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Localization and Expression of TRPV6 in all Intestinal Segments and Kidney of Laying Hens

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Abstract

In mammalian intestine, the passive entry of Ca^{2+} into enterocytes via the transient receptor potential vanilloid channel type 6 (TRPV6) is recognized to be an important rate-limiting entry step in maintaining Ca^{2+} homeostasis. There is, however, little information on the expression patterns of TRPV6 in the laying hen and therefore this study investigated TRPV6 localization and expression in different intestinal segments and kidney of laying hens during peak lay. Immunohistochemical analysis of the intestine indicated that TRPV6 was localized to the brush-border membranes of the duodenum, jejunum, ileum, cecum, and rectum. Expression was weaker in the rectum and little or no expression was found in crypt and goblet cells. In addition, TRPV6 mRNA was quantified amongst different intestinal segments and expression was highest in the duodenum and jejunum. Furthermore, western blotting indicated that the duodenum expressed the greatest amount of TRPV6 and the rectum the least with the other segments expressing intermediate levels. In the kidney, distinct immunopositive staining for TRPV6 was detected at the apical domain of the distal convoluted tubules (DCT) and medullary connecting tubules (CNT). Interestingly, distribution of TRPV6 extended to the proximal convoluted tubules (PCT), which is not generally implicated in active Ca^{2+} transcellular reabsorption. Furthermore, the kidney expressed lower TRPV6 mRNA ($P<0.05$) and protein levels compared to duodenum possibly implicating an important role for the TRPV5 homolog in the kidney. In conclusion, the epithelial Ca^{2+} channel TRPV6 is strongly expressed in the apical cells of the entire intestine and the renal tubules suggesting that active Ca^{2+} transcellular transport plays a crucial role in dietary calcium (re)absorption in laying hens.

Keywords: laying hens; TRPV6; intestine; kidney

Introduction

Active Ca^{2+} (re)absorption is of great importance in the maintenance of Ca^{2+} homeostasis for many physiological functions. It is also essential for skeletal hydroxyapatite formation and skeletal mineralization and to satisfy the high Ca^{2+} requirement for eggshell calcification in the laying hen (Bianco et al., 2007; Sang-Hwan et al., 2009; Vander et al., 2005). In modern commercial strains of laying hens, 2 to 3 g of calcium (equivalent to 10% of total body calcium) is transferred daily for deposition as eggshell calcium (Miller, 1992; Sugiyama and Kusuhara, 2001). The major source of calcium is from intestinal dietary Ca^{2+} absorption, renal Ca^{2+} reabsorption and skeletal stores. Such intensive transport imposes severe demands on Ca^{2+} homeostasis, and activates at least two extremely effective mechanisms for Ca^{2+} transfer (Bar, 2008a).

Epithelial Ca^{2+} absorption involves two distinct pathways: the saturated process corresponding to active Ca^{2+} transcellular transport, and the unsaturated process relating to diffusion through a paracellular route. In mammalian intestine, the transcellular Ca^{2+} transport system plays a crucial role in maintaining Ca^{2+} homeostasis, which is a three-step process, comprising the passive entry of Ca^{2+} into enterocytes via the transient receptor potential vanilloid channel type 5 or 6 (TRPV5, TRPV6); the cytosolic transfer of Ca^{2+} bound to the protein calbindin D_{9k} (avian and other lower species' tissues contain calbindin D_{28K}); and the extrusion of Ca^{2+} across the basolateral membrane via Ca^{2+}-ATPase (PMCA 1b) and/or a Na^{+}-Ca^{2+} exchanger (NCX 1b) (van Abel et al., 2005). A similar mechanism exists in tissues such as the kidney, uterus, and placenta (Belkacemi et al., 2005; Hirnet et al., 2003; Hoenderop et al., 2005; Kim et al., 2006; Suzuki et al., 2008; Lee and Jeung, 2007).

The ion channel TRPV6 is likely to function as an epithelial calcium channel in different tissues, such as intestine, kidney, bone, skin, placenta, and exocrine organs of mammals, all of which are characterized by high Ca^{2+} transport requirements (Nijenhuis et al., 2003; Hoenderop et al., 2005). Recently, TRPV6 has been reported to exert significant effects in facilitating the entry of Ca^{2+} into the epithelial cells, and there is a significant correlation between TRPV6 expression and Ca^{2+} transport

(Brown et al., 2005; Bianco et al., 2007). Intestinal Ca^{2+} malabsorption and renal Ca^{2+} reabsorption disorders are characteristics of the TRPV6$^{-/-}$ mouse and these observations have led to the suggestion that TRPV6 is a important rate-limiting entry step in maintaining Ca^{2+} homeostasis (Bianco et al., 2004; Bianco et al., 2007).

However this Ca^{2+} transport model may not be strictly applicable to avian tissues. Bar (2008a) postulated that Ca^{2+} absorption via paracellular transport predominated in the intestine of laying hens with the transcellular pathway utilized only during low Ca^{2+} dietary conditions. Nevertheless, the accumulating evidence has indicated that high expression levels of calbindins and PMCA 1b exist in the intestine and the kidney, as well as the eggshell gland (ESG) of laying hens. However, the presence of TRPV6 in avian tissues is unclear, despite the recognized importance of efficient Ca^{2+}-transporters in the laying hen (Bar, 2008a; Wasserman and Talyor, 1966; Taylor and Wasserman, 1967; Melancon and DeLuca, 1970; Davis et al., 1987; Qin and Klandorf, 1993a,b).

