ELSEVIER

Bone 40 (2007) S102 – S107

BONE

www.elsevier.com/locate/bone

Abstracts

Comparative Endocrinology of Calcium Regulation—Sunday

Comparative Endocrinology

Sunday, June 24, 2007

CE01

Calcium storage in sarcoplasmic reticulum: The role of calcium pumps and binding proteins during elevated influx in calcifying crayfish

M. Wheatly[1], Y. Gao[1], C. Gillen[2], Z. Jamal[1]
[1]Wright State University, Dayton, OH
[2]Kenyon College, Gambier, OH, USA

The sarcoplasmic reticulum (SR) is the primary intracellular calcium (Ca) store (ICS). Mineralization events typically require mass vectorial Ca flux across epithelial cells. The role of the ICS in sequestering Ca at these times can be explored through examining expression of associated proteins including the Sarco/Endoplasmic Reticulum Ca ATPase (SERCA) and the Sarcoplasmic Ca binding Protein (SCP). The comparative model employed in our laboratory is the molting cycle of the freshwater crayfish where whole animal Ca influx is heightened in postmolt to effect cuticular mineralization as opposed to intermolt when the organism exhibits Ca balance. Epithelial tissues (antennal gland, renal; hepatopancreas, digestive) were compared with non-epithelial tissues (abdominal and cardiac muscle). Techniques of analysis involved real time PCR, Northern blotting, Western analysis, in situ, and immunofluorescence. SERCA and SCP expression were both downregulated in postmolt compared with intermolt stage, suggesting that their cellular functions are linked. Other studies in our lab have shown that Ca importers (epithelial Ca channel, ECaC) and basolateral exporters (Plasma Membrane Ca ATPase, PMCA; NCX, Sodium/Calcium eXchanger) both exhibit upregulation during postmolt vectorial Ca influx compared with intermolt. Collectively this suggests that there is reciprocal regulation between Ca transporting mechanisms on external (plasma) membranes and internal (SR) membranes. Seemingly the need for Ca sequestration in SR decreases when Ca is moving rapidly through epithelial cells en route to the extracellular fluid and thence to mineralization sites. Supported by US National Science Foundation grant IBN 0445202.

M. Wheatly, None.

CE02

The comparative endocrinology of STC-1

Graham Wagner, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, Ontario, Canada

The polypeptide hormone, stanniocalcin-1 (STC-1) was originally identified in bony fishes as a systemic regulator of mineral metabolism, and is best known for its regulatory effects on calcium/phosphate transport by the gills, gut and kidneys. The discovery of mammalian forms of STC-1 has resulted in progressively growing interest as to its possible role in humans. Moreover, new discoveries related to the mammalian hormone are resulting in significant reappraisals as to its role in fishes. This review covers the comparative endocrinology of STC-1 from the perspectives of structure, function and regulation. It also delves into one of the more intriguing aspects of its biology; receptor-mediated sequestration of STC-1 by target cell organelles. New information of fish STC-1 receptors will also be presented. Based on our currently state of knowledge, it is apparent that STC-1 has an ancient lineage and has significant roles in metabolism, reproduction and development.

CE03

Effect of physical stress on the osteoblasts and osteoclasts: Analysis of bone metabolism using goldfish scale as a model for bone

N. Suzuki
Noto Marine Laboratory, Institute of Nature and Environmental Technology, Kanazawa University, Noto-cho, Ishikawa 927-0553, JAPAN

Teleost scale is a calcified tissue that contains osteoblasts, osteoclasts, and bone matrix, all of which are similar to those found in human bones. Recently, a new in vitro model system using goldfish scale was developed. This system can be used to simultaneously detect the activities of both scale osteoclasts and osteoblasts with tartrate-resistant acid phosphatase and alkaline phosphatase as the respective markers and precisely analyze the co-relationship between osteoblasts and osteoclasts. Using this system, we analyzed the bone metabolism by physical stress: acceleration and ultrasound (US) stimulation.

The bone metabolism under various degrees of acceleration (0.5-, 1-, 2-, 4-, and 6-G) with a G-load apparatus was examined.

