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Calcium-binding protein regucalcin mRNA expression in the kidney cortex is suppressed by saline ingestion in rats

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Abstract

The effect of adrenalectomy (ADX) or saline ingestion, which is a hypertensive factor, on the expression of calcium-binding protein regucalcin mRNA in the kidney cortex of rats was investigated. The change of regucalcin mRNA levels was analyzed by Northern blotting using rat liver regucalcin complementary DNA (0.9 kb of open-reading frame). Regucalcin mRNA was expressed in the kidney cortex but not the medulla. Rats were adrenalectomized, and 48 h later they were sacrificed. ADX caused a reduction of regucalcin mRNA levels in the kidney cortex, suggesting that adrenal glands participate in the regulation of the mRNA expression. This reduction was not restored by the subcutaneous administration of dexamethasone with an effective dose (1 mg/kg body weight), which can stimulate kidney regucalcin mRNA expression. Regucalcin mRNA levels in the kidney cortex of rats were markedly suppressed by the ingestion of saline for 7 days. The ADX-induced decrease of renal cortex regucalcin mRNA levels was not appreciably restored by saline ingestion. Moreover, regucalcin mRNA levels in the kidney cortex of spontaneous hypertensive rats (SHR) were clearly decreased as compared with that of control (Wistar-Kyoto) rats. Meanwhile, calcium content in the kidney cortex was not significantly decreased by ADX or saline ingestion. The present study suggests that the expression of regucalcin mRNA in the kidney cortex of rats is suppressed by saline administration. (Mol Cell Biochem **162**: 139–144, 1996)

Key words: regucalcin, calcium-binding protein, gene expression, saline ingestion, hypertensive rats, kidney cortex

Introduction

Calcium ion (Ca^{2+}) plays an important role in the regulation of many cell functions, and it is an intracellular mediator of hormonal action [1–3]. The Ca^{2+} effect is amplified by calmodulin and protein kinase C, which are related to a signal transduction in cells [4, 5]. The Ca^{2+} signaling action is known to be regulated by a novel Ca^{2+}-binding protein regucalcin, which can inhibit the activations of Ca^{2+}/calmodulin-dependent protein kinase [6], protein kinase C [7] and Ca^{2+}-activated DNA fragmentation [8], due to binding of Ca^{2+}. Presumably, regucalcin plays an important role in the regulation of cell functions related to Ca^{2+} signaling [9].

Recently, we have identified a complete nucleotide and amino acid sequences of regucalcin from the cloning of a complementary deoxyribonucleic acid (cDNA) coding for the protein in rat liver [10]. The regucalcin gene is localized on rat chromosome Xqll.1–12 proximal end [11]. The expression of regucalcin mRNA is stimulated by a signaling through Ca^{2+}/calmodulin in rat liver [12]. The supply of calcium in rats can induce an elevation of regucalcin concentration in the liver as measured by an enzyme-linked immunoadsorbent assay [13]. Although regucalcin is predominantly localized in rat liver, the protein is also expressed in the kidney of rats [14, 15].

The kidney plays a physiologic role in the regulation of

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calcium homeostasis in blood by reabsorption of urinary calcium. Regucalcin may play a role in the regulation of mineral metabolism in the kidney, but this is not known presently. More recently, it has been demonstrated that regucalcin mRNA is expressed in the kidney cortex but not the medulla of rats, and that the expression may be mediated through Ca^{2+}/calmodulin action induced by calcium administration [16]. This suggests a role for regucalcin in calcium metabolism of kidney cells. Also, the previous study showed that regucalcin mRNA expression in the kidney cortex is suppressed by aldosterone and that the expression is stimulated by dexamethasone administration in rats [17].

The present investigation was undertaken to clarify whether renal regucalcin mRNA expression is regulated by saline ingestion which may be a hypertensive state. It was found that adrenalectomy (ADX) induces a remarkable suppression of regucalcin mRNA expression in rat kidney cortex, and that the expression is also suppressed by saline ingestion. Moreover, regucalcin mRNA expression was clearly suppressed in the kidney cortex of spontaneous hypertensive rats (SHR).

