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Ouabain-sensitive H,K-ATPase: tissue-specific expression of the mammalian genes encoding the catalytic α subunit

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Abstract Human ATP1AL1 and corresponding genes of other mammals encode the catalytic α subunit of a non-gastric ouabain-sensitive H,K-ATPases, the ion pump presumably involved in maintenance of potassium homeostasis. The tissue specificity of the expression of these genes in different species has not been analyzed in detail. Here we report comparative RT-PCR screening of mouse, rat, rabbit, human, and dog tissues. Significant expression levels were observed in the skin, kidney and distal colon of all species (with the exception of the human colon). Analysis of rat urogenital organs also revealed strong expression in coagulating and preputial glands. Relatively lower expression levels were detected in many other tissues including brain, placenta and lung. In rabbit brain the expression was found to be specific to choroid plexus and cortex. Prominent similarity of tissue-specific expression patterns indicates that animal and human non-gastric H,K-ATPases are indeed products of homologous genes. This is also consistent with the high sequence similarity of non-gastric H,K-ATPases (including partial sequences of hitherto unknown cDNAs for mouse and dog proteins).

Key words: Non-gastric H,K-ATPase; Catalytic α subunit; Isoform; Tissue-specific expression

1. Introduction

Different animal K^+-dependent ATPases (X,K-ATPases) are the most closely related ion-transporting P-type ATPases. All known X,K-ATPases function as cation exchangers which pump K^+ into the cell and Na^+ or H^+ out of the cell; they exhibit a much higher level of sequence homology between their catalytic α subunits (~110 kDa) than with other P-ATPases, and contain a glycosylated β subunit (core protein ~35 kDa) [1,2]. The X,K-ATPase family combines three distinct groups of ion pumps which can be distinguished on the basis of their structure-functional properties. The first group consists of universal components of the plasma membrane of animal cells, the Na,K-ATPases (four isoforms for the α and three isoforms for the β subunit). The gastric H,K-ATPase represents a second group. Recently discovered catalytic subunits of non-gastric or ouabain-sensitive H,K-ATPases comprise the third distinct group of X,K-ATPases [2].

The existence of non-gastric H,K-ATPases, which are involved in K^+ absorption and proton secretion in kidney and distal colon, was predicted in many previous physiological, biochemical and pharmacological studies (for review, see [3,4]). However, none of these ATPases has been isolated, and the β subunits specific for non-gastric H,K-ATPase have not yet been identified. To date, only cDNAs encoding α subunits of mammalian non-gastric H,K-ATPases from human kidney and skin [5,6] and from distal colons of rat [7], guinea pig [8], and rabbit (GenBank accession number AF023128) have been cloned. The protein product of the human ATP1AL1 gene (Atp1al1) and its homologs are structurally equally distant (63–64% amino acid identity) from the Na,K-ATPase α subunit isoforms and the gastric H,K-ATPase α subunit. Functional properties of the Atp1al1 and rat colonic protein have been studied through heterologous expression in combination with gastric H,K-ATPase β subunit as a surrogate for the unknown real β subunit [9–11]. The data obtained therein demonstrated that these proteins are constituents of a new, non-gastric type of H,K-ATPase which, in contrast to the gastric H,K-ATPase, are sensitive to cardiotonic steroids such as ouabain. Thus, significant differences in structure and in sensitivity to inhibitors between gastric and non-gastric H,K-ATPases clearly indicated that these enzymes represent separate groups within the family of X,K-ATPases.

Available data on the expression of these genes are rather limited. The Atp1al1 mRNA was detected in human kidney, skin, and brain [5,6,12]. The mRNA of rat H,K-ATPase was detected in distal colon and at a lower level in kidney, uterus, heart and forestomach [7]. In the present study, we aimed to elucidate the pattern of tissue-specific expression of mRNAs for α subunits of non-gastric H,K-ATPases in several mammalian species. The data obtained herein demonstrate similar patterns of expression of human and animal mRNAs. These observations are consistent with the view that animal and human non-gastric H,K-ATPases are products of homologous genes [12].

