Regulation by 1α,25-dihydroxyvitamin D_3 of expression of stanniocalcin messages in the rat kidney and ovary

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Abstract Regulation by vitamin D_3 of expression of the genes for stanniocalcins 1 and 2 (STC-1 and STC-2) was studied and their levels were shown to be oppositely regulated in the kidney and to remain unaffected in the ovary. Female rats were treated with calcitriol, the active form of vitamin D_3, and alterations in the levels of STC-1 and STC-2 mRNA were determined by Northern blot analysis in the kidney and ovary where the STC-1-expressing cells have previously been identified by in situ hybridization histochemistry. In the kidney, calcitriol treatment increased the STC-1 mRNA levels more than 3-fold, but decreased the STC-2 message to trace levels. In the ovary, however, both STC-1 and STC-2 mRNA levels were not significantly affected by the calcitriol treatment. These results support the hypotheses that (1) STC-1 and STC-2 have opposite effects on calcium and phosphate homeostasis, namely anti-hypercalcemic and anti-hypocalcemic actions, respectively, and (2) the mammalian stanniocalcin system acquired, in addition to the role in the systemic mineral metabolism, a role in the reproduction system that operates independently of the systemic condition.

Key words: Stanniocalcin; Calcitriol; Stanniocalcin-related protein; Hypercalcemia

1. Introduction

Stanniocalcins are a group of glycoprotein hormones that are involved in the maintenance of calcium and phosphate homeostasis [1]. Stanniocalcin was first (1) identified as a disulfide-linked homodimeric hormone secreted from the corpuscles of Stannius, endocrine glands associated with the kidneys of the bony fishes, (2) shown to prevent hypercalcemia by reducing Ca^{2+} influx from the gill, by promoting renal reabsorption of phosphate which chelates excess Ca^{2+} and stimulates its deposition to bones and scales, and by inhibiting Ca^{2+} uptake from the intestine, and (3) considered to be a regulator unique to fishes for a long time until the discovery of its mammalian counterparts, human [2,3] and mouse [4] stanniocalcin. The mammalian stanniocalcin is similar to the fish hormone in its amino acid sequence and anti-hypercalcemic actions but exhibits quite different tissue distribution; in mammals, stanniocalcin occurs in a wide variety of tissues including the kidney, brain, heart, lung, bone, and ovary [2–13].

Recently, a related protein of ~300 amino acid residues was cloned by three groups [14–16] based on the sequence information (AA497118, AA195455, AA298636, AA456244, AA497040, AA223369, AA423593) in the EST database dbEST and named stanniocalcin 2 (STC-2) [14,16] or stanniocalcin-related protein (STCrP) [15]. In the case of the STC-2 nomenclature, stanniocalcin was renamed stanniocalcin 1 (STC-1). Although STC-1 and STC-2 share a moderate sequence similarity (~40%) in their N-terminal region, they are quite divergent in the C-terminal region. Furthermore, STC-1 and STC-2 have been suggested to exert opposite effects on the phosphate uptake mediated by the Na^+/phosphate cotransporter 3 [16].

In contrast to the detailed characterization of the biochemical and physiological properties of stanniocalcins, their regulation of expression is poorly understood. While the mode of regulation of expression of the fish stanniocalcin gene in the corpuscles of Stannius has been studied to some extent [17], that of the mammalian genes for STC-1 and STC-2 remains poorly characterized. We report here the effects of 1α,25-dihydroxyvitamin D_3 (calcitriol, the hormonal form of vitamin D) on the levels of stanniocalcin mRNA in the rat kidney and ovary.

2. Materials and methods

2.1. Animal treatment

Female Sprague-Dawley rats (6 weeks old) were obtained from the Tokyo Laboratory Animals Co. and housed 2–3 per cage, given food and water ad libitum, and maintained on a normal 12-h light/dark cycle. Calcitriol (1 μg/kg/day) or saline was injected intraperitoneally for 2 and 6 days, 1 h before the dark cycle. At the end of the experimental period, animals were killed by decapitation, and the kidneys and ovaries were removed, immediately frozen in liquid nitrogen, and stored at −80°C until use.