The objective of the present study was to determine the distribution and expression patterns of TRPV6 in the intestine and kidney of the laying hen. This information is important to fully understand the physiological role of this Ca^{2+} channel during eggshell mineralization in health and disease. It also has implications for pathological bone mineralization, the development of avian osteoporosis and general skeletal health in the laying hen.

Materials and Methods

Animals

Ten ISA laying hens of 1.5–2.0 kg in weight at the peak egg laying stage (220 days old and 90% egg laying rate) were obtained from Qinglongshan Husbandry Co. (Nanjing, China). They were housed in individual cage with a light-controlled (16L:8D) and air-conditioned (20 °C ± 2, humidity: 50 ± 5%) environment. Each hen was allocated to a wire cage equipped with a nipple drinker and given a commercial standard layer diet. Production records of all hens were kept during the two weeks

before sacrifice.

Six hens with analogous egg sequences were decapitated after egg laying, and samples of the duodenum, jejunum, ileum, cecum, rectum, and kidney were taken within 10 min after death. Samples for RNA isolation and Western blotting were rinsed with ice-cold phosphate buffered saline (PBS; pH 7.4). Intestinal epithelia were separated manually from the underlying submucosal layer of the duodenum, jejunum, and ileum, using glass slides on ice. All samples were immediately frozen in liquid nitrogen and stored at –80 °C. In addition, fresh central portions of intestinal tissues and kidney for immunohistochemistry were fixed in 10% buffered formalin (pH 7.4) and stored at 4°C for 24–48h. Samples were dehydrated and processed through to paraffin wax using standard methods.

All animal and experimental procedures were undertaken following the guidelines of the Animal Ethics Committee of Nanjing Agricultural University, China.

Immunohistochemistry

Paraffin sections (5 μm) were cut and placed on poly-L-lysine-coated glass slides were prepared according to a standard procedure. After dewaxing and rehydration, sections were rinsed in 0.02 M PBS (pH 7.4) and endogenous peroxidase activity was quenched by incubation with 0.2% H_2O_2 in 0.1 M phosphate buffer (pH 7.3) containing 0.2% Triton X-100 for 25 min. After rinsing in PBS, the sections were placed in 10 mM citrate target retrieval buffer and brought to boiling in a microwave oven for 5–10 min. The sections were then blocked with 5% BSA to prevent unspecific binding, incubated with anti-TRPV6 polyclonal antibody (ACC-036, Alamone Labs, Jerusalem, Israel) diluted 1:100 in PBS containing 0.3% Triton X-100, 0.05% Tween 20 and 1% BSA, and then placed in a humidified chamber at 4°C overnight. After washing in PBS, sections were incubated with biotinylated goat anti-rabbit IgG (Boster Bio-Technology Co., Ltd., Wuhan, China) at a dilution of 1:400 for 45 min at room temperature. The slides were washed three times in PBS and then finally incubated with streptavidin-biotin complex (SA2002, Boster Bio-Technology Co., Ltd) for 20 min. After washing, the immunoreaction was

revealed using a DAB kit buffer (AR1022, Boster Bio-Technology Co., Ltd., Wuhan, China) before being lightly counterstained with haematoxylin and mounted. Sections incubated with PBS or with primary antibody pre-incubated with TRPV6 antigen instead of primary antiserum or solely with conjugated secondary antibodies acted as negative controls and all preperations were devoid of positive staining. Photographs were taken with a Nikon Eclipse 5*i* (H5505, Nikon, Japan) microscope equipped with Digital Sight Camera System (DS-Fi1 Nikon, Japan).

RNA extraction and reverse transcriptase (RT)-PCR

Total RNA was isolated from 100 mg of samples using TRIzol Reagent (Tiangen Biotech Co., Ltd., Beijing, China), according to the manufacturer's protocol. After Dnase I treatment to eliminate genomic DNA, 2 μg of total RNA was reverse-transcribed into cDNA by incubating at 37 °C for 1 h, in 50 pmol oligo(dT)18 primer, 100 U reverse transcriptase M-MLV, 1mM dNTP mixture and 20 U Rnase inhibitor in a 20 μl final volume. The reaction was terminated by heating at 95 °C for 5 min and then quickly cooled on ice.

Qualitative PCR

In order to amplify a cDNA fragment of 426 bp, a pair of primers (forward primer: 5'- ACGAGGATGAGTTGAGGC-3'; reverse primer: 5'-GTCCAAGGTTTT CAGGGT-3') were designed according to the mRNA sequence of Gallus Gallus TRPV6 (GenBank accession no. XM416530, 1145-1570 bp) to span two introns in the genomic DNA. RT-PCR was carried out using standard PCR conditions (1 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 μM forward primer, 0.4 μM reverse primer, 0.05 U TaKaRa Taq^{TM}) and protocol (5 min at 94 °C; 5 cycles of 45s at 94 °C, 45s at 54 °C, 45s at 72 °C; then 30 cycles of 45s at 94 °C, 45s at 50 °C, 45s at 72 °C; with a final extension at 72 °C for 10 min). No template controls (NTC) were also completed to monitor possible genomic DNA contamination. The PCR product was cloned into the pMD18-T vector and the sequence was determined by Invitrogen Biotechnology Ltd. (Shanghai, China), which was verified as TRPV6 by NCBI Blast.

