รายงานวิจัยฉบับสมบูรณ์

โครงการ การวิจัยที่ใช้แนวทางการวิจัยวิทยาศาสตร์ชีวภาพพื้นฐานเพื่อนำไปสู่การศึกษาสาเหตุของโรค การวินิจฉัยและการรักษา Metabolic Bone Disease ที่พบมากในประเทศไทย

โดย ศาสตราจารย์ ดร.นทีทิพย์ กฤษณามระ

ตุลาคม 2550

สัญญาเลขที่ RTA 478008

รายงานฉบับสมบูรณ์

โครงการ การวิจัยที่ใช้แนวทางการวิจัยวิทยาศาสตร์ชีวภาพพื้นฐานเพื่อนำไปสู่การศึกษาสาเหตุของโรค การวินิจฉัยและการรักษา Metabolic Bone Disease ที่พบมากในประเทศไทย

ผู้วิจัย ศาสตราจารย์ ดร.นทีทิพย์ กฤษณามระ
ภาควิชาสรีรวิทยา
เครือข่ายวิจัยด้านแคลเซียมและกระดูก
คณะวิทยาศาสตร์
มหาวิทยาลัยมหิดล

สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

สารบัญ

Abstract

From our earlier reports of the stimulatory effect of prolactin (PRL) on the *in vivo* intestinal calcium absorption in nonmated pregnant and lactating rats, we hypothesized that prolactin might have a role in the regulation of calcium and bone metabolism especially during the reproductive phase. In the present study, we investigated the mechanism of action of prolactin in the intestine as well as explored prolactin action on the electrolyte transport in the colon and endometrium, and calcium handling in the mammary cells.

We were able to show that PRL had a regulatory role in supplying calcium for developing bone and for milk production by stimulating calcium absorption and accelerating bone turnover. We found that long term exposure to PRL increased the duodenal calcium absorption by stimulating the transcellular active calcium transport in adult rats, while stimulating both the transcellular and solvent drag-induced active calcium transport in young rats. Increased calcium transport was explained by enhanced brush border uptake of calcium and increased activities of the basolateral Na^{+}-K^{+}-ATPase and Ca^{2+}-ATPase. The intracellular PRL signal transduction involved the PI3kinase and MAPKinase pathways. As for the electrolyte transport, PRL inhibited the Ca^{2+}-dependent Cl^{-} and K^{+} secretion in the colon by interfering with the intracellular Ca^{2+} signaling. In contrast, PRL stimulated the anion secretion in the endometrium via the JAK-STAT pathway, this could contribute to provision of appropriate uterine fluid environment for implantation and embryo development. We also found that PRL stimulated bone remodeling in adult rats and the action was estrogen dependent.

In the second project, we studied the effect of chronic metabolic acidosis on calcium-phosphorus and bone metabolism in cats with chronic renal failure (CRF). Although bone mineral density was not significantly reduced, CRF cats had hyperparathyroidism and high bone resorption with lower bone formation. Metabolic acidosis appeared to be a potentiating factor in the induction of these changes. Since there was no correlation between blood pH and parathyroid hormone levels, the cause of change in bone remodeling may be multifactorial and probably involved a direct effect of acid on bone cells. Thus, close monitoring of cats with CRF and early correction of acidosis were necessary to prevent osteopenia and hyperparathyroidism with its consequences. Measurement of bone mineral density was not sensitive enough to detect early changes in bone, so it should be used with other biochemical tests.

บทคัดย่อ

เนื่องจากกลุ่มวิจัยของเราได้พบว่าโพรแลคตินมีผลกระตุ้นการดูดซึมแคลเซียมในหนูขาวธรรมดา หนูท้องและหนูให้นมลูก เราจึงตั้งสมมติฐานว่าโพรแลคตินน่าจะมีหน้าที่ควบคุมเมตาบอลิสมของแคลเซียมและกระดูก โดยเฉพาะในแม่หนูท้องและให้นม ในการวิจัยนี้ เรามุ่งศึกษากลไกการออกฤทธิ์ของโพรแลคตินต่อการดูดซึมแคลเซียม ผลต่อการขนส่งสารอิเล็กโทรไลท์และของเหลวที่ลำไส้ใหญ่และผนังมดลูก และการขนส่งแคลเซียมในเซลล์เต้านม

จากการวิจัยเราพบว่า โพรแลคตินเป็นฮอร์โมนที่มีส่วนในการควบคุมการขนถ่ายแคลเซียมโดยกระตุ้นการดูดซึมแคลเซียมและกระตุ้นวงจรการสร้าง-สลายกระดูกเพื่อให้มีแคลเซียมเพียงพอสำหรับการสร้างกระดูกในวัยเจริญเติบโต และสำหรับการผลิตน้ำนมเลี้ยงลูกอ่อน ผลการวิจัยแสดงให้เห็นว่าการมีระดับโพรแลคตินในเลือดสูงเป็นเวลานาน มีผลเพิ่มการดูดซึมแคลเซียมที่ลำไส้ส่วนต้น โดยมีผลกระตุ้นการขนส่งแบบ transcellular active ในหนูเจริญวัย และกระตุ้นการขนส่งแบบ transcellualr active และ solvent drag-induced active transport ในหนูอายุน้อย การขนส่งแคลเซียมที่เพิ่มขึ้นมีกลไกมาจากการนำเข้าแคลเซียมจากโพรงลำไส้ผ่านเยื่อเซลล์ที่เพิ่มขึ้น และการกระตุ้นการทำงานของ Na^{+}-K^{+}-ATPase และ $Ca^{2=}$-ATPase ที่เยื่อเซลล์ด้านเลือด โพรแลคตินออกฤทธิ์ที่เซลล์ลำไส้ผ่านกลไก PI3Kinase และ MAPKinase สำหรับการขนส่งอิเล็กโทรไลท์และของเหลวนั้น พบว่าโพรแลคตินมีผลยับยั้งการขับหลั่ง Cl^{-} และ K^{+} ประเภทที่ต้องใช้ Ca^{2+} ที่เซลล์ลำไส้ใหญ่ โดยมีผลยับยั้งกลไกการส่งสัญญาณภายในเซลล์ผ่านทาง Ca^{2+} แต่ที่เซลล์ผนังมดลูกโพรแลคตินกลับมีผลกระตุ้นการขับหลั่งอิออนประจุลบโดยออกฤทธิ์ผ่านกลไก JAK-STAT ผลดังกล่าวบ่งบอกหน้าที่ของโพรแลคตินในการควบคุมให้ของเหลวในมดลูกมีคุณสมบัติเหมาะสมสำหรับการฝังตัวและการเจริญเติบโตของตัวอ่อน นอกจากนั้นเรายังพบว่าโพรแลคตินมีผลกระตุ้นวงจรสร้าง-สลายกระดูกในหนูเจริญวัย ซึ่งการออกฤทธิ์ที่กระดูกนี้ต้องอาศัยฮอร์โมนเพศหญิงเอสโตรเจนด้วย

ในโครงการวิจัยที่ 2 เราศึกษาผลของภาวะเลือดเป็นกรดระยะยาวต่อเมตาบอลิสมของแคลเซียมฟอสฟอรัสและกระดูกในแมวไทยที่เป็นโรคไตวายเรื้อรัง (CRF) จากการทดลองพบว่าถึงแม้ความหนาแน่นของกระดูกจะไม่ลดลงอย่างมีนัยสำคัญ แต่แมว CRF ก็เกิดภาวะพาราไทรอยด์ฮอร์โมนสูง มีการสลายกระดูกมากขึ้น และการสร้างกระดูกลดลง ภาวะเลือดเป็นกรดดูจะเป็นตัวแปรสำคัญที่ทำให้เกิดการเปลี่ยนแปลงเหล่านี้ เนื่องจากไม่พบความสัมพันธ์ระหว่างความเป็นกรดของเลือดกับระดับพาราไทรอยด์ฮอร์โมนในเลือด การเพิ่มการสลายกระดูกหรือลดการสร้างกระดูกน่าจะมาจากหลายสาเหตุ เช่น ผลโดยตรงของกรดต่อเซลล์กระดูก ดังนั้นสัตวแพทย์ไม่ควรใช้การเปลี่ยนแปลงของความหนาแน่นของกระดูกในการวินิจฉัยในช่วงแรกแต่ควรติดตามดูแลแมว CRF อย่างใกล้ชิด และลดภาวะกรดในเลือดโดยเร็วที่สุด เพื่อป้องกันไม่ให้เกิดภาวะมวลกระดูกลดหรือภาวะพาราไทรอยด์ฮอร์โมนสูง ซึ่งจะนำไปสู่ความผิดปกติอื่นๆ

สรุปโครงการ (EXECUTIVE SUMMARY)

ทุนส่งเสริมกลุ่มวิจัย (เมธีวิจัยอาวุโส สกว.)
ประจำปี พ.ศ. 2547

Title: Research Program : Basic biological science approach to the study of etiology, diagnosis, and treatment of metabolic bone diseases of high incidence in Thailand

โปรแกรมการวิจัยที่ใช้แนวทางการวิจัยวิทยาศาสตร์ชีวภาพพื้นฐานเพื่อนำไปสู่การศึกษาสาเหตุของโรค การวินิจฉัย และการรักษา Metabolic Bone Diseases ที่พบมากในประเทศไทย

Project Director :

Professor Nateetip Krishnamra, Ph.D.

Institution : Department of Physiology
Consortium for Calcium and Bone Research
Faculty of Science, Mahidol University
Rama VI Road, Rajthevee, Bangkok 10400

Telephone : 0-2201-5629 Facsimile : 0-2354-7154
E-mail : scnks@mahidol.ac.th

Field of Research : Directed Basic Biomedical Research

Duration : 3 years (2005-2007)

BACKGROUND AND RATIONALE

At present there is very little basic and clinical research on calcium metabolism and bone disorders in Thailand. One of the reasons is that bone diseases are mostly chronic and not easily diagnosed in the early stages. However, incidences of bone diseases have been known to increase dramatically with increase in life extectancy and the proportion of elderly population. Undoubtedly, bone diseases will ultimately create a negative socioeconomic impact in Thailand in the near future. It is thus necessary for Thailand to encourage the development of research in this field for better understanding of

the etiology and mechanisms of bone diseases, better and earlier diagnoses, and effective treatment and prevention. Multidisciplinary research approach can make use of the basic fundamental knowledge and clinical findings for the benefit of patients and also for improving the quality of life of the general population.

Prolactin has been demonstrated by our laboratory to be a novel calcium-regulating hormone during pregnancy and lactation (Lotinun et al. 1998; Piyabhan et al. 2000; Charoenphandhu et al. 2001; Lotinun et al. 2003). We have shown that prolactin stimulated the in vivo absorption of calcium in the small intestine. Calcium absorption can be classified into passive and active (dependent on the Ca-ATPase) absorption. We have further identified three components of the active calcium transport and have shown that they were enhanced by prolactin (Tanrattana et al. 2004). The fact that basal endogenous prolactin had a role in the regulation of normal intestinal calcium absorption in nonlactating rats (Piyabhan et al. 2000), it was believed that endogenous prolactin, albeit low concentrations (7-8 ng/mL), had a physiological significance in normal female rats.

Because prolactin receptors on rat bone-forming cells, osteoblasts, have been reported (Coss et al. 2000), prolactin might have a direct action on bone cells. Previous investigations reported high prolactin levels during lactation affecting the primary trabecular sites (Ritchie et al. 1998), such as vertebrae and sternum and the trabecular portions of cortical bones (Clement-Lacroix et al. 1999). Moreover, there were reports of pathological hyperprolactinemia-induced decrease in bone mass (Klibanski et al. 1988; Biler et al. 1992; Naliato et al. 2005). However, it was not known whether hyperprolactinemia affected bone directly, or via inducing estrogen deficiency. Therefore, we were interested in investigating the role of prolactin in the regulation of calcium metabolism and bone turnover during growth, in nonmated rats, as well as in pregnant and lactating rats. **Our hypothesis was that prolactin orchestrated the transport of calcium in the various calcium handling target organs namely, the intestine, bone, and mammary gland, so as to channel absorbed calcium for growth and development of young, and milk production in lactation.** Bone remodeling was also accelerated under prolactin influence to provide calcium if dietary calcium was not adequate. However, excess prolactin may result in accelerated bone remodeling and loss of bone mass. There appear to be a delicate balance of prolactin actions which is dependent on age, sex, and reproductive phases.

Metabolic acidosis can have drastic effect on the organ systems including bones. **Distal renal tubular acidosis (dRTA)** which is caused by the inability of kidney to excrete ammonia and acid, is one example of the cause of chronic metabolic acidosis in human. Chronic renal failure (CRF) which is the most

common renal diseases in dogs and cats in Thailand also results in chronic metabolic acidosis. It was not known how chronic metabolic acidosis affect the calcium regulating hormones and calcium-phosphorus homeostasis. **It would be interesting to use Siamese cat model to understand the impact of chronic metabolic acidosis on calcium and bone metabolism.**

Objectives

Our goal was to understand possible causes and mechanisms underlying metabolic bone disorders and calcium imbalance which may ultimately lead to a decrease in bone mass. To reach our goal, the strategy was to use the basic research in calcium and bone metabolism to familiarize ourselves with experimental approaches and technicalities necessary for indepth investigations. The multidisciplinary approach was essential for translating the clinical problems into basic science experiments, and vice versa.

Research Strategy

The research projects involved multidisciplinary approaches at whole body, organ, cellular and molecular levels of organization. Models for the study of metabolic bone diseases were hyperprolactinemia and chronic metabolic acidosis-induced bone change.

Research Projects

1. **Investigation on prolactin (PRL) and its novel role as a calcium regulating hormone**

    1.1. Study of the mechanism of action and signal transduction of PRL in the regulation of calcium and fluid transport in the intestine.
        1.1.1 Study of the mechanism and signal transduction pathway of acute prolactin action on the enhancement of the transepithelial calcium transport in rat duodenum and CaCo2-monolayer.
        1.1.2 Study of the acute effect, mechanism of action and signal transduction of prolactin in the regulation of ion transport in the large intestine.
    1.2. Role of prolactin in fluid and electrolyte regulation in the endometrium
    1.3. Investigation of the role of PRL in calcium handling in the mammary gland

1.3.1 Expression and localization of calcium transporters other than P-types Ca^{2+} ATPase in mammary tissue during the mammary development and lactation.

1.3.2 The effect of prolactin on calcium metabolism in mammary cells.

2. **Study of the etiology, mechanism of diseases, and possible treatment of the metabolic bone diseases using the Siamese cat model.**

The role of metabolic acidosis on changes in PTH, vitamin D, calcium and phosphate homeostasis and bone changes in Siamese cats with chronic renal failure.

Outcome

1. **International Publications**

1.1 Amnattanakul S, Charoenphandhu N, Limlomwongse L, **Krishnamra N**. Endogenous prolactin modulated the calcium absorption in the jejunum of suckling rats. Can J Physiol Pharmacol 2005; 83: 1-10. *(Impact Factor 1.603)*

1.2 Tudpor K, Charoenphandhu N, Saengumnart W, **Krishnamra N**. Long-term prolactin exposure differentially stimulated the transcellular and solvent drag-induced calcium transport in the duodenum of ovariectomized rats. Explt Biol Med 2005; 230: 836-844. *(Impact Factor 2.369)*

1.3 Charoenphandhu N, Limlomwongse L, **Krishnamra N**. Prolactin directly enhanced Na^{+}/K^{+} and Ca^{2+}-ATPase activities in the duodenum of female rats. Can J Physiol Pharmacol 2006, 84: 555-563. *(Impact Factor 1.603)*

1.4 Anantamongkol U, Takemura H, Suthiphongchai T, **Krishnamra N**, Horio Y. Regulation of Ca^{2+} mobilization by prolactin in mammary gland cells : Possible role of secretory pathway Ca^{2+}-ATPase type 2. Biochem Biophys Res Comm 2007; 352(2): 537-542. *(Impact Factor 3.0)*

1.5 Puntheeranurak S, Schreiber R, Spitzner M, Sausbier M, Ruth P, **Krishnamra N**, Kunzelmann K. Control of ion transport in mouse proximal and distal colon by prolactin. Cell Physiol Biochem 2007; 19: 77-88. *(Impact Factor 4.1)*

1.6 Jantarajit W, Thongon N, Pandaranandaka J, Teerapornpuntakit J, **Krishnamra N**, Charoenphandhu N. Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by

the phosphaenositide-3-kinase pathway. Am J Physiol Endocrinol Metab 2007; 293 : E372-384. *(Impact Factor 4.456)*

1.7 Charoenphandhu N, **Krishnamra N**. Prolactin : an important regulator of intestinal calcium transport . Can J Physiol Pharmacol 2007; 85: 569-581. *(Impact Factor 1.603)*

2. Manuscripts in preparation

2.1 Siriwetwiwat J, Vitayakritsirikul V, Anantamongkol U, Prapong T, Charoenphandhu N, Krishnamra N, Prapong S. Expression and localization of selective calcium channels, TRPV5 and TRPV6 in active mammary tissues. (in preparation)

2.2 Regulation of prolactin on electrolyte transport across porcine endometrial epithelial cells.

2.3 The effects of metabolic acidosis on parathyroid hormone secretion, calcium-phosphorus homeostasis and bone remodeling in cats with natural occurring chronic renal failure.

3. Academic Position Achievement

- Assist. Prof. to Assoc. Prof. Dr. Chatsri Dechapunya
  Srinakariwirot University
- Assoc. Prof. to Prof. Somnuek Damrongkitchaiporn
  Mahidol University

4. Graduate Students

M.Sc. 5	Miss Suwimol Amnattanakul	(Mahidol University)
	Mr.Kukiat Tudpor	(Mahidol University)
	Miss Kanogwan Thongchote	(Mahidol University
	Miss Orasmee Wimuktanundha	(Chulalongkorn University)
	Mr. Yongyut Pongprachachoen	(Chulalongkorn University)
Ph.D. 4	Miss Supaporn Puntheeranurak	(Mahidol University)
	Miss Dutmanee Seriwatanachai	(Mahidol University)
	Miss Atchariya Anantamongkol	(Mahidol University)

Miss Wipawan Wittayakritsirikul (Kasetsart University)

5. **Research Assistantship**

Miss Wasana Saengamnart
Miss Wilaiwan Tipsingh
Miss Jeerawan Thongboonchu

6. **Organized Academic Meeting, Seminar, Invited Lecture and Workshop**

- TRF Senior Scholar annual academic meetings 3 (2005, 2006, 2007)
- Consortium for Calcium and Bone Research (COCAB) organized workshop 2 (2006, 2007)
- Special lecture by Prof. Karl Kunzelmann, University of Regensburg, Germany (2005)
- Special seminar "Prolactin : its role in the regulation of calcium and bone metabolism" at the University of Western Ontario Canada (2007), presented by Prof. Nateetip Krishnamra and Dr. Narattaphol Charoenphandhu
- Poster presentations at international academic meetings 5
- Oral presentations at international academic meetings 2
- Oral presentations at local academic meetings 3
- Special lectures at local academic meetings 10
- COCAB group seminars 14 (2007 3, 2006 4, 2005 5, 2004 2)

References

- Lotinun S, Limlomwongse L, Krishnamra N. The study of a physiological significance of prolactin in the regulation of calcium metabolism during pregnancy and lactation in rats. Can J Physiol Pharmacol1998; 76: 218-228.
- Piyabhan P,Krishnamra N, Limlomwongse L. Changes in the regulation of calcium metabolism and bone calcium content during growth in the absence of endogenous prolactin and during hyperprolactinemia : a longitudinal study in male and female Wistar rats. Can J Physiol Pharmacol 2000; 78: 757-765.
- Lotinun S, Limlomwongse L, Sirikulchayanonta V, Krishnamra N. Bone calcium turnover, formation, and resorption in bromocryptine – and prolactin-treated lactating rats. Endocrine 2003; 20: 163-170.