2.2. Preparation of cDNA probes

To obtain STC-1 and STC-2 cDNA probes, we first amplified by PCR a part of the human STC-1 and rat STC-2 sequence covering the coding region, using human and rat kidney cDNA as templates, which was performed using ExTaq (Takara) DNA polymerase and the following oligonucleotide primers: 5′-TGTGAATAACCTCTCCCTGG-3′ (STC-1, antisense), 5′-TAGCGGAAACTTCTCAGAGA-3′ (STC-1, sense), 5′-CTTTCATTTCACCTCCGGAT-3′ (STC-2, antisense) and 5′-ATACCAAGAACCATGTGTGC-3′ (STC-2, sense). The PCR was run for 35 cycles by repeating denaturation at 94°C for 30 s, annealing at 60°C for 1 min and polymerization at 72°C for 2 min. Products of expected sizes were isolated by agarose gel electrophoresis, purified, and then subcloned into the *Eco*RV site of pBluescript and sequenced. The novel rat STC-2 sequence covering all the coding region has been submitted to the DDBJ/GenBank/EMBL Data Bank with accession number AB030707. Rat STC-2 consists of 296 amino acid residues and shares ~96% amino acid sequence identity with mouse STC-2 (296 amino acid residues) and ~84% identity with human STC-2 (302 amino acid residues). Our original intention in isolating human STC-1 cDNA was to raise monospecific

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antisera against the recombinant protein, but here we used it for quantification of rat STC-1 mRNA since it is highly homologous to the rat sequence (~92%).

2.3. Total RNA isolation and Northern blot analysis

Total RNA was isolated by the acid guanidinium thiocyanate/phenol/chloroform method. For Northern analysis, 20 μg of total RNA was electrophoresed in 1% agarose gel containing formaldehyde and transferred to Magna MT nylon membrane (Micron Separation) by capillary blotting overnight using 20×SSC (SSC: 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) as the transfer buffer. After transfer, membranes were baked for 2 h at 80°C and prehybridized for 30 min at 37°C in 5×SSPE (SSPE: 0.15 M NaCl, 1 mM EDTA, and 10 mM NaH_2PO_4, pH 7.4) containing 50% formaldehyde, 5×Denhardt's solution (Denhardt's: 0.1% each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin), and 0.5% SDS. The above PCR-derived human STC-1 and rat STC-2 cDNA was labeled with [α-^{32}P]dCTP (3000 Ci/mmol, Amersham Pharmacia Biotech) using a Ready-To-Go DNA labeling kit (Amersham Pharmacia Biotech), and the unincorporated nucleotides were removed by passage through a Sephadex G-50 column (Amersham Pharmacia Biotech). Hybridization was performed at 42°C for 16 h. After hybridization, the membranes were rinsed twice with 2×SSC and 0.1% SDS for 30 min at 50°C, washed with 1×SSC and 0.1% SDS for 30 min at 55°C and twice with 0.5×SSC and 0.1% SDS for 1 h at 55°C, and exposed to imaging plates (Fuji Film) in a cassette for 1 week. The results were analyzed using a Fujix BAS2000 Bio-imaging analyzer (Fuji Film).

3. Results

3.1. Verification of calcitriol treatment

The effectiveness of the calcitriol treatment was verified by measuring the plasma levels of Ca^{2+}. Following the treatment, plasma concentration of Ca^{2+} was increased from 2.47 ± 0.16 mM (n = 4) to 3.13 ± 0.19 (n = 5, day 2) and 3.10 ± 0.18 mM (n = 5, day 6), demonstrating the well established hypercalcemic effect of calcitriol.