8756-3282/$ - see front matter
doi:10.1016/j.bone.2007.04.163

After loading for 5 and 10 min, the scales were incubated for 6 and 24 h. The osteoblastic and osteoclastic activities were then measured. The osteoblastic activities gradually increased corresponding to 1-G to 6-G acceleration. In addition, ER mRNA expression was the highest under 6-G acceleration. On the other hand, the osteoclastic activity decreased at 24 h of incubation under low acceleration (0.5- and 1-G). This change coincided with TRAP mRNA expression. Under 2-G acceleration, the strength of suppression in osteoclastic activity was the highest. At both 6 and 24 h of incubation, the osteoclastic activity decreased under 2-G acceleration. The strength of the inhibitory action under 4- and 6-G acceleration was lower than that under 2-G acceleration.

Osteoblastic activity significantly increased by pulsed low-density US (1 MHz, 60 mW/cm^2 I_{SATA}, 50% duty factor at 0.5 Hz, 180 pulses) in 18 h of incubation at 15 °C after US treatment but not in shorter incubation periods, while osteoclastic activity did not change in the same incubation period. To examine the mechanism of US in osteoblasts, estrogen receptor (ER) and insulin-like growth factor-I (IGF-I) mRNA expressions in the cultured scales were analyzed by RT-PCR. ER mRNA expression was found to be higher in the US-treated scales than in the control scales in 18 h of incubation at 15 °C after treatment, although ER mRNA expression did not change in 3 h of incubation. On the other hand, IGF-I mRNA expression increased in 3 h of incubation at 15 °C after US treatment. Therefore, IGF-I mRNA expression was more rapid to respond to US than ER mRNA expression, and IGF-I may have an important function in activation of osteoblasts by US treatment.

In our co-culture system, osteoblasts and osteoclasts reacted sensitively to several degrees of acceleration and US stimulation. Therefore, we strongly believe that our in vitro co-culture system is useful for the analysis of bone metabolism under physical stress.

N. Suzuki, None.

CE04

Parathyroid hormone-related protein-like genes in the cartilaginous fish, *Callorhinchus milii*

A.S. Ibrahim[1], T.I. Walker[2], J. Bell[2], S. Brenner[3], B. Venkatesh[3], **J.A. Danks**[1]
[1]RMIT University, Bundoora, AUSTRALIA
[2]Marine and Freshwater Resources Insititute, Queenscliff, AUSTRALIA
[3]Institute of Molecular and Cell Biology, Singapore, SINGAPORE

Parathyroid hormone-related protein (*pthrp*) genes have been isolated and cloned in a number of vertebrates, including teleosts. Cartilaginous fishes are the most basal living jawed vertebrates. They comprise of two groups: the elasmobranchs (sharks and rays) and the holocephalans. We have identified 3 pthrp-like genes (designated *EFpthrp1,2 and 3*) in the genome of a holocephalan cartilaginous fish, *Callorhinchus millii*, commonly known as elephant fish or elephant shark These animals are confined to south-eastern waters of Australia and New Zealand. *C. milli* has been recently targeted for whole-genome sequencing since its genome is the smallest known among cartilaginous fishes (Venkatesh et al., Science 314:1892, 2006). The expression patterns of the three genes were analyzed by RT-PCR. None of the three genes code for parathyroid hormone although *EFpthrp3* gene appeared to be confined to the brain.

J.A. Danks, TeeleOstin Pty Ltd; TeeleOstin Pty Ltd; TeeleOstin Pty Ltd.

