonine which were increased by the addition of calcium chloride (25 μM). Trifluo-

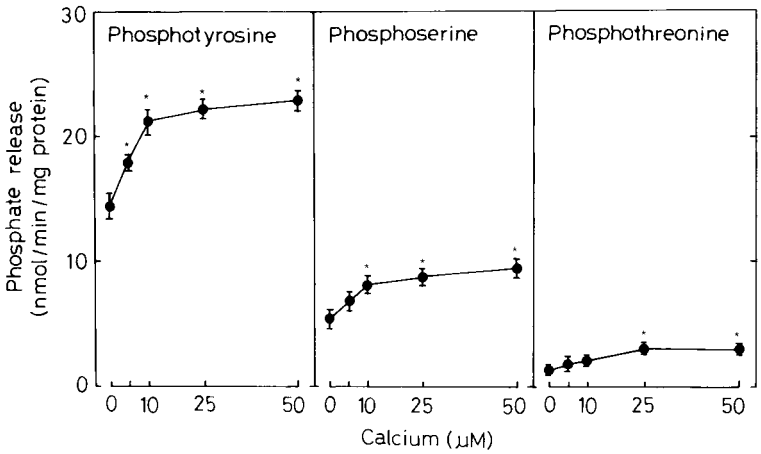

Fig. 1. Effect of calcium addition on protein phosphatase activity towards various phosphoamino acids in the nuclei of rat kidney cortex. The enzyme reaction mixture contained either vehicle or calcium chloride (5, 10, 25, or 50 μM) without calmodulin addition. Each value represents the mean±SEM of five experiments with different rats. *$P < 0.01$, compared with the control (none) value.

perazine (10 μM) had a significant inhibitory effect on protein phosphatase activity towards phosphoserine or phosphothreonine in the presence of calcium chloride (25 μM).

The effect of various inhibitors on nuclear protein phosphatase activity without calcium or calmodulin addition is shown in Figure 3. Protein phosphatase activity towards phosphotyrosine was significantly decreased in the presence of vanadate (10^{-5} M) in the enzyme reaction mixture, while the enzyme activity was not significantly altered by okadaic acid (10^{-6} M) or cyclosporin A (10^{-6} M). Protein phosphatase activity towards phosphoserine or phosphothreonine was significantly decreased by the addition of cyclosporin A (10^{-6} M), while the enzyme activity was not significantly changed by vanadate (10^{-5} M) or okadaic acid (10^{-6} M).

Effect of Endogenous Regucalcin on Protein Phosphatase Activity in Renal Cortex Nuclei

The effect of regucalcin addition on protein phosphatase activity in the nuclei of rat kidney cortex is shown in Figure 4. The addition of regucalcin (25, 50, or 100 nM) in the enzyme reaction mixture caused a significant decrease in protein phosphatase activity towards phosphotyrosine or phosphoserine. The enzyme activity towards phosphothreonine was significantly decreased by the addition of regucalcin with 50 or 100 nM.

The effect of regucalcin on the calcium-increased protein phosphatase activity towards phosphoamino acids in the nuclei of rat kidney cortex is shown in Figure 5. The addition of calcium chloride (25 μM) in the enzyme reaction mixture caused a significant increase in protein phosphatase activity towards phosphotyrosine, phosphoserine, or phosphothreonine. This effect was not significantly enhanced in the presence of calmodulin (5 μg/ml). The effect of calcium chloride (25 μM) without or with calmodulin (5 μg/ml) in increasing protein phosphatase activity towards phosphotyrosine, phosphoserine, and phosphothreonine was completely prevented by the addition of regucalcin (100 nM).