3.2. Up-regulation of STC-1 and down-regulation of STC-2 gene expressions in the rat kidney on calcitriol treatment

For Northern blot analyses, we chose the kidney and ovary as the sources of mRNA since they have been demonstrated to contain relatively high levels of stanniocalcin mRNA and furthermore they are so far the only mammalian tissues in which the sites of STC-1 expression have been clarified at the cellular level by autoradiographic in situ hybridization histochemistry: the cortical and medullary collecting duct cells of the kidney [11] and the secondary interstitial cells and theca-interna cells of the ovary [12] have been established as the major sites of STC-1 mRNA expression. As shown in Fig. 1, the STC-1 message levels in the kidney increased about 3-fold on calcitriol treatment (lanes 5 and 6 compared with lane 4). In marked contrast, STC-2 mRNA expression was reduced to about 20% of the level found before calcitriol treatment (Fig. 2, lanes 5 and 6 compared with lane 4). This tendency is somewhat surprising but consistent with the recent observation by Ishibashi et al. [16] that the effect of STC-2 on the Na^+/phosphate cotransporter 3 was opposite to that of STC-1 and strongly suggests that although STC-1 and STC-2 belong to the same stanniocalcin family, they act as anti-hypercalcemic and anti-hypocalcemic agents, respectively, to restore normocalcemia. Together with previous reports from other laboratories describing modulations by STC-1 of renal phosphate excretion [3,18] and of intestinal Ca^{2+} and phosphate uptake [19], the calcitriol sensitivity of STC-1 and STC-2 expression, demonstrated here in the mammalian system for the first time, indicates that the mammalian stanniocalcin system also plays important roles in maintaining systemic calcium and phosphate homeostasis by working in harmony with other calcium-mobilizing hormones.

3.3. Stanniocalcin levels in the rat ovary are regulated independently of the systemic calcium levels

Using the mouse, Varghese et al. [12] have shown that the ovary contains the highest levels of STC-1 mRNA. We therefore determined STC-1 and STC-2 message levels in the ovary of calcitriol-treated rats by Northern blot analysis (lanes 1–3 in Figs. 1 and 2). Our results indicated that the STC-2 gene was also highly expressed in the ovary but neither STC-1 nor STC-2 mRNA levels were significantly altered by the treatment, suggesting that the ovary has an autocrine/paracrine stanniocalcin system working independently of the calcium levels in the systemic circulation. The function of the ovarian stanniocalcins, for example whether they are involved in the maintenance of Ca^{2+} and phosphate homeostasis within the ovary or whether they gained a completely different reproductive role during evolution of the mammalian species, remains to be clarified.

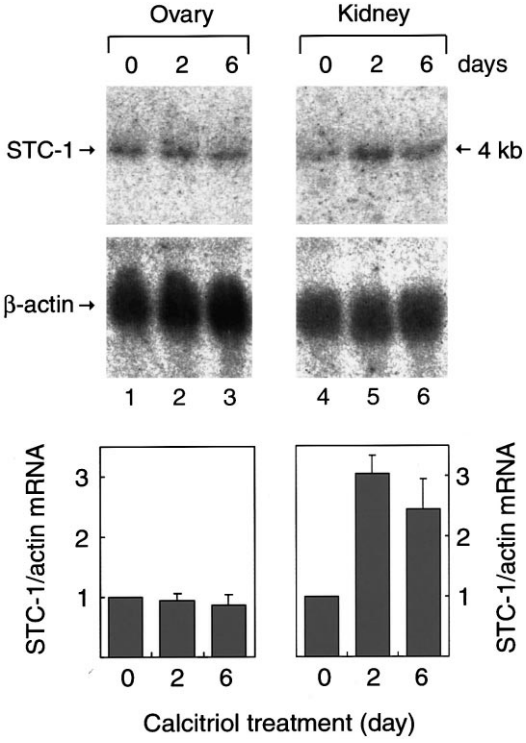

Fig. 1. Northern blot analysis of STC-1 mRNA in the ovary (A) and kidney (B) of the control and calcitriol-treated rats. Upper panels: STC-1 mRNA levels probed with ^{32}P-labeled STC-1 cDNA. Middle panels: loading controls showing the levels of β-actin mRNA. Total RNA was prepared from the tissues on day 0 (lanes 1 and 4), 2 (lanes 2 and 5), and 6 (lanes 3 and 6) after calcitriol treatment. Each lane contains 20 μg of total RNA preparations. Lower panels: densitometric analysis. Quantitation was performed by measuring the photostimulated luminescence (PSL) values using a Fuji Film bioimage analyzer (Model 2000) and by comparing STC-1 transcript levels with those of β-actin. The ratio of STC-1 transcript to β-actin at day 0 was set at 1 (n = 3).