Quantitative RT-PCR

TRPV6 mRNA expression in the kidney and different intestinal segments was

quantified and normalised to β-actin expression using an ABI-prism 7300 Sequence Detection System. The primers for TRPV6 (forward primer: 5'-TGGAACGGACTAA GTCAGAAGTTG-3'; reverse primer: 5'-CGTTATGGCTG GGATGTTGTT-3', 141 bp, GenBank accession no. XM416530, 49-197 bp) and for β-actin (forward primer: 5'-TGCGTGACATCAAGGAGAAG-3' and reverse primer: 5'-TGCCAGGTACA TTGTGGTA-3' (Hu et al., 2008) were designed by Prime Premier 5.0 and synthesized by Invitrogen Biotechnology Ltd. (Shanghai, China).

Aliquots of the cDNA samples were used for real-time PCR in a final volume of 20 μL, containing 10 μL SYBR Premix Ex Taq (2×), 0.4 μL ROX Reference Dye (50×) and 0.8 μM of each forward and reverse primers. The PCR protocols included an initial denaturation (30s at 95 °C), and a two-step amplification program (5 s at 95 °C, 31 s at 62 °C, repeated 40 times). The method of $2^{-\Delta\Delta Ct}$ was used to analyze the real-time RT-PCR data (Livak et al., 2001). No template control (NTC) was also completed. All samples were amplified during the same RT-PCR run and repeated at least three times.

All reagents were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China), unless otherwise specified.

Protein isolation and Western blotting analysis

Quick-frozen tissue samples (~100mg) were mechanically homogenized during thawing in ice-cold RIPA buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium Deoxycholate, 0.1% SDS and 1% protease inhibitor PMSF/isopropanol (10 μg/ml, added prior to lysing)] using a tissue grinder (Polytron, Polytron PT1200E; Brinkman Instruments, Littau, Switzerland). After incubation on ice for 30 min, the homogenate was centrifuged at 25,000 g for 30 min at 4 °C to remove all insoluble material. The supernatant was collected, and the protein concentrations were determined by the BCA Protein Assay Kit (Boster Bio-Technology Co., Ltd., Wuhan, China) according to the manufacturer's instructions. Samples were boiled for 5 min in SDS-PAGE sample loading buffer and 80 μg of protein extracts were electrophoresed on a 10% polyacrylamide gel and transferred onto nitrocellulose membranes at 100 V for 80 min using a tank blotting

system (Bio-Rad, USA). The membranes were then cut to isolate the TRPV6 band (80 kDa~110 kDa) and GAPDH band (37 kDa) according to prestained SDS-PAGE standards. After blocking with 5% skimmed milk in TBS for 2 h at room temperature, membranes were incubated in blocking solution with rabbit polyclonal anti-TRPV6 antibodies (ACC-036, Alamone Labs, Jerusalem, Israel; 1:200) or mouse monoclonal anti-GAPDH antibodies (KC-5G4, Kangchen Bio-tech Inc., Shanghai, China; 1:10000) at 4 °C overnight. After washing 4×10 min with TBS-0.1% Tween 20 (TBS-T), the blots were incubated with HRP-conjugated goat anti-rabbit IgG (GGHL-15P, Immunology Consultants Laboratory, Inc., USA; 1:5000) and HRP-conjugated goat anti-mouse IgG (BS50350, Bioworld Technology Co., Ltd., USA; 1:10000) for 1 h at room temperature, respectively. Finally, the blotted membranes were washed and detected by enhanced chemiluminescence (ECL) (PA112, Tiangen Biotech Co., Ltd., Beijing, China). ECL signals recorded on x-ray film were scanned and analyzed with Kodak 1D Electrophoresis Documentation and Analysis System 120 (Kodak Photo Film Co., Ltd., United Stated). The band density of TRPV6 was normalized to GAPDH.

Statistics

All statistical analysis was performed with SPSS 11.0 for Windows (SPSS Inc., Chicago, IL). Values of mRNA abundance and peptide/protein content were expressed as the fold-change relative to the control group. Data were presented as mean ± S.E.M. The differences were tested by one-way analysis of variances (ANOVA) for independent samples. A *P*-value less than 0.05 was considered significant.

Results

Immunohistochemistry

To elucidate the localization of TRPV6 in the intestine and kidney of laying hens, immunostaining of different intestinal segments and kidney sections with an anti-TRPV6 antibody was performed. In the intestine, TRPV6 was abundantly present in brush-border membranes of the duodenum, jejunum, ileum, cecum, and rectum (Figs. 1 A - G). Intense staining was observed in absorptive enterocytes along the

entire tip-crypt lamina axis in the villi (Figs. 1 A - D), whereas the localization of TRPV6 in the rectum was weaker and restricted to epithelial cells of the upper villi (Figs. 1 E - G). Little or no staining was found in crypt and goblet cells (Figs. 1 F and G). In control sections, no immunoreactive staining was observed (Figs. 1 H).

In the kidney, distinct immunopositive staining for TRPV6 was detected in apical cells of the tubules of the renal cortex and medulla, which was predominantly confined to the cortical proximal convoluted tubules (PCT), distal convoluted tubules (DCT), and medullary connecting tubules (CNT) (Figs. 1 I - K). Staining was not observed in glomeruli and collecting tubules (Figs. 1 I). The control sections were negative (Fig. 1 L).