CE06

Calcium metabolic strategies in egglaying crocodilian (*Alligator mississippiensis*) archosaurs

C.G. Dacke[1], R.M. Elsey[2], P.L. Trosclair, III[2], M.H. Schweitzer[3]
[1]University of Portsmouth, Portsmouth, UNITED KINGDOM
[2]Louisiana Department of Wildlife and Fisheries, Rockefeller Wildlife Refuge, Grand Chenier, LA
[3]Dept. of Marine, Earth, Atmospheric Sciences, Raleigh, NC, USA

The close relationship between extant crown group archosaurs Crocodylia and Aves, and extinct non-avian archosaurs, Dinosauria, is unquestioned. Medullary bone is a highly mineralized, bony reproductive tissue present in the endosteal cavities of all extant egglaying birds, and has recently been reported in *Tyrannosaurus rex* [1]. The evolutionary significance of this tissue was tested by examining ground sections of femora from two reproducing and one non-reproducing female alligators, and a male for comparison. No substantial histological difference, and no evidence of medullary bone, was observed among any specimens on the endosteal surfaces of the long bones, supporting the evolutionary origin of this tissue after the divergence of dinosaurs from other archosaurs, and uniting dinosaurs and birds, with crocodilians as the sister group to this lineage [2]. However, because alligators produce hard shelled eggs, they require a labile calcium source for rapid calcification, a role supplied by medullary bone in birds and probably *T rex*. One possible source for this calcium is endolymphatic sacs. To test this possibility, we conducted preliminary radiographic examinations of skulls from three alligators, including a recently nested young female, an immature female and a young male; all specimens were approximately the same size and length. Unlike anuran amphibians and some reptiles, this limited skull series does not show evidence of well developed X-ray opaque endolymphatic sacs. However, an area associated with these sacs in some lizards (within the parietal foramen) shows evidence of resorption in the nested female alligator, compared with the male and immature female. X-ray examination of bony scutes on the dorsal integument of the same animals also suggests that those from the nested female are less mineralised than in the male, but similar to the immature female. It is possible that in the absence of medullary bone, crocodilian archosaurs use alternative strategies including mobilization of dermal calcium deposits to meet the substantial metabolic stresses of calcium metabolism during egglay.

References

[1] Schweitzer, et al. Science 2005; 308:1456–60.
[2] Schweitzer, et al. Bone in press.

C.G. Dacke, None.

CE07

Expression and localization of vitamin D receptor in all intestinal segments of egg-laying hens

T. Sugiyama, Y. Takizawa, S. Kusuhara
Niigata University, Niigata, JAPAN

Calcium metabolism in egg-laying hens is extraordinary compared with all other classes of vertebrates, because they lay an egg with a hard shell that consists of 5.7 g calcium carbonate about 2.3 g that is calcium. Modern strains of the domestic hen can produce up to 300 eggs per year. Therefore, egg-laying hen requires a large amount of calcium to support eggshell formation. Dietary calcium is transported through enterocyte cytoplasm by calcium binding protein (calbindin; CaBP). CaBP expression is as follows, duodenum > jejunum > cecum > ileum > colon, which also corresponds to the potency of calcium absorption. The expression of CaBP is stimulated by $1,25(OH)_2D_3$, which binds to vitamin D receptor (VDR) and transcribes the promoter genes of CaBP. Therefore, it is accepted that CaBP synthesis depends on the expression levels of VDR in enterocytes. To clarify the mechanism of calcium absorption in intestines of egg-laying hen, this study investigated the expression and localization of VDR in all intestinal segments of egg-laying hens.

All intestinal segments (duodenum, ileum, jejunum, caecum and colon) were dissected from White Leghorn hens (260–300 days of age), and the expression and localization of VDR were detected with semiquantitative RT-PCR and immunohistochemistry. The expression levels of VDR mRNA did not differ in all intestines with RT-PCR analysis. Immunohistochemical study represented that VDR was extensively localized in the nuclei of enterocytes in duodenum and jejunum. Cecum and colon represented the diffuse localizations of VDR in both cytoplasm and nuclei.

In conclusion, CaBP synthesis is not dependent on the expression level of VDR, but the translocation of cytoplasmic VDR into the nucleus regulates the synthesis of CaBP.

