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Nongastric H-K-ATPase in rodent prostate: lobe-specific expression and apical localization

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Pestov, Nikolay B., Tatyana V. Korneenko, Gail Adams, Manoranjani Tillekeratne, Mikhail I. Shakhparonov, and Nikolai N. Modyanov. Nongastric H-K-ATPase in rodent prostate: lobe-specific expression and apical localization. *Am J Physiol Cell Physiol* 282: C907–C916, 2002. First published December 12, 2001; 10.1152/ajpcell.00258.2001.—The molecular basis of active ion transport in secretory glands such as the prostate is not well characterized. Rat nongastric H-K-ATPase is expressed at high levels in distal colon surface cell apical membranes and thus is referred to as "colonic." Here we show that the ATPase is expressed in rodent prostate complex in a lobe-specific manner. RT-PCR and Western blot analyses indicate that rat nongastric H-K-ATPase α-subunit (α_{ng}) mRNA and protein are present in coagulating gland (anterior prostate) and lateral and dorsal prostate and absent from ventral lobe, whereas Na-K-ATPase α-subunit is present in all lobes. RT-PCR analysis shows that Na-K-ATPase α_4 and α_3 and gastric H-K-ATPase α-subunit are not present in significant amounts in all prostate lobes. Relatively low levels of Na-K-ATPase α_2 were found in lateral, dorsal, and anterior lobes. α_{ng} protein expression is anteriodorsolateral: highest in coagulating gland, somewhat lower in dorsal lobe, and even lower in lateral lobe. Na-K-ATPase protein abundance has the reverse order: expression in ventral lobe is higher than in coagulating gland. α_{ng} protein abundance is higher in coagulating gland than distal colon membranes. Immunohistochemistry shows that in rat and mouse coagulating gland epithelium α_{ng} protein has an apical polarization and Na-K-ATPase α_1 is localized in basolateral membranes. The presence of nongastric H-K-ATPase in rodent prostate apical membranes may indicate its involvement in potassium concentration regulation in secretions of these glands.

ATP1AL1; *ATP12A*; hydrogen-potassium-adenosinetriphosphatase; sodium-potassium-adenosinetriphosphatase; X-potassium-adenosinetriphosphatase; male accessory glands; potassium transport

THE FAMILY OF X-K-ATPASES is responsible for inward potassium transport in exchange for sodium (Na-K-ATPase) or protons (gastric and nongastric H-K-ATPases). Mammalian X-K-ATPases consist of a catalytic α-subunits of ~110 kDa for which six genes have been identified and a glycosylated β-subunit of ~35 kDa for which five genes are known (6, 25, 27, 45). Almost all tissues have Na-K-ATPase α_1- and β_1-subunits (especially abundant in kidney and brain), whereas other X-K-ATPase isoforms are restricted to particular tissues and cell types. The gastric H-K-ATPase α- and β-subunits are specific for parietal cells of the stomach mucosa, X-K-ATPase β_{muscle} (β_m) for striated muscle, and Na-K-ATPase α_4 for male germ cells; Na-K-ATPase α_2, α_3, and β_2 are characteristic for excitable tissues (muscle, brain, retina, etc). Na-K-ATPase β_3 has a peculiar pattern of expression: its mRNA is abundant in testis, brain, and adrenals, whereas significant amounts of the protein have been detected in lung, testis, and liver (6).

The α-subunit of the nongastric ouabain-sensitive H-K-ATPase (α_{ng}), which was cloned from rat colon (17) and thus is also referred to as "colonic" H-K-ATPase (also HKα_{2-6}; Ref. 27), is encoded by the gene *ATP12A* (alternative name *ATP1AL1*). Full-length mRNA sequences for the rat (17), human (25), guinea pig (3), rabbit (8, 21), and toad (29) enzymes and partial sequences for the mouse and dog (46) enzymes are known. Noncanonical transcripts that include parts of the first intron from rat (30) and rabbit (8) genes have also been reported.

Although the role of the nongastric H-K-ATPase in H^+ secretion and K^+ reabsorption under normal conditions has not yet been demonstrated directly, its adaptive regulation to pathophysiological conditions such as potassium depletion, NaCl deficiency, and renal acidosis argues in favor of an important role of this ion pump in disease states associated with dysfunctions of electrolyte homeostasis (for review, see Refs. 53 and 56).