Materials and methods

Chemicals

Dexamethasone was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Deoxycytidine 5′ -[α-^{32}P] triphosphate ([^{32}P] dCTP; 110 TBq/nmol) and nylon membranes (Hybond N$^+$) for Northern hybridization were obtained from Amersham (Buckinghamshire, UK). A human β-actin gene fragment (0. 43 kb) as an internal standard was obtained from Wako Pure Chemical Co. (Osaka, Japan). Molecularsize standards (0.24–9.5 kb RNA Ladder) for electrophoresis of RNA were purchased from Bethesda Research Laboratories (Gaithersburg, MD, USA). Other reagents were obtained from Wako Pure Chemical Co. Any water and solutions used for RNA preparation were treated with chemical diethylpyrocarbonate (DEPC, Sigma) to inhibit RNase activity.

Animals and administration procedures

Male Wistar rats, 4 (weighing 80–90 g) or 40 weeks old, purchased from Japan SLC Inc. (Hamamatsu, Japan), were fed commercial laboratory chow (solid, Oriental Yeast Co., Ltd., Tokyo, Japan) containing 57.5% carbohydrate, 1.1% calcium and 1.1% phosphorus, and distilled water, *ad libitum*. The adrenal glands were removed with fine forceps under light anesthesia with ether. At 48 h after adrenalectomy (ADX), the rats received the administration of dexamethasone or saline solution. Dexamethasone was dissolved in 99.5% ethanol as a concentration of 0.2 mg/ml. The steroid solution (0.5 ml/100 g body weight; BW) with an effective dose [17] was subcutaneously administered in rats. Rats were sacrificed by bleeding at 3 or 6 h after the steroid administration. Sodium chloride (0.9%, saline), which was dissolved in distilled water, was freely ingested as drinking water for 7 days in normal (or sham operated) or ADX rats. In separate experiments, Wistar-Kyoto rats (control) and spontaneous hypertensive rats (SHR; 5 weeks old) were obtained from Japan SLC Inc., and they were fed 4 days with the commercial laboratory chow described above and sacrificed. Control animals received vehicle solution. Animals were killed by bleeding. The kidney was immediately removed after bleeding, and the kidney cortex was separated. Kidney cortex were frozen at –80°C until use.

Isolation of RNA

Total RNAs in the kidney cortex were prepared as described previously [14,16]. Kidney cortex was rinsed with ice-cold 0.25 M sucrose solution, and homogenized in buffer solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarcosyl, 0.1 M 2-mercaptoethanol and 2 M sodium acetate. Total RNAs were extracted by vigorous shaking in a mixture of phenol, chloroform and isoamyl alcohol, and the phases were separated by centrifugation at 10,000 × g for 20 min at 4°C. RNA located in the aqueous phase was precipitated with isopropanol at –20°C. RNA precipitates were pelleted by centrifugation, and the pellets were redissolved in 50 μl of DEPC-treated 0.5% sodium dodecyl sulfate (SDS).

Northern blotting

Fifteen micrograms of total RNAs extracted from kidney cortex were electrophoresed in 1.2% agarose denaturing gels containing 2.2 M formaldehyde in MOPS buffer (pH 7, containing 20 mM 3-N-morpholino-propanesulfonic acid, 5 mM sodium acetate, and 1 mM EDTA) with 3 V/cm for 3 h [16, 18]. The electrophoresed gels were transferred to nylon membranes by blotting. Total regucalcin cDNA (the 0.9 kb, *Kpn*I-*Pst*I insert) was labeled with [^{32}P] dCTP by random primers with the DNA polymerase Klenow fragment. This radioactive probe was used for hybridization detection of RNAs on blots. The membranes were prehybridized, and hybridized in buffer solution containing 50% formamide, 5 × SSPE (1 × SSPE; 1.15 M NaCl, 10 mM NaH_2PO_4, 1 mM EDTA), 5 × Denhardt's reagent(1 × Denhardt's reagent; 0.02% w/v each of bovine serum albumin, Ficoll, polyvinylpyrrolidine) and 0.5% SDS with ^{32}P-labeled regucalcin cDNA in a sealed plastic bag at 42°C for 16 h. After hybridiza-

tion the membranes were washed as follows: 2 × SSPE and 0.1% SDS at 40°C (twice, each for 15 min), followed by 0.1 × SSPE and 0.1% SDS at room temperature (twice, each for 15 min), and then membranes were exposed to X-ray film for 12 h.