Qualitative and quantitative RT-PCR

Standard RT-PCR with TRPV6 primers yielded a single amplified fragment of the expected size of 426 bp from all intestinal segments and the kidney (Fig. 2). Sequence analysis revealed a 100% homology at the nucleotide level with the predicted mRNA sequence of Gallus Gallus TRPV6. Real-time PCR analysis demonstrated that, of the tissues examined, the highest level of TRPV6 expression was in the duodenum. The expression of TRPV6 in the other intestinal segments and kidney were lower in comparison; jejunum ($P<0.05$), kidney ($P<0.05$) and ileum ($P<0.01$) (Fig. 3). The lowest level of expression was found in the cecum and rectum samples ($P<0.01$).

Western blotting analysis

Immunoblotting for TRPV6 expression in the various intestinal segments and kidney revealed one band at approximately 80 kDa in all intestinal segments and in the kidney (Fig. 4A). Densitometric quantification of the band normalized to GAPDH expression indicated that the strongest expression was detected in the duodenum (Fig. 4B). TRPV6 expression was less in all other tissues examined but this only reached significance in the rectum ($P<0.01$).

Discussion

The current study demonstrated that the epithelial Ca^{2+} channel TRPV6 was expressed in absorptive or re-absorptive epithelia of laying hen tissues. As a gatekeeper protein responsible for Ca^{2+} entry, the expression of TRPV6 in the small

intestine, cecum and rectum, and the apical domain of the PCT, DCT, and CNT of the kidney are consistent with a role for this active Ca^{2+} channel in active Ca^{2+} transport in intestinal and renal epithelia of laying hens.

Immunohistochemical analysis of the intestine indicated that TRPV6 was abundantly localized in the entire tip-crypt lamina axis of villi in the duodenum, jejunum, ileum, cecum, and rectum, while it was weakly expressed in crypt cells and not at all in goblet cells of the rectum. This result is in line with previous reports that immunoreactive CaBP-D28k was localised in the enterocyte cytoplasm of all intestinal segments in laying hen (Sugiyama et al., 2007). Thus, it is concluded that TRPV6 is expressed strongly in all segments of the intestine of laying hens, and active Ca^{2+} transcellular transport may exert significant effects in Ca^{2+} absorption from the intestinal lumen during the peak egg-laying stage.

Quantitative PCR indicated precise and different levels of TRPV6 expression among the various intestinal segments. The duodenum expressed the greatest amount of TRPV6 and the rectum the least; TRPV6 expression in the other intestinal segments was intermediate between these extremes. TRPV6 protein expression was also the lowest in the rectum and significantly less than the expression levels in the duodenum. These findings are consistent with the upper intestine (duodenum and jejunum) being the main site of Ca^{2+} absorption in birds (Hurwitz et al., 1967). However, the abundant presence of TRPV6 mRNA and protein in the ileum, is in contrast to observations in rat, human and sheep (Peng et al., 2000; Nijenhuis et al., 2003; Wilkens et al., 2009) and suggests that the ileum may also have a significant role in intestinal Ca^{2+} absorption in laying hens. This assertion is consistent with data showing that the uptake of calcium is a saturable process at low calcium concentrations in the ileum of chicken *in vitro* (Wong and Norman, 1975). However the low TRPV6 gene expression noted in the cecum and rectum noted in this present study are at variance with observations in other species (Karbach and Feldmeier, 1993; Nijenhuis et al., 2003; Peng et al., 2000;Wilkens et al., 2009). In the rat and mouse the cecum was not only the site of the greatest rate of transcellular Ca^{2+} transport, but also contained the highest expression of TRPV6 mRNA in the intestines of rat and mouse

(Karbach and Feldmeier, 1993; Peng et al., 2000; Nijenhuis et al., 2003). In humans, TRPV6 expression is most abundant in the duodenum and jejunum with very low levels present in the ascending colon and no expression detected in the ileum or distal segments of the large intestine (Peng et al., 2000).

A recent study indicated that the three groups of proteins (TRPV6 and TRPV5, Calbindins and PMCA) that facilitate the transcellular mechanism of Ca^{2+} transport are vitamin D-dependent (Bar, 2008b). Also, vitamin D response elements (VDRE) have been detected on the putative promoter regions of TRPV5 and TRPV6 genes in both human and mouse (Hoenderop et al., 2001; Weber et al., 2001). Experimental evidence does indeed support a regulatory role of $1,25(OH)_2D_3$ in TRPV6 mediated transcellular Ca^{2+} transport. Van Cromphaut and co-workers reported reduced duodenal expression of TRPV6 in VDR null mice. Furthermore, in laying hens, the varying protein concentrations of CaBP-D28k in the different regions of the intestine were correlated to the number of $1,25(OH)_2D_3$ receptors in each segment (van Cromphaut et al., 2001; Sugiyama et al., 2007). Therefore, it has been proposed that the different expression levels of TRPV6 in the various intestinal segments is related to the number of $1,25\text{-}(OH)_2D_3$ receptors in each segment.