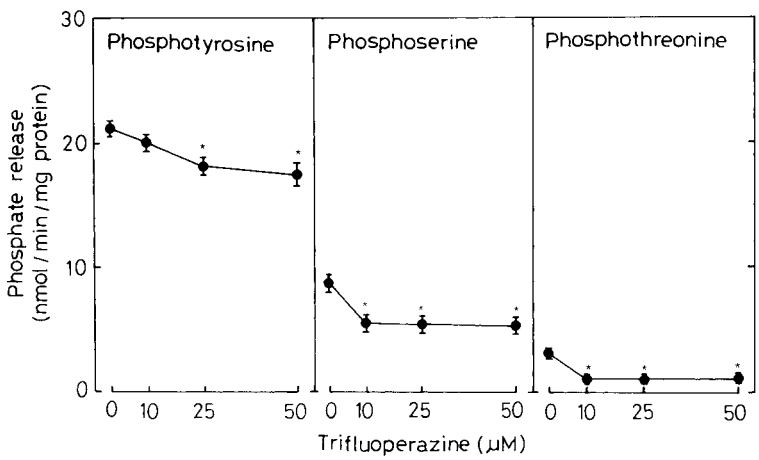

Fig. 2. Effect of trifluoperazine on the calcium-increased protein phosphatase activity towards various phosphoamino acids in the nuclei of rat kidney cortex. The enzyme reaction mixture contained either vehicle or trifluoperazine (10, 25, or 50 μM) with calcium chloride (25 μM) addition. Each value represents the mean±SEM of five experiments with different rats. *$P < 0.01$, compared with the control (none) value.

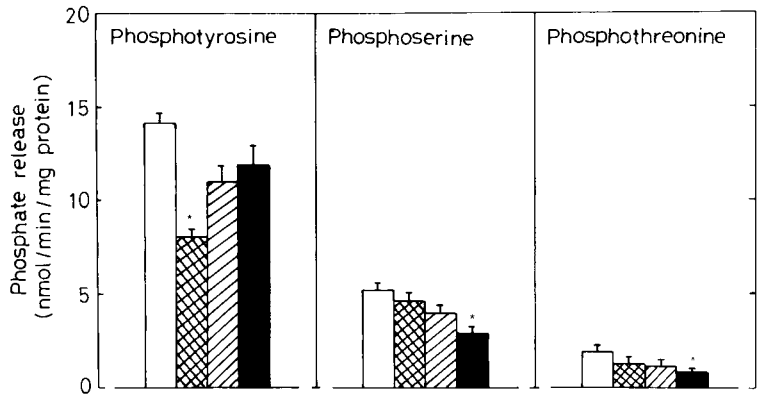

Fig. 3. Effect of various inhibitors on protein phosphatase activity towards various phosphoamino acids in the nuclei of kidney cortex. The enzyme reaction mixture contained either vehicle, vanadate (10^{-6} M), okadaic acid (10^{-6} M), or cyclosporin A (10^{-6} M) without calcium and calmodulin. Each value represents the mean±SEM of five experiments with different rats. *$P < 0.01$, compared with the control (none) value. White bars, control; double hatched bars, vanadate; hatched bars, okadaic acid; black bars, cyclosporin A.

The effect of anti-regucalcin monoclonal antibody on protein phosphatase activity towards phosphoamino acids in the nuclei of rat kidney cortex is shown in Figure 6. Protein phosphatase activity towards phosphotyrosine, phosphoserine, and phosphothreonine was significantly increased in the presence of anti-regucalcin monoclonal antibody (25 or 50 ng/ml). The effect of anti-regucalcin monoclonal antibody (25 ng/ml) in increasing protein phosphatase activity towards three phosphoamino acids was completely prevented by the addition of regucalcin (100 nM) in the enzyme reaction mixture (Fig. 7).

The effect of various inhibitors on the anti-regucalcin monoclonal antibody-increased protein phosphatase activity towards phosphoamino acids in the nuclei of rat kidney cortex is shown in Figure 8. Anti-regucalcin monoclonal antibody (25 ng/ml) increased protein phosphatase activity towards phosphotyrosine was significantly inhibited by the addition of vanadate (10^{-4} M) in the enzyme reaction mixture, while okadaic acid (10^{-5} M) or cyclosporin A (10^{-5} M) did not have an effect. Protein phosphatase activity towards phosphoserine and phosphothreonine which was raised in the presence of anti-regucalcin monoclonal antibody (25 ng/ml) was significantly inhibited by the addition of cyclosporin A (10^{-5} M), while vanadate (10^{-4} M) or okadaic acid (10^{-5} M) did not have an appreciable effect.