Quantity and integrity of mRNA were monitored by rehybridizing with a radioactive cDNA from β-actin gene fragment under identical conditions. The size of the hybridizing RNA was determined by running the standard RNA molecules of known sizes in parallel. The density of the autoradiographic data was quantified by densitometer scanning (Dual wavelength Flying-spot Scanner, CS-9000, Shimadzu Co., Japan).

Calcium determination

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 [16]. Calcium content was expressed as μg per gram wet tissue.

Statistical methods

The significance of differences between values was estimated by using Student *t*-test; P values of less then 0.05 were considered to indicate a statistically significant difference.

Results

The effect of adrenalectomy (ADX) on regucalcin mRNA expression in the kidney cortex of rats is shown in Fig. 1. Rats were sacrificed 48 h after ADX. Northern blot analysis using the regucalcin cDNA probe showed that regucalcin mRNA was expressed in the kidney cortex but not the medulla, as showed previously [16]. Also, two distinct mRNA isoforms of 1.8 and 1.6 kb which were generated by alternative splicing of common RNA precursor molecules were seen in the tissues, as reported previously [14]; the 1.6 kb mRNA including the open-reading frame (0.9 kb) may be an intermediate molecule of the mature mRNA of regucalcin [10, 14]. ADX caused a remarkable decrease of regucalcin mRNA levels in the kidney cortex as compared with that of normal (sham-operated) rats. The renal cortex mRNA levels were not appreciably altered by increasing ages (40 weeks old). The effect of ADX on renal cortex mRNA levels was also seen in aged rats. Now, β-actin mRNA levels in the same RNA samples were not appreciably altered by ADX; β-actin mRNA levels in the kidney cortex of 4 weeks old rats were 92.5 ± 8.4% of control level.

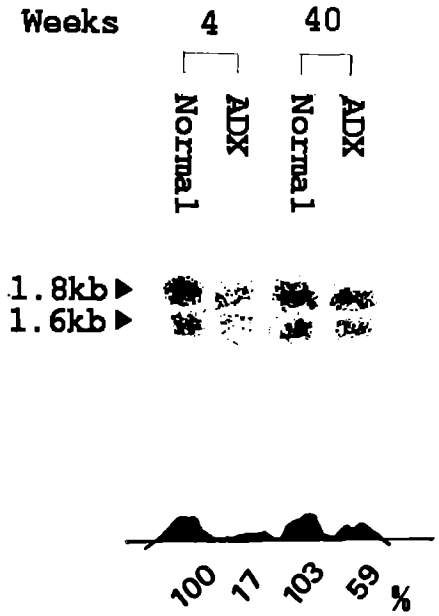

Fig. 1. The alteration of regucalcin mRNA levels in the kidney cortex of adrenalectomized (ADX) rats. Rats (4 or 40 weeks old) were surgically removed adrenal glands, and 48 h later they were sacrificed by bleeding. Total RNAs (15 μg) isolated from the kidney cortex were subjected to Northern blot analysis. The pattern of hybridization obtained with rat liver regucalcin cDNA (0.9 kb of open-reading frame) is shown. The arrowhead indicates hybridizing bands corresponding to mRNA encoding the regucalcin. The mature mRNA (1.8 kb) of regucalcin was used for the quantitation. The figure shows one of four experiments with separate rats. The densitometric data (% of control with 4 weeks old rats; mean ± S.E.M.) for each group were 21 ± 5.1 ($p < 0.01$), 113 ± 10.1 and 58 ± 5.3, respectively. β-Actin mRNA levels in the same samples were not appreciably altered by ADX.