- Charoenphandhu N, Limlomwongse L, Krishnamra N. Prolactin directly stimulates transcellular active calcium transport in the duodenum of female rats. Can J Physiol Pharmacol 2001; 79: 430-438.
- Tanrattana C, Charoenphandhu N, Limlomwongse L, Krishnamra N. Prolactin directly stimulated the solvent drag-induced calcium transport in the duodenum of female rats. Biochim Biophys Acta 2004; 1665: 81-91.
- Coss D, Yang L, Kuo CB, Xu X, Luben RA, Walker AM. Effects of prolactin on osteoblasts alkaline phosphatase and bone formation in the developing rat. Am J Physiol Endocrinol Metab 2000; 279: E1216-1225.
- Ritchie LD, Fung EB, Halloran BP, Turnlund JR, Van Loan MD, Cann CE, King LC. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. Am J Clin Nutr 1998; 67: 693-701.
- Clement-Lacroix P, Ormandy C, Lepescheux L, Ammann P, Damotte D, Goffin V et al. Osteoblasts are a new target for prolactin : analysis of bone formation in prolactin receptor knockout mice. Endocrinology 1999; 140: 96-105.
- Klibanski A, Biller BM, Rosenthal DI. Schoenfeld DA, Saxe V. Effects of prolactin and estrogen deficiency in amenorrheic bone loss. J Clin Endocrinol Metab 1988; 67: 124-130.
- Biller BM, Baum HB. Rosenthal DI, Saxe VC, Charpie PM, Klibanski A. Progressive trabecular osteopenia in women with hyperprolactinemic amenorrhea. J Clin Endocrinol Metab 1992; 75: 692-697.
- Naliato EC, Farias ML, Braucks GR, Costa FS. Zylberberg D, Violante AH. Prevalence of osteopenia in men with prolactinoma. J Endocrinol Invest 20005; 28: 12-17.

รายงานโครงการวิจัย

ทุนส่งเสริมกลุ่มวิจัย (เมธีวิจัยอาวุโส สกว.)

ประจำปี พ.ศ. 2547

การวิจัยที่ใช้แนวทางการวิจัยวิทยาศาสตร์ชีวภาพพื้นฐานเพื่อนำไปสู่การศึกษาสาเหตุของโรค การวินิจฉัย และการรักษา Metabolic Bone Disease ที่พบมากในประเทศไทย

Basic biological science approach to the study of etiology, diagnosis, and treatment of metabolic bone diseases of high incidence in Thailand

หัวหน้าโครงการวิจัย ศาสตราจารย์ ดร.นทีทิพย์ กฤษณามระ
ภาควิชาสรีรวิทยา
เครือข่ายวิจัยด้านแคลเซียมและกระดูก
คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

Field of Research

Directed Basic biomedical Resarch

Duration

3 years (2005-2007)

หัวหน้าโครงการวิจัยย่อย

- ศาสตราจารย์ นพ.สมนึก ดำรงกิจชัยพร
  หน่วยไต ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี
  มหาวิทยาลัยมหิดล
- รองศาสตราจารย์ ดร.ฉัตรศรี เดชะปัญญา
  ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
- ผู้ช่วยศาสตราจารย์ สพญ.ดร.ศิริวรรณ พราพงษ์
  ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์
- รองศาสตราจารย์ สพญ.ดร. รสมา ภู่สุนทรธรรม
  ภาควิชาอายุรศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ผู้ร่วมวิจัย

- นพ.ดร.นรัตถพล เจริญพันธุ์
  ภาควิชาสรีรวิทยา เครือข่ายวิจัยด้านแคลเซียมและกระดูก
  คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
- รองศาสตราจารย์ สพญ.ดร.สุทธาสินี ปุญญโชติ
  ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

ผู้ช่วยวิจัย

- นางวาสนา แสงอำนาจ (ภาควิชาสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล)
- นส.วิไลวรรณ ทิพสิงห์ (2547-2549)
- นส.จิรวรรณ ทองบุญชู (2549-2550)

นักศึกษาบัณฑิตผู้ร่วมวิจัย

หลักสูตรสรีรวิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล

- นส.สุภาพร พันธุ์ธีรานุรักษ์ (ป.เอก)
- นส.อัจฉริยา อนันตมงคล (ป.เอก)
- นส.ดุษมณี เสรีวัฒนาชัย (ป.เอก)
- นส.สุวิมล อำนาจธนากุล (ป.โท)
- นายกู้เกียรติ ทุดปอ (ป.โท)
- นส.กนกวรรณ ทองโชติ (ป.โท)

หลักสูตรสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

- นส.อรรัศมี วิมุกตะนันท์ (ป.โท)
- นายยงยุทธ์ พงษ์ประชาชื่น (ป.โท)

หลักสูตรพันธุวิศวกรรม คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเกษตรศาสตร์

- นส.วิภาวรรณ วิทยกฤตศิริกุล (ป.เอก)

ประเภทของงานวิจัย

การวิจัยวิทยาศาสตร์ชีวภาพพื้นฐาน

โครงการวิจัย

1. Investigation on prolactin (PRL) and its novel role as a calcium regulating hormone

1.1 Study of the mechanism of action and signal transduction of PRL in the regulation of calcium and fluid transport in the intestine.

1.1.1 Study of the mechanism and signal transduction pathway of acute prolactin action on the enhancement of the transepithelial calcium transport in rat duodenum and CaCo2-monolayer.

1.1.2 Study of the acute effect, mechanism of action and signal transduction of prolactin in the regulation of ion transport in the large intestine.

1.2 Role of prolactin in fluid and electrolyte regulation in the endometrium

1.3 Investigation of the role of PRL in calcium handling in the mammary gland

1.3.1 Expression and localization of calcium transporters other than P-types Ca^{2+} ATPase in mammary tissue during the mammary development and lactation.

1.3.2 The effect of prolactin on calcium metabolism in mammary cells.

2 **Study of the etiology, mechanism of diseases, and possible treatment of the metabolic bone diseases using the Siamese cat model**

The role of metabolic acidosis on changes in PTH, vitamin D, calcium and phosphate homeostasis and bone changes in Siamese cats with chronic renal failure.

(1) Investigation on prolactin (PRL) and its novel role as a calcium regulating hormone

Background and Rationale

Prolactin has been demonstrated to be a novel calcium-regulating hormone during pregnancy and lactation (Lotinun et al. 1998; Piyabhan et al. 2000; Charoenphandhu et al. 2001; Krishnamra et al. 2001; Lotinun et al. 2003). Both conditions place a stress on maternal calcium metabolism as a result of a high calcium loss for the intrauterine fetal development and lactogenesis. Because $1,25(OH)_2D_3$, a putative calcium-regulating hormone, does not contribute to an increase in the intestinal calcium absorption during these periods (Halloran and DeLuca 1980; Boass et al. 1981), the enhanced calcium absorption should be under the influence of other calcium-regulating hormones, particularly prolactin(Mainoya 1975; Pahuja and DeLuca 1981; Krishnamra et al. 1993; Krishnamra et al. 1998). The physiological significance of prolactin has recently been demonstrated by our finding which showed reductions in the intestinal calcium absorption and vertebral mineral density in growing rats during bromocriptine-induced suppression of endogenous prolactin (Piyabhan et al. 2000).

Acute effect of prolactin on the intestinal calcium absorption was shown in pregnant and lactating rats by Krishnamra and colleagues (1990). Moreover, the elevated duodenal calcium absorption of pregnant and lactating rats was found to correlate with changes in the plasma level of prolactin (Boass et al. 1992). Generally, small intestine has two mechanisms of calcium transport, known as gradient-dependent passive transport and metabolically energized active calcium transport (Nellans and Kimberg 1978; Bronner 2003). It is apparent that duodenum possesses both types of transport whereas the remaining parts possess mostly the passive transport (Bronner et al. 1986; Karbach 1991, 1992). Duodenum is a site where calcium absorption is tightly controlled, especially during increased calcium requirement (Boass et al. 1992; Karbach 1991, 1992). To gain further insight into effects of prolactin on each mechanism, our pioneer investigations provided evidence that 0.2 mg/kg prolactin injected intraperitoneally (i.p.) enhanced the passive calcium transport by *in situ* perfused small intestine (Krishnamra et al. 1998) and the active calcium transport by *in vitro* everted intestinal sacs (Krishnamra and Taweerathitam 1995) within an hour. Its effect was confined to the duodenum and the proximal jejunum (Krishnamra et al. 1998) where highly expressed prolactin receptors were reported (Dusanter-Fourt et al. 1992; Ouhtit et al. 1994). We also showed that both exogenous and endogenous

prolactin significantly enhanced the gradient-dependent passive calcium transport in the small intestine of female rats (Krishnamra et al. 1998; Piyabhan et al. 2000).

In the absence of the calcium gradient, the metabolically energized active calcium transport could be measured (Bronner et al. 1986; Bronner 1990), and its three fractions, namely transcellualr active, solvent drag-induced-and voltage-dependent calcium transport, could be studied separately (Charoenphandhu et al. 2001). Transcellular active calcium transport is a primarily active process composed of apical facilitated calcium entry, cytoplasmic calcium translocation and basolateral Ca^{2+}-ATPase-dependent calcium extrusion (Slepchenko and Bronner 2001; Bronner 2003). In 2001, Charoenphandhu and coworkers elucidated that prolactin directly and acutely stimulated the transcellular active calcium transport in a dose-response manner.

The solvent drag-induced and voltage-dependent calcium transports are secondary to the transcellular active sodium transport which produces a convective water flow (Diamond and Bossert 1967; Karbach 1991; Spring 1998) and an electrodiffusive force from transepithelial potential difference (Clarkson and Toole 1964; Rose and Schultz 1971; Sten-Knudsen and Ussing 1981), respectively, for paracellular calcium movement. In the duodenum, the voltage-dependent mechanism is presumptive but has been reported to be negligible and could be considered absent under both control and prolactin-exposed condition (Charoenphandhu et al. 2001). However, the presence and importance of the solvent drag-induced duodenal calcium transport has not been studied, and the direct action of prolactin on this component of calcium transport has not been investigated yet.

The perijunctional actomyosin-regulated widening of the tight junction has been postulated to increase the tight junction permeability as well as the solvent drag (Madara et al. 1987; Perez et al. 1997). Some inert chemicals such as mannitol, inulin and polyethyleneglycol were utilized to determine the widening of tight junction (Madara et al. 19887; Krugliak et al. 1994; Perez et al. 1997; Lloyd 1998). Nevertheless, a growing number of tight junction-associated proteins have been reported, and the tight junction was evinced to share physiological properties with conventional charge-selective ion channels (Simon et al. 1999; Weber et al. 2001; Tang and Goodenough, 2003). The regulation of solvent drag through tight junction may, therefore, not solely be regulated by the widening of the paracellular pores. We hypothesized that, under the influence of prolactin, the widening of tight junction was not sufficient to enhance the solvent drag-induced duodenal calcium transport. To prove the hypothesis, we administered cytochalasin E to induce the widening of duodenal tight junction. The jejunum was used as a reference because changes in the permeability of tight junction have been intensively studied in the rat jejunum (O'Rourke et al. 1995; Perez et al. 1997; Krishnamra et al. 1998).

We recently demonstrated in the *in vitro* system that the duodenum responded to the acute direct actions of prolactin by increasing the solvent drag-induced component of the active calcim transport (Tanrattana et al, 2004). We also found that the widening of tight junction was not required for the prolactin-induced increase in calcium transport, and that the convective force alone was sufficient to enhanced calcium movement. We would like to speculate that the properties of the tight junction in limiting the paracellular calcium traversing the epithelia are charge-selective and not size-selective since there was no association between the widening of tight junction and the solvent drag-induced calcium transport. It is possible that the tetraspan proteins claudins in the intercellular fibrils may be the critical proteins involved in paracellular transport of calcium (Colegio et al. 2002).

References

1. Boass, A., Lovdal, J.a., and Toverud, s.U. 1992. Pregnancy- and lactation-induced changes in active intestinal calcium transport in rat. Am. J. Physiol. 263: G127-G134.
2. Boass, A., and Toverud, S.U. 1997. Enhanced nonsaturable calcium transport in the jejunum of rats during lactation, but not during pregnancy. J. Bone Miner. Res. 12: 1577-1583.
3. Boass, A., Toverud, S.U., Pike, J.W., and Haussler, M.R. 1981. Calcium metabolism during lactation : enhanced intestinal calcium absorption in vitamin D-deprived hypocalcemic rats. Endocrinology, 109: 900-907.
4. Bronner, F. 1990. Intestinal calcium transport : the cellular pathway. Miner. Electrolyte Metab. 16 :94-100.
5. Bronner, F. 2003. Mechanisms and functional aspects of intestinal calcium absorption. J. Exp. Zoology. 300: 47-52.
6. Bronner, F., Pansu, D., and Stein, W.D. 1986. An analysis of calcium transport across the rat intestine. Am. J. Physiol. 250: G561-G56.
7. Charoenphandhu, N., Limlomwongse, L., and Krishnamra, N. 2001. Prolactin directly stimulates transcellular active calcium transport in the duodenum of female rats. Can. J. Physiol. Pharmacol. 79: 430-438.
8. Clarkson, T.W., and Toole, S.R. 1964. Measurement of short-circuit current and ion transport across the ileum. Am. J. Physiol. 206: 658-668.

9. Colegio, O.R., Van Itallie, C.M., McCrea, H.J., Rahner, C., and Anderson, J.M. 2002. Claudins create charge-selective channels in the paracellular pathway between epithelial cells. Am. J. Physiol. 283: C142-C147.
10. Diamond, J.M., and Bossert, W.H. 1967. Standing-gradient osmotic flow : a mechanism for coupling of water and solute transport in epithelia. J. Gen. Physiol. 50: 2061-2083.
11. Dusanter-Fourt, I., Belair, L., Gespach, C., and Djiane, J. 1992. Expression of prolactin (PRL) receptor gene and PRL-binding sites in rabbit intestinal epithelial cells. Endocrinology, 130: 2877-2882.
12. Halloran, B.P., and DeLuca, H.F. 1980. Calcium transport in small intestine during pregnancy and lactation. Am. J. Physiol. 239: E64-E68.
13. Karbach, U. 1991. Segmental heterogeneity of cellular and paracellular calcium transport across the rat duodenum and jejunum. Gastroenterology, 100: 47-58.
14. Karbach, U. 1992. Paracellular calcium transport across the small intestine. J. Nutr. 122: 672-677.
15. Krishnamra, N., Lotinun, S., and Limlomwongse, L. 1993. Acute effect and mechanism of action of prolactin on the in situ passive calcium absorption in rat. Bone Miner. 23: 253-266.
16. Krishnamra, N., Ousingsawat, J., and Limlomwongse, L. 2001. Study of acute pharmacologic effects of prolactin on calcium and water transport in the rat colon by an in vivo perfusion technique. Can. J. Physiol. Pharmacol. 79: 415-421.
17. Krishnamra, N., and Taweerathitam, P. 1995. Acute effect of prolactin on active calcium absorption in rats. Can. J. Physiol. Pharmacol. 73: 1185-1189.
18. Krishnamra, N., Thumchai, R., and Limlomwongse, L. 1990. Acute effect of prolactin on the intestinal calcium absorption in normal, pregnant and lactating rats. Bone Miner. 11: 31-41.
19. Krishnamra, N., Wirunrattanakij, Y., and Limlomwongse, L. 1998. Acute effects of prolactin on passive calcium absorption in the small intestine by in vivo perfusion technique. Can. J. Physiol. Pharmacol. 76: 161-168.
20. Krugliak, P., Hollander, D., Schlaepfer, C.C., Nguyen, H., and Ma, T.Y. 1994. Mechanism and sites of mannitol permeability of small and large intestine in the rat. Dig. Dis. Sci. 39: 796-801.

21. Lloyd, J.B. 1998. Intestinal permeability to polyethyleneglycol and sugars : a re-evaluation. Clin. Sci. 95: 107-110.
22. Lotinun, S., Limlomwongse, L., and Krishnamra, N. 1998. The study of a physiological significance of prolactin in the regulation of calcium metabolism during pregnancy and lactation in rats. Can. J. Physiol. Pharmacol. 76: 218-228.
23. Lotinun, S., Limlomwongse, L., Sirikulchayanonta, V., and Krishnamra, N. 2003. Bone calcium turnover, formation, and resorption in bromocriptine- and prolactin-treated lactating rats. Endocrine. 20: 163-170.
24. Madara, J.L., and Pappenheimer, J.R. 1987. Structural basis for physiological regulation of paracellular pathways in intestinal epithelia. J. Membr. Biol. 100: 149-164.
25. Mainoya, J.R. 1975. Effects of bovine growth hormone, human placental lactogen and ovine prolactin on intestinal fluid and ion transport in the rat. Endocrinology, 96: 1165-1170.
26. Nellans, H.N., and Kimberg, D.V. 1978. Cellular and paracellular calcium transport in rat ileum : effect of dietary calcium. Am. J. Physiol. 235: E726-E727.
27. O'Rourke, M., Shi S., Gisolfi, C., and Schedl,H. 1995. Effect of absorption of D-glucose and water on paracellular transport in rat duodenum-jejunum. Am. J. Med. Sci. 309: 146-151.
28. Ouhtit, A., Kelly, P.A., and Morel, G. 1994. Visualization of gene expression of short and long forms of prolactin receptor in rat digestive tissues. Am. J. Physiol. 266: G807-G815.
29. Pahuja, D.N., and DeLuca, H.F. 1981. Stimulation of intestinal calcium transport and bone calcium mobilization by prolactin in vitamin D-deficient rats. Science, 214: 1038-1039.
30. Peng, J.B., Chen, X.Z., Berger, U.V., Vassilev, P.M., Tsukaguchi, H., Brown, E.M., and Hediger, M.A. 1999. Molecular cloning and characterization of a channel-like transporter mediating intestinal calcium absorption. J. Biol. Chem. 274: 22739-22746.
31. Perez, M., Barber, A., and Ponz, F. 1997. Modulation of intestinal paracellular permeability by intracellular mediators and cytoskeleton. Can. J. Physiol. Pharmacol. 75: 287-292.