2. Materials and methods

2.1. Tissues

Red New Zealand 40–50 day old rabbits, 6 month old C57 Black mice and Sprague-Dawley rats were used. Mice were killed by cervical dislocation, rats by decapitation, rabbits by decapitation under anesthesia with ether. Tissues of rats fed a potassium-deficient diet were a

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Abbreviations: CSF, cerebrospinal fluid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription and polymerase chain reaction

The nucleotide sequences reported here have been submitted to the GenBank/EMBL Data Bank with accession numbers AF094823 and AF1001691.

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kind gift of Dr. J. Shapiro. Dog kidneys were purchased from Pel-Freez Inc. (Rogers, AR). Samples of human tissues were obtained 5 h post mortem from a 75 year old male (heart failure) at Sklifosovsky Ambulance Institute (Moscow). Human placentas were obtained from the Medical Center for Gynecology, Obstetrics and Perinatology (Moscow) and St. Vincent Mercy Medical Center (Toledo, OH). Human tissues were also provided by Cooperative Human Tissue Network (Columbus, OH), Harvard Brain Tissue Resource Center (Belmont, MA) and Proctology State Scientific Center (Moscow).

2.2. RT-PCR

Total RNA was isolated from about 30 mg tissue using the SV Total RNA Isolation System (Promega), which includes DNase treatment. To 5 μg of the RNA in water 400 ng dT_{15} (Promega) was added, heated at 65°C 2 min and placed on ice. Reverse transcription was carried out in 1 mM dNTP, 75 mM KCl, 5 mM DTT, 50 mM Tris-HCl pH 8.0 (at 37°C), 6 mM $MgCl_2$, 0.002% BSA, 25 units ribonuclease inhibitor (Fermentas, Lithuania), 200 units M-MLV reverse transcriptase (Promega) in 45 μl total volume at 37°C for 2 h. The reaction mixture was extracted with phenol-chloroform followed by ethanol precipitation. The pellet was dissolved in 50 μl TE.

PCR was performed in 20 μl 67 mM Tris-HCl, 16.6 mM ammonium sulfate, 1.5 mM $MgCl_2$, 0.25 mM dNTP, 0.01% Tween-20, 5 pmol of each primer, 1 μl cDNA and 0.1 unit *Taq* DNA polymerase (Laboratory of Biotechnology, Moscow) on MC-2 (DNK Tekhnologia, Moscow) or GeneAmp-9600 (Perkin Elmer) thermal cyclers with the following settings: 94°C for 1.5 min; 40 cycles at 94°C 45 s, 55°C 45 s, 72°C 60 s; 72°C for 5 min. PCR products were analyzed by gel electrophoresis in 1.5% agarose. Quality control of RT-PCR was made by amplification (30 cycles) of GAPDH cDNA with the unispecies primers FUGAP (TGCACCACCAACTGCTTAGC) and BUGAP (CRTTGTCATACCAGGAGGAAATGAGC).

2.3. DNA sequencing

PCR products were purified from agarose gel with the GeneClean II kit (Bio 101, Vista, CA) and sequenced in both directions with the use of corresponding primers at the University of Michigan Sequencing Core Lab (Ann Arbor, MI).

3. Results and discussion

RT-PCR was used to elucidate the tissue-specific expression patterns of non-gastric H,K-ATPase α subunit in several mammalian species. Primers were designed using pair-wise alignments of the known cDNAs in order to find stretches with high homology between the non-gastric H,K-ATPase genes from different species and low homology with gastric H,K- and Na,K-ATPases. The antisense primer BAU-1 (GGNRCRGCCACAAACCAGTACTG) is complementary to all sequenced mammalian non-gastric ATPase cDNAs (coordinates 3004–3026 for the coding part of the human cDNA). Two sense primers were used: F3-BHRA, specific for rabbit and human – CATYATGAACAGGAAGCCTCG; and F2-RG, specific for rat and guinea pig genes – CAGCTKGCCATCTACTCTTACC. F3-BHRA and F2-RG were found to be complementary also to dog and mouse cDNAs, respectively.