Catalytic properties of the rat, guinea pig, and human nongastric H-K-ATPases have been studied extensively by several groups using different heterologous expression systems [*Xenopus* oocytes (12, 14–16,

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22, 29, 41), kidney cell line HEK (3, 23, 24, 30, 51), and insect cells (1, 35)], different β-subunits [gastric H-K-ATPase β-subunit (1, 23, 24, 41), β_1 (12, 14–16), β_3 (51), all known X-K-ATPase β-subunits (22), and without a β-subunit partner (35)] and different methods of functional evaluation (^{86}Rb uptake, measurements of intra- and extracellular ions, and ATP-hydrolyzing activity). As a result of these different experimental approaches, there is currently no consensus on the catalytic properties of the enzyme. It has been characterized either as ouabain-sensitive H^+-K^+-ATPase (3, 12, 16, 22, 23, 41) or ouabain-insensitive K-ATPase (51). Its function may not be limited to H^+/K^+ exchange but might include transport of Na^+ (15, 24) and NH_4^+ (14). Also, ATPase activity has been detected in nominally K^+-free medium (1). Nevertheless, it should be borne in mind that in the presence of physiological concentrations of K^+, Na^+, and H^+ the enzyme is expected to act primarily as an H-K-ATPase (1).

The initial studies on rat *ATP12A* by Crowson and Shull (17) demonstrated that the gene has a high level of expression in rat distal colon and a lower level in kidney, uterus, and forestomach, whereas the human gene is expressed in skin, kidney, and brain (25, 42). Later studies revealed more information on the cell type-specific expression and regulation of the gene. In mammalian kidney, this ATPase was detected in distal parts of the nephron (20, 21, 32, 36, 57) and is increased by stresses such as ischemia-reperfusion (58) or dietary potassium depletion (11, 18, 21, 50). In rat colon this ATPase is specific for surface cells (28, 47) and undergoes upregulation by dietary or hormonal disturbances of ion homeostasis (13, 50). Under some pathological conditions [chronic diarrhea in Na/H exchanger (NHE)3-deficient mice], its expression in distal colon is also elevated and becomes detectable in proximal colon (52). Ablation of the ATPase by gene targeting results in an increased fecal potassium loss under potassium-deprived conditions (38).

Because the cellular location of the ATPase in distal colon and kidney was found to be the apical membranes (20, 32, 47, 57), it is usually thought to be a specific apical marker in some ion-transporting epithelia. However, when heterologously expressed, the human α_{ng} is localized in renal epithelial cells differentially: predominantly apically in the MDCK cell line and laterally in the LLC-PK_1 cell line (48). Moreover, at least in some tissues like uterus, the ATPase transcripts were detected not in the luminal epithelium but instead in "blood vessel-rich areas" (28).

Because the initial studies on the tissue-specific expression of the human and rat genes (17, 25) suggested that they may encode different isoforms (27, 30, 52), in a previous paper (46) we described a broader screening of the expression in different tissues of several mammalian species (mouse, rat, rabbit, human, and dog) by RT-PCR. It was demonstrated that the tissue specificity of the expression in these species is almost identical. Thus these mammalian genes have not only structural but also functional homology (25, 27, 46, 54). Another interesting observation was that a significant level of expression was detected in some rat male accessory organs, the preputial gland and a part of the prostate complex—the coagulating gland (46).

Studies on X-K-ATPases in prostate are very limited despite the significant biomedical importance of this organ, and most of these studies have been carried out using only the ventral lobe of the rat prostate (2, 19, 59). Also, the coagulating gland is a convenient model for studies of apocrine secretion (4). Thus we decided to investigate the expression of α_{ng} in rodent prostate in more detail compared with Na-K-ATPase.

MATERIALS AND METHODS

RT-PCR and cDNA cloning. Primer sequences for the detection of rat and mouse α_{ng} transcripts were described previously (46). For transcripts of X-K-ATPase α-subunits the following primer pairs were used: FR123 (gaagctcatcatygtggagggctg) and BR123 (kkggctgcctcttcatgatgtc) for α_1, α_2, and α_3; AF1 (yctytctctsatccttgagtacac) and AB1 (gttagcctgaaacactgccctgt) for α_1; AF2 (gayctggaggacagctatggaca) and AB2 (aggrtaccgccgcaggatgagc) for α_2; AF3 (accagctatccatccatgagac) and AB3 (ggagggtcgatcatggacatga) for α_3; A4MRF (acaactttgcctccattgtgac) and A4MRB (ttcatgccctgcttgaagagt) for α_4; and GAMRHF (ycagattggtgccattcagtc) and GAMRHB (gyttccggatctcatcatagac) for gastric H-K-ATPase α-subunit. Conditions of RT-PCR were essentially as described previously (46) with the exception that the annealing temperature for α_1 primers was set at 50°C. Agarose gels were imaged with the help of a Typhoon 8600 laser scanner (Amersham Pharmacia, Piscataway, NJ).