32. Piyabhan, P., Krishnamra, N., and Limlomwongse, L. 2000. Changes in the regulation of calcium metabolism and bone calcium content during growth in the absence of endogenous prolactin and during hyperprolactinemia : A longitudinal study in male and female Wistar rats. Can. J. Phyisol. Pharmacol.78: 757-765.
33. Rose, R.C., and Schultz, S.G. 1971. Studies on the electrical potential profile across rabbit ileum. Effects of sugars and amino acids on transmural and transmucosal electrical potential differences. J. Gen. Physiol. 57: 639-663.
34. Sekine, N., Ullrich, S., Regazzi, R., Pralong, W.F., and Wollheim, C. 1996. Postreceptor signaling of growth hormone and prolactin and their effects in the differentiated insulin-secreting cell line INS 1. Endocrinology 137: 1841-1850.
35. Simon, D.B., Lu, Y., Choate, K.A., Velazquez, H., Al-Sabban, E., Praga, M., Cassari, G., Bettinelli, A., Colussi, G., Rodriguez-Soriano, J., McCredie, D., Milford, D., Sanjad, S., and Lifton, R.. 1999. Paracellin-1, a renal tight junction protein required for paracellualr Mg^{2+} resorption. Science. 285: 103-106.
36. Slepchenko, B.M., and Bronner, F. 2001. Modeling of transcellular Ca transport I rat duodenum points to coexistence of two mechanisms of apical entry. Am. J. Physiol. Cell Physiol. 281: C270-C281.
37. Spring, K.R. 1998. Routes and mechanism of fluid transport by epithelia. Annu. Rev. Physiol. 60: 105-119.
38. Sten-Knudsen, O., and Ussing, H.H. 1981. The flux ratio equation under nonstationary condition. J. Membr. Biol. 63: 233-242.
39. Tang, V.W., and Goodenough, D.A. 2003. Paracellular ion channel at the tight junction. Biophys. J. 84: 1660-1673.
40. Tanrattana C., Charoenphandhu, N., Limlomwongse, L., and Krishnamra, N. 2004. Prolactin directly stimulated the solvent drag-induced calcium transport in the duodenum of female rats. Biochim Biophys Acta 2004; 1665: 81-91.
41. Van Cromphaut, s.J., Dewerchin, M., Hoenderop, J.G., Stockmans, I., Van Herck, E., Kato, S., Bindels, R.J., Collen, D., Carmeliet, P., Bouillon, R., and Carmeliet, G. 2001. Duodenal calcium absorption in vitamin D receptor-knockout mice : functional and molecular aspects. Proc. Natl. Acad Sci. USA 98: 13324-13329.
42. Weber, S., Schlingmann, K.P., Peters, M., Nejsum, L.N., Nilsen, s., Engel, H., Grzeschik, K.H., Seyberth, H.W., Grone, H.J., Nusing, R., and Konrad, M. 2001.

Primary gene structure and expression studies of rodent paracellin-1. J. Am. Soc. Nephrol. 12: 2664-2672.

43. Weber, K., Erben, R.G., Rump, a., and Adamski, J. 2001. Gene structure and regulation of the murine epithelial calcium channels ECaC 1 and 2. Biochem Biophys Res Commun 289: 1287-1294.

1.1 Study of the mechanism of action and signal transduction of prolactin (PRL) in the regulation of calcium and fluid transport in the intestine

Present Findings

The present research first established the effect of endogenous prolactin on calcium absorption in various intestinal segments of the suckling rats. Before measuring the calcium fluxes, 9-day-old rats were administered for 7 days with 0.9% NaCl, s.c. (control), 3 mg/kg bromocriptine, i.p., twice daily to abolish secretion of endogenous prolactin, or bromocriptine plus exogenous 2.5 mg/kg prolactin, s.c. Thereafter, the 16-day-old rats were experimented upon by instilling the ^{45}Ca-containing solution into the intestinal segments. The results showed that, under a physiological condition, the jejunum had the highest rate of calcium absorption compared with other segments (1.4 ± 0.35 μmol.$^{-1}$, $p<0.05$). The duodenum and ileum also manifested calcium absorption, whereas the colon showed calcium secretion. Lack of endogenous prolactin decreased lumen-to-plasma and net calcium fluxes in jejunum from 2.07 ± 0.31 to 1.19 ± 0.12 and 1.40 ± 0.35 to 0.88 ± 0.18 μmol.h^{-1},cm^{-1} ($p<0.05$), respectively, and exogenous prolactin restored the jejunal calcium absorption to the control value. Endogenous prolactin also had an effect on the duodenum but, in this case, exogenous prolactin did not reverse the effect of bromocriptine. However, neither ileal nor colonic calcium fluxes were influenced by prolactin. Because luminal sodium concentration has been demonstrated to affect calcium absorption in mature rats, the effect of varying luminal sodium concentrations on calcium fluxes in suckling rats was evaluated. The jejunum was used due to its highest rate of calcium absorption. After filling the jejunal segments with 124 (control), 80, 40 mmol/L Na^+-containing or Na^+-free solution, increases in calcium absorption were found to be inversely related to luminal sodium concentrations in both control and

bromocriptine-treated rats. The plasma concentration of ^{45}Ca under luminal sodium free condition was also higher than that of the control condition (2.26%±0.07% vs. 2.01%±0.09% administered dose, $p<0.05$). However, ^{3}H-mannitol, a marker of the widening of tight junction that was introduced into the lumen, had a stable level in the plasma during an increase in plasma ^{45}Ca, suggesting that the widening of tight junction was not required for enhanced calcium absorption. **In conclusion, calcium absorption in suckling rats was of the highest rate in the jejunum where endogenous prolactin modulated calcium absorption without increasing the paracellular transport of mannitol.**

We further investigated the effect of prolactin on the duodenal calcium absorption and found that 200, 400, and 800 ng/mL prolactin produced a significant increase in the total ATPase activity of duodenal crude homogenate in a dose-dependent manner within 60 min (i.e., from a control value of 1.53±0.13 to 2.29±0.21 ($p<0.05$), 2.68±0.19 ($p<0.01$), and 3.92±0.33 ($p<0.001$) μmol P_i (mg protein)$^{-1}$.min^{-1}, respectively. Activity of Na^+/K^+-ATPase was increased by 800 ng/mL prolactin from 0.17±0.03 to 1.18±0.29 μmol P_i (mg protein)$^{-1}$.min^{-1} ($p<0.01$). Prolactin at doses of 400 and 600 ng/mL also significantly increased the activities of Ca^{2+}-ATPase in crude homogenate from a control value of 0.84 ±0.03 to 1.75±0.29 ($p<0.05$, and 2.30±0.37 ($p<0.001$) μmol P_i (mg protein)$^{-1}$.min^{-1}. When the crude homogenate was purified for the basolateral membrane, the Na^+/K^+-ATPase activities were elevated 10-fold. In the purified homogenate, 800 ng/mL prolactin increased Na^+/K^+-ATPase activity from 1.79±0.38 to 2.63±0.44 μmol P_i (mg protein)$^{-1}$.min^{-1} ($p<0.05$), and Ca^{2+}-ATPase activity from 0.08±0.14 to 0.23±0.23 μmol P_i (mg protein)$^{-1}$.min^{-1} ($p<0.001$). Because the apical calcium entry was the first important step for the transcellualr active calcium transport, the brush border calcium uptake was also investigated in this study. We found that, 8 min after being directly exposed to 800 ng/mL proalctin, the brush border calcium uptake into the duodenal epithelial cells was increased from 0.31±0.02 to 0.80±0.28 μmol P_i (mg protein)$^{-1}$.min^{-1} ($p<0.05$). **It was concluded that prolactin directly and rapidly enhanced the brush border calcium uptake as well as the activities of the basolateral Na^+/K^+- and Ca^{2+}/K^+-ATPase in the duodenal epithelium of female rats.** These findings explained the mechanisms by which prolactin stimulated duodenal active calcium absorption. We next studied the mechanism

and signaling pathway by which PRL enhanced calcium transport in the rat duodenum and Caco-2 monolayer. Both epithelia strongly expressed mRNAs and proteins of PRL receptors. Ussing chamber technique showed that the duodenal active calcium fluxes were increased by PRL in a dose-response manner with the maximal effect dose of 800 ng/ml. This response diminished after exposure to LY-294002, a phosphoinositide 3-kinase (PI3K) inhibitor. Caco-2 monolayer gave similar response to PRL with the maximal effective dose of 600 ng/ml. By nullifying the transepithelial potential difference, we showed that the voltage-dependent paracellular calcium transport did not contribute to the PRL-enhanced flux in Caco-2 monolayer. In contrast, the calcium gradient-dependent paracellular transport and calcium permeability were increased by PRL. Effects of PRL on Caco-2 monolayer were abolished by PI3K inhibitors (LY-294002 and wortmannin), but not by inhibitors of MEK (U-0126) or JAK2 (AG-490). To investigate whether the PRL-enhanced parcellular transport was linked to changes in the epithelial charge selectivity, the permeability ratio of sodium and chloride (P_{Na}/P_{Cl}) was determined. We found that PRL elevated the (P_{Na}/P_{Cl}) in both epithelia, and the effects were blocked by PI3K inhibitors. In conclusion, PRL directly and rapidly stimulated the active and passive calcium transport in the rat duodenum and Caco-2 monolayer via the nongenomic PI3K-signaling pathway. This PRL-enhanced paracellular calcium transport could have resulted from altered charge selectivity.

Since there has been no report pertaining a down regulation of active duodenal calcium transport in estrogen deficient female rats, we next investigated the long term effect of prolactin on the active duodenal calcium transport in ovariectomized rats, The aim of the study was therefore, to demonstrate the effects of long-term prolactin exposure produced by anterior pituitary (AP) transplantation on the duodenal calcium transport in young (9-week-old) and adult (22-week-old) ovariectomized rats. We found that ovariectomy did not alter the transcellular active duodenal calcium transport in young and adult rats fed normal calcium diet (1.0% w/w Ca) but decreased the solvent drag-induced duodenal calcium transport from 75.50±10.12 to 55.75±4.77 $nmol.hr^{-1}.cm^{-2}$ ($p<0.05$) only in adult rats. Long-term prolactin exposure stimulated the transcellular active calcium transport in young and adult AP-grafted ovariectomized rats fed with normal calcium diet by more than 2-fold from 7.56±0.79 to 16.54±2.05 ($p<0.001$) and 9.78±0.72 to 15.99±1.75 ($p<0.001$) $nmol.hr^{-1}.cm^{-2}$, respectively. However, only the

solvent drag-induced duodenal calcium transport in young rats was enhanced by prolactin from 95.51±10.64 to 163.20±18.03 nmol.hr^{-1}.cm^{-2} ($p<0.001$) whereas that in adult rats still showed a decreased flux from 75.50±10.12 to 47.77±5.42 nmol.hr^{-1}.cm^{-2} ($p<0.05$). Because oral calcium supplement has been widely used to improve calcium balance in estrogen-deficient animals, the effect of a high-calcium diet (2.0% w/w Ca) was also investigated. The results showed that stimulatory action of long-term prolactin on the transcellular active duodenal calcium transport in both young and adult rats was diminished after being fed a high-calcium diet. The same diet also abolished prolactin-enhanced solvent drag-induced duodenal calcium transport in young and further decreased that in adult AP-grafted ovariectomized rats. We concluded that the solvent drag-induced duodenal calcium transport in adult rats was decreased after ovariectomy. **Long-term prolactin exposure stimulated the transcellular active duodenal calcium transport in both young and adult rats whereas enhancing the solvent drag-induced duodenal calcium transport only in young rats. Effects of prolactin were abolished by a high-calcium diet.**

Most of the previous work was on the small intestine but prolactin has been known to increase fluid and NaCl absorption in the rat proximal colon too. Therefore we analyzed ion transport in mouse proximal and distal colon, and acute changes induced by PRL. In the proximal colon, carbachol activated a Ca^{2+} dependent Cl^- secretion that was sensitive to DIDS and NFA. In the distal colon, both ATP and carbachol activated K^+ secretion. Ca^{2+}-activated KCl transport in proximal and distal colon was inhibited by PRL (200 ng/ml), while amiloride sensitive Na^+ absorption and cAMP induced Cl^- secretion remained unaffected. Luminal large conductance Ca^{2+}-activated K^+ (BK) channels were largely responsible for Ca^{2+}-activated K^+ secretion in the distal colon, and basolateral BK channels supported Ca^{2+}-activated Cl^- secretion in the proximal colon. Ca^{2+} chelating by BAPTA-AM attenuated effects of carbachol and abolished effects of PRL. Both inhibition of P13 kinase with wortmannin and blockage of MAP kinases with SB 203580 or U 0126, interfered with the acute inhibitory effect of PRL on ion transport, while blocking of Jak/Stat kinases with AG 490 was without effects. PRL attenuated the increase in intracellular Ca^{2+} that was caused by stimulation of isolated colonic crypts with carbachol. **Thus prolactin inhibits Ca^{2+} dependent Cl^-**

and K^+ secretion in the colon by interfering with intracellular Ca^{2+} signaling and probably by activating P13 kinase and MAP kinase pathways.

In summary, we have found stimulatory effect of prolactin on the transcellular and solvent drag-induced active calcium absorption and the passive calcium absorption in the small intestine as shown in Figure 1.

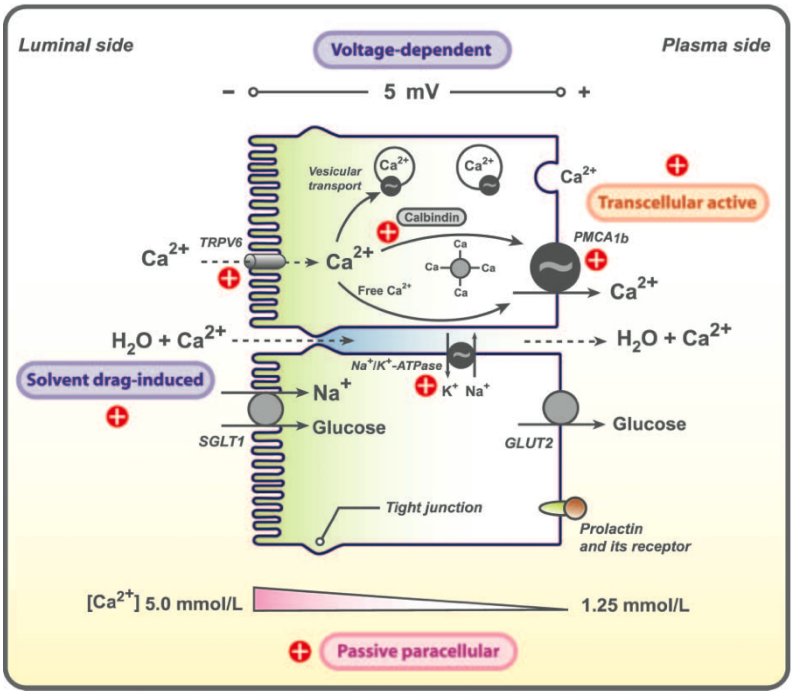


Fig. 1 Schematic diagram shows active and passive calcium transport in the duodenum of female rats. Active transport consists of 3 components : transcellular active, solvent drag-induced, and voltage-dependent transport. Passive paracellular calcium transport is dependent on the calcium gradient across the epithelial sheet. The "plus" signs (+) represent the sites of prolactin stimulation (Bruns et al. 1983; Charoenphandhu et al, 2001, 2006a; Tanrattana et al. 2004). TRPV6, transient receptor potential vanilloid family channel 6; $PMCA_{1b}$, plasma membrane Ca^{2+}-ATPase isoform 1b;SGLT1, Na^+/glucose cotransporter 1; GLUT2, glucose transporter 2. (Charoenphandhu and Krishnamra 2007)

1.2 Role of prolactin in fluid and electrolyte regulation in the endometrium

Background and Rationale

The endometrium is responsible for the transfer of nutrients, fluid and electrolytes between the circulatory system and the uterine lumen. The transport activities of the endometrial epithelium is thus essential for the maintenance of the composition of fluid and electrolytes, and the suitable environment for fertilization, implantation and embryo development. Several signaling molecules such as hormones, prostaglandins, growth factors, regulatory peptide and cytokines have been shown to regulate ion transport processes across the endometrial epithelial cells (Vetter and O'Grady, 1996; Deachapunya and O'Grady, 1998). These molecules are produced by endometrial epithelial cells under the influence of ovarian steroid hormones and act as autocrine and paracrine mediators to regulate proliferation, differentiation and secretion including ion transport activities (Jacobs and Carson, 1993; Chen et al., 1995).

Prolactin (PRL) is synthesized and secreted from anterior pituitary gland and extrapituitary tissues including myometrium, deciduas, mammary epithelial cells. It exerts a wide variety of biological actions such as water and electrolyte balance, growth of mammary gland, milk production and secretion. Recently, PRL has been considered as a novel calcium regulating hormones for its effect on increased intestinal calcium absorption (Krishnamra et al., 1997; Charoenphandhu et al., 2001), especially under conditions where high calcium is needed during pregnancy and lactation.

In the endometrium, PRL is synthesized by decidualized endometrial stromal cells from the late secretory phase of the menstrual cycle and throughout pregnancy. The level of PRL is much higher in blood and amniotic fluid during pregnancy (Golander et al., 1978; Daly et al., 1983). After conception, a continuous increase of PRL production in decidual cells leads to an accumulation of PRL in the amniotic fluid up to 2-3 μg/ml. (Golander et al., 1978; Daly et al., 1983). In addition, PRL receptors (PRL-R) and its mRNA as well as the JAK-STAT proteins have been identified in human glandular epithelial cells and stromal cells (Tseng and Zhu, 1998; Jabbour et al., 1998). Levels of PRL receptor mRNA are much higher in glandular cells compared with stromal cells. Although the exact role of PRL in the human endometrium remains to be clarified; however, the pattern of secretion and expression supports a role for PRL during implantation and placentation this hypothesis is supported by the findings that the blastocyst implantation and the maintenance of pregnancy are impaired in PRL and PRL-R knockout mice (Jikihara *et al.,* 1996). PRL also has mitogenic effect on endometrial cells. It increases endometrial cell growth at physiological concentrations and inhibits cell growth and attachment at higher concentrations (>100 ng/ml) (Nagami

and Tominaga, 1991; Tseng and Mazella, 1999). In the rhesus monkey, PRL regulates the amniotic and fetal extracellular fluid and electrolyte balance by decreasing the water flux from the amniotic side of fetal membrane (Josimovich et al., 1977). These lines of evidence strongly suggest the role of PRL in the regulation of endometrial cell transport functions. Due to the presence of PRL and its roles on fluid and electrolyte regulation of the amniotic fluid, it is likely that the local production of PRL exerts autocrine and paracrine actions to regulate volume and composition of fluid and electrolytes within uterine cavity, to provide optimal conditions for implantation and development of embryo.

There have been a few reports on the effect of PRL on the regulation of water and electrolyte transport in various epithelial cells. Previous studies using mouse mammary epithelial cells grown on floating collagen gels have demonstrated that PRL treatment for at least 3 days in the culture media produces an increase in short circuit current (Isc) and transepithelial potential difference (PD), corresponding to an increase in net active Na^+ absorption and a small net Cl^- secretion (Bisbee *et al*., 1978). In mouse mammary epithelial cell line HC 11, PRL acutely increases Cl^- transport through the JAK-STAT system (Selvaraj et al., 2000). In rabbit mammary glands, PRL decreases the membrane permeability to sucrose, suggesting a decrease in permeability of tight junction (Linzell et al., 1975). Studies in mammalian intestinal tissues using everted sac technique have shown the effect of PRL on increasing the fluid and NaCl absorption in rat, hamster and quinea pig jejunum, but not in quinea pig ileum and rat colon (Mainoya *et al*., 1974). These studies suggest an important role of PRL in the regulation of transepithelial ion transport in a variety of epithelia. However, little is known about the effect of PRL on the transport of water and ions by endometrial epithelial cells.