Saline Administration–Induced Decrease in Protein Phosphatase Activity in Renal Cortex Nuclei

Regucalcin mRNA expression in the kidney cortex of rats is significantly decreased by the intake of saline for 7 days [Shinya et al., 1996], suggesting that endogenous regucalcin is decreased in the kidney cortex of saline-administrated rats. The analysis with Western blotting of regucalcin protein in the nuclei of renal cortex from normal rats and saline-administered rats is shown in Figure 9. Regucalcin level was markedly ($P < 0.01$) lowered in the renal cortex

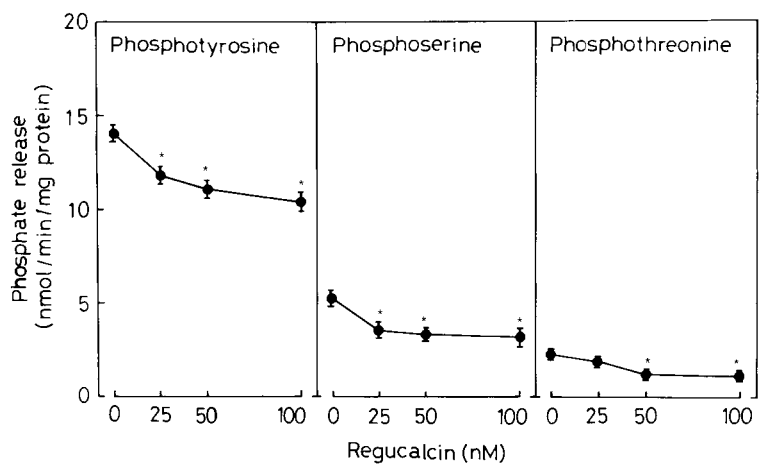

Fig. 4. Effect of regucalcin addition on protein phosphatase activity towards various phosphoamino acids in the nuclei of rat kidney cortex. The enzyme reaction mixture contained either vehicle or regucalcin (25, 50, or 100 nM) without calcium and calmodulin addition. Each value represents the mean±SEM of five experiments with different rats. *$P < 0.01$, compared with the control (none) value.

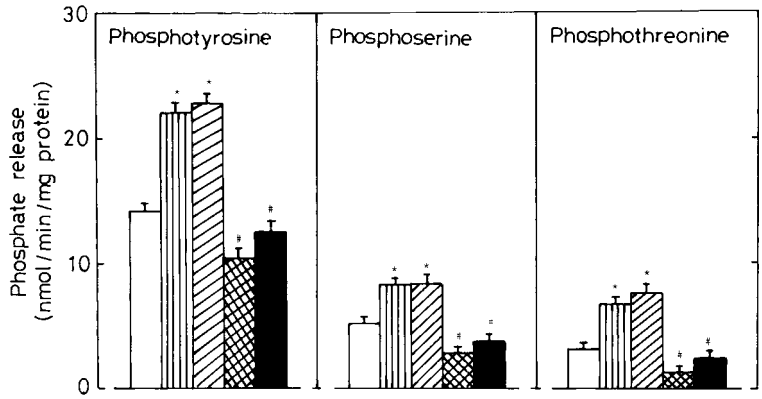

Fig. 5. Effect of regucalcin on the calcium–or calcium plus calmodulin-increased protein phosphatase activity towards various phosphoamino acids in the nuclei of rat kidney cortex. The enzyme reaction mixture contained either vehicle, calcium chloride (25 μM), calcium chloride (25 μM) plus calmodulin (5 μg/ml), calcium chloride (25 μM) plus regucalcin (250 nM), calcium chloride (25 μM), and calmodulin (5 μg/ml) plus regucalcin (100 nM). Each value represents the mean±SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value. White bars, control; lined bars, calcium; hatched bars, calcium and calmodulin; double hatched bars, calcium plus regucalcin; black bars, calcium and calmodulin plus regucalcin.

nuclei of rats administered saline for 7 days, as compared with that of control (normal) rats; the densitometric data showed 85.8±4.5 or 40.1±2.8 (percent of control; mean±SEM of four rats) at 2 or 7 days with saline administration, respectively.