T. Sugiyama, None.

CE08

Molecular cloning and expression of calcitonin receptor in chicken medullary bone

Y. Teruchi[1], **T. Sugiyama**[1], Y. Egawa[1], N. Suzuki[2], Y. Sano[1], C.V. Gay[3], S. Kusuhara[1]
[1]Niigata University, Niigata, JAPAN
[2]Kanazawa University, Ishikawa, JAPAN
[3]The Pennsylvania State University, State College, PA, USA

Calcitonin (CT) regulates serum calcium concentration by the inhibition of osteoclastic bone resorption and renal calcium absorption. Medullary bone is a specific tissue of female birds, and functions as a calcium reservoir for eggshell formation. Calcium is released from medullary bone by osteoclastic bone resorption, a process which is closely correlated with eggshell formation. However, it is unknown whether the calcitonin receptor (CTR) is present in avian medullary bone and other tissues. Therefore, we tried to clone chicken CTR complementary deoxyribonucleic acid (cDNA) and to detect the expression of CTR mRNA in various tissues.

Putative partial cCTR cDNA, a 426 bp fragment, was obtained from medullary bone of White Leghorn hens (15 h after oviposition) with degenerate PCR, and a 548 bp fragment of the 5′ end and a 1540 bp fragment of the 3′ end were also obtained with RLM-RACE. Sequence alignment of these fragments revealed a putative full-length cCTR cDNA of 2428 bp. The cDNA including the initiation codon ATG at nucleotides 197–199 and the termination codon TAG at 1607–1609. The predicted amino acid sequence of cCTR contained 469 amino acid residues which was highly homologous to frog CTR (84%), human CTR (63%), pig CTR (60%) and rat CTR (59%). Also, an extracellular amino acid N-terminal region, which contained multiple glycosylation sites and conserved cysteine residues, was found. RT-PCR analysis also suggested that the expression of cCTR mRNA was detected in medullary bone, brain, kidney, skeletal muscle and oviduct, but not in heart, liver, spleen, lung, stomach and gizzard of egg-laying hens. In this study, we obtained a putative cDNA sequence for cCTR encoding a protein of 469 amino acid residues which had a high degree of homology with CTR of other species. It is also suggested that medullary bone osteoclasts express CTR, and that CT inhibits osteoclastic resorption of medullary bone.

T. Sugiyama, None.

CE09

Carbon dioxide rich water bathing increase local VEGF, Angiopoietin-I secretions in ischemic lower limbs of DM, ASO patients

K. Saito[1], M. Nonomura[2]
[1]Kyoto Katsura Hospital, Hemodialysis Center, Nephrology, Kyoto, JAPAN
[2]Kyoto Katsura Hospital, Hemodialysis Center, Urology, Kyoto, JAPAN

Background: Carbon dioxide rich water bathing (COWB) known to dilate local veins, increase local vascular blood flow, decrease local tissue pressure by increasing NO synthesis. COWB improves numbness, edema, skin ulcer of ischemic tissues in Diabetes Mellitus (DM), and necrosis of Arteriosclerosis Obliterans (ASO) patients. Through LDL-Apheresis (LDL-A) is known to increase vascular endothelial growth factor (VEGF) in ASO patients, we evaluate VEGF, Angiopoietin-I and CD34/CD33 (2-color).

Methods: About 36 °C, 1100–1200 ppm carbon dioxide enriched water has made by CO2 diffusion method using follow fiber, which CO2 gas inside, and circulating warm water outside. In 16 DM or ASO patients, we execute COWB of lower limbs for 15 min. We collect blood samples from the local vein

where COWB executed, and evaluate plasma NOx, VEGF, Angiopoietin-I and populations of CD34+CD33+ endothelial progenitor cells.

Results: After once COBW, VEGF are significantly increased (1.6-folds; $p<0.05$), also NOx, Angiopoietin-I are increased. Daily COBW cause dose-dependent increment of VEGF (3.1-folds at day 14; $p<0.05$). CD34+ population seems to become decrease according to ages. Correspondingly CD34+ CD33+ endothelial progenitor cells increased. Patients who resist COWB therapy, VEGF and populations of CD34+CD33+ endothelial progenitor cells are low.

Conclusion: COWB, which increase local VEGF, Angiopoietin-I secretion and CD34+CD33+ endothelial progenitor cells, is suggested to improve clinical symptoms of DM, ASO.