The endometrial epithelial cells from mammalian species have been isolated and grown as a primary cell in culture (Nagami and Tominaga, 1991; Jacobs and Carson 1993; Deachapunya and O'Grady, 1998). These cells possess the morphological and functional polarity in culture, providing a useful system for studying transepithelial electrolyte transport. Several hormones, growth factors and cytokines have been shown to affect the transport-related activities of endometrial epithelial cell in culture. Our preliminary study demonstrates that treatment of cell monolayers with PRL for 2 days causes an increase in basal Isc which is markedly inhibited by amiloride, Na^+ channel blockers. This finding suggests a possible role of PRL in the regulation of electrolyte transport in the endometrium. **Therefore the objectives of this study were to identify the mechanism and regulation of PRL on water and electrolyte transport in the primary culture of endometrial epithelial cells.**

Objectives of the Research Project

1. To characterize the possible effect of PRL on water and electrolyte transport in the primary culture of glandular endometrial epithelial cells
2. To study the transport mechanisms of PRL action on the regulation of electrolyte transport in endometrial epithelial cells
3. To characterize the expression of PRL receptor in endometrial epithelial cells under normal and estrogen-treated conditions
4. To investigate the mechanism of action and the intracellular signaling pathway of PRL in endometrium

References

1. Bisbee, C.A., Machen, T.E. and Bern, H.A. (1979) Mouse mammary epithelial cells on floating collagen gels: Transepithelial ion transport and effects of prolactin. *Proc. Natl. Acad. Sci.* USA 76: 536-540.
2. Chan, H.C., Liu, C.Q., Fong, S.K., Law, S.H., Wu, L.J., So, E., Chung, Y.W., Ko, W.H. and Wong, P.Y.D. 1997. Regulation of Cl^- secretion by extracellular ATP in cultured mouse endometrial epithelium. *J. Membrane Biol.* 156:45-52.
3. Chan, H.C., S.K. Fong, Y.W. Chung, and P.Y.D. Wong. 1997. Stimulation of anion secretion by β-adrenoreceptors in the mouse endometrial epithelium. *J. Physiol.* 501:517-525.
4. Chen, D.B., Yang, Z.M., Hilsenrath, R.,Le,S.P. and Harper, M.J.K. (1995) Stimulation of prostaglandin $F_{2\alpha}$ and PGE_2 release by tumour necrosis factors-α in cultured human luteal phase endometrial cells. *Hum. Reprod*. 10: 2773-2780.
5. Charornphandhu, N., Limlomwingse, L. and Krishnamra, N. (2001) Prolactin directly stimulates transcellular active calcium transport in the duodenum of female rats. *Can. J. Physiol. Pharmacol*. 79: 430-438.
6. Golander, A., Hurley, T., Barrett, J., Hizi, A. and Handwerger, S. (1978) Prolactin synthesis by human choriondecidual tissue: A possible source of prolactin in the amniotic fluid. *Science* 202: 311-313.
7. Daly, D.C., Maslar, I.A. and Riddick, D.H. (1983) Prolactin production during in vitro decidualization of proliferative endometrium. *Am. J. Obstet. Gynecol.* 145: 672-678.
8. Deachapunya, C. and O'Grady, S.M. (1998) EGF regulates amiloride-sensitive Na absorption across glandular endometrium. *FASEB Journal* 12(5): A591.
9. Deachapunya, C., and O'Grady, S.M. (1998) Regulation of chloride secretion across porcine endometrial epithelial cells by prostaglandin E_2. *J. Physiol.* 508:31-47.

10. Jacobs, A.L. and Carson, D.D. (1993) Uterine epithelial cell secretion of interleukin-α induces prostaglandin E_2 and $PGF_{2\alpha}$ secretion by uterine stromal cells in vitro. *Endocrinology* 312: 300-308.
11. Jabbour, H.N., Critchley, H.O. and Boddy, S.C. (1998) Expression of functional prolactin receptors in nonpregnant human endometrium: janus kinase-2, signal transducer and activator of transcription-1 (STAT1), and STAT5 proteins are phosphorylated after stimulation with prolactin. *J. Clin. Endocrinol. Metab.* 83(7): 2545-53.
12. Jikihara, H., Kessler, C.A., Cedars, M.I., Bra, A.K. (1996) Up-regulation of the human prolactin receptor in the endometrium. *J. Endocrinol*. 5: 157-162.
13. Josimovich, J.B., Merisko, K. and Boccella, L. (1977) Amniotic prolactin control over amniotic and fetal extracellular fluid water and electrolytes in the rhesus monkey. *Endocrinology* 100: 564-570.
14. Krishnamra, N., Wirunrattanakij, Y. and Limlomwongse, L. (1997) Acute effects of prolactin on passive calcium absorption in the small intestine by vivo perfusion technique. *Can. J. Physiol. Pharmacol.* 76: 161-168.
15. Linzell, J.L., Peaker, M. and Taylor, J.C. (1975) The effects of prolactin and oxytocin on milk secretion and on the permeability of the mammary epithelium in the rabbit. *J. Physiol.* 253(2): 547-63.
16. Mainoya, J.R., bern, H.A. and Regan, J.W. (1974) Influence of ovine prolactin on transport of fluid and sodium chloride by the mammalian intestine and gall bladder. *J. Endocrinol.* 63: 311-317.
17. Selvaraj, N.G, Omi, E., Gibori, G., Rao, M.C. (2000) Janus kinase 2 (JAK2) regulates prolactin-mediated chloride transport in mouse mammary epithelial cells through tyrosine phosphorylation of Na^+-K^+-$2Cl^-$ cotransporter. *Mol. Endocrinol.* 14(12): 2054-65.
18. Nagami, A.I. and Tominaga, T. (1991) Effects of prolactin on cultured human endome-trial cells. *Horm. Res*. 35: 50-57.
19. Tseng, L. and Zhu, H.H. (1998) Progestin induces prolactin receptor in human endometrial stromal cells. *J. Soc. Gynecol. Invest.* 5: 149-155.
20. Tseng, L. and Mazella, J. (1999) Prolactin and its receptor in human endometrium. *Seminars in reproductive endocrinology* 17(1): 23-27.
21. Vetter, A.E., and O'Grady, S.M. (1996) Mechanisms of electrolyte transport across the endometrium I. Regulation by $PGF_{2\alpha}$ and cAMP. *Am. J. Physiol.* 270:663-672.

Present Findings

The regulation of prolactin(PRL) on electrolyte transport was investigated in primary culture of glandular endometrial epithelial cells. Porcine endometrial epithelial cells was isolated, cultured on permeable filters and mounted in Ussing chambers with the use of voltage clamp circuitry to measure short circuit current

(Isc). PRL produced a peak and sustained increase in Isc in a concentration dependent manner with a maximum effect at 1 μg/ml and an EC_{50} value of 120 ng/ml. The Isc increased by PRL was mostly inhibited by pretreatment with an apical addition of 200 μM NPPB or 1 mM diphenylamine-2-carboxylic acid (DPC), Cl^- channel blockers, but not by 10 μM amiloride, a Na^+ channel blocker. In addition, replacement of Cl^- and HcO_3^- or pretreatment with 200 μM bumetanide, a Na^+-K^+-$2Cl^-$ cotransporter inhibitor, in the basolateral solution mostly abolished the PRL-stimulated Isc. Pretreatment with 10 μM indomethacin also abolished the PRL-induced increase in Isc. Addition of 50 μM AG490, an inhibitor of JAK2 activity, to both apical and basolateral solutions completely abolished the PRL-increased Isc. Western blot analysis and immunocytochemistry revealed the expression of short isoform PRL receptors. These results demonstrated the PRL stimulation of anion secretion in endometrial epithelial cells, which may be mediated by JAK-STAT dependent pathway or through the release of cyclooxygenase metabolites.

(Full manuscript is in the Appendix)

References

1. Bisbee, C.A., Machen, T.E. and Bern, H.A. (1979) Mouse mammary epithelial cells on floating collagen gels: Transepithelial ion transport and effects of prolactin. *Proc. Natl. Acad. Sci.* USA 76: 536-540.
2. Chan, H.C., Liu, C.Q., Fong, S.K., Law, S.H., Wu, L.J., So, E., Chung, Y.W., Ko, W.H. and Wong, P.Y.D. 1997. Regulation of Cl^- secretion by extracellular ATP in cultured mouse endometrial epithelium. *J. Membrane Biol.* 156:45-52.
3. Chan, H.C., S.K. Fong, Y.W. Chung, and P.Y.D. Wong. 1997. Stimulation of anion secretion by β-adrenoreceptors in the mouse endometrial epithelium. *J. Physiol.* 501:517-525.
4. Chen, D.B., Yang, Z.M., Hilsenrath, R.,Le,S.P. and Harper, M.J.K. (1995) Stimulation of prostaglandin $F_{2\alpha}$ and PGE_2 release by tumour necrosis factors-α in cultured human luteal phase endometrial cells. *Hum. Reprod*. 10: 2773-2780.
5. Charornphandhu, N., Limlomwingse, L. and Krishnamra, N. (2001) Prolactin directly
   stimulates transcellular active calcium transport in the duodenum of female rats. *Can. J. Physiol. Pharmacol*. 79: 430-438.
6. Golander, A., Hurley, T., Barrett, J., Hizi, A. and Handwerger, S. (1978) Prolactin synthesis by human choriondecidual tissue: A possible source of prolactin in the amniotic fluid. *Science* 202: 311-313.

7. Daly, D.C., Maslar, I.A. and Riddick, D.H. (1983) Prolactin production during in vitro decidualization of proliferative endometrium. *Am. J. Obstet. Gynecol*. 145: 672-678.
8. Deachapunya, C. and O'Grady, S.M. (1998) EGF regulates amiloride-sensitive Na absorption across glandular endometrium. *FASEB Journal* 12(5): A591.
9. Deachapunya, C., and O'Grady, S.M. (1998) Regulation of chloride secretion across porcine endometrial epithelial cells by prostaglandin E_2. *J. Physiol.* 508:31-47.
10. Jacobs, A.L. and Carson, D.D. (1993) Uterine epithelial cell secretion of interleukin-α induces prostaglandin E_2 and $PGF_{2\alpha}$ secretion by uterine stromal cells in vitro. *Endocrinology* 312: 300-308.
11. Jabbour, H.N., Critchley, H.O. and Boddy, S.C. (1998) Expression of functional prolactin receptors in nonpregnant human endometrium: janus kinase-2, signal transducer and activator of transcription-1 (STAT1), and STAT5 proteins are phosphorylated after stimulation with prolactin. *J. Clin. Endocrinol. Metab.* 83(7): 2545-53.
12. Jikihara, H., Kessler, C.A., Cedars, M.I., Bra, A.K. (1996) Up-regulation of the human prolactin receptor in the endometrium. *J. Endocrinol*. 5: 157-162.
13. Josimovich, J.B., Merisko, K. and Boccella, L. (1977) Amniotic prolactin control over amniotic and fetal extracellular fluid water and electrolytes in the rhesus monkey. *Endocrinology* 100: 564-570.
14. Krishnamra, N., Wirunrattanakij, Y. and Limlomwongse, L. (1997) Acute effects of prolactin on passive calcium absorption in the small intestine by vivo perfusion technique. *Can. J. Physiol. Pharmacol.* 76: 161-168.
15. Linzell, J.L., Peaker, M. and Taylor, J.C. (1975) The effects of prolactin and oxytocin on milk secretion and on the permeability of the mammary epithelium in the rabbit. *J. Physiol.* 253(2): 547-63.
16. Mainoya, J.R., bern, H.A. and Regan, J.W. (1974) Influence of ovine prolactin on transport of fluid and sodium chloride by the mammalian intestine and gall bladder. *J. Endocrinol.* 63: 311-317.
17. Selvaraj, N.G, Omi, E., Gibori, G., Rao, M.C. (2000) Janus kinase 2 (JAK2) regulates prolactin-mediated chloride transport in mouse mammary epithelial cells through tyrosine phosphorylation of Na^+-K^+-$2Cl^-$ cotransporter. *Mol. Endocrinol.* 14(12): 2054-65.
18. Nagami, A.I. and Tominaga, T. (1991) Effects of prolactin on cultured human endometrial cells. *Horm. Res*. 35: 50-57.
19. Tseng, L. and Zhu, H.H. (1998) Progestin induces prolactin receptor in human endometrial stromal cells. *J. Soc. Gynecol. Invest.* 5: 149-155.
20. Tseng, L. and Mazella, J. (1999) Prolactin and its receptor in human endometrium. *Seminars in reproductive endocrinology* 17(1): 23-27.

21. Vetter, A.E., and O'Grady, S.M. (1996) Mechanisms of electrolyte transport across the endometrium I. Regulation by $PGF_{2\alpha}$ and cAMP. *Am. J. Physiol.* 270:663-672.

1.3 Investigation of the role of prolactin in calcium handing in the mammary gland

Background and Rationale

Huge amount of calcium (Ca) is transported from plasma to be stored in the mammary gland cells via unclear mechanisms before eventually to be secreted into milk (5, 6). In cows, this process, if very active, can be dangerous because it leads to nearly fatal hypocalcemia before parturition (7). These changes in cellular activities during pregnancy and lactation are thought to be under the influences of a number of hormones and growth factors.

The mechanisms of calcium transport across the mammary epithelial cells are not well understood. However, the drastic changes in cellular activities commence at the beginning of pregnancy and continue consistently through lactation. Ca enters the mammary gland cells from the serosal side via unknown mechanism, which is proposed to involve some as yet unidentified Ca channels (8). The huge amount of the Ca influx is kept in the intracellular calcium storages or pumped out of the cell to prevent causing cytotoxicity due to abnormally high level of the cytosolic calcium. These intracellular calcium storages are endoplasmic reticulum, mitochondria and Golgi apparatus, the last of which, being different from the other organs, is the major storage (9, 10). Ca is delivered into milk via a number of pathways. The intracellular calcium is bound to casein, lactose and inorganic anions (eg. phosphate and citrate) and transported by the Golgi apparatus. They form the micelle complex in milk. However, two-third of these Ca vesicles bind to casein and form the casein micelle complex (11, 12). From this evidence, it could be implied that the regulatory mechanism of Ca transport may link to that of casein. There might be Ca channel localized at the apical membrane of the mammary gland cell, but it has not been investigated.

In addition, the cytosolic calcium concentration can be maintained at low level (10^{-7} M) by the activity of plasma membrane Ca^{2+}-ATPases (PMCA) in the mammary gland cells. There are three types of PMCA, namely, PMCA1, 2 and 4. PMCA1 and PMCA4 are housekeeping form, which are found ubiquitously in many organs. They are located mostly at the basolateral side of the cells and in minimal amount at the apical side. Their numbers are increased in pregnancy and keep rising through the lactating period (13, 14). PMCA2, which is originally found in the brain only (15), is also localized chiefly at the apical side of the mammary gland cells and is drastically increased on the first day of lactation and continue to increase over the whole period (13, 14). PMCA2 is thus proposed to be the effective mechanism for Ca secretion into milk. The elevated expression of PMCA2 starting at the beginning of lactation also indicates its association with PRL.

Nevertheless, the direct action of PRL on the expression of these PMCAs has not been demonstrated.

Factors affecting milk Ca concentration

The output of Ca into milk is much greater than the intake unless the mother drink a large amount of milk with adequate intake of vitamin D. It has been shown that dietary Ca per se is not an important factor affecting milk Ca content (16). If dietary Ca is not sufficient for milk production, then Ca will be made available from bone of the lactating mothers (17). The parathyroid hormone is also involved with a role in supplying the required Ca by maintaining the plasma Ca level. Milk production has been shown to be regulated by a number of hormones, such as 1, 25-$(OH)_2D_3$ (18) and parathyroid hormone-related peptide (PTHr) (19). However, the mechanism of how the concentration of Ca in milk is controlled or whether it is controlled at all has not been studied.

Prolactin

PRL has direct effects on growth and activities of the mammary gland during lactation. In human, its level greatly increases from the non-pregnant level of 1-10 ng/ml to about 200 ng/ml during pregnancy and decreases by 50% to 100 ng/ml in the first week of lactation. After 2-3 months postpartum, the basal level declines to 40-50 ng/ml but elevates to about ten- to twenty-fold during suckling. (20)

During pregnancy and lactation, the highly elevated level of PRL regulates the growth and differentiation of mammary gland cells including the synthesis of many significant substances such as casein, lactose, and fatty acids, which are secreted into milk (21). Its effect on the synthesis and transport of the milk-containing substances has been studied extensively. PRL also affects the transport of electrolytes notably phosphate and iodide (22, 23, 24). Lactose synthesis by mammary gland cells and transport into milk during parturition are under combined effect of PRL and growth hormone since they enhance the expression of GLUT1 transporter required for uptake of glucose which is a precursor of lactose (25). In the cultured mouse tissue studies, PRL was also found to stabilize and promote the transcription of casein mRNA, which was expressed maximally at 10 ng PRL/ml (26, 27). The study in rabbit mammary epithelial cells demonstrated that casein secretion is stimulated by PRL through the activation of arachidonic acid which is the non-genomic signaling pathway (28).

Since PRL is the lactating hormone that has stimulating effects on the secretion of many major constituents of milk especially casein, it was hypothesized that PRL should also enhance the secretion of Ca in milk. However, the effects of

PRL on Ca transport in the mammary gland cells were still not clearly understood. In this study, we aimed to investigate both genomic and non-genomic actions of PRL on calcium metabolism in the mammary gland cells. The effect of PRL on the expression of PMCA will be used to represent the genomic action, whereas acute changes in the cellular calcium uptake will represent the non-genomic action. Possible genomic effect of PRL on Ca transport proteins will also be examined. Finally, the signaling pathway of PRL involved in the regulation of calcium metabolism in mammary gland will be evaluated. These investigations will be performed on breast cancer cell lines, MCF-7. However, at the end of this experimental plan, mammary gland explants would also be used to demonstrate the physiological role of PRL.

Relationship of PRL and calcium metabolism

From our laboratory investigations, we found a strong relationship between PRL and Ca metabolism in the gastrointestinal tract and bone. Ca channels allow the passive movement of Ca from the lumen into intestinal cells (31). Ca is then translocated across the cell and transported via Ca-ATPase across the basolateral membrane into the blood. This transcellular active transport of Ca was found to be stimulated by PRL. Passive absorption of Ca along the paracellular pathway was also enhanced by PRL (32). Bone Ca turnover rate and Ca accumulation were increased after 7 day treatment with PRL (33, 34).