The change in protein phosphatase activity towards phosphoamino acids in the nuclei of kidney cortex of rats administered saline for 2 or 7 days is shown in Figure 10. Protein phosphatase activity towards phosphotyrosine or phosphoserine was significantly decreased by saline administration for 2 or 7 days, while the enzyme activity towards phosphothreonine was not significantly decreased by saline administration for 7 days. The effect of anti-regucalcin monoclonal antibody (25 ng/ml) in increasing protein phosphatase activity towards three phosphoamino acids was seen in the renal cortex nuclei of rats administrated saline for 7 days (Fig. 11). However, the enhancing effect of anti-regucalcin monoclonal antibody on protein phosphatase activity was clearly weakened in the renal cortex nuclei of saline-administered rats, because the nuclear regucalcin levels were reduced by saline administration.

DISCUSSION

Regucalcin is mainly present in liver and kidney cortex of rats [Shimokawa and Yamaguchi, 1992; Yamaguchi and Isogai, 1993]. This protein plays a role in the regulation of Ca^{2+}-related cellular function in liver and kidney cortex cells [Yamaguchi, 2000a,b, in review]. Previous study showed that regucalcin has a

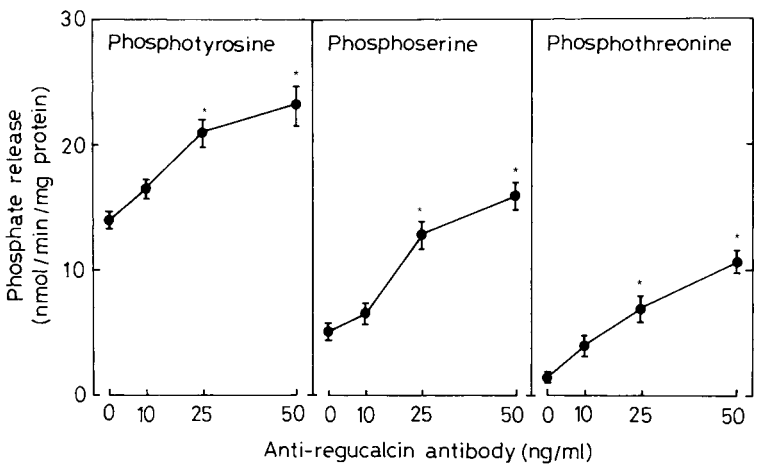

Fig. 6. Effect of anti-regucalcin monoclonal antibody on protein phosphatase activity towards various phosphoamino acids in the nuclei rat kidney cortex. The reaction mixture contained either vehicle or anti-regucalcin monoclonal antibody (10, 25, or 50 ng/ml). Each value represents the mean±SEM of five experiments with different rats. $*P < 0.01$, compared with the control (none) value.

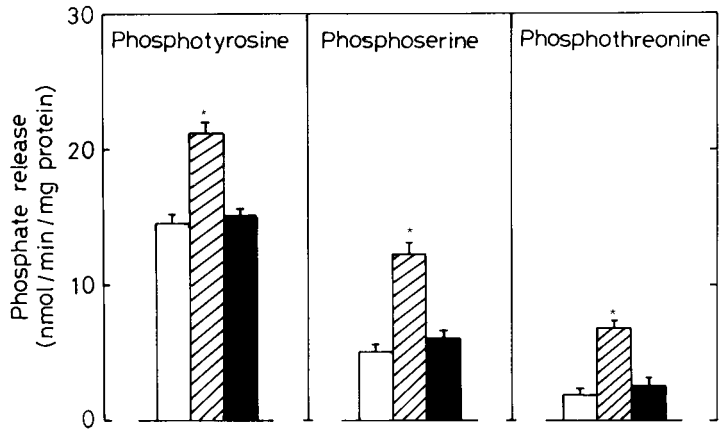

Fig. 7. Effect of regucalcin on the anti-regucalcin monoclonal antibody-increased protein phosphatase activity towards various phosphoamino acids in the nuclei of rat kidney cortex. The enzyme reaction mixture contained either vehicle, anti-regucalcin monoclonal antibody (25 ng/ml), or anti-regucalcin monoclonal antibody (25 ng/ml) plus regucalcin (100 nM). Each value represents the mean±SEM of five experiments with different rats. $^{*}P < 0.01$, compared with the control (none) value. White bars, control; hatched bars, antibody; black bars, antibody plus regucalcin.