The kidney plays an essential role in Ca^{2+} balance by regulating the Ca^{2+} excretion from the body (Hoenderop et al., 2005; Lambers et al., 2006). To satisfy the requirement for shell calcification, Ca^{2+} filtered at the glomeruli is extensively reabsorbed when it passes through the individual nephron segments. In this study, the mRNA expression of TRPV6 in kidney was significantly lower than that in the duodenum. These findings indirectly support the hypothesis that TRPV5 may be the primarily epithelial Ca^{2+} channel responsible for renal transcellular Ca^{2+} transport as the relative mRNA expression of TRPV5 is 100 times more abundant than that of TRPV6 in the kidney (van Abel et al., 2005). Whether TRPV5 is involved in Ca^{2+} reabsorption in laying hens tissues remains to be investigated. Furthermore, TRPV6 was detected in the cortical PCT, DCT and medullary CNT but not in the glomeruli and collecting tubules which is in agreement with studies in other species (Hoenderop et al., 2002; Reilly and Ellison, 2000; Loffing et al., 2001; Wilkens et al., 2009).

However, the majority of Ca^{2+} reabsorption in PCT is regarded to be energetically passive (Suki, 1979; Hoenderop et al., 2005) and therefore the positive staining in the PCT suggests that TRPV6 has other functions, rather than simply in Ca^{2+} absorption.

In conclusion, this study further substantiated the postulated function of TRPV6 as the gatekeeper of active Ca^{2+} (re)absorption in tissues of laying hens. Firstly, TRPV6, responsible for dietary Ca^{2+} absorption, is located on the apical membrane along all intestinal segments, but with different expression levels. Secondly, the expression of TRPV6 in kidney was lower than that in the duodenum. Furthermore, the distribution in renal tubules, especially PCT, demonstrated that the Ca^{2+} selective channel is widely distributed suggesting other function for this protein. Finally, our results lead to new possible areas for further study of the Ca^{2+} transfer mechanism in the laying hen. These include the examination of the differential expression patterns of TRPV6 between laying and non-laying hens, coexpression with other vitamin-dependent proteins, such as CaBP D28k, PMCA 1b, and their expression in other important Ca^{2+} transport tissues, such as eggshell gland and medullary bone.

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Figure Legends

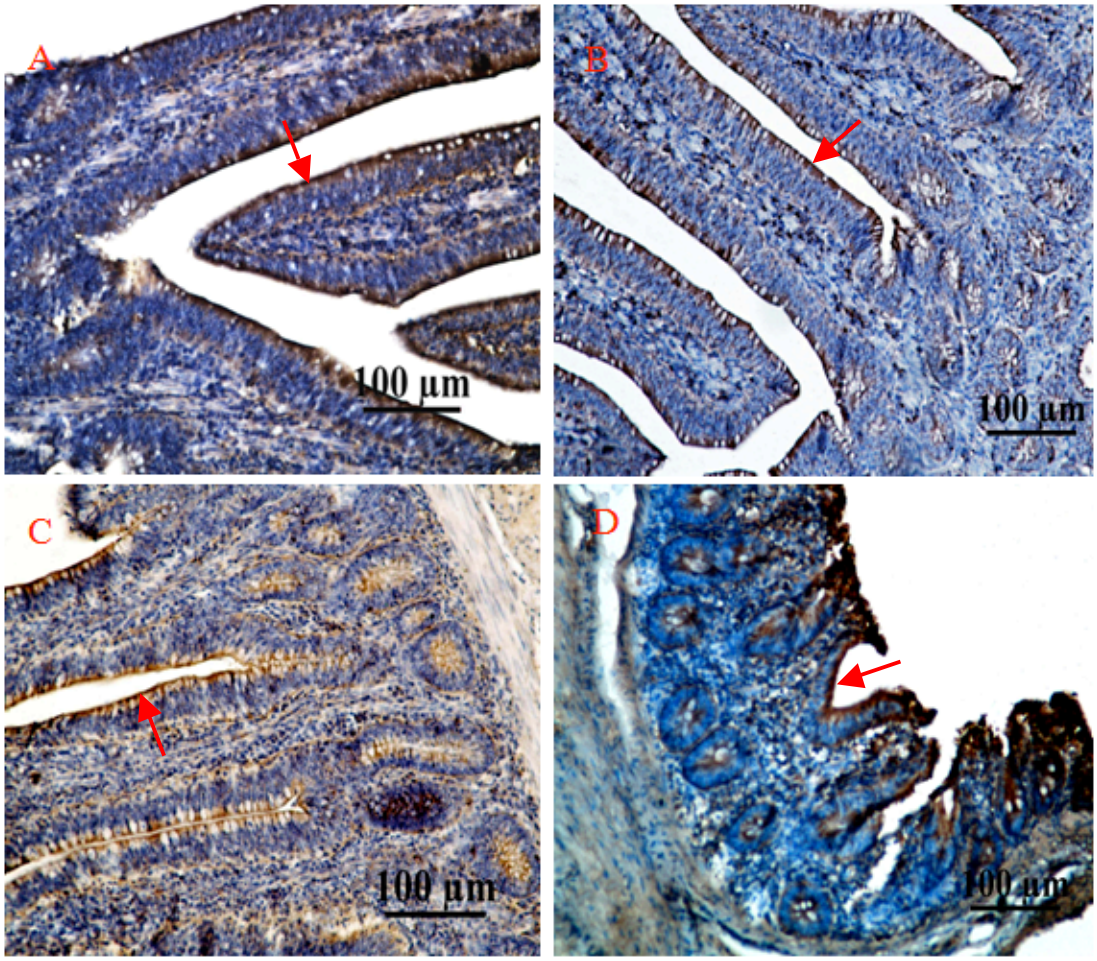
Fig. 1. Photomicrographs illustrating immunohistochemical labels using anti-TRPV6 antibody (Figs. A – L). Intense staining was distributed in absorptive epithelial cells of the duodenum, jejunum, ileum, and cecum (A – D, seen red arrow). Weak straining was observed on the rectal epithelium and crypt (E – G, seen red arrow). In addition, staining was also positive in different tubular segments of the kidney, including cortical proximal convoluted tubules (PCT, arrow (right) in I), distal convoluted tubules (DCT, arrow (left) in I), and medullary connecting tubules (CNT, arrow in J and K). No stain was observed in the negative control (H and L).