The effect of dexamethasone (DEX) administration on regucalcin mRNA level in the kidney cortex of ADX rats (4 weeks old) is shown in Fig. 2. Dexamethasone (100 ug/100 g body weight) was subcutaneously administered in ADX rats, and 3 or 6 h later they were sacrificed. Regucalcin mRNA levels in the kidney cortex of ADX rats were negligible as compared with that of normal (sham-operated) rats. This reduction was not appreciably restored by the administration of dexamethasone. Also, such restoration was not clearly seen at 24 h after the administration of dexamethason (100 ug/100 g) in ADX rats (data not shown). The vehicle administration (0.5 ml of 99.5% ethanol/100 g) had no effect on renal cortex regucalcin mRNA levels (data not shown).

The effect of saline administration on regucalcin mRNA levels in the kidney cortex of young (4 weeks old) and elderly (40 weeks old) rats was examined, and the result is shown in Fig. 3. Rats were freely given saline as drinking water for 7 days. The administration of saline caused a significant decrease of regucalcin mRNA levels in the kidney cortex of young rats. Thus decrease was also seen in the kidney cortex of elderly rats. Meanwhile, β-actin mRNA levels in the

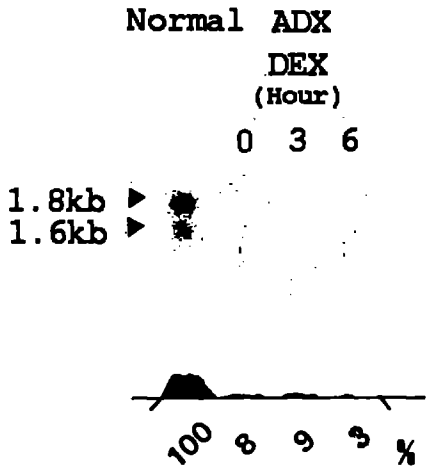

Fig. 2. Effect of dexamethasone administration on regucalcin mRNA levels in the kidney cortex of adrenalectomized rats. Rats were adrenalectomized (ADX), and 48 h later they received a single subcutaneous administration of dexamethasone (100 μg/100 g body weight). The animals were sacrificed at 3 or 6 h after the administration. Total RNAs (15 μg) isolated from the kidney cortex were subjected to Northern blot analysis. The pattern of hybridization obtained with rat liver regucalcin cDNA (0.9 kb of open reading frame) is shown. The arrowhead indicates hybridizing bands corresponding to mRNA encoding the regucalcin. The mature mRNA (1.8 kb) of regucalcin was used for the quantitation. The figure shows one of four experiments with separate rats. Dexamethasone administration in ADX rats had no effect on regucalcin mRNA levels. RNA electrophoressed on the gels was not degradated.

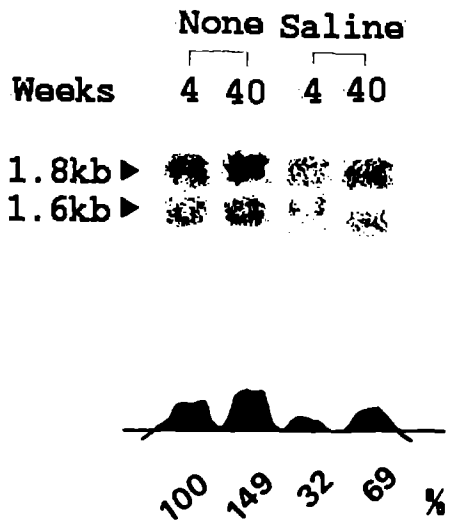

Fig. 3. The alteration of regucalcin mRNA levels in the kidney cortex of rats ingested with saline. Rats were freely given saline as drinking water for 7 days. Total RNAs (15 μg) isolated from the kidney cortex were subjected to Northern blot analysis. The pattern of hybridization obtained with rat liver regucalcin cDNA (0.9 kb of open-reading frame) is shown. The arrowhead indicates hybriding bands corresponding to mRNA encoding the regucalcin. The mature mRNA (1.8 kb) of regucalcin was used for the quantitation. The figure shows one of four experiments with separate rats. The densitometric data (% of control with 4 weeks old rats; mean ± S.E.M.) for each group were 135 ± 15.1, 35 ± 4.3 and 62 ± 5.7, respectively. β-Actin mRNA levels in the same samples were not appreciably altered by saline ingestion.

same RNA samples were not altered by saline ingestion; the mRNA levels were 97.9 ± 6.4% of control level.