suppressive effect on protein phosphatase activity in the cytoplasm of rat kidney cortex cells [Morooka and Yamaguchi, 2001]. The role of regucalcin in the nuclei of rat kidney cortex, however, is not yet known. The present study clearly demonstrates that regucalcin can inhibit protein phosphatase activity in the nuclei of rat kidney cortex, suggesting that regucalcin plays a role in the regulation of the nuclear function in renal cortex cells.

Protein phosphatase activity towards phosphotyrosine, phosphoserine, and phosphothreonine was found in the nuclei of kidney cortex cells. The enzyme activity towards three phosphosamino acids was increased by calcium addition in the enzyme reaction mixture, and this increase was clearly inhibited in the presence of trifluoperazine, an antagonist of calmodulin [Vincenzi, 1982]. Ca^{2+}/calmodulin-dependent protein phosphatase may be localized in the nuclei of kidney cortex. Protein phosphatase activity towards phosphoserine and phosphothreonine was significantly inhibited by cyclosporin A, an inhibitor of calcineurin which is a Ca^{2+}-dependent and calmodulin-stimulated phosphatase [Pallen and Wang, 1983]. Calcineurin may be present in the nuclei of rat kidney cortex. Protein phosphatase activity towards phosphotyrosine was markedly inhibited by vanadate, an inhibitor of protein tyrosine phosphatase [Hunter, 1995] suggesting that protein tyrosine phosphatase is localized in renal cortex nucleus. Protein tyrosine phosphatase activity showed relatively higher levels than that of protein serine/threonine phosphatase in the nuclei of kidney cortex.

Regucalcin is found in the nuclei isolated from rat liver [Omura and Yamaguchi, 1999; Tsurusaki et al., 2000]. Also, this protein was

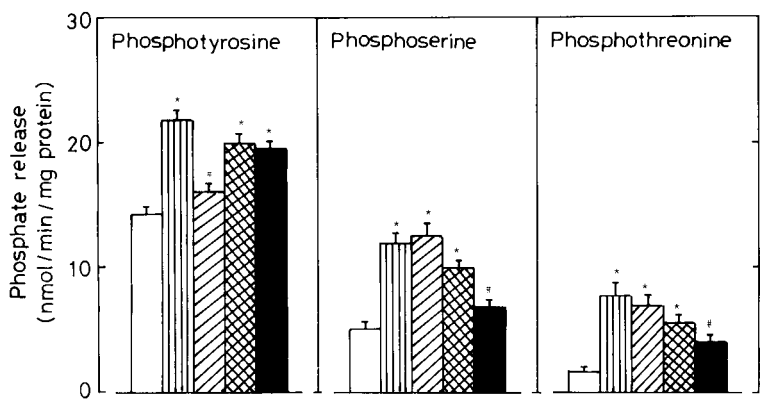

Fig. 8. Effect of various inhibitors on the anti-regucalcin monoclonal antibody-increased protein phosphatase activity towards various phosphoamino acids in the nuclei of rat kidney cortex. The enzyme reaction mixture contained either vehicle, vanadate (10^{-4} M), okadaic acid (10^{-5} M), or cyclosporin A (10^{-5} M) without or with anti-regucalcin monoclonal antibody (25 ng/ml). Each value represents the mean±SEM of five experiments with different rats. $^{*}P < 0.01$, compared with the control (none) value. $^{\#}P < 0.01$, compared with the value for antibody. White bars, control; lined bars, antibody; hatched bars, antibody plus vanadate; double hatched bars, antibody plus okadaic acid; black bars, antibody plus cyclosporin A.