The results of RT-PCR are presented in Fig. 1. The products were of the expected sizes (440 bp in the case of rat templates, and 492 bp in the case of human and rabbit tem-

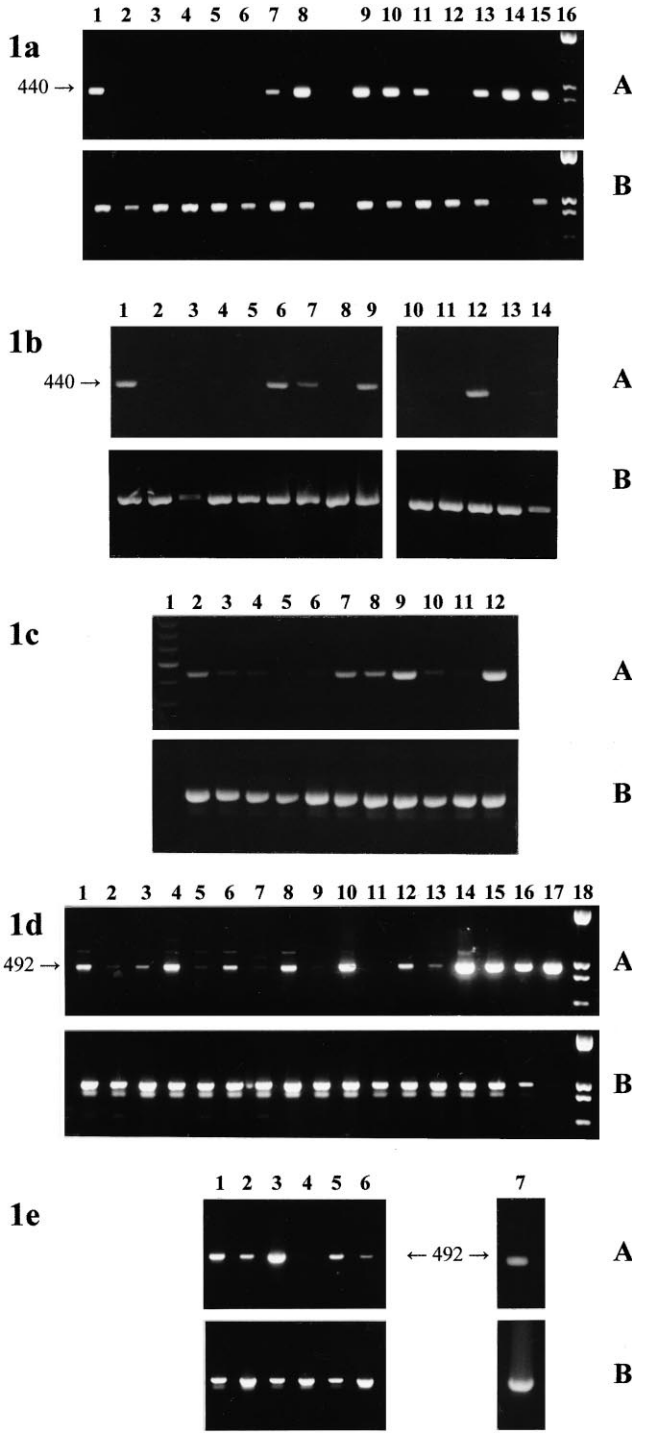

Fig. 1. RT-PCR analysis of the ouabain-sensitive (non-gastric) H,K-ATPase gene expression in tissues of several mammalian species. A: Amplification products of the non-gastric H,K-ATPase α subunit mRNA. B: Control amplification of GAPDH. 1a: Mouse: 1, brain; 2, submandibular gland; 3, thyroid; 4, heart; 5, liver; 6, pancreas; 7, gastric mucosa; 8, rectal epithelium; 9, ovary and oviduct; 10, uterus; 11, vagina; 12, testis; 13, epididymis, ductus deferens and male accessory glands; 14, penis; 15, skin (face); 16, pUC18/*Hin*fI digest. 1b: Rat: 1–9, adult tissues; 10–14, neonatal tissues. 1, skin (face); 2, skeletal muscle; 3, brain; 4, thymus; 5, diaphragm; 6, lung; 7, heart; 8, adrenal; 9, kidney; 10, stomach; 11, pancreas; 12, skin (face); 13, skeletal muscle; 14, brain. 1c: Rat male genital tract: 1, 100 bp DNA ladder; 2, bladder; 3, testis; 4, caput epididymidis; 5, cauda epididymidis; 6, ductus deferens; 7, vesicular gland; 8, prostate; 9, coagulating gland; 10, bulbourethral gland; 11, urethra; 12, preputial gland. 