The administration of saline in ADX rats did not cause an appreciable alteration of regucalcin mRNA levels in the kidney cortex (Fig. 4), when ADX rats (4 weeks old) were freely given saline for 7 days. Thus, the ADX-induced reduction of regucalcin mRNA levels in the kidney cortex was not restored by saline ingestion.

The alteration of calcium content in the kidney cortex was examined. The administration of saline for 7 days did not cause a significant decrease of renal cortex calcium content in comparison with that of normal rats (data not shown). Also, the calcium content was not changed by ADX. Presumably, ADX or saline ingestion induces a decrease of regucalcin mRNA expression in the kidney cortex of rats independent on the decrease in renal cortex calcium content, since the increase in renal cortex calcium can stimulate regucalcin mRNA expression in rat kidney cortex [16].

The expression of regucalcin mRNA in the kidney cortex of spontaneous hypertensive rats (SHR) was examined, and the result is shown in Fig. 5. Regucalcin mRNA levels in the kidney cortex of SHR were clearly decreased as compared with that of control (Wistar-Kyoto) rats. β-Actin mRNA levels in the same RNA samples were not altered in SHR; the mRNA levels were 95.3 ± 7.2% of control. Also, the RNA electrophoresed on the gels was not degradated.

Discussion

Calcium-binding protein regucalcin mRNA is expressed in the kidney cortex but not the medulla of rats [16]. This expression is stimulated by the renal cortex calcium which is increased by calcium administration in rats [16]. The kidney cortex constitutes nephron including glomerullar reabsorption of calcium. Regucalcin in the kidney cortex may be involved in a physiological function in the glomerullar filtration and tubular reabsorption of calcium, although this is not clarified fully. The administration of aldosterone in rats caused an appreciable decrease of regucalcin mRNA expression in the kidney cortex [17], suggesting that the mRNA expression is partly regulated by the adrenal glands or the steroid hormone-stimulated renal sodium reabsorption [19]. In the present study, it was found that adrenalectomy (ADX) or saline ingestion induces a remarkable decrease of regucalcin mRNA expression in the kidney cortex of rats.

A synthetic glucocorticoid dexamethasone with an effective dose (1 mg/kg) may have a stimulatory effect on regucalcin mRNA expression in the kidney cortex of rats [17]. ADX may deplete steroid hormones which are secreted from adrenal glands. To clarify a possible involvement of steroid hormones, dexamethasone was administered to ADX rats.

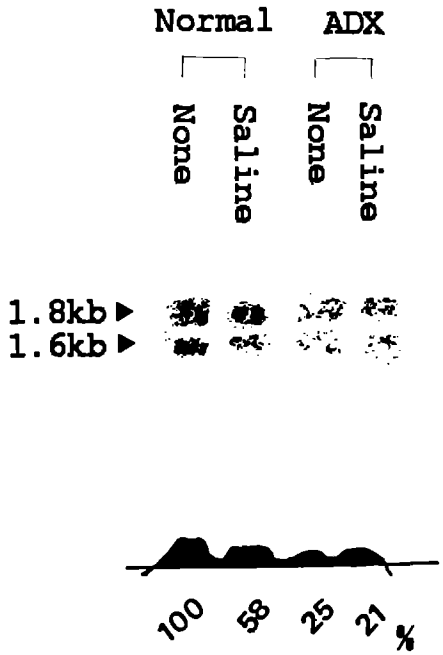

Fig. 4. The alteration of regucalcin mRNA levels in the kidney cortex of ADX rats ingested with saline. ADX rats were freely given saline as drinking water for 7 days. Total RNAs (15 μg) isolated from the kidney cortex were subjected to Northern blot analysis. The pattern of hybridization obtained with rat liver regucalcin cDNA (0.9 kb of open-reading frame) is shown. The arrowhead indicates hybridizing bands corresponding to mRNA encoding the regucalcin. The mature mRNA (1.8 kb) of regucalcin was used for the quantitation. The figure shows one of four experiments with separate rats. The densitometric data (% of control with normal rats; mean ± S.E.M.) for each group were 52 ± 4.7, 21 ± 3.6 and 18 ± 2.9, respectively. β-Actin mRNA levels in the same samples were not appreciably altered by saline ingestion.