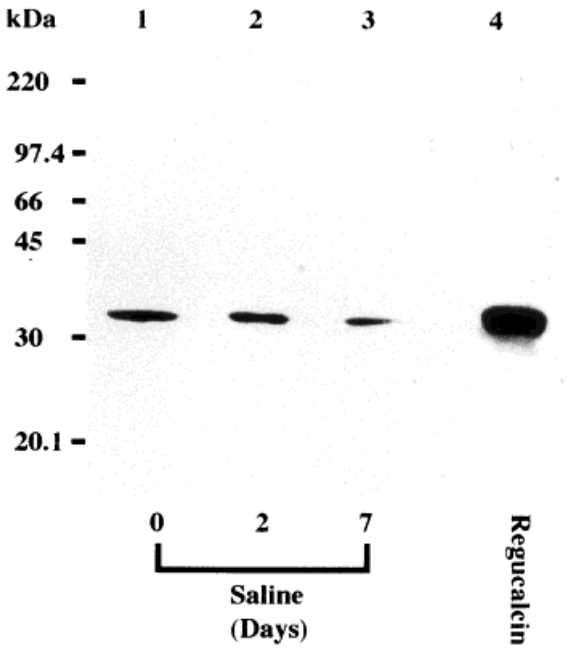

Fig. 9. Quantitative analysis of regucalcin protein in the nuclei of rat renal cortex. Rats were fed with daily intake of saline as a drinking water for 2 or 7 days. Western blot analysis was carried out on the extracts (100 μg nuclear proteins) obtained from the kidney cortex of normal or saline-administered rats. **Lane 1**, normal rat kidney cortex; **lane 2**, saline-administered rats (2 days); **lane 3**, saline-administered rats (7 days); **lane 4**, regucalcin (0.01 μg) as the marker. The figure shows one of four experiments with separate samples.

localized in the nuclei of rat kidney cortex, suggesting its role in the nuclear function. Regucalcin could inhibit protein phosphatase activity towards phosphotyrosine, phosphoserine, and phosphothreonine in the nuclei isolated from rat kidney cortex. Regucalcin may have an inhibitory effect on protein tyrosine phosphatase and protein serine/threonine phosphatase activities in renal cortex nuclei. Regucalcin is a kind of Ca^{2+}-binding protein [Yamaguchi, 2000a]. The effect of regucalcin in inhibiting Ca^{2+}-dependent protein phosphatase activity in renal cortex nucleus is not resulted from its Ca^{2+} binding. Presumably, regucalcin directly binds to protein phosphatases and it reveals an inhibitory effect on protein phosphatase activity in kidney cortex nucleus. Calmodulin and other Ca^{2+}-binding proteins may not have an inhibitory effect on protein phosphatase activity in kidney cortex nucleus.

The presence of anti-regucalcin monoclonal antibody caused a significant increase in protein phosphatase activity towards three phosphoamino acids in the enzyme reaction mixture containing the nuclei of rat kidney cortex, indicating an involvement of endogenous regucalcin. This increase was completely blocked by the addition of regucalcin. Endogenous regucalcin may suppress protein phosphatase activity towards three phosphoamino acids in the nuclei of rat kidney cortex. The effect of antibody in increasing nuclear protein phosphatase activity towards phosphotyrosine was clearly inhibited by vanadate. This result suggests that endogenous regucalcin has a suppressive effect on vanadate sensitive-protein tyrosine phosphatase activity in kidney cortex nucleus. In addition, the effect of anti-regucalcin antibody on protein phosphatase activity towards phosphoserine and phosphothreonine was significantly prevented by cyclosporin A. Endogenous regucalcin may partly inhibit Ca^{2+}-dependent protein serine/threonine phosphatase activities in the nuclei.

Regucalcin mRNA levels were measured in the kidney cortex of rats, and the levels were