1d: Rabbit: 1, cortex; 2, white matter; 3, olfactory bulbs; 4, choroid plexus of lateral ventricle; 5, thalamus; 6, hypothalamus; 7, brain stem; 8, retina; 9, heart; 10, kidney; 11, liver; 12, pancreas; 13, gastric mucosa; 14, rectal epithelium; 15, penis; 16, skin (back); 17, skin (face); 18, pUC18/*Hin*fI digest. 1e: Human: 1, CACO-2 cells; 2, brain; 3, kidney; 4, liver; 5, placenta; 6, sigmoid colon epithelium. Dog: 7, kidney.

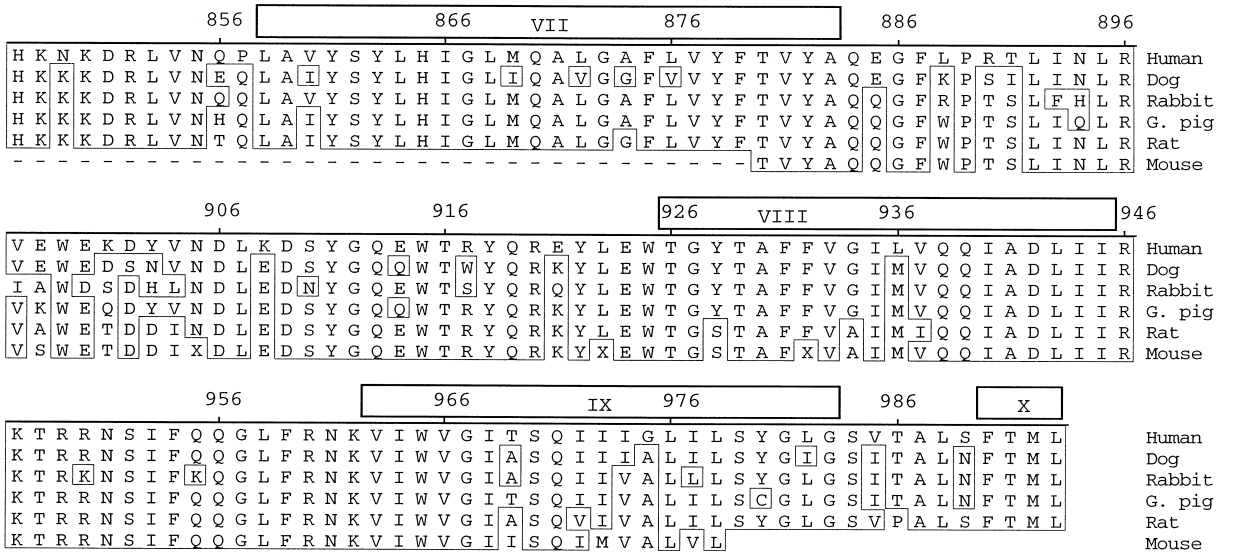

Fig. 2. Sequence comparison of the C-terminal parts of the human [6], dog, rabbit (GenBank accession number AF023128), guinea pig [8], rat [7], and mouse α subunits of non-gastric H,K-ATPases. The amino acids are numbered according to the human Atp1al1 sequence. Boxes indicate identity to corresponding residues of Atp1al1. Transmembrane segments are marked by rectangles.

plates). Precise quantification of the ATPase mRNA was beyond the aims of this work. Still, one can make a qualitative estimation of the expression level from the band intensities: no expression detected, low or high level.

As shown in Fig. 1a, the product of PCR amplification of the mouse gene homologous to the human ATP1AL1 was detected not only in colon but also in skin, female reproductive organs (vagina, uterus, fallopian tubes and/or ovary), and in the male reproductive system. The mRNA was also detected in brain. Negative results were obtained for heart, liver, salivary glands and thyroid.