Present Finding

Regulatory role of prolactin(PRL) on Ca^{2+} mobilization in human mammary gland cell line MCF-7 was examined. Direct addition of PRL did not affect cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]$); however, treatment with PRL for 24 h significantly decreased the peak level and duration time of ($[Ca^{2+}]$) elevation evoked by ATP or thapsigargin(TG). Intracellular Ca^{2+} release by IP_3 or TG in permeablized cells was not decreased after PRL-treatment, indicating that the Ca^{2+} release was not impaired by PRL treatment. Extracellular Ca^{2+} entry evoked by ATP or TG was likely to be intact, because entry of extracelualr Ba^{2+} was not affected by PRL treatment. Among Ca^{2+}-ATPase expressed in MCF-7 cells, we found significant increase of secretory pathway Ca^{2+}-ATPase type 2 (SPCA2) mRNA in PRL-treated cells by RT-PCR experiments including quantitative RT-PCR. Knockdown of SPCA2 by siRNA in PRL-treated cells showed similar Ca^{2+} mobilization to that in PRL-untreated cells. **The present results suggest that PRL facilitates Ca^{2+} transport into Golgi apparatus and may contribute the supply of Ca^{2+} to milk.**

(Full manuscript is in the appendix)

References

1. McManaman JL and Neville MC. Mammary physiology and milk secretion: review. Advanced Drug Delivery Reviews 2003, 55: 629-641.
2. Kuhn NJ. Progesterone withdrawal as the lactogenic trigger in the rat. J Endocrinol 1969, 44: 39-54.
3. Hobbs AA, Richards DA, Kessler DJ and Rosen JM. Complex hormonal regulation of rat casein gene expression. J Biol Chem 1982, 257: 3598-3605.
4. Nguyen DA, Parlow AF and Neville MC. Hormonal regulation of tight junction closure in the mouse mammary epithelium during the transition from pregnancy to lactation. J Endocrinol 2001, 170: 347-356.
5. Neville MC and Peaker M. Calcium fluxes in mouse mammary tissue in vitro: intracellular and extracellular calcium pools. J Physiol (Lond.) 1982, 323: 497-517.
6. Baumrucker CR. Calcium transport in lactation. In: Lactation: A Comprehensive Treatise, edited by Larson BL. New York: Academic 1195: 110-114, 1994.
7. Horst RL, Goff JP and Reinhardt TA. Calcium and vitamin D metabolism during lactation. J Mammary Biol Gland Neoplasia 1997, 2: 253-263.
8. Shennan DB and Peaker M. Transport of milk constituents by the mammary gland: review. Physiol Rev 2000, 80: 925-951.
9. Neville MC, Selker F, Semple K and Watters C. ATP-dependent calcium transport by a Golgi-enriched membrane fraction from mouse mammary gland. Membrane Biol 1981, 61: 97-105.
10. Virk SS, Kirk CJ and Shears SB. Ca^{2+} transport and Ca^{2+}-dependent ATP hydrolysis by Golgi vesicles from lactating rat mammary glands. Biochem J 1985, 226: 741-748.
11. Neville MC, Keller RP, Casey C and Allen JC. Calcium partitioning in human bovine milk. J Dairy Sci 1994, 77: 1964-1975.
12. Neville MC and Watters CD. Secretion of calcium into milk: review. J Dairy Sci 1983, 66: 371-380.
13. Reinhardt TA and Horst RL. Ca^{2+}-ATPases and their expression in the mammary gland of pregnant and lactating rats. Am J Physiol 1999, 276: C796-C802.
14. Reinhardt TA, Filoteo AG, Pennistion JT and Horst RL. Ca^{2+}-ATPase protein expression in mammary tissue. Am J Physiol Cell Physiol 2000, 279: C1595-C1602.
15. Carafoli E, Garcia-Martin E and Guerini D. The plasma membrane calcium pump: recent developments and future perspectives. Experientia 1996, 52: 1091-1100.

16. Prentice A, Jarjou LMA, Cole TJ, Stirling DM, Dibba B and Fairweather-Tait S. Calcium requirements of lactating Gambian mothers: effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. Am J Clin Nutr 1995, 62:58-67.
17. Wysolmerski JJ. The evolutionary origins of maternal calcium and bone metabolism during lactation. Mammary Gland Biol Neoplasia. 2002 Jul; 7(3): 267-76.
18. Mezzetti, Monti MG, Casolo LP, Piccinini and Moruzzi. 1,25-Dihydroxycholecalciferol-Dependent Calcium Uptake by Mouse Mammary Gland in Culture. Endocrinology 1988, 122(2): 389-394
19. Barlet J-P, Champredon C,Coxam V, Davicco MJ and Tressol JC. Parathyroid hormone-related peptide might stimulate calcium secretion into the milk of goats. J Endocrinol 1992, 132: 353-359.
20. Matthew Peterson C. Human Reproduction: Clinical, Pathologic and Pharmacologic Correlations. Knowledge Weavers ,Spencer S. Eccles Health Science Library ,University of Utah 1997.
21. Neville MC, McFadden TB, Forsyth I. Hormonal regulation of mammary differentiation and milk secretion. J Mammary Gland Biol Neoplasia. 2002, 7(1): 49-66.
22. Rillema JA, Collins S and Williams CH. Prolcatin stimulation of iodide uptake and incorporation into protein is polyamine-dependent in mouse mammary gland explants. P.S.E.B.M. 2000, 224:41-44.
23. Rillema JA, Yu TX and Jhiang SM. Effect of prolactin o sodium iodide symporter expression in mouse mammary gland explants. Am J Physiol Endorinol Metab 2000, 279: E769-E772.
24. Rillema JA. Effect of prolactin on phosphate transport and incorporation in mouse mammary gland explants. Am J Physiol Endorinol Metab 2002, 283: E132-E137.
25. Hudson ER, Wilde ML, Flint DJ and Baldwin SA. Regulation of GLUT1 expression in the mammry gland. Biochem Soc Trans 1997, 25: 92.
26. Rillema JA, Smolinski SM and Fan G. Temporal effects of PRL on casein kinase activity, casein synthesis and casein mRNA accumulation in mouse mammary gland explants. Horm Metab Res 1994, 26 (8): 367-70.
27. Hennighausen L, Robinson GW, Wagner KU and Liu XW. Prolactin signaling in mammary gland development. J Biol Chem 1997, 272(12): 7567-7569.
28. Blachier F, Lacroix MC, Ahmed AM, Leger C and Ollivier-Bousquet M. Arachidonic acid metabolism and casein secretion in lactating rabbit mammary epithelial cells: effects of inhibitors of prostaglandins and leukotrienes synthesis. Prostaglandins 1998, 35: 259-276.

Summary of the role of prolactin as a calcium regulating hormone

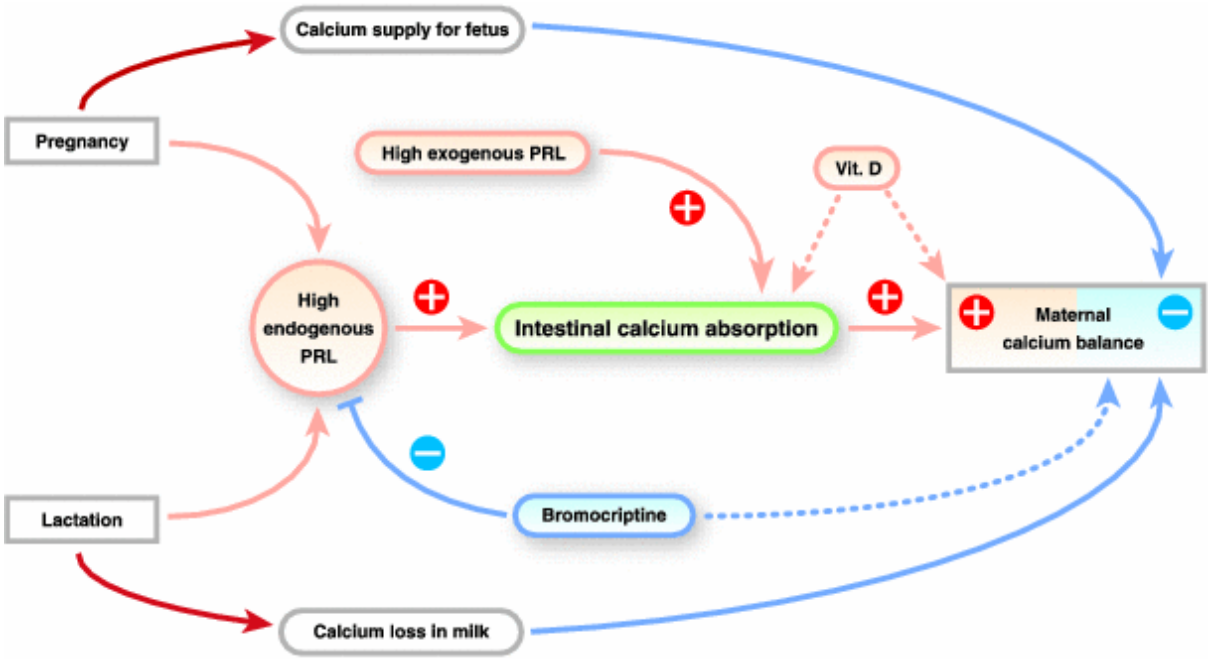


Fig. 2 Diagram shows possible actions of prolactin (PRL) on calcium metabolism. During pregnancy and lactation, maternal calcium is utilized for fetal growth and milk production, respectively, thereby inducing negative calcium balance. High endogenous prolactin, which stimulates intestinal calcium absorption (as well as turnover and renal calcium reabsorption), may help to alleviate calcium loss during these reproductive periods. These effects of endogenous prolactin can be abolished by bromocriptine, a dopaminergic agonist that suppresses prolactin secretion. In addition, exogenous prolactin administration mimics high endogenous prolactin by enhancing intestinal calcium absorption, thus maintaining calcium balance. Nonetheless, pregnant and lactating animals may still require the presence of vitamin D (Vit. D) to modulate intestinal calcim absorption. (Charoenphandhu and Krishnamra 2007)

(2) Study of the etiology, mechanism of diseases, and possible treatment of the metabolic bone disease using the Siamese cat model

Background and Rationale

Chronic renal failure (CRF) is the most common renal disease in dogs and cats in Thailand. CRF is defined as primary renal failure that has persisted for an extended period, usually months to years. Regardless of the causes(s) of nephron loss, irreversible renal structural lesions characterize CRF (1). In United States, DiBartola et al. (1987) found that CRF is a common disease especially in older cats. In his study, he found that 53% of affected cats were older than 7 years, but animals ranged in age from 9 months to 22 years (2). A survey of 36 feline patients with CRF indicated that the mean age of cats with CRF was 7.4 years (3). In a study of the age distribution of renal failure in cats, 37% of cats were younger than 10 years, 31% were between 10 and 15, and 32% were older than 15 (4). In Thailand, CRF was commonly observed especially in middle-aged cats with the mean age of 6.0 years old (5). Various reports also indicated that feline chronic renal failure was recognized with increased frequency in Maine coon, Abyssinian, Siamese, Russian blue, and Burmese cats (6). The increase incidence of CRF in middle-age Siamese cats may be due to genetic predisposition of this breed to chronic renal failure.

Metabolic acidosis is a well-recognized component of CRF in dogs and human. It results primarily from the limited ability of failing kidneys to excrete hydrogen ions, secondary to disordered ammoniagenesis, decreased filtration of phosphate and sulphate compounds, and decreased maximal renal tubular proton secretion (7). Bicarbonate wasting may also contribute. Bicarbonate wasting and chloride retention result in hyperchloremic (normal anion gap) acidosis. When phosphate and organic acid (uric acid, hippuric acid, lactic acid) retention is sufficient, high-anion-gap acidosis results. In retrospective case series, 63% and 80% of cats with CRF had metabolic acidosis (2,4). From our study of feline chronic renal failure at Chulalongkorn University Veterinary Teaching Hospital between January 2001 and December 2003, we followed CRF cats prospectively for 2 months and found that 100% of those cats with end-stage CRF developed acidosis at the end of the study. CRF Cats with metabolic acidosis had decreased survival rate when compared with other CRF cats(5).The role of metabolic acidosis in cats with CRF remain to be investigated.

From one study in UK, eighty cats with chronic renal failure (CRF) were evaluated in a prospective study to investigate the prevalence and etiopathogenesis of renal secondary hyperparathyroidism (RHPTH), using routine plasma biochemistry and assays of parathyroid hormone (PTH), blood ionized calcium and 1,25 dihydroxycholecalciferol (1,25[OH]2D3). Hyperparathyroidism was a frequent sequela of

CRF in that study, affecting 84 per cent of cats with CRF, the severity and prevalence of RHPTH increasing with the degree of renal dysfunction (9). However, significant ionized hypocalcemia was present only in cats with end-stage renal failure. A number of cats were hyperparathyroid in the absence of abnormalities in the parameters of calcium homeostasis measured in that study(9).

Feline chronic renal failure causes much concern to cats' owners in Thailand and remain one of the most common renal problems for cats with advanced age. More than 70% of cats presented to Chulalongkorn University Veterinary Teaching Hospital with chronic renal failure died within one year after first diagnosis(5). Recent report suggested that Siamese cats may have breed predisposition for feline chronic renal failure (6). The effect of metabolic acidosis on parathyroid hormone secretion, vitamin D metabolites, calcium-phosphorus homeostasis, and changes in bone of Siamese cats with naturally occurring chronic renal failure remain to be investigated.

Present Findings

2.1 The role of metabolic acidosis on parathyroid hormone secretion and change in electrolytes in cats with naturally occurring chronic renal failure

The purpose of this study was to investigate the effect of acid – base status on plasma parathyroid hormone level, calcium-phosphorus homeostasis, and changes in electrolytes in cats with naturally occurring chronic renal failure. Thirty-one cases presented to the Small Hospital, Faculty of Veterinary Science, Chulalongkorn University were followed prospectively. Cats were categorized into three groups. Control group consisted of healthy cats which were presented to the same hospital for vaccinated at the same time with CRF cats. Two chronic renal failure groups diagnosed by veterinarians on duty which had more than 50 mg/dl of blood urea nitrogen (BUN) and more than 2.1 mg/dl of serum creatinine level. Chronic renal failure groups were divided into non-acidosis group (n=11) and acidosis group (n=8) whose blood pH level were higher and lower than 7.3 respectively. Blood collection was taken for hematology, blood chemical profile, acid-base status and plasma parathyroid hormone level analysis on day 0, 14, 30 and 60 of the study. The result of this study revealed that both of the chronic renal failure groups had anemia indicated by the decreasing of red blood cells, hemoglobin concentration and packed cell volume compared to the control group ($p<0.05$). Parathyroid hormone and phosphorus level of chronic renal failure group with metabolic acidosis was significantly higher than group of chronic renal failure without metabolic acidosis on Day 0 of the study ($p<0.05$). **Chronic renal failure cats with metabolic acidosis had hyperparathyroidism and hyperphosphatemia.** However, there was no significant correlation between blood pH and parathyroid

hormone level which may be due to low number of chronic renal failure cats with metabolic acidosis in the study.

2.2 The effect of metabolic acidosis on calcium-phosphorus homeostasis and bone remodeling in cats with natural occurring chronic renal failure.

The purpose of this study was to investigate the effect of acid-base status on calcium-phosphorus homeostasis and bone remodeling in cats with naturally occurring chronic renal failure. Twenty-nine cats presented to the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University were studied. Cats were categorized into three groups. The control group was a group of healthy cats which were presented at the same time of study (n=6). Two chronic renal failure groups were diagnosed by the veterinarians and were based on more than 50 mg/dl of blood urea nitrogen (BUN) and more than 2.1 mg/dl of serum creatinine level. Chronic renal failure groups were divided into non-acidosis group (n=9) and acidosis group (n=12) whose blood pH levels were lower than 7.3. Blood collections were taken for hematology, blood chemical profile, acid-base status, vitamin D, and plasma parathyroid hormone level analysis on day 0, 30, 60, 90, 120, and 150 of the study. Bone formation and bone remodeling were measured using bone alkaline phosphatase, Dpd level in urine samples, and bone density in all groups of cats. The result of this study revealed that cats with chronic renal failure and metabolic acidosis had hyperphosphatemia and hyperparathyroidism throughout the study. However, there were no significant changes in the level of total calcium, adjusted calcium, or ionized calcium levels. Cats with chronic renal failure and acidosis had significantly lower level of bone alkaline phosphatase which indicated less bone formation and higher levels of Dpd in urine which indicated more bone resorption than control cats. There were no significant difference of bone mineral density in all three groups of cats that may be due to less severity of chronic renal failure stage and a shorter duration of metabolic acidosis that occurred during the study. **Metabolic acidosis seems to be the potentiating factor to promote hyperparathyroidism and induced calcium-phosphorus imbalance and bone changes in cats with chronic renal failure.** Close monitoring in cats with chronic renal failure and correction of acidosis help to prolong live and give better quality of life to cats with naturally occurring chronic renal failure.

References

1. Fine LG, Orphanides C, Norman JT. Progressive renal disease: The chronic hypoxia hypothesis. Kidney Int 1998; 53(suppl 65): s-74-s-78.
2. DiBartola SP, Rutgers HC, Zack PM, et al. Clinicopathologic findings associated with chronic renal disease in cats: 74 cases (1973-1984). J Am Vet Med Assoc 1987; 190:1196-1202.
3. Polzin DJ, Osborne CA, O'Brien TD: Diseases of the kidneys and ureters. In: Ettingers
   SJ (ed.): Textbook of Veterinary Internal Medicine, 3 rd ed. Philadelphia, WB Saunders, 1989, pp 1963-2046.
4. Lulich JP, O'Brien TD, Osborne CA, et al. Feline renal failure: Questions, answers, questions. Compend Contin Educ Pract Vet 1992; 14:127-152.
5. Pusoonthornthum R, Pusoonthornthum P, Yibchok-anun S. Calcium – Phosphorus Homeostasis and Changes in Parathyroid Hormone Secretion in Siamese Cats with Various Stages of Naturally Occurring Chronic Renal Failure. 2004 Am J Vet Res (To be published).
6. Polzin DJ, Osborne CA, Jacob F, Ross S. Chronic renal failure. In: Ettingers SJ (ed.): Textbook of Veterinary Internal Medicine, 5 th ed. Philadelphia, WB Saunders, 2000, pp 1634-1662.
7. Kimmel P. Management of the patient with chronic renal disease. In: Greenberg A (ed.): Primer on Kidney Diseases. San Diego, Academic Press, 1998. pp 433-440.
8. Barber PJ, Elliott J. Feline chronic renal failure: calcium homeostasis in 80 cases diagnosed between 1992 and 1995. J Small Anim Pract 1998 Mar;39(3):108-116.
9. Elliot J, Barber PJ. Feline chronic renal failure: clinical findings in 8 cases diagnosed between 1992-1995. J Small Anim Pract 1998; 39:78-85.
10. Barber, PJ, Torrance AG, Elliot J. Carboxyl fragment interference in assay of feline parathyroid hormone. J Vet Int Med 1994; 8:168.
11. Barber PJ, Elliot J, Torrance AG. Measurement of feline intact parathyroid hormone: assay validation and sample handling studies. J Small Anim Pract 1993;34:614-620.
12. Barsanti JA, Lees GE, Willard MD, Green R. Urinary disorders. In: Willard MD, Tvedten H, Turnwald GH (ed.): Small Animal Clinical Diagnosis by Laboratory Methods, 3 rd edi. Philadelphia, WB Saunders, 1999. pp 108-135.
13. DiBartola SP, Green R, Willard MD. Electrolyte and acid-base. In: Willard MD, Tvedten H, Turnwald GH (ed.): Small Animal Clinical Diagnosis by Laboratory Methods, 3 rd ed. Philadelphia, WB Saunders, 1999. pp 93-107.
14. Freeman LM, Kehayias JJ, Roubenoff R. Use of dual-energy x-ray absorptiometry (DEXA) to measure lean body mass, body fat, and bone mineral content (BMC) in dogs and cats. J Vet Intern Med. 1996 Mar-Apr;10(2):99-100.