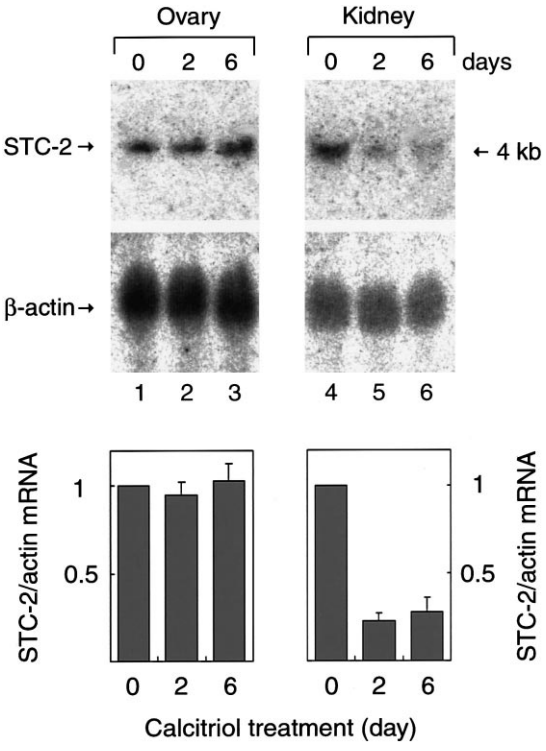

Fig. 2. Northern blot analysis of STC-2 mRNA in the ovary (A) and kidney (B) of the control and calcitriol-treated rats. β-Actin mRNA levels (middle panels) were used as loading controls. Total RNA was prepared from the tissues after 0 (lanes 1 and 4), 2 (lanes 2 and 5), and 6 (lanes 3 and 6) days of calcitriol treatment. Each lane contains 20 μg of total RNA preparations. Quantitation was performed as in Fig. 1 (lower panels, $n = 3$).

4. Discussion

In the present study, we determined the effects of calcitriol on the message levels of STC-1 and STC-2 in the rat kidney and ovary. The major findings are as follows. (1) STC-1 mRNA levels were increased in the kidney of calcitriol-treated rats; this is consistent with the anti-hypercalcemic nature of STC-1. (2) In the case of STC-2, however, a marked reduction occurred. (3) In the ovary, both STC-1 and STC-2 mRNA levels remained unaffected by the treatment, suggesting the presence of an independently operating ovarian stanniocalcin system. Of special interest is the second finding, which supports the recent communication by Ishibashi et al. [16] reporting an inhibitory effect of STC-2 on the Na^+/phosphate cotransporter activity (cf. STC-1 is a stimulator of the transporter) and strongly suggests that STC-1 and STC-2 are counteracting molecules, STC-1 being anti-hypercalcemic and STC-2 anti-hypocalcemic. In this context, structural features unique to STC-2 may deserve comment: Chang and Reddel [14] have noted the presence of a cluster of histidine residues (HHxxxxHH) in its C-terminal portion and pointed out its potential for interaction with metal ions; and Hulova and Kawauchi [20] have shown that although all 10 cysteine residues involved in intramolecular disulfide bonds are completely conserved, including their spacings, among the stanniocalcin molecules so far sequenced, the position of the 11th cysteine residue that is responsible for the disulfide-linked homodimer formation is not spatially conserved between STC-1 and STC-2.

Calcitriol has been established to be hypercalcemic and there are several reports that suggest that increased Ca^{2+} levels stimulate the STC-1 gene expression or stabilize the STC-1 message. For example, Chang et al. [2] have demonstrated using a human cell line that the STC-1 mRNA levels are positively regulated by Ca^{2+} in the medium; Srivastav et al. [21] have shown that plasma Ca^{2+} and stanniocalcin levels in tilapia are increased by calcitriol treatment; Sato et al. [7] have identified stanniocalcin as a protein whose mRNA is strongly inducible in vascular endothelial cells treated with lysophosphatidylcholine which is known to increase Ca^{2+} influx [22]; and Ellis and Wagner [23] have demonstrated using primary culture of trout corpuscles of Stannius cells that STC-1 mRNA is stabilized by Ca^{2+}. It is therefore likely that the above mentioned effects of calcitriol on the STC-1 and STC-2 mRNA levels are mediated through Ca^{2+}. Whether the regulations occur at the level of transcription or, like the fish corpuscles of Stannius [23], at the level of post-transcription or both remains to be determined.