Among the rat tissues tested we observed strong signals in skin, lung and kidney (Fig. 1b). No expression was observed in skeletal muscle, thymus, diaphragm, adrenal, neonatal stomach and pancreas, and adult brain. A faint band was detected in neonatal brain. No other differences were observed between adults and neonates. The expression in the rat skin is not changed under dietary potassium depletion, neither is it induced in the brain (data not shown). The most interesting finding was the detection of the mRNA in the male urogenital system (Fig. 1c). Weak expression was detected in many sections, but strong signals only in coagulating and preputial glands.

In the rabbit (Fig. 1d) we detected remarkable non-gastric H,K-ATPase gene expression in colon, different areas of the skin, and in penis. Less pronounced signals were observed in kidney, and weak signals were observed in brain, retina and pancreas. Liver, gastric mucosa and heart were negative. For a more detailed analysis, rabbit brain was dissected into several parts (Fig. 1d, lanes 1–7). Expression was found in choroid plexus and cortex while no signal was observed in the whole brain stem, thalamus and white matter of the pallium.

Analysis of human tissues (Fig. 1e) showed that the non-gastric H,K-ATPase mRNA is present at a significant level in kidney. Moderate levels were observed in brain, placenta, low levels in sigmoid colon and colon carcinoma CACO-2 cells while no expression was observed in liver.

Thus, patterns of tissue-specific expression of non-gastric H,K-ATPases exhibit prominent similarity in all species tested. The transcripts were always detected in skin, rectum and kidney. The liver was always negative. Differences in tissue-specific expression among species seem not to be considerable. Only one apparent exception is the rat, being unique and different even from the mouse – no expression in the adult brain but a positive signal in heart. More detailed consideration of our results obtained with particular tissues from different species is outlined below.

3.1. Kidney

The expression of the human and rat genes of interest in kidney is well established [3,5,13–15]. Here, we detected the presence of ATP1AL1 homologs also in mouse, rabbit and dog kidney. Recent data on upregulation of rat mRNA in kidney of potassium-restricted animals support the role of the ouabain-sensitive H,K-ATPase in maintenance of potassium homeostasis [3,13].

3.2. Colon

Rat mRNA of non-gastric H,K-ATPase α subunit is abundantly expressed in distal colon [7]. We also detected strong signals from corresponding mRNAs in mouse and rabbit colons. In contrast, a much lower level of the ATP1AL1 mRNA was detected in human large intestine. The observation may indicate a considerably lower importance of this ion pump in potassium absorption reflecting anatomical and physiological differences of the gastrointestinal tracts. Unfortunately, data on the capacity of potassium absorption in human large intestine are very limited [16]. The low level of expression in available human specimens derived from tissues surrounding colon tumors may be due to the degeneration of mucosal epithelium associated with the underlying disease [12].

3.3. Skin

The presence of mRNA of Atp1al1 in human skin has been reported earlier [6,12]. Here, we detected transcripts of the corresponding genes also in rodent and rabbit skin. Thus,

expression in skin is a common feature of the non-gastric H,K-ATPase genes. Considering the role of the skin in maintenance of ion homeostasis in mammals, we suggest that these ion pumps are components of sweat glands. The most probable location of the non-gastric H,K-ATPase is the ductal gland region where a highly efficient ouabain-sensitive process of electrolyte reabsorption takes place to render final hypotonic sweat [17,18]. Interestingly, an ouabain-sensitive K-dependent *p*-nitrophenylphosphatase activity was detected along the entire peripheral ductal cell membrane and interpreted as unpolarized distribution of Na,K-ATPases [19]. Since ouabain can no longer be considered to be specific for Na,K-ATPase [9,10], the above observations may reflect, in fact, the presence of the ouabain-sensitive H,K-ATPase in apical membrane and the Na,K-ATPase in basolateral membrane. Functionally, the ducts of sweat glands are also capable of acidifying sweat [18]. The Atp1al1 and other non-gastric H,K-ATPases might be responsible for such acidification.