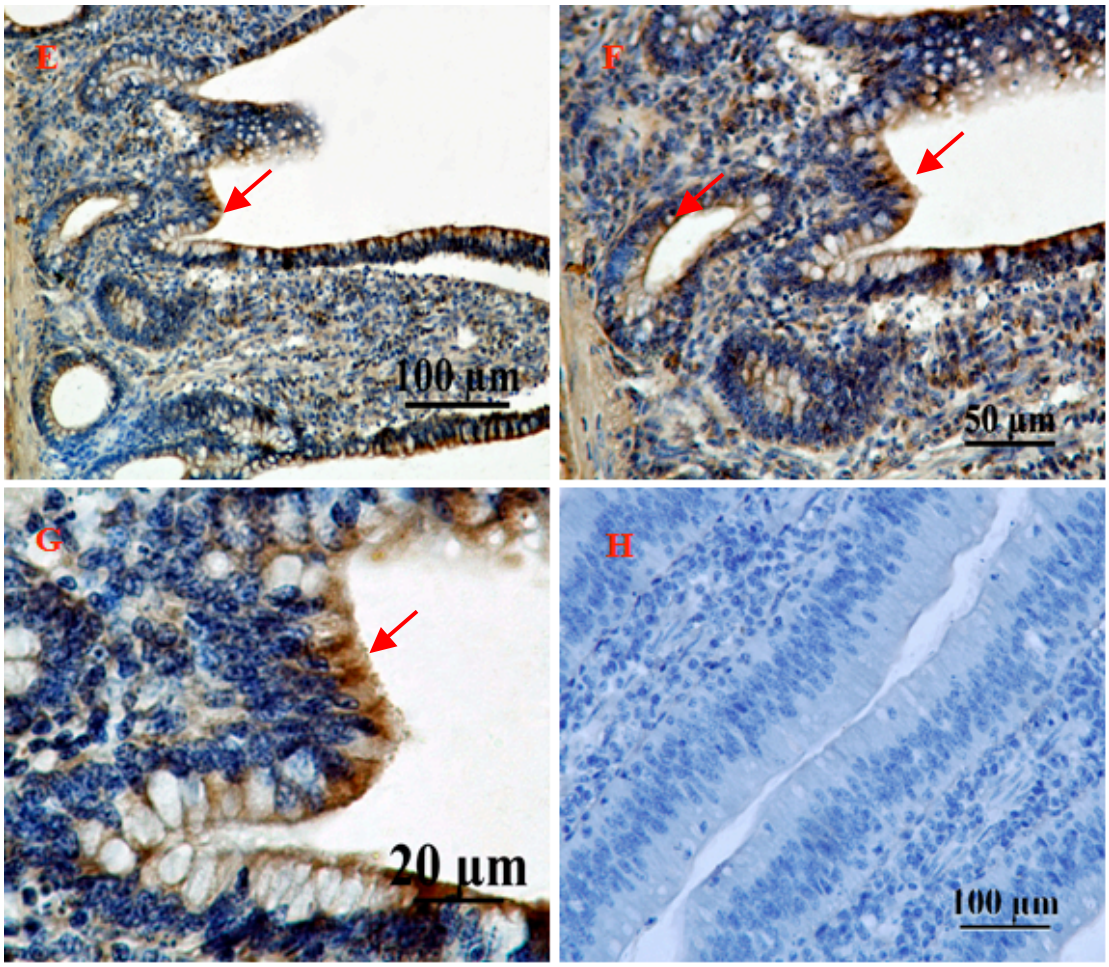
Fig. 2. Reverse transcription-PCR analysis of TRPV6 mRNA expression in intestinal segments and kidney. The amplified fragment has the expected length of 426 bp. NTC, no template control; water was added instead of cDNA

Fig. 3. Expression of TRPV6 mRNA in the duodenum, jejunum, colon, cecum, rectum, and kidney quantified with real-time PCR. β-actin was used as a reference gene. Values are the fold-change relative to that in the duodenum and are expressed as means ± S.E.M., n=6. "*" indicates statistical difference between tissues (P<0.05). "**" indicates significant statistical difference between tissues (P<0.01)

Fig. 4. Quantification of TRPV6 content in different intestinal segments and kidney by Western blotting analysis. A) Immunoreactive bands for TRPV6 of ~80 kDa and GAPDH protein are shown. B) Statistical analysis of TRPV6. Values are the fold-changes relative to that in the duodenum, expressed as means ± S.E.M., n=6. Results are the mean of at least three separate Western blots. "**" indicates significant statistical difference between tissues (P<0.01).