15. Turner AS, Norrdin RW, Gaarde S, Connally HE, Thrall MA. Bone mineral density in feline mucopolysaccharidosis VI measured using dual-energy X-ray absorptiometry. Calcif Tissue Int. 1995 Sep;57(3):191-5.

ผลงานที่ได้จากโครงการวิจัย

1. ผลงานตีพิมพ์ในวารสารวิชาการนานาชาติ

1.1 Amnattanakul S, Charoenphandhu N, Limlomwongse L, **Krishnamra N**. Endogenous prolactin modulated the calcium absorption in the jejunum of suckling rats. Can J Physiol Pharmacol 2005; 83: 1-10. *(Impact Factor 1.603)*

1.2 Tudpor K, Charoenphandhu N, Saengumnart W, **Krishnamra N**. Long-term prolactin exposure differentially stimulated the transcellular and solvent drag-induced calcium transport in the duodenum of ovariectomized rats. Explt Biol Med 2005; 230: 836-844. *(Impact Factor 2.369)*

1.3 Charoenphandhu N, Limlomwongse L, **Krishnamra N**. Prolactin directly enhanced Na^{+}/K^{+} and Ca^{2+}-ATPase activities in the duodenum of female rats. Can J Physiol Pharmacol 2006, 84: 555-563. *(Impact Factor 1.603)*

1.4 Anantamongkol U, Takemura H, Suthiphongchai T, **Krishnamra N**, Horio Y. Regulation of Ca^{2+} mobilization by prolactin in mammary gland cells : Possible role of secretory pathway Ca^{2+}-ATPase type 2. Biochem Biophys Res Comm 2007; 352(2): 537-542. *(Impact Factor 3.0)*

1.5 Puntheeranurak S, Schreiber R, Spitzner M, Sausbier M, Ruth P, **Krishnamra N**, Kunzelmann K. Control of ion transport in mouse proximal and distal colon by prolactin. Cell Physiol Biochem 2007; 19: 77-88. *(Impact Factor 4.1)*

1.6 Jantarajit W, Thongon N, Pandaranandaka J, Teerapornpuntakit J, **Krishnamra N**, Charoenphandhu N. Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by the phosphaenositide-3-kinase pathway. Am J Physiol Endocrinol Metab 2007; 293 : E372-384. *(Impact Factor 4.456)*

1.7 Charoenphandhu N, **Krishnamra N**. Prolactin : an important regulator of intestinal calcium transport . Can J Physiol Pharmacol 2007; 85: 569-581. *(Impact Factor 1.603)*

2. ผลงานวิจัยที่เตรียมเพื่อส่งตีพิมพ์ในวารสารวิชาการนานาชาติ

2.1 Siriwetwiwat J, Vitayakritsirikul V, Anantamongkol U, Prapong T, Charoenphandhu N, Krishnamra N, Prapong S. Expression and localization of selective calcium channels, TRPV5 and TRPV6 in active mammary tissues. (in preparation)

2.2 Regulation of prolactin on electrolyte transport across porcine endometrial epithelial cells.

2.3 The effects of metabolic acidosis on parathyroid hormone secretion, calcium-phosphorus homeostasis and bone remodeling in cats with natural occurring chronic renal failure.

3. การเลื่อนตำแหน่งทางวิชาการของผู้วิจัยหลัก

- ผศ.ดร.ฉัตรศรี เดชะปัญญา เป็น รองศาสตราจารย์
- รศ. นพ. สมนึก ดำรงกิจชัยพร เป็น ศาสตราจารย์

4. นักศึกษาบัณฑิต

ระดับปริญญาโท	นส.สุวิมล อำนาจฉนากุล	(มหิดล)	จบปี พ.ศ. 2547
	นายกู้เกียรติ ทุดปอ	(มหิดล)	จบปี พ.ศ. 2547
	นส.กนกวรรณ ทองโชติ	(มหิดล)	จบปี พ.ศ. 2550
	นส.อรรัศมี วิมุกตะนันท์	(จุฬาลงกรณ์)	จบปี พ.ศ. 2550
	นายยงยุทธ์ พงศ์ประชาชื่น	(จุฬาลงกรณ์)	
ระดับปริญญาเอก	นส.สุภาพร พันธุ์ธีรานุรักษ์	(มหิดล)	จบปี พ.ศ. 2549
	นส.ดุษมณี เสรีวัฒนาชัย	(มหิดล)	จะจบปี พ.ศ. 2551
	นส.อัจฉริยา อนันตมงคล	(มหิดล)	จะจบปี พ.ศ. 2551
	นส.วิภาวรรณ วิทยกฤตศิริกุล	(เกษตร)	จะจบปี พ.ศ. 2552

5. ผู้ช่วยวิจัย

นส. วาสนา แสงอำนาจ
นส.วิไลวรรณ ทิพสิงห์
นส.จีรพรรณ ทองบุญชู

6. กิจกรรมทางวิชาการ

- "ประชุมวิชาการ เมธีวิจัยอาวุโส ศาสตราจารย์ นทีทิพย์ กฤษณามระ" ประจำปี พ.ศ. 2548, 2549 และ 2550 จัดที่คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล
- ประชุมอบรมเชิงปฏิบัติการ ซึ่งจัดโดยเครือข่ายวิจัยด้านแคลเซียมและกระดูก (COCAB) 2 ครั้ง ในปี พ.ศ. 2549 และ พ.ศ. 2550
- จัดการบรรยายพิเศษ โดย Prof. Dr. Karl Kunzelmann จากมหาวิทยาลัย Regensburg ประเทศเยอรมัน ในปี พ.ศ. 2548

- ศาสตราจารย์ ดร.นทีทิพย์ กฤษณามระ บรรยายพิเศษเรื่อง “Prolactin : its role in the regulation of calcium and bone metabolism” และ นพ.ดร.นรัตถพล เจริญพันธุ์ บรรยายพิเศษเรื่อง “Claudin expression in osteoblasts” ที่ University of Ontario Canada ในปี พ.ศ. 2550
- เสนอผลงานวิจัยประเภทโปสเตอร์ที่การประชุมวิชาการนานาชาติ 5 ครั้ง
- เสนอผลงานวิจัยประเภท oral ที่การประชุมวิชาการนานาชาติ 2 ครั้ง
- เสนอผลงานวิจัยประเภท oral ที่การประชุมวิชาการภายในประเทศ 3 ครั้ง
- บรรยายพิเศษในประเทศ 10 ครั้ง
- จัดสัมมนาวิชาการของเครือข่ายวิจัยฯ 14 ครั้ง

ภาคผนวก (Appendix)

บทความเพื่อการเผยแพร่

งานวิจัยของเรามีวัตถุประสงค์ที่จะศึกษาค้นคว้าเพื่อให้ได้องค์ความรู้ใหม่ที่เป็นองค์รวมเกี่ยวกับการควบคุมสมดุลแคลเซียมและเมตาบอลิสมของกระดูก องค์ความรู้ที่ได้นี้จะสามารถใช้เป็นฐานต่อยอดความรู้ทางการแพทย์สำหรับค้นหาสาเหตุความผิดปกติของกระดูกใน metabolic bone diseases และศึกษาหาวิธีวินิจฉัยและป้องกัน การวิจัยของเราใช้วิธีศึกษาที่ทุกระดับตั้งแต่ในระดับร่างกายของสัตว์ทดลอง ระบบอวัยวะ เนื้อเยื่อ เซลล์ สู่ระดับชีววิทยาโมเลกุล หัวข้อวิจัยคือ **การศึกษาผลของฮอร์โมนโพรแลคตินในฐานะฮอร์โมนที่ช่วยควบคุมสมดุลแคลเซียมและเมตาบอลิสมของกระดูกในภาวะตั้งครรภ์และให้นมบุตร**

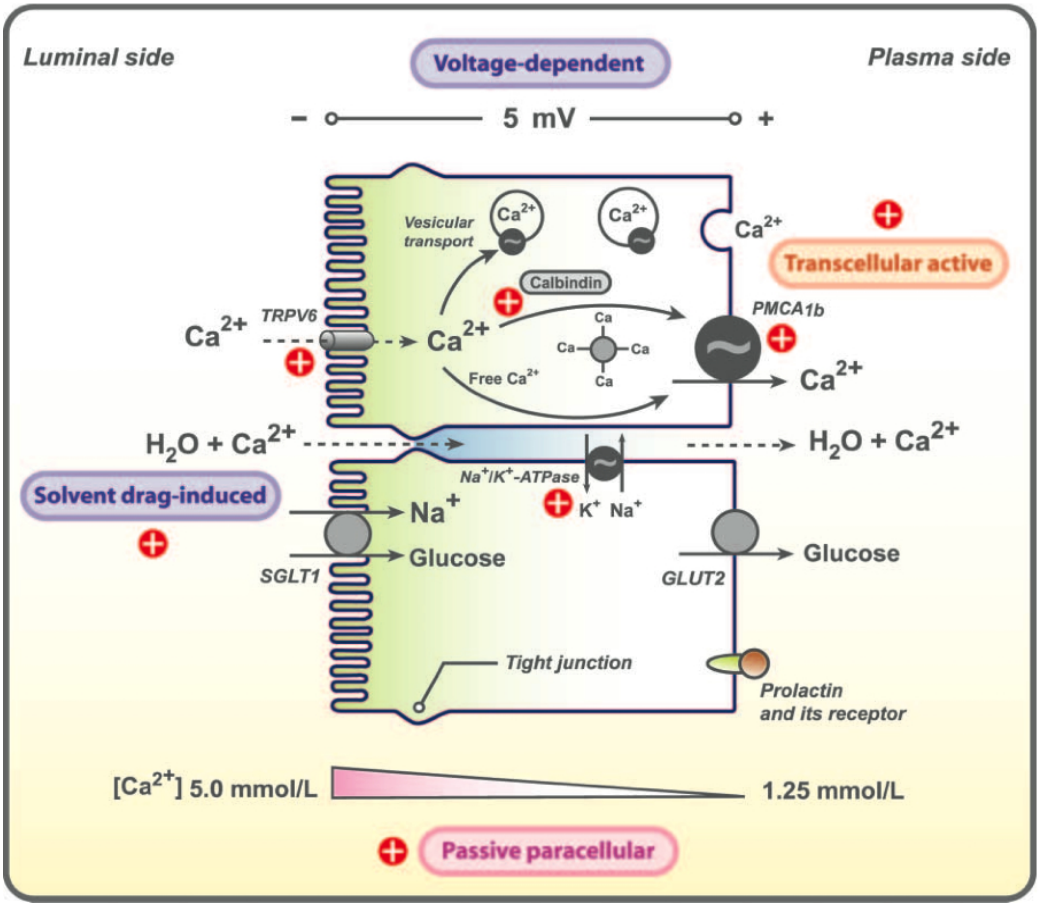
โพรแลคตินเป็นฮอร์โมนจากต่อมใต้สมองส่วนหน้า เป็นที่รู้จักทั่วไปจากหน้าที่หลักคือกระตุ้นการผลิตน้ำนมในเซลล์เต้านม แต่จากงานวิจัยของเราพบว่า โพรแลคตินสมารถกระตุ้นการดูดซึมแคลเซียมทั้งแบบพาสซีพไม่ใช้พลังงาน (passive paracellular calcium absorption) และแบบแอคทีฟที่ต้องใช้ ATP (active calcium absorption) ซึ่งแบบแอคทีฟนี้ยังแบ่งได้อีกคือ โพรแลคติน
กระตุ้นการขนส่งแคลเซียมทั้งแบบผ่านเซลล์ (transcellular transport) และผ่านช่องระหว่างเซลล์ตามการไหลของของเหลว (solvent drag-induced paracellular active transport) ดังที่แสดงในรูปที่ 1 การขนส่งแคลเซียมที่เพิ่มขึ้นนี้เป็นผลมาจากโพรแลคตินกระตุ้นการนำเข้าแคลเซียมจากโพรงลำไส้ผ่านเยื่อเซลล์และเพิ่มการทำงานของ Na^{+}-K^{+}-ATPase ที่เป็นตัวทำให้เกิด solvent drag-induced paracellular transport และการทำงานของ Ca^{2+}-ATPase ที่ช่วยขนส่งแคลเซียมออกจากเซลล์สู่เลือด โดยโพรแลคตินออกฤทธิ์ผ่านทางกลไกส่งสัญญาณ PI3-kinase และ MAPkinase

เป็นที่ทราบกันดีว่าโพรแลคตินมีผลต่อการขนส่งอิเล็กโทรไลท์โดยเฉพาะ Na^{+} และ Cl^{-} ในเนื้อเยื่อหลายชนิด จากการวิจัยโดยใช้เทคนิค in vitro Ussing chamber พบว่าโพรแลคตินมีผลยับยั้งการขับหลั่ง Cl^{-} และ K^{+} ชนิดที่ต้องอาศัย Ca^{2+} โดยออกฤทธิ์ผ่านทางกลไก PI3kinase และ MAPkinase เช่นเดียวกัน แต่ที่ผนังมดลูกโพรแลคตินกลับมีผลกระตุ้นการขับหลั่งอิออนประจุลบผ่านทางกลไก JAK-STAT ผลดังกล่าวนี้มีความสำคัญต่อการควบคุมคุณสมบัติของของเหลวภายในมดลูก ซึ่งมีความสำคัญมากต่อกระบวนการปฏิสนธิ การฝังตัวและการเจริญเติบโตของตัวอ่อนในครรภ์

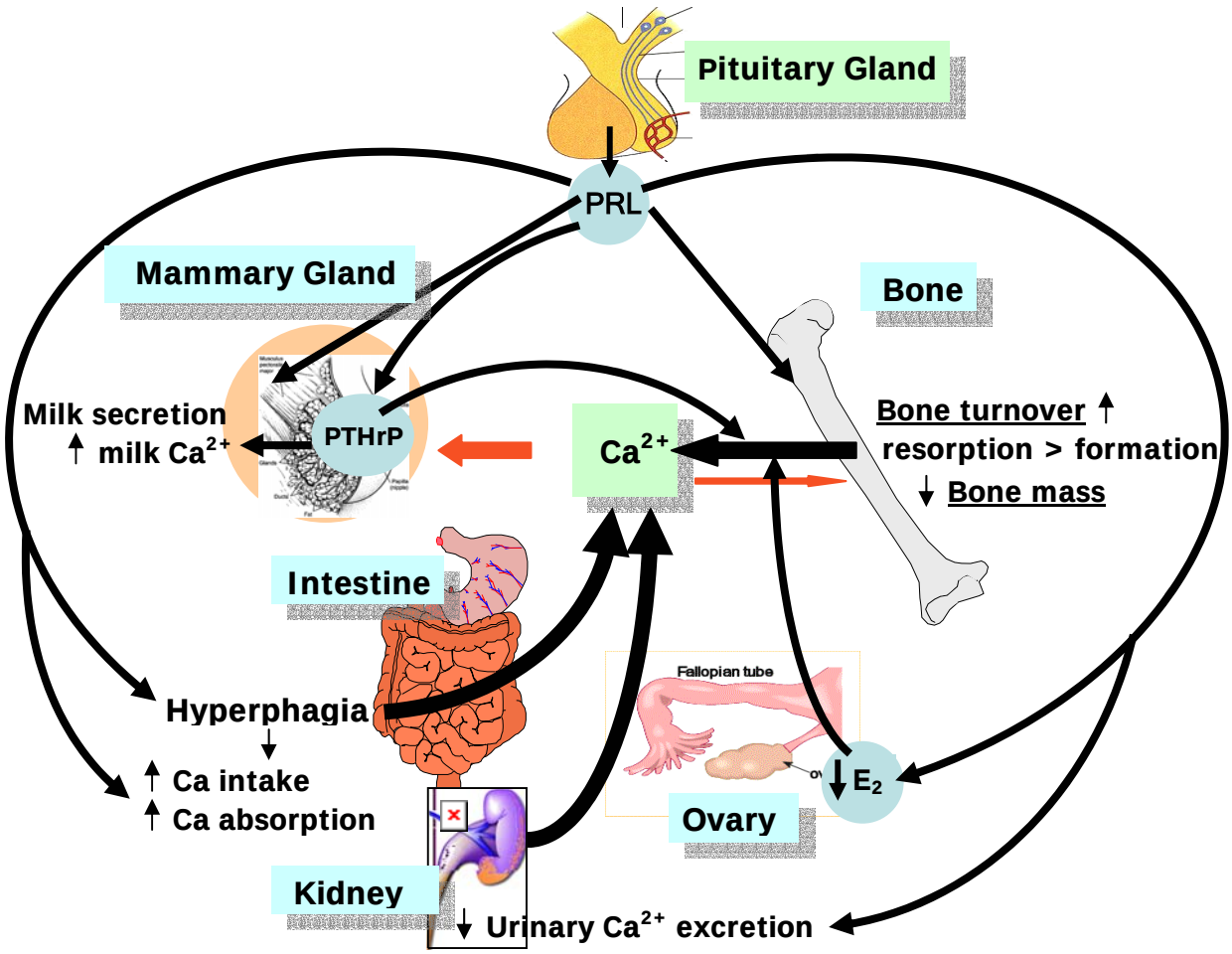
นอกจากนั้นเรายังพบอีกว่าเมื่อร่างกายมีระดับของโพรแลคตินในเลือดสูง เช่นในช่วงตั้งครรภ์หรือหลังคลอดซึ่งโพรแลคตินมีระดับในเลือดเพิ่มขึ้นเป็น 20 เท่า โพรแลคตินมีผล

กระตุ้นการสลายกระดูก และลดการขับแคลเซียมทิ้งในปัสสาวะ ทำให้มีการปล่อยแคลเซียมกลับสู่เลือดเป็นผลให้มีแคลเซียมในเลือดในระดับที่สูงเพียงพอสำหรับส่งผ่านรกเพื่อการเจริญเติบโตของตัวอ่อนในครรภ์และสำหรับเซลล์เต้านมใช้ในการผลิตน้ำนม ดังนั้นไม่ว่าจะเป็นสัตว์ประเภทปลา นก หรือสัตว์เลี้ยงลูกด้วยนม โพรแลคตินเป็นฮอร์โมนที่เรียกว่า “Maternal hormone” คือมีหน้าที่หลักในการเตรียมร่างกายของแม่ทั้งทางกายภาพและพฤติกรรมให้มีความพร้อมในการดูแลเลี้ยงลูกอ่อน (รูปที่ 2)