3.4. Urogenital tracts

The rodent ATP1AL1 homolog is expressed in female and male urogenital systems (Fig. 1a,c). Its significance in the coagulating gland, a part of the prostate complex, is difficult to understand because data on its secretion composition are not available [20]. From the detection of the transcripts in the preputial gland, a large sebaceous gland, we can speculate that the non-gastric H,K-ATPase may be present also in sebaceous glands of the skin.

3.5. Placenta

Regarding the ATP1AL1 gene expression in full-term placenta (predominantly in villous tissue), we suggest that ouabain-sensitive H,K-ATPase is involved in materno-fetal ion exchange and that syncytiotrophoblast, a polarized epithelial layer which forms the main barrier between mother and fetus [21], is the most probable place of this ion pump location.

3.6. Brain

Reliable detection of Atp1al1 mRNA expression in human brain has been reported earlier [5]. The amount of Atp1al1 homologous mRNAs in animal brains is obviously very low and it was not detected at all in the rat brain. The non-gastric H,K-ATPase may be physiologically important in brain only if the expression is tightly regulated or limited to a small population of cells. Recently, an H,K-ATPase activity was reported in rat microglia [25]. But the absence of the rabbit ATP1AL1 homolog mRNA in white matter is very unfavorable to the hypothesis that this activity may be attributed to the non-gastric H,K-ATPase. In the rabbit the expression was reliably detected only in cortex (or in adjacent meninges) and choroid plexus. From these data a speculation can be made that the expression is a feature of particular epithelia of the meninges and choroid plexus. If this is true, the physiological significance of the ATPase in the brain may lie in CSF potassium level maintenance. CSF is known to contain less potassium than plasma [22] and this level is strictly controlled under plasma hypo- and hyperkalemia [23,24]. Indeed, in hyperkalemia an increase of α1 Na,K-ATPase in rat choroid plexus has been reported [24], so there is a possibility that Atp1al1 expression might also be upregulated under this condition.

The cDNAs encoding mouse and dog non-gastric ATPases have not yet been characterized; therefore, the corresponding PCR fragments were sequenced. All nucleotide sequences revealed high homology with previously reported non-gastric H,K-ATPase cDNAs: the mouse/rat pair exhibits ∼90% identity over 340 bp, and the human/dog pair ∼87% identity over 410 bp. The alignment of the deduced amino acid sequences with previously established proteins [6–8] is shown in Fig. 2. These sequences represent C-terminal domains of polypeptide chain which include transmembrane segments (TM) VII, VIII, IX and part of TM X. The cytoplasmic sequences preceding TM VII and the loop connecting TM VII and TM IX are the most conservative. The majority of replacements is concentrated in the extracellular loop between TM VII and TM VII. On average, pair-wise comparison of the sequences presented revealed about 85% identity. The known complete human, rat, guinea pig and rabbit sequences also share ∼85% identical amino acids [6–8]. Such a level of similarity is typical for distinct isoforms of the Na,K-ATPase (86–87% identity), whereas the human and rat homologous Na,K-ATPase isoforms exhibit 97–99% sequence identity. Therefore, it was suggested that non-gastric H,K-ATPase α subunits may be products of closely related, but distinct genes [6,7]. However, the similarity between 3′ untranslated regions of the human and rat genes [12] clearly demonstrated that the genes are indeed homologs. The similarity of tissue-specific expression of the genes reported here provides additional support for this conclusion. Therefore, the greater degree of structural diversity may reflect that sequence requirements for non-gastric H,K-ATPase were not as strict during evolution as in the case of the extremely well conserved gastric H,K- and Na,K-ATPases [12].

Recent findings on modulation of the rat α subunit mRNA expression by potassium and acid/base balance and aldosterone status [13–15], which are in good accordance with physiological regulation of K^+ absorption reported in distal colon and kidney [3,4], provide additional support for the role of the ouabain-sensitive H,K-ATPase in the maintenance of potassium homeostasis [26]. The functional status of this ion pump may be important to diverse disease processes such as cardiovascular and renal disorders, in which dysfunctions of ion homeostasis are involved.

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