Fig. 1




E
100 μm
F
50 μm
G
20 μm
H
100 μm

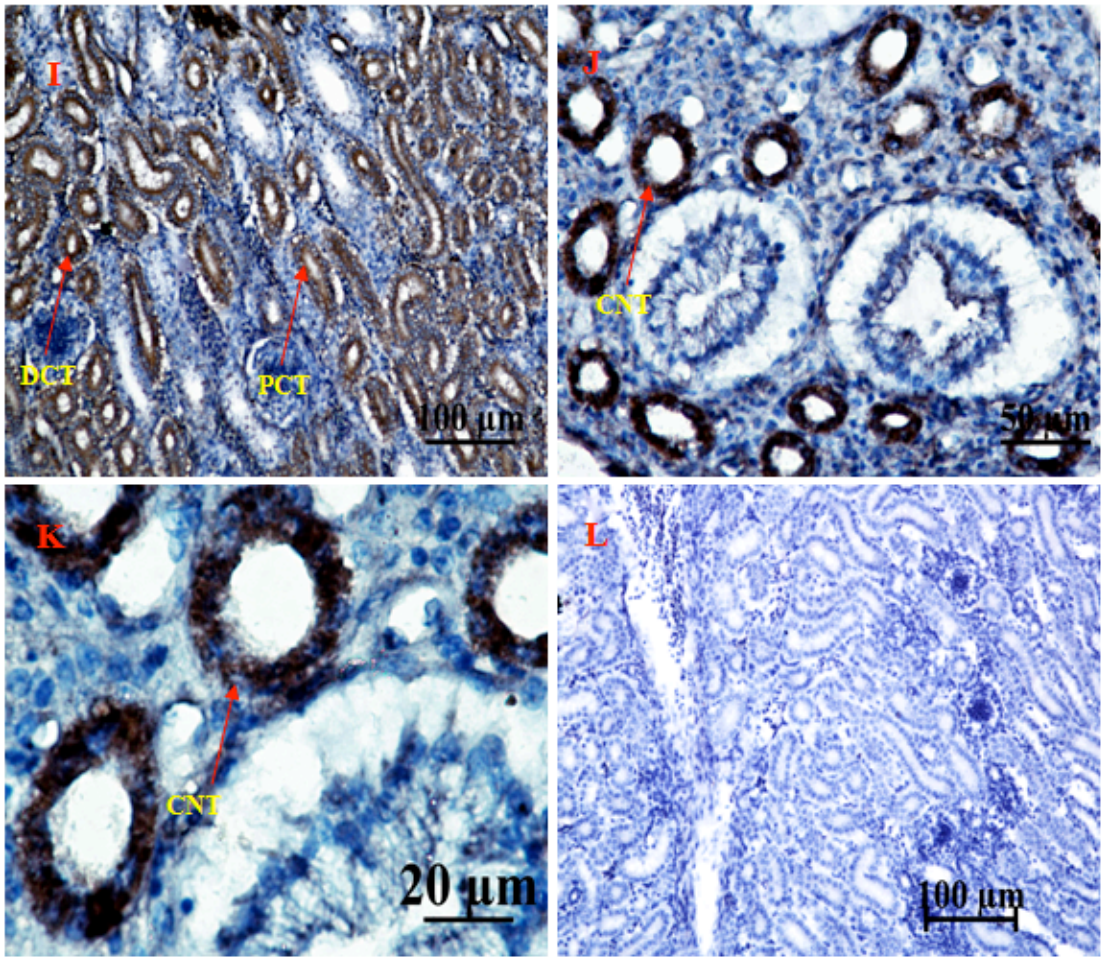


Fig. 1

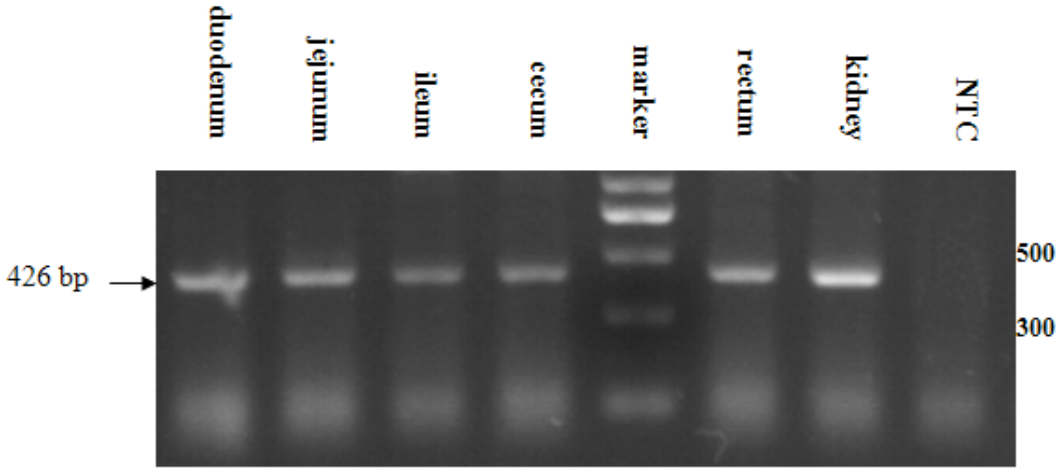

duodenum
jejunum
ileum
cecum
marker
rectum
kidney
NTC
426 bp
500
300

Fig. 2

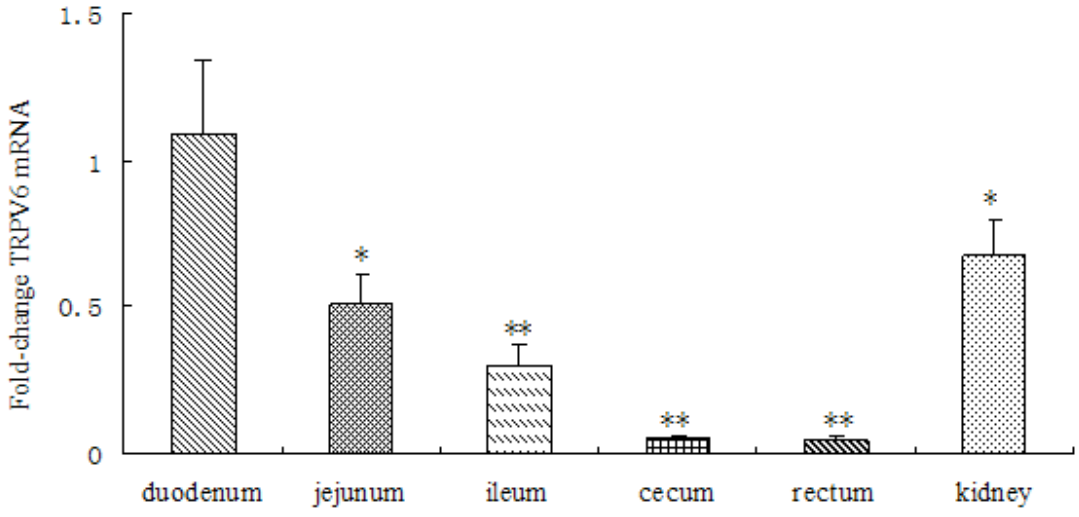

Fold-change TRPV6 mRNA
1.5
1
0.5
0
*
**
**
**
*