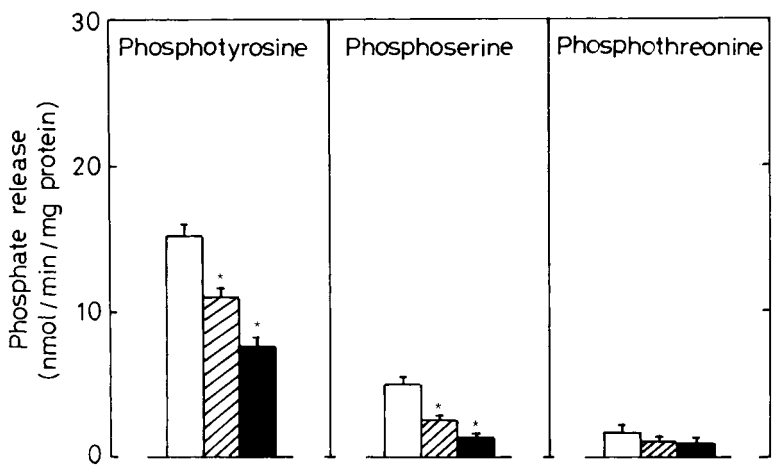

Fig. 10. Alteration in protein phosphatase activity towards various phosphoamino acids in the renal cortex nuclei of rats administered saline. Renal cortex was obtained at 2 or 7 days with daily intake of saline as drinking water. The enzyme activity was measured in a reaction mixture without calcium and calmodulin addition. Each value represents the mean±SEM 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.

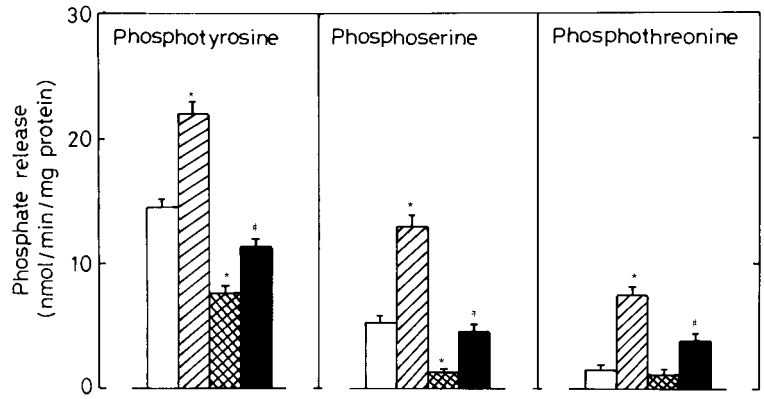

Fig. 11. Effect of anti-regucalcin monoclonal antibody on protein phosphatase activity towards various phosphoamino acids in the renal cortex nuclei of saline-administered rats. Renal cortex was obtained at 7 days with daily intake of saline as drinking water. The enzyme reaction mixture contained either vehicle or anti-regucalcin monoclonal antibody (25 ng/ml). Each value represents the mean±SEM of five experiments with different rats. $^{*}P < 0.01$, compared with the control (none) value. $^{\#}P < 0.01$, compared with the value for saline administration without antibody addition. White bars, control; hatched bars, antibody; double hatched bars, saline administration; black bars, antibody addition with saline administration.

decreased by saline administration for 7 days to rats [Shinya et al., 1996]. Also, saline administration for 7 days produced a significant reduction of regucalcin levels in the cytoplasma of renal cortex [Morooka and Yamaguchi, 2001]. The present study clearly demonstrated that regucalcin in the nuclei of kidney cortex is decreased by saline administration to rats. In addition, saline administration caused a remarkable decrease in protein phosphatase activity towards three phosphoamino acids in the nuclei. Meanwhile, the effect of anti-regucalcin monoclonal antibody in increasing protein phosphatase activity towards three phosphoamino acids in the nuclei was weakened by saline administration. Protein phosphatases, which are suppressed by endogenous regucalcin in the nuclei, may be impaired by saline administration to rats. At present, a pathophysiologic significance of saline administration-induced decrease in protein phosphatase activity in kidney cortex nuclei is unknown. If saline intake leads to hypertensive condition, a decrease in protein phosphatase activity in kidney cortex nuclei may be a part in the development of hypertension with renal impairment.

Dephosphorylation of many phosphorylated proteins is regulated by protein phosphatase in cells [Hunter, 1995]. Protein kinase may be implicated to transcriptional regulation in the nuclei of cells. Regucalcin could inhibit protein phosphatase activity in the nuclei of rat kidney cortex. This finding suggests that regucalcin plays an important role in the regulation of nuclear signaling which is related to gene expression in renal cortex cells. This, however, remains to be elucidated.

In conclusion, it has been demonstrated that endogenous regucalcin has a suppressive effect on protein phosphatase activity in the nuclei of rat kidney cortex cells.

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