ดังนั้นผลงานวิจัยของเราจึงสนับสนุนสมมติฐานที่ว่า **“โพรแลคตินเป็นฮอร์โมนที่ช่วยควบคุมสมดุลแคลเซียมและเมตาบอลิสมของกระดูก เพื่อให้แม่มีแคลเซียมในเลือดเพียงพอสำหรับการสร้างกระดูกของตัวอ่อนและผลิตน้ำนมเลี้ยงลูกแรกเกิด** กล่าวคือ ถ้าแม่ได้รับแคลเซียมจากอาหารไม่พอ แคลเซียมในเลือดของแม่จะถูกนำไปใช้สำหรับลูกในครรภ์หรือในการผลิตน้ำนมจนระดับแคลเซียมในเลือดอาจมีค่าลดลง ซึ่งจะเป็นอันตรายได้ ดังนั้นร่างกายของแม่จึงใช้โพรแลคตินเป็นสัญญานสั่งให้มีการสลายกระดูกมากขึ้น เพื่อปล่อยแคลเซียมออกมาในเลือด โพรแลคตินเป็นฮอร์โมนที่ไม่เพียงแต่จะมีความสำคัญในแม่เท่านั้น เรายังพบอีกว่าโพรแลคตินในระดับปกติ(ซึ่งมีค่าต่ำ)ในสัตว์อายุน้อยที่ยังอยู่ในวัยเจริญเติบโต ก็มีผลกระตุ้นการดูดซึมแคลเซียมและกระตุ้นกระบวนการสร้างและสลายกระดูกด้วย แต่มีผลต่างจากในสัตว์อายุมาก คือในสัตว์อายุน้อยทำให้มีมวลกระดูกเพิ่มขึ้น ดูเหมือนว่าโพรแลคตินจะเป็นฮอร์โมนที่ช่วยควบคุมสมดุลแคลเซียมและเมตาบอลิสมของกระดูกในทุกวัย แต่ผลของโพรแลคตินจะมีความแตกต่างกันขึ้นอยู่กับวัยด้วย ซึ่งรายละเอียดเกี่ยวกับกลไกการออกฤทธิ์ของโพรแลคติน และทำไมโพรแลคตินมีผลต่างกันในสัตว์อายุน้อยและสัตว์อายุมาก หรือเป็นไปได้หรือไม่ที่จะพัฒนาโพรแลคตินให้ใช้เป็นยาเพื่อเพิ่มประสิทธิ ภาพการดูดซึมแคลเซียม และควบคุมวงจรการสร้าง-สลายกระดูก (Bone remodeling) จะต้องศึกษาวิจัยต่อไป



รูปที่ 1 โพรแลคตินมีผลกระตุ้นการดูดซึมแคลเซียมที่ลำไส้เล็กทั้งแบบพาสซีฟตามตามลาดความเข้มข้นและแบบแอคทีฟที่ต้องใช้เอทีพี สำหรับการขนส่งแคลเซียมแบบแอคทีฟ โพรแลคตินกระตุ้นการขนส่งแบบแอคทีฟ 2 ชนิดได้แก่ (1) ชนิดผ่านเซลล์ (transcellular active transport) โดยใช้โปรตีน TRPV6 calbindin และ PMCA1b และ (2) การขนส่งแคลเซียมแบบแอคทีฟที่เกิดจากการไหลของๆเหลว ผ่านช่องระหว่างเซลล์ (solvent drag-induced paracellular transport) ซึ่งอาศัยการทำงานของ Na^{+}-K^{+}-ATPase



รูปที่ 2 โพรแลคติน (PRL) ในฐานะฮอร์โมนที่ควบคุมสมดุลแคลเซียมและเมตาบอลิสมของกระดูก โดยมีผลกระตุ้นการดูดซึมแคลเซียมที่ลำไส้ลดการขับถ่ายแคลเซียมในปัสสาวะ และกระตุ้นวงจรการสร้าง-สลายกระดูก (Bone remodeling) ทำให้มีแคลเซียมออกมาในเลือดมากขึ้น โพรแลคตินระดับสูงในเลือดยังมีผลยับยั้งการหลั่งฮอร์โมนเอสโตรเจน (E_2) และกระตุ้นการหลั่งพาราไทรอยด์ฮอร์โมนรีเลเท็ดเพปไทด์ (PTHrP) ซึ่งทำให้มีการสลายกระดูกมากขึ้น นอกจากนั้นโพรแลคตินยังมีผลต่อเซลล์เต้านมทำให้มีการขับหลั่งแคลเซียมออกมาในน้ำ

Reprints

International Publication

1.1. Amnattanakul S, Charoenphandhu N, Limlomwongse L, **Krishnamra N**. Endogenous prolactin modulated the calcium absorption in the jejunum of suckling rats. Can J Physiol Pharmacol 2005; 83: 1-10. *(Impact Factor 1.603)*

1.2. Tudpor K, Charoenphandhu N, Saengumnart W, **Krishnamra N**. Long-term prolactin exposure differentially stimulated the transcellular and solvent drag-induced calcium transport in the duodenum of ovariectomized rats. Explt Biol Med 2005; 230: 836-844. *(Impact Factor 2.369)*

1.3. Charoenphandhu N, Limlomwongse L, **Krishnamra N**. Prolactin directly enhanced Na^{+}/K^{+} and Ca^{2+}-ATPase activities in the duodenum of female rats. Can J Physiol Pharmacol 2006, 84: 555-563. *(Impact Factor 1.603)*

1.4. Anantamongkol U, Takemura H, Suthiphongchai T, **Krishnamra N**, Horio Y. Regulation of Ca^{2+} mobilization by prolactin in mammary gland cells : Possible role of secretory pathway Ca^{2+}-ATPase type 2. Biochem Biophys Res Comm 2007; 352(2): 537-542. *(Impact Factor 3.0)*

1.5. Puntheeranurak S, Schreiber R, Spitzner M, Sausbier M, Ruth P, **Krishnamra N**, Kunzelmann K. Control of ion transport in mouse proximal and distal colon by prolactin. Cell Physiol Biochem 2007; 19: 77-88. *(Impact Factor 4.1)*

1.6. Jantarajit W, Thongon N, Pandaranandaka J, Teerapornpuntakit J, **Krishnamra N**, Charoenphandhu N. Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by the phosphaenositide-3-kinase pathway. Am J Physiol Endocrinol Metab 2007; 293 : E372-384. *(Impact Factor 4.456)*

1.7. Charoenphandhu N, **Krishnamra N**. Prolactin : an important regulator of intestinal calcium transport . Can J Physiol Pharmacol 2007; 85: 569-581. *(Impact Factor 1.603)*

Endogenous prolactin modulated the calcium absorption in the jejunum of suckling rats

Suwimol Amnattanakul, Narattaphol Charoenphandhu, Liangchai Limlomwongse, and Nateetip Krishnamra

Abstract : Prolactin has been reported to stimulate intestinal calcium absorption in young and mature, but not aging rats. The present study was performed on suckling rats to elucidate the actions of endogenous prolactin on calcium absorption in various intestinal segments. Before measuring the calcium fluxes, 9-day-old rats were administered for 7 days with 0.9% NaCl, s.c. (control), 3 mg/kg bromocriptine, i.p., twice daily to abolish secretion of endogenous prolactin, or bromocriptine plus exogenous 2.5 mg/kg prolactin, s.c. Thereafter, the 16-day-old rats were experimented upon by instilling the ^{45}Ca-containing solution into the intestinal segments. The results showed that, under a physiological condition, the jejunum had the highest rate of calcium absorption compared with other segments (1.4 ± 0.35 μmol·h^{-1}·cm^{-1}, $p < 0.05$). The duodenum and ileum also manifested calcium absorption, whereas the colon showed calcium secretion. Lack of endogenous prolactin decreased lumen-to-plasma and net calcium fluxes in jejunum from 2.07 ± 0.31 to 1.19 ± 0.12 and 1.40 ± 0.35 to 0.88 ± 0.18 μmol·h^{-1}·cm^{-1} ($p < 0.05$), respectively, and exogenous prolactin restored the jejunal calcium absorption to the control value. Endogenous prolactin also had an effect on the duodenum but, in this case, exogenous prolactin did not reverse the effect of bromocriptine. However, neither ileal nor colonic calcium fluxes were influenced by prolactin. Because luminal sodium concentration has been demonstrated to affect calcium absorption in mature rats, the effect of varying luminal sodium concentrations on calcium fluxes in suckling rats was evaluated. The jejunum was used due to its highest rate of calcium absorption. After filling the jejunal segments with 124 (control), 80, 40 mmol/L Na^+-containing or Na^+-free solution, increases in calcium absorption were found to be inversely related to luminal sodium concentrations in both control and bromocriptine-treated rats. The plasma concentration of ^{45}Ca under luminal sodium free condition was also higher than that of the control condition ($2.26\% \pm 0.07\%$ vs. $2.01\% \pm 0.09\%$ administered dose, $p < 0.05$). However, ^{3}H-mannitol, a marker of the widening of tight junction that was introduced into the lumen, had a stable level in the plasma during an increase in plasma ^{45}Ca, suggesting that the widening of tight junction was not required for enhanced calcium absorption. In conclusion, calcium absorption in suckling rats was of the highest rate in the jejunum where endogenous prolactin modulated calcium absorption without increasing the paracellular transport of mannitol.

Key words: calcium absorption, intestinal segments, prolactin, suckling rats.

Résumé : On a constaté que la prolactine stimule l'absorption de calcium intestinal chez les rats jeunes et matures, mais pas chez les rats vieillissants. La présente étude a été effectuée sur des rats à la mamelle dans le but de comprendre les actions de la prolactine endogène sur l'absorption de calcium dans divers segments intestinaux. Avant de mesurer le flux calcique, on a administré à des rats âgés de 9 jours, pendant 7 jours, 0,9% de NaCl s.c. (témoin), 3 mg/kg de bromocriptine i.p., deux fois par jour pour supprimer la sécrétion de prolactine endogène, ou de la bromocriptine plus 2,5 mg/kg de prolactine s.c. Ensuite, on a instillé la solution contenant le ^{45}Ca dans les segments intestinaux des rats âgés de 16 jours. Les résultats ont montré qu'à l'état physiologique, le jéjunum a eu le plus haut taux d'absorption de calcium ($1,4 \pm 0,35$ μmol·h^{-1}·cm^{-1}, $p < 0,05$). Le duodénum et l'iléon ont aussi montré une absorption de calcium alors que le colon a démontré une sécrétion. L'absence de prolactine endogène a diminué le flux de calcium de la lumière au plasma ainsi que le flux net de $2,07 \pm 0,31$ à $1,19 \pm 0,12$ et de $1,40 \pm 0,35$ à $0,88 \pm 0,18$ μmol · h^{-1} · cm^{-1} ($p < 0,05$), respectivement, et la prolactine exogène a ramené l'absorption de calcium jéjunal à la valeur témoin. La prolactine endogène a aussi eu un effet sur le duodénum, mais, dans ce cas, la prolactine exogène n'a pas renversé l'effet de la bromocriptine. Toutefois, la prolactine n'a pas eu d'effet sur les flux de calcium iléal et colique. Des travaux antérieurs ayant démontré que la concentration de sodium luminal influe sur l'absorption de calcium chez les rats matures, on a évalué l'effet d'une variation des concentrations de sodium luminal sur les flux de calcium chez les rats à la mamelle. On a utilizé le jéjunum en raison de son très haut taux d'absorption de calcium. Après avoir rempli les segments du jéjunum avec une solution contenant 124 (témoin), 80, 40 mmol/L de Na^+ ou une solution ne contenant pas Na^+, on a observé que les augmentations d'absorption de calcium étaient inversement liées aux concentrations de

Received 9 February 2005. Published on the NRC Research Press Web site at http://cjpp.nrc.ca on 15 July 2005.

S. Amnattanakul, N. Charoenphandhu, L. Limlomwongse, and N. Krishnamra.[1] Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

[1]Corresponding author (e-mail: scnks@marhidol.ac.th).

 doi: 10.1139/Y05-045

Long-Term Prolactin Exposure Differentially Stimulated the Transcellular and Solvent Drag-Induced Calcium Transport in the Duodenum of Ovariectomized Rats

KUKIAT TUDPOR, NARATTAPHOL CHAROENPHANDHU, WASANA SAENGAMNART, AND NATEETIP KRISHNAMRA[1]

Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand

Prolactin, having been shown to stimulate transcellular active and solvent drag-induced calcium transport in the duodenum of female rats, was postulated to improve duodenal calcium transport in estrogen-deficient rats. The aim of the present study was, therefore, to demonstrate the effects of long-term prolactin exposure produced by anterior pituitary (AP) transplantation on the duodenal calcium transport in young (9-week-old) and adult (22-week-old) ovariectomized rats. We found that ovariectomy did not alter the transcellular active duodenal calcium transport in young and adult rats fed normal calcium diet (1.0% w/w Ca) but decreased the solvent drag-induced duodenal calcium transport from 75.50 ± 10.12 to 55.75 ± 4.77 nmol·hr^{-1}·cm^{-2} ($P < 0.05$) only in adult rats. Long-term prolactin exposure stimulated the transcellular active calcium transport in young and adult AP-grafted ovariectomized rats fed with normal calcium diet by more than 2-fold from 7.56 ± 0.79 to 16.54 ± 2.05 ($P < 0.001$) and 9.78 ± 0.72 to 15.99 ± 1.75 ($P < 0.001$) nmol·hr^{-1}·cm^{-2}, respectively. However, only the solvent drag-induced duodenal calcium transport in young rats was enhanced by prolactin from 95.51 ± 10.64 to 163.20 ± 18.03 nmol·hr^{-1}·cm^{-2} ($P < 0.001$) whereas that in adult rats still showed a decreased flux from 75.50 ± 10.12 to 47.77 ± 5.42 nmol·hr^{-1}·cm^{-2} ($P < 0.05$). Because oral calcium supplement has been widely used to improve calcium balance in estrogen-deficient animals, the effect of a high-calcium diet (2.0% w/w Ca) was also investigated. The results showed that stimulatory action of long-term prolactin on the transcellular active duodenal calcium transport in both young and adult rats was diminished after being fed a high-calcium diet. The same diet also abolished prolactin-enhanced solvent drag-induced duodenal calcium transport in young and further decreased that in adult AP-grafted ovariectomized rats. We concluded that the solvent drag-induced duodenal calcium transport in adult rats was decreased after ovariectomy. Long-term prolactin exposure stimulated the transcellular active duodenal calcium transport in both young and adult rats whereas enhancing the solvent drag-induced duodenal calcium transport only in young rats. Effects of prolactin were abolished by a high-calcium diet. Exp Biol Med 230:836–844, 2005.

Key words: calcium transport; duodenum; ovariectomy; prolactin; solvent drag

Introduction

Prolactin, as a novel calcium-regulating hormone, has been demonstrated to stimulate active calcium transport in the duodenum of rats (1–3). Three components of active duodenal calcium transport were identified, that is, transcellular active, solvent drag-induced, and voltage-dependent calcium transport (4). However, the latter portion was considered negligible (4). By using the Ussing chamber technique, we reported that 200 to 800 ng/ml prolactin directly stimulated both transcellular active (4) and solvent drag-induced (5) calcium transport in the duodenum of sexually mature female rats in a dose-response manner. Stimulatory action of prolactin was not dependent on the prolactin-induced production of 1α,25-dihydroxycholecalciferol [1,25$(OH)_2D_3$] because such effect was observed within 20 mins after direct exposure to prolactin (3, 6).

Long-term estrogen deficiency caused by either bilateral oophorectomy in the young or menopause in adult places a stress on human calcium metabolism, thus resulting in osteopenia and osteoporosis (7, 8). Besides putative estrogen depletion-induced osteoporosis, a decrease in intestinal calcium absorption was also reported by Kalu *et al.* in 1989 (9). However, quantitative study of the active duodenal calcium transport after estrogen depletion has

This research was supported by the Thailand Research Fund (TRF) and the National Center for Genetic Engineering and Biotechnology (BIOTEC).

[1] To whom correspondence should be addressed at Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand. E-mail: scnks@mahidol.ac.th

Received May 13, 2005.
Accepted July 29, 2005.

1535-3702/05/23011-0836$15.00

Prolactin directly enhanced Na^+/K^+- and Ca^{2+}-ATPase activities in the duodenum of female rats

Narattaphol Charoenphandhu, Liangchai Limlomwongse, and Nateetip Krishnamra

Abstract: Prolactin has recently been shown to directly stimulate 2 components of the active duodenal calcium transport in female rats, i.e., solvent drag-induced and transcellular-active calcium transport. Since the basolateral Na^+/K^+- and Ca^{2+}-ATPases, respectively, play important roles in these 2 transport mechanisms, the present study aimed to examine the direct actions of prolactin on the activities of both transporters in sexually mature female Wistar rats. The results showed that 200, 400, and 800 ng/mL prolactin produced a significant increase in the total ATPase activity of duodenal crude homogenate in a dose-dependent manner within 60 min (i.e., from a control value of 1.53 ± 0.13 to 2.29 ± 0.21 ($p < 0.05$), 2.68 ± 0.19 ($p < 0.01$), and 3.92 ± 0.33 ($p < 0.001$) μmol P_i·(mg protein)$^{-1}$·min^{-1}, respectively). Activity of Na^+/K^+-ATPase was increased by 800 ng/mL prolactin from 0.17 ± 0.03 to 1.18 ± 0.29 μmol P_i·(mg protein)$^{-1}$·min^{-1} ($p < 0.01$). Prolactin at doses of 400 and 600 ng/mL also significantly increased the activities of Ca^{2+}-ATPase in crude homogenate from a control value of 0.84 ± 0.03 to 1.75 ± 0.29 ($p < 0.05$), and 2.30 ± 0.37 ($p < 0.001$) μmol P_i·(mg protein)$^{-1}$·min^{-1}. When the crude homogenate was purified for the basolateral membrane, the Na^+/K^+-ATPase activities were elevated 10-fold. In the purified homogenate, 800 ng/mL prolactin increased Na^+/K^+-ATPase activity from 1.79 ± 0.38 to 2.63 ± 0.44 μmol P_i·(mg protein)$^{-1}$·min^{-1} ($p < 0.05$), and Ca^{2+}-ATPase activity from 0.08 ± 0.14 to 2.03 ± 0.23 μmol P_i·(mg protein)$^{-1}$·min^{-1} ($p < 0.001$). Because the apical calcium entry was the first important step for the transcellular active calcium transport, the brush border calcium uptake was also investigated in this study. We found that, 8 min after being directly exposed to 800 ng/mL prolactin, the brush border calcium uptake into the duodenal epithelial cells was increased from 0.31 ± 0.02 to 0.80 ± 0.28 nmol·(mg protein)$^{-1}$ ($p < 0.05$). It was concluded that prolactin directly and rapidly enhanced the brush border calcium uptake as well as the activities of the basolateral Na^+/K^+- and Ca^{2+}-ATPases in the duodenal epithelium of female rats. These findings explained the mechanisms by which prolactin stimulated duodenal active calcium absorption.

Key words: Ca^{2+}-ATPase, calcium transport, duodenum, Na^+/K^+-ATPase, prolactin.