duodenum
jejunum
ileum
cecum
rectum
kidney

Fig. 3

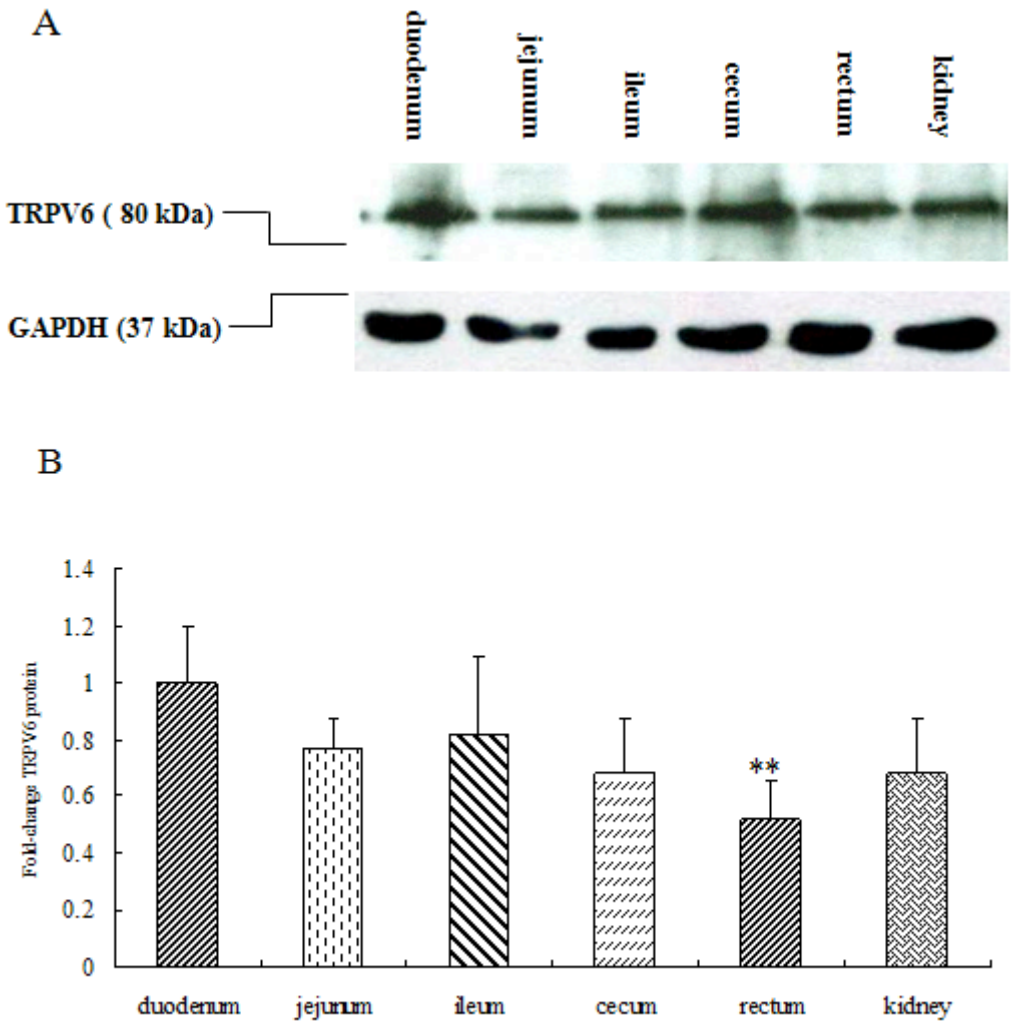

A
duodenum
jejunum
ileum
cecum
rectum
kidney
TRPV6 (80 kDa)
GAPDH (37 kDa)
B
Fold-change TRPV6 protein
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
duodenum
jejunum
ileum
cecum
rectum
kidney

Fig. 4