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References

[1] Wagner, G.F. (1994) in: Molecular Endocrinology of Fish (Farrel, A.P. and Randall, D.J., Eds.), Fish Physiology, Vol. XIII, pp. 273–306, Academic Press, San Diego, CA.
[2] Chang, A.C., Janosi, J., Hulsbeek, M., de Jong, D., Jeffrey, K.J., Noble, J.R. and Reddel, R.R. (1995) Mol. Cell. Endocrinol. 112, 241–247.
[3] Olsen, H.S., Cepeda, M.A., Zhang, Q.Q., Rosen, C.A., Vozzolo, B.L. and Wagner, G.F. (1996) Proc. Natl. Acad. Sci. USA 93, 1792–1796.
[4] Chang, A.C., Dunham, M.A., Jeffrey, K.J. and Reddel, R.R. (1996) Mol. Cell. Endocrinol. 124, 185–187.
[5] Liang, P., Averboukh, L., Keyomarsi, K., Sager, R. and Pardee, A.B. (1992) Cancer Res. 52, 6966–6968.
[6] Haddad, M., Roder, S., Olsen, H.S. and Wagner, G.F. (1996) Endocrinology 137, 2113–2117.
[7] Sato, N., Kokame, K., Shimokado, K., Kato, H. and Miyata, T. (1998) J. Biochem. (Tokyo) 123, 1119–1126.
[8] De Niu, P., Olsen, H.S., Gentz, R. and Wagner, G.F. (1998) Mol. Cell. Endocrinol. 137, 155–159.
[9] Zhang, K.Z., Westberg, J.A., Paetau, A., von Boguslawsky, K., Lindsberg, P., Erlander, M., Guo, H., Su, J., Olsen, H.S. and Andersson, L.C. (1998) Am. J. Pathol. 153, 439–445.
[10] Yoshiko, Y. and Maeda, N. (1998) Mol. Cell. Endocrinol. 141, 37–40.
[11] Wong, C.K., Ho, M.A. and Wagner, G.F. (1998) J. Endocrinol. 158, 183–189.
[12] Varghese, R., Wong, C.K., Deol, H., Wagner, G.F. and DiMattia, G.E. (1998) Endocrinology 139, 4714–4725.
[13] Yoshiko, Y., Son, A., Maeda, S., Igarashi, A., Takano, S., Hu, J. and Maeda, N. (1999) Endocrinology 140, 1869–1874.
[14] Chang, A.C. and Reddel, R.R. (1998) Mol. Cell. Endocrinol. 141, 95–99.
[15] DiMattia, G.E., Varghese, R. and Wagner, G.F. (1998) Mol. Cell. Endocrinol. 146, 137–140.
[16] Ishibashi, K., Miyamoto, K., Taketani, Y., Morita, K., Takeda, E., Sasaki, S. and Imai, M. (1998) Biochem. Biophys. Res. Commun. 250, 252–258.
[17] Wagner, G.F. and Jaworski, E. (1994) Mol. Cell. Endocrinol. 99, 315–322.

[18] Wagner, G.F., Vozzolo, B.L., Jaworski, E., Haddad, M., Kline, R.L., Olsen, H.S., Rosen, C.A., Davidson, M.B. and Renfro, J.L. (1997) J. Bone Miner. Res. 12, 165–171.
[19] Madsen, K.L., Tavernini, M.M., Yachimec, C., Mendrick, D.L., Alfonso, P.J., Buergin, M., Olsen, H.S., Antonaccio, M.J., Thomson, A.B. and Fedorak, R.N. (1998) Am. J. Physiol. 274, G96–102.
[20] Hulova, I. and Kawauchi, H. (1999) Biochem. Biophys. Res. Commun. 257, 295–299.
[21] Srivastav, A.K., Flik, G. and Wendelaar Bonga, S.E. (1998) Gen. Comp. Endocrinol. 110, 290–294.
[22] Chen, Y., Morimoto, S., Kitano, S., Koh, E., Fukuo, K., Jiang, B., Chen, S., Yasuda, O., Hirotani, A. and Ogihara, T. (1995) Atherosclerosis 112, 69–76.
[23] Ellis, T.J. and Wagner, G.F. (1995) J. Biol. Chem. 270, 1960–1965.