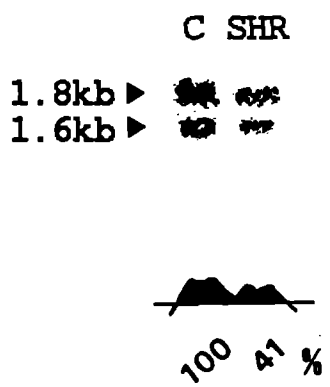

Fig. 5. The alteration of regucalcin mRNA expression in the kidney cortex of Wistar-Kyoto rats (C) and spontaneous hypertensive rats (SHR). Total RNAs (15 μg) isolated from the kidney cortex were subjected to Northern blot analysis. The pattern of hybridization obtained with rat liver regucalcin cDNA (0.9 kb of open-reading frame) is shown. The arrowhead indicates hybridizing bands corresponding to mRNA encoding the regucalcin. The mature mRNA (1.8 kb) of regucalcin was used for the quantitation. The figure shows one of four experiments with separate rats. The densitometric data (% of control; mean ± S.E.M.) were 39 ± 3.7. β-Actin mRNA levels in the same samples were not appreciably altered in SHR.

This treatment did not prevent the ADX-induced suppression of regucalcin mRNA expression in the kidney cortex of rats. The suppressive effect of ADX on renal regucalcin mRNA expression was intensive. Presumably, adrenal glands have an important role in the regulation of regucalcin mRNA expression in the kidney cortex of rats. Conversely, aldosterone can stimulate renal sodium reabsorption [19]. The steroid hormone administration in normal rats caused a significant decrease of regucalcin mRNA expression in the kidney cortex [17]. From this result, it is assumed that regucalcin mRNA expression in the kidney cortex is partly involved in an alteration of renal sodium transport. However, the ingestion of saline caused a clear suppression of regucalcin mRNA expression in the kidney cortex of rats. Moreover, the supply of saline in ADX rats did not restore the ADX-induced suppression of renal regucalcin mRNA expression, suggesting that ADX reveals a suppressive effect on regucalcin mRNA expression independent on sodium transport in the kidney cortex of rats.

The aldosterone administration-induced suppression of regucalcin mRNA expression in the kidney cortex of rats, as reported previously [17], may be related to renal sodium reabsorption because saline ingestion can decrease the kidney regucalcin mRNA expression. At present, the mechanism is unknown. Sodium-calcium exchangeable transport system is existed in kidney cortex [20]. Regucalcin mRNA expression in the kidney cortex is stimulated by calcium administration in rats, which can increase the tissue calcium content [16]. However, saline administration to rats did not cause a significant decrease of calcium content in the kidney cortex, suggesting that renal calcium does not participate in the saline ingestion-induced suppression of the regucalcin mRNA expression. Presumably, the increase in renal sodium following saline ingestion induces the suppression of regucalcin mRNA expression in the kidney cortex of rats. It is possible, however, that sodium movement influences on Ca^{2+} signal transduction that stimulates transcriptional activity in kidney cells.

Regucalcin mRNA expression was suppressed in the kidney cortex of spontaneous hypertensive rats (SHR). Saline ingestion, which may generate hypertensive condition, clearly decreased regucalcin mRNA expression in the kidney cortex. Presumably, an alteration in renal sodium transport following saline ingestion influences on the expression of regucalcin mRNA in the kidney cortex of rats. Whether the suppressed expression of regucalcin mRNA is involved in hypertensive state, however, remains to be clarified.

In conclusion, it has been demonstrated that mRNA expression of the Ca^{2+}-binding protein regucalcin in the kidney cortex is suppressed by administration of saline in normal or adrenalectomized rats, and that the expression is also decreased in spontaneous hypertensive rats (SHR).

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