K. Saito, None.

CE10

Regulation of mammalian calcium and bone metabolism during pregnancy and lactation

C. Kovacs
Memorial University of Newfoundland, St. John's, NF, CANADA

The skeleton fulfills several obvious and important roles: it protects the vital organs, it is the scaffold from which organs and tissues are hung, it enables locomotion on land in a biped or quadruped posture, and it houses the bone marrow. Common clinical concerns about the skeleton include traumatic fractures, fragility fractures from osteoporosis, and a multitude of disorders that crowd the marrow space. Far less well appreciated is the function of the skeleton to serve as a storehouse of minerals and alkali, even to the point of compromising its integrity and strength. Among the times of great demand for calcium and other minerals are pregnancy, lactation, egg laying, and antler formation. In each of these time periods, the skeleton undergoes a significant, rapid and reversible demineralization in order to provide mineral respectively to the fetus, neonate, egg shell, and antlers. This presentation will review our present understanding of the adaptations in mineral metabolism that occur during pregnancy and lactation, and how these adaptations will uniquely affect the presentation, diagnosis and management of disorders of calcium and bone metabolism such as primary hyperparathyroidism, hypoparathyroidism and vitamin D deficiency. Maternal adaptations to pregnancy and lactation have evolved differently over time, such that dietary calcium absorption dominates in pregnancy, whereas the temporary borrowing of calcium from the skeleton dominates during lactation. Lactation programs an obligatory skeletal calcium loss irrespective of maternal calcium intake, but the calcium is completely restored to the skeleton after weaning through mechanisms that are not understood. While some women will experience fragility fractures as a consequence of pregnancy or lactation, the vast majority of women can be reassured that the changes in calcium and bone metabolism during pregnancy and lactation are normal, healthy, and without adverse consequences in the long-term.

C. Kovacs, None.

CE11

Molecular cloning and mRNA expression of genes involved in transepithelial calcium transport in horses

K.M. Rourke, C.W. Kohn, T.J. Rosol, R.E. Toribio
The Ohio State University, Columbus, OH, USA

Gene expression analysis has improved our understanding of various physiological processes, including mineral homeostasis. Horses have unique features with regard to calcium regulation including high serum ionized and total calcium concentrations, a high parathyroid gland calcium set-point, high intestinal absorption and renal excretion of calcium, and low serum vitamin D concentrations. Limited information on the molecular mechanisms of equine calcium regulation is available. The goal of this project was to clone and determine the expression of genes that are important in transepithelial calcium transport in the equine gastrointestinal tract and kidney. The mRNAs for the following genes were cloned: epithelial calcium channel 1 (ECaC1 or TRPV5), calcium transporter 1 (CaT1 or TRPV6), transient receptor melastatin 7 (TRPM7), calbindin D9k (CDB9), calbindin D28k (CBD28), plasma membrane ATPase (PMCA), sodium/calcium exchanger (NCX), vitamin D receptor (VDR), and 1 alpha hydroxylase. The quantitative expression of TRPV5, TRPV6, CBD9, CBD28, and VDR was evaluated in various segments of the intestinal tract and the kidney. Expression analysis of the remaining genes is ongoing. We hypothesized that horses would have relatively high expression of CBD9, TRPV5, TRPV6, and VDR in the small intestine compared to the large colon. We anticipated that mRNA expression for CBD28 and TRPV5 would be higher in the kidney, while expression for CBD9 and TRPV6 would be higher in the intestinal tract. Because VDR regulates genes involved on calcium transport, we theorized that VDR expression would coincide with the expression of TRPV5 and CBD9. We found CBD9 mRNA expression in the proximal small intestine (duodenum, jejunum) to be 100–1000 fold higher than in the distal small intestine (ileum) and large colon. TRPV5 expression was 10 fold higher in the proximal small intestine and kidney than in the distal small intestine and large colon. In contrast to other species, we found that VDR expression was higher (100 fold) in the distal intestinal tract (ileum, large colon) compared to the proximal intestine. VDR mRNA expression in the kidney was similar to that in the distal intestinal tract. Unexpectedly, VDR expression followed an opposite pattern compared to those observed for TRPV5 and CBD9. Completing and integrating mRNA expression analysis of the remaining calcium-regulating genes will improve our understanding of equine and comparative calcium regulation.