Résumé : De récents travaux ont montré que la prolactine stimule directement 2 composantes du transport actif de calcium dans le duodénum des rats femelles, à savoir le transport par convection de soluté et le transport actif transcellulaire de calcium. Étant donné que les Na^+/K^+- et Ca^{2+}-ATPases basolatérales jouent un rôle important dans ces 2 mécanismes de transport, la présente étude a eu pour but d'examiner les actions directes de la prolactine sur les activités des 2 transporteurs chez des rats Wistar femelles adultes. Les résultats ont montré que des doses de 200, 400 et 800 ng/mL de prolactine ont entraîné une augmentation significative de l'activité ATPase totale de l'homogénat brut duodénal, en fonction de la dose utilisée, en moins de 60 min, d'une valeur témoin de 1,53 ± 0,13 à 2,29 ± 0,21 ($p < 0,05$), 2,68 ± 0,19 ($p < 0,01$), et 3,92 ± 0,33 ($p < 0,001$) μmol P_i·(mg protéine)$^{-1}$·min^{-1}, respectivement. Une dose de 800 ng/mL de prolactine a augmenté l'activité de la Na^+/K^+-ATPase de 0,17 ± 0,03 à 1,18 ± 0,29 μmol P_i·(mg protéine)$^{-1}$·min^{-1} ($p < 0,01$). La prolactine, aux doses de 400 et 600 ng/mL, a aussi augmenté de manière significative les activités de la Ca^{2+}-ATPase dans l'homogénat brut, d'une valeur témoin de 0,84 ± 0,03 à 1,75 ± 0,29 ($p < 0,05$) et 2,30 ± 0,37 ($p < 0,001$) μmol P_i·(mg protéine)$^{-1}$·min^{-1}. Lorsqu'on a purifié l'homogénat brut de la membrane basolatérale, les activités Na^+/K^+-ATPase ont augmenté d'un facteur 10. Dans l'homogénat purifié, 800 ng/mL de prolactine ont augmenté l'activité Na^+/K^+-ATPase de 1,79 ± 0,38 à 2,63 ± 0,44 μmol P_i·(mg protéine)$^{-1}$·min^{-1} ($p < 0,05$) et l'activité Ca^{2+}-ATPase de 0,08 ± 0,14 à 2,03 ± 0,23 μmol P_i·(mg protéine)$^{-1}$·min^{-1} ($p < 0,001$). Étant donné que l'entrée apicale de calcium a été la première étape importante pour le transport actif transcellulaire de calcium, on a aussi examiné la capture de calcium dans la membrane de bordure en brosse. On a constaté que, 8 min après avoir été exposée directement à 800 ng/mL de prolactine, la capture de calcium dans la membrane de bordure en brosse des cellules épithéliales duodénales a augmenté de 0,31 ± 0,02 à 0,80 ± 0,28 nmol·(mg protéine)$^{-1}$ ($p < 0,05$). On a conclu que la prolactine stimule directement et rapidement la capture de calcium dans la membrane de bordure en brosse et les activités des Na^+/K^+- et Ca^{2+}-ATPases basolatérales dans l'épithélium duodénal des rats femelles. Ces résultats ont expliqué les mécanismes par lesquels la prolactine stimule l'absorption active de calcium dans le duodénum.

Mots clés : Ca^{2+}-ATPase, transport de calcium, duodénum, Na^+/K^+-ATPase, prolactine.

[Traduit par la Rédaction]

Received 19 May 2006. Published on the NRC Research Press Web site at http://cjpp.nrc.ca on 6 July 2006.

N. Charoenphandhu, L. Limlomwongse, and N. Krishnamra.[1] Department of Physiology, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

[1]Corresponding author (e-mail: scnks@mahidol.ac.th).

 doi:10.1139/Y05-161

Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 352 (2007) 537–542
BBRC
www.elsevier.com/locate/ybbrc

Regulation of Ca^{2+} mobilization by prolactin in mammary gland cells: Possible role of secretory pathway Ca^{2+}-ATPase type 2

Utchariya Anantamongkol [a,1], Haruo Takemura [c,*,1], Tuangporn Suthiphongchai [b], Nateetip Krishnamra [a], Yoshiyuki Horio [c]

[a] Department of Physiology, Faculty of Sciences, Mahidol University, Rama VI Road, Bangkok 10400, Thailand
[b] Department of Biochemistry, Faculty of Sciences, Mahidol University, Rama VI Road, Bangkok 10400, Thailand
[c] Department of Pharmacology, Sapporo Medical University, South 1, West 17, Sapporo 060-8556, Japan

Received 9 November 2006
Available online 20 November 2006

Abstract

Regulatory role of prolactin (PRL) on Ca^{2+} mobilization in human mammary gland cell line MCF-7 was examined. Direct addition of PRL did not affect cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$); however, treatment with PRL for 24 h significantly decreased the peak level and duration time of $[Ca^{2+}]_i$ elevation evoked by ATP or thapsigargin (TG). Intracellular Ca^{2+} release by IP_3 or TG in permeablized cells was not decreased after PRL-treatment, indicating that the Ca^{2+} release was not impaired by PRL treatment. Extracellular Ca^{2+} entry evoked by ATP or TG was likely to be intact, because entry of extracellular Ba^{2+} was not affected by PRL treatment. Among Ca^{2+}-ATPases expressed in MCF-7 cells, we found significant increase of secretory pathway Ca^{2+}-ATPase type 2 (SPCA2) mRNA in PRL-treated cells by RT-PCR experiments including quantitative RT-PCR. Knockdown of SPCA2 by siRNA in PRL-treated cells showed similar Ca^{2+} mobilization to that in PRL-untreated cells. The present results suggest that PRL facilitates Ca^{2+} transport into Golgi apparatus and may contribute the supply of Ca^{2+} to milk.

Keywords: Mammary gland cells; Prolactin; Ca^{2+}-ATPase; Ca^{2+} entry; Ca^{2+} release

Golgi apparatus participates in protein sorting and trafficking, endosome function and protein processing. Ca^{2+} is indispensable for these functions. Ca^{2+} is pumped from the cytoplasm into the Golgi apparatus by Ca^{2+}-ATPases and distributes as a bound form with various Ca^{2+} binding proteins and also as a free ionized form [1]. Secretory pathway Ca^{2+}-ATPases (SPCAs) are principal for the transport of Ca^{2+} into Golgi apparatus and two isoformes, SPCA1 and SPCA2, are found [2]. In contrast to the widespread tissue distribution pattern of SPCA1, SPCA2 is confined to a limited number of tissues such as mammary and salivary glands, brain, and epithelial cells of gastrointestinal organs [3]. However, the functional roles and regulating mechanisms of SPCAs are still unknown.

PRL is a polypeptide hormone, which is indispensable for the growth and development of mammary glands, synthesis of milk, and maintenance of milk secretion [4]. PRL is necessary for lactogenesis, because both PRL [5] and PRL receptor [6] knockout mice fail to produce milk. PRL was reported to increase cytoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) in the hepatocytes of lactating rats [7], human T-cells [8], human glioma cells [9] and CHO cells expressing PRL receptors [10,11], although there has been no report about Ca^{2+} mobilization by PRL in mammary epithelial cells. Suckling of mammary gland increases not only PRL secretion but also milk secretion. By mechanical stimulation, mammary epithelial cells release ATP, UTP, and UDP [12], which bind to purinergic receptors and increase $[Ca^{2+}]_i$ [13]. The elevation of $[Ca^{2+}]_i$ has been

* Corresponding author. Fax: +81 11 612 5861.
E-mail address: takemura@sapmed.ac.jp (H. Takemura).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.11.055

Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2007;19:77-88

Accepted: November 28, 2006

Control of Ion Transport in Mouse Proximal and Distal Colon by Prolactin

Supaporn Puntheeranurak[2], Rainer Schreiber[1], Melanie Spitzner[1], Jiraporn Ousingsawat[1], Nateetip Krishnamra[2*] and Karl Kunzelmann[1*]

[1]Institut für Physiologie, Universität Regensburg, [2]Department of Physiology, Faculty of Science, Mahidol University, Bangkok, *KK and NK share senior authorship

Key Words

Prolactin • BK channels • CFTR • EnaC • Ca^{2+}-activated Cl^- channels • Epithelial transport • Secretion • Colon • Ion channels

Abstract

The lactogenic hormone prolactin (PRL) has been known to affect Ca^{2+} and electrolyte transport in the intestinal epithelium. In the present study we analyzed ion transport in mouse proximal and distal colon, and acute changes induced by PRL. In the proximal colon, carbachol activated a Ca^{2+} dependent Cl^- secretion that was sensitive to DIDS and NFA. In the distal colon, both ATP and carbachol activated K^+ secretion. Ca^{2+}-activated KCl transport in proximal and distal colon was inhibited by PRL (200ng/ml), while amiloride sensitive Na^+ absorption and cAMP induced Cl^- secretion remained unaffected. Luminal large conductance Ca^{2+}-activated K^+ (BK) channels were largely responsible for Ca^{2+}-activated K^+ secretion in the distal colon, and basolateral BK channels supported Ca^{2+}-activated Cl^- secretion in the proximal colon. Ca^{2+} chelating by BAPTA-AM attenuated effects of carbachol and abolished effects of PRL. Both inhibition of PI3 kinase with wortmannin and blockage of MAP kinases with SB 203580 or U 0126, interfered with the acute inhibitory effect of PRL on ion transport, while blocking of Jak/Stat kinases with AG 490 was without effects. PRL attenuated the increase in intracellular Ca^{2+} that was caused by stimulation of isolated colonic crypts with carbachol. Thus PRL inhibits Ca^{2+} dependent Cl^- and K^+ secretion by interfering with intracellular Ca^{2+} signaling and probably by activating PI3 kinase and MAP kinase pathways.

Introduction

Regulation of salt and water balance is essential to maintain homeostasis in most organisms. The lactogenic hormone, prolactin (PRL) is involved in water and electrolyte balance in most vertebrates. PRL receptors (PRL-R) are present in kidney and other epithelial tissues involved in salt balance. Thus PRL has been shown to control salt and water balance in teleosts and amphibians

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/07/0194-0077$23.50/0

Accessible online at:
www.karger.com/journals/net

Karl Kunzelmann
Institut für Physiology, Universität Regensburg
Universitätsstr. 31, D-93053 Regensburg (Germany)
Tel +49 941/943-4302, Fax +49 941/943-4315
E-Mail uqkkunze@mailbox.uq.edu.au

Am J Physiol Endocrinol Metab 293: E372–E384, 2007.
First published May 8, 2007; doi:10.1152/ajpendo.00142.2007.

Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by the phosphoinositide 3-kinase pathway

Walailuk Jantarajit,[1]* Narongrit Thongon,[1]* Jantarima Pandaranandaka,[2,3] Jarinthorn Teerapornpuntakit,[1] Nateetip Krishnamra,[1,2] and Narattaphol Charoenphandhu[1,2]

[1]*Department of Physiology and* [2]*Consortium for Calcium and Bone Research, Faculty of Science, Mahidol University, Bangkok; and* [3]*Faculty of Medicine, Thammasat University, Pathumthani, Thailand*

Submitted 3 March 2007; accepted in final form 18 April 2007

Jantarajit W, Thongon N, Pandaranandaka J, Teerapornpuntakit J, Krishnamra N, Charoenphandhu N. Prolactin-stimulated transepithelial calcium transport in duodenum and Caco-2 monolayer are mediated by the phosphoinositide 3-kinase pathway. *Am J Physiol Endocrinol Metab* 293: E372–E384, 2007. First published May 8, 2007; doi:10.1152/ajpendo.00142.2007.—Prolactin (PRL) has been shown to stimulate intestinal calcium absorption but the mechanism was still unknown. This study aimed to investigate the mechanism and signaling pathway by which PRL enhanced calcium transport in the rat duodenum and Caco-2 monolayer. Both epithelia strongly expressed mRNAs and proteins of PRL receptors. Ussing chamber technique showed that the duodenal active calcium fluxes were increased by PRL in a dose-response manner with the maximal effective dose of 800 ng/ml. This response diminished after exposure to LY-294002, a phosphoinositide 3-kinase (PI3K) inhibitor. Caco-2 monolayer gave similar response to PRL with the maximal effective dose of 600 ng/ml. By nullifying the transepithelial potential difference, we showed that the voltage-dependent paracellular calcium transport did not contribute to the PRL-enhanced flux in Caco-2 monolayer. In contrast, the calcium gradient-dependent paracellular transport and calcium permeability were increased by PRL. Effects of PRL on Caco-2 monolayer were abolished by PI3K inhibitors (LY-294002 and wortmannin), but not by inhibitors of MEK (U-0126) or JAK2 (AG-490). To investigate whether the PRL-enhanced paracellular transport was linked to changes in the epithelial charge selectivity, the permeability ratio of sodium and chloride (P_{Na}/P_{Cl}) was determined. We found that PRL elevated the P_{Na}/P_{Cl} in both epithelia, and the effects were blocked by PI3K inhibitors. In conclusion, PRL directly and rapidly stimulated the active and passive calcium transport in the rat duodenum and Caco-2 monolayer via the nongenomic PI3K-signaling pathway. This PRL-enhanced paracellular calcium transport could have resulted from altered charge selectivity.

charge selectivity; dilution potential; paracellular transport; prolactin receptor; tight junction; transcellular transport

AS ONE OF THE CALCIUM-REGULATING HORMONES during pregnancy and lactation, prolactin (PRL) has been shown to stimulate intestinal calcium absorption (38), thereby protecting against development of negative calcium balance during these reproductive periods. Further investigations in nonmated female rats also revealed stimulatory actions of PRL on intestinal calcium absorption (11, 54), especially in the duodenum which was the most efficient site for calcium transport (21, 32). Although the presence of PRL receptor (PRLR) proteins in duodenal enterocytes of rats was controversial, expression of rat PRLR (rPRLR) transcripts in the duodenal mucosa, demonstrated by in situ hybridization technique (45), implicated a direct action of PRL on the duodenal epithelial cells.

Calcium traversed the duodenal epithelium by both active and passive pathways with the former being negligible in the more distal segments of the small intestine (26, 41). Three components of the active calcium transport, which was cellular energy-dependent, were identified, namely transcellular, solvent drag-induced paracellular, and voltage-dependent paracellular transport (11, 13). Passive calcium transport, on the other hand, was independent of cellular energy and occurred entirely through the paracellular channel (41). We (12) previously reported that PRL stimulated the transcellular active calcium transport by increasing apical calcium uptake and basolateral Ca^{2+}-ATPase activity in isolated duodenal enterocytes. However, the mechanisms and signaling pathways by which PRL enhanced the calcium transport in the rat duodenum had not been investigated.

Regarding the paracellular calcium transport, which constituted the major route of calcium absorption (9, 46), it was previously thought that the passive component of the paracellular transport was not regulated and was entirely determined by the calcium concentration gradient across the epithelia (41). However, recent evidence suggested that paracellular transport was determined by the size- and charge-selective properties of the tight junction and could be altered by the activities of perijunctional actomyosin complex and charge-selective tight junction proteins of the claudin family, respectively (43, 58). In situ perfusion experiments in the rat duodenum suggested that PRL increased paracellular passive calcium flux in the presence of 20 mmol/l luminal calcium (34). However, cytochalasin E, which disrupted actomyosin functions, thereby resulting in widening of the tight junction, did not alter PRL-enhanced paracellular calcium transport in the duodenum (54). Therefore, we hypothesized that PRL regulated the paracellular calcium transport by altering the charge selectivity rather than size selectivity of the paracellular channels.

Nothing was known regarding the intracellular signal transduction of PRL in the duodenum. The putative genomic signaling pathways of PRL in mammary glandular epithelia, neurons and liver appeared to involve Janus kinase-2 (JAK2) signal transducers and activator of transcription (STAT)5, phosphoinositide 3-kinase (PI3K), and mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK) pathways (6, 30, 42, 55, 62). However, nongenomic cascades of JAK2, PI3K, and MEK have also been demonstrated as sig-

* W. J. and N. T. contributed equally to this study.

Address for reprint requests and other correspondence: N. Charoenphandhu, Dept. of Physiology, Faculty of Science, Mahidol Univ., Rama VI Road, Bangkok 10400, Thailand (e-mail: naratt@narattsys.com).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

REVIEW / SYNTHÈSE

Prolactin is an important regulator of intestinal calcium transport

Narattaphol Charoenphandhu and Nateetip Krishnamra

Abstract: Prolactin has been shown to stimulate intestinal calcium absorption, increase bone turnover, and reduce renal calcium excretion. The small intestine, which is the sole organ supplying new calcium to the body, intensely expresses mRNAs and proteins of prolactin receptors, especially in the duodenum and jejunum, indicating the intestine as a target tissue of prolactin. A number of investigations show that prolactin is able to stimulate the intestinal calcium transport both in vitro and in vivo, whereas bromocriptine, which inhibits pituitary prolactin secretion, antagonizes its actions. In female rats, acute and long-term exposure to high prolactin levels significantly enhances the (*i*) transcellular active, (*ii*) solvent drag-induced, and (*iii*) passive calcium transport occurring in the small intestine. These effects are seen not only in pregnant and lactating animals, but are also observed in non-pregnant and non-lactating animals. Interestingly, young animals are more responsive to prolactin than adults. Prolactin-enhanced calcium absorption gradually diminishes with age, thus suggesting it has an age-dependent mode of action. Although prolactin's effects on calcium absorption are not directly vitamin D-dependent; a certain level of circulating vitamin D may be required for the basal expression of genes related to calcium transport. The aforementioned body of evidence supports the hypothesis that prolactin acts as a regulator of calcium homeostasis by controlling the intestinal calcium absorption. Cellular and molecular signal transductions of prolactin in the enterocytes are largely unknown, however, and still require investigation.

Key words: calcium transport, paracellular transport, prolactin, small intestine, transcellular transport.

Résumé : On a montré que la prolactine stimule l'absorption de calcium intestinal, augmente le renouvellement osseux et diminue l'excrétion de calcium rénal. L'intestin grêle, qui est le seul organe réapprovisionnant le corps en calcium, exprime fortement les ARNm et les protéines des récepteurs de la prolactine, en particulier dans le duodénum et le jéjunum, ce qui indique que l'intestin est un tissu cible de la prolactine. De nombreuses recherches montrent que la prolactine peut stimuler le transport de calcium intestinal tant in vitro qu'in vivo, alors que la bromocriptine, qui inhibe la sécrétion de prolactine hypophysaire, antagonise ses actions. Une exposition aiguë et prolongée à de hauts taux de prolactine stimule grandement le transport de calcium actif transcellulaire, induit par convection de soluté et passif dans le grêle des rats femelles. On observe ces effets non seulement chez les femelles gravides ou lactantes, mais aussi chez les femelles normales. Fait intéressant, les jeunes animaux sont plus sensibles à la prolactine que les adultes, l'absorption de calcium stimulée par la prolactine diminuant graduellement avec l'âge, ce qui autorise à penser que son action est fonction de l'âge. De plus, les effets de la prolactine sur l'absorption de calcium ne dépendent pas directement de la vitamine D; toutefois, un certain taux de vitamine D circulante pourrait être nécessaire pour l'expression basale des gènes associés au transport de calcium. Le faisceau d'arguments énoncés ci-dessus viennent conforter l'hypothèse que la prolactine agit comme un régulateur de l'homéostasie du calcium en régulant l'absorption du calcium intestinal. Toutefois, la transduction des signaux cellulaires et moléculaires de la prolactine dans les entérocytes est très peu connue et devra faire l'objet d'autres études.

Mots-clés : transport de calcium, transport paracellulaire, prolactine, intestin grêle, transport transcellulaire.

[Traduit par la Rédaction]

Received 3 December 2006. Published on the NRC Research Press Web site at cjpp.nrc.ca on 1 August 2007.

N. Charoenphandhu[1] and N. Krishnamra.[2] Department of Physiology, and Consortium for Calcium and Bone Research (COCAB), Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.

[1]Corresponding author (e-mail: naratt@narattsys.com).
[2]Corresponding author (e-mail: scnks@mahidol.ac.th).

 doi:10.1139/Y07-041