K.M. Rourke, None.

CE12

Prevalence of hypovitaminosis D And secondary hyperparathyroidism in a sample of postmenopausal women in Buenos Aires

Z. Man, M.S. Larroude, M.S. Moggia, R.D. Diaz, J.C. Morgenstern, M.P. Yantorno, M.G. Torres Cerino,

G.A. Macías, M. Pérez Sáinz
Centro TIEMPO, C.A.Buenos Aires, ARGENTINA

Vitamin D (VD) has multiple biological effects, as it plays a key role in the serum calcium homeostasis.

The definition of normal values was based on Gaussian distributions of the concentrations seen in apparently healthy subjects, but it did not take into account race, age, latitude, poor dietary VD intake, use of sunscreens.

The cutoff point has been a matter of debate; the optimal value would be the one that, below that point, PTH levels starts to increase.

Objective: To evaluate the prevalence of 25-hydroxivitamin D [25(OH)D] in a sample of postmenopausal women and its correlation with PTH levels, using two different 25(OH)D cutoff values considered as desirable by two different classifications.

Materials and methods: 196 postmenopausal women from Buenos Aires and its metropolitan area (Lat: 35°S), mean age: 65 years (51–85), were evaluated between October and December 2005. Women with thyroid, liver or renal diseases or those treated with corticosteroids, antiepileptic and anticoagulants were excluded. 25(OH)D levels were measured by a RIA assay using the Diasorin® kit, and PTH levels by chemiluminescence.

VD status was categorized using the Hollis and McKenna classifications. We also evaluated the prevalence of secondary hyperparathyroidism (PTH levels > 65 pg/ml).

***Bruce Hollis classification*:** 25(OH)D deficiency < 10 ng/ml; insufficiency 10–20 ng/ml; hypovitaminosis 20–30 ng/ml; desirable > 30 ng/ml.

McKenna classification: 25(OH)D deficiency < 10 ng/ml (< 25 nmol/l); insufficiency 10–20 ng/ml (25–50 nmol/l): hypovitaminosis 20–40 ng/ml (50–100 nmol/l); desirable > 40 ng/ml (> 100 nmmol/l).

Results: No VD deficiency was detected. Using the Hollis classification, 70 women (35.7%) had VD insufficiency; 90 (46%) had hypovitaminosis D; and only 36 (18.3%) had desirable values. Secondary hyperparathyroidism was detected in 1.5% of the women included.

The McKenna classification revealed that 121 women (61.8%) had hypovitaminosis D, and 2,6% had desirable values. No cases of secondary hyperparathyroidism were detected under this classification.

Conclusions: We found that the prevalence of secondary hyperparathyroidism was 1.5% and 0% by using a cutoff value for sufficiency of 30 ng/ml and 40 ng/ml. Therefore, we recommend a 25(OH)D cutoff value higher than 40 ng/ml.

Vitamin D status	*n* = 196	%	PTH > 65 ng/ml
			n = 37 (18.9%)
Deficiency < 10 ng/ml	0	0.0	0 (0)
Insufficiency 10–20 ng/ml	70	35.7	19 (9.7)
Hypovitaminosis 20–30 ng/ml (Hollis)	90	46.0	15 (7.7)
Desirable > 30 ng/ml	36	18.3	3 (1.5)
Hypovitaminosis 20–40 ng/ml (McKenna)	121	61.8	18 (9.2)
Desirable > 40 ng/ml (McKenna)	5	2.6	0 (0)

Z. Man, None.

CE13

Defining new Vitamin D normal range based upon bone turnover markers, calcium and PTH levels

M.M. Sarquis[1], E.P. Dias[2], B.C. Silva[3], **B.M. Camargos**[4]
[1]UFMG, Belo Horizonte, BRAZIL
[2]Hospital Felício Rocho, Belo Horizonte, BRAZIL
[3]Santa Casa de Miseric¡½rdia de Belo Horizonte, Belo Horizonte, BRAZIL
[4]Osteon BH—Estudos em osteometabolismo, Belo Horizonte, BRAZIL

Vitamin D (VD) is important in many physiological processes, especially those relating to bone metabolism. Serum VD is produced in proportion to the amount of sun exposure and VD ingested. Serum 25(OH) vitamin D (25OHVD) is the preferred test to measure nutritional status and body stores of VD. Vitamin D deficiency has been an under-recognized problem because of suboptimal assays and inappropriately low levels considered "normal range" for blood VD concentration. Consequences of VD deficiency include secondary hyperparathyroidism, osteomalacia (rickets in children), osteoporosis and myopathy. Population-based reference ranges for VD vary widely, depending on ethnic background, age, geographic location of the studied populations, and the sampling season. Marked variability for VD assays has been demonstrated between laboratory measurements.

As a result, a universal definition of an optimal serum 25OHVD concentration is not possible, and whether an individual 25OHVD is low or normal may be a function of the laboratory method used. To determine the sufficiency of VD we measured parathyroid hormone (PTH) and bone turnover markers (BTM) in a population derived from our clinical practice. Its effects on bone health were evaluated by dual X-ray absorptiometry (DXA).

Keywords: Vitamin D; Parathyroid hormone; Bone; Turnover markers; Telopeptides

B.M. Camargos, None.

CE14

Estrogen is a negative regulator of klotho expression in mouse kidney

O.K. Oz, A. Hajibeigi, W. Siyambalapitiyage, C. Cummins, M. Kuro-o, J.E. Zerwekh
UT Southwestern Medical Center at Dallas, Dallas, TX, USA

Klotho is a glycoprotein predominantly expressed in the kidney, parathyroid gland, reproductive organs and choroids plexus in the brain. Studies have shown that decreased expression of the klotho gene in mice leads to multiple disorders, such as arteriosclerosis, skin atrophy, abnormal calcium homeostasis and shortened life span. The klotho gene encodes two forms of the protein, a single pass-membrane 130 Kda glycoprotein with proteolytic activity and a splice variant without the transmembrane domain which is termed secreted or

soluble form. The klotho protein functions as a cofactor essential for activation of FGF signaling by FGF23 and exhibits beta-glucoronidase enzymatic activity. Recent research studies have documented the co-localization of klotho with TRPV5 in the distal renal tubule and shown that the klotho protein hydrolyses the extracellular sugar residues of TRPV5, entrapping the channel in plasma membrane and increasing calcium translocation. Up-regulation of klotho and TRPV5 by vitamin-D has been reported. To our knowledge there have been no investigations of a potential regulatory role for estradiol in klotho expression. We examined a potential role in vitro and in vivo study of klotho expression was conducted at the mRNA and protein level. The kidney cell line (MDCK) was cultured in DMEM supplemented with either 10% regular FBS or charcoal stripped FBS (csFBS). The klotho protein expression as detected by Western blot (WB) was significantly higher in cells grown in csFBS compared to regular serum. However, the addition of estradiol at 10(−8)M in 10% csFBS restored klotho expression to the same level as cells grown in 10%FBS. To determine the effects of estrogen in vivo, we used wild type (WT) and aromatase deficient mice (ArKO) treated with estrogen (20 ug/mouse 3×/week) or vehicle for 3 weeks. RNA and protein were prepared from the kidneys for real time PCR and WB analysis, respectively. Our results showed a significantly higher expression of klotho both at the mRNA and protein levels in ArKO animals compared to WT and ArKO treated with estrogen. Interestingly, urinary klotho protein levels, detected by WB, were also higher in the ArKO group. Based on our observations we conclude that estrogen down-regulates the klotho in the murine kidney. Determination of the effect of the estrus cycle on urinary klotho levels is underway.

This work was supported by NIH grant P01 DK20543.

O.K. Oz, None.