To produce NH_2-terminal fragment of rat α_{ng} (amino acid coordinates 13–102) the corresponding DNA fragment was amplified from rat colon cDNA (46) by using the primers FRAT (cgtgggatccaacggaactaaagacgtcaag) and BRAT (ccttaagcttaatctctggagtttgcttgg) with the use of the high-fidelity *Pfu* DNA polymerase (Stratagene, La Jolla, CA). The PCR product was digested with *Bam*HI and *Hin*dIII and cloned at the same sites of pQE30 expression vector (Qiagen, Valencia, CA).

Antibodies. Recombinant protein expression in *Escherichia coli,* purification by immobilized metal affinity chromatography, and immunization of rabbits were achieved essentially as described previously (31). Affinity purification of antibodies was performed with the antigens absorbed on polyvinylidene difluoride (PVDF) membrane according to the method of Rucklidge et al. (49).

Mouse monoclonal antibody α6F against Na-K-ATPase α_1-subunit (55) was obtained from Developmental Studies Hybridoma Bank (Iowa City, IA). Affinity-purified rabbit polyclonal antibodies against COOH-terminal peptide KETYY (which is specific for all Na-K-ATPase isoforms; Ref. 5) were kindly provided by Dr. J. Kyte (University of California, San Diego, CA).

Preparation of membranes. Sprague-Dawley rats weighing 200–300 g were killed by decapitation. Tissue samples were frozen in liquid nitrogen, powdered with a hammer, and homogenized with a Polytron homogenizer at 12,000 rpm for 1 min in *buffer A* [10 mM HEPES-Na, 1 M KCl, 5 mM Na-EDTA, 0.25 M sucrose, protease inhibitor cocktail (P2714, Sigma), 100 μM phenylmethylsulfonyl fluoride (PMSF), 0.1% methanol, pH 7.0]. The homogenate was centrifuged at 9,000 *g* for 20 min, and the microsomes were pelleted at 150,000 *g* for 30 min. The membranes were washed with *buffer A* without KCl, resuspended in a small

volume of the buffer, freed from remaining debris by centrifugation for 5 min at 4,000 *g*, and stored at −70°C.

Western blotting. Membranes were dissolved in SDS sample loading buffer (34), and the protein concentration was measured by a modification of the Bradford procedure that includes coprecipitation of proteins with calcium phosphate (43). Proteins (10 μg/well) were electrophoresed in 8% polyacrylamide gels and blotted onto PVDF membrane (Amersham Pharmacia). The membrane was washed in methanol and stained in 50% methanol-1% acetic acid-0.03% Coomassie brilliant blue G-250 followed by washes with 50% methanol. The membrane was then cut, destained in methanol, washed in 50% methanol followed by incubation in 50 mM Tris-(pH 6.8)-100 mM mercaptoethanol-2% SDS for 15 min at room temperature, washed in Tris-buffered saline (TBS), and blocked in TBS containing 5% nonfat milk. The membrane was consequently incubated with the affinity-purified rabbit antibodies and then either with peroxidase-conjugated anti-rabbit antibodies (Amersham Pharmacia) or, for maximal sensitivity, subsequently with biotin-conjugated anti-rabbit antibodies (Amersham Pharmacia) and streptavidin-peroxidase (S9420, Sigma) for 1 h each with thorough washes in TBS containing 0.1% Tween 20 between incubations. The immunoblots were visualized with a chemiluminescent substrate (ECL+Plus, Amersham Pharmacia). For negative controls, the same reagents were used except that affinity-purified rabbit antibodies against a nonrelated His-tagged protein (45) were substituted for anti-α_{ng} antibodies. Molecular mass standards were the 10-kDa protein ladder (Life Technologies, Rockville, MD). Densitometry was performed with a Bio-Rad Model GS-690 imaging densitometer (Bio-Rad Laboratories, Hercules, CA).

Immunohistochemistry. Tissues were frozen in isopentane-liquid nitrogen and cut at 15-μm thickness. Sections were fixed with 5% paraformaldehyde in PBS, dehydrated in graded ethanols, and air dried. The sections were treated for 1 h with 1% hydrogen peroxide in PBS followed by PBS containing 5% normal porcine serum, 0.2% Triton X-100, 0.02% saponin, 0.05% sodium azide, and 50 mM glycine pH 7.5 at room temperature for 1 h and then incubated with the primary antibodies in PBS containing 1% pig serum overnight. The sections were washed in PBS and developed using Vectastain ABC anti-rabbit kit (Vector Laboratories, Burlingame, CA) and NovaRED peroxidase substrate (Vector Laboratories).

For labeling with anti-Na-K-ATPase antibodies (both rabbit anti-KETYY and mouse monoclonal anti-α1 antibody α6F), fixation with formaldehyde resulted in loss of staining. For this reason a milder fixation procedure was used: sections were incubated in methanol-acetone (5:3 vol/vol) at −15°C for 30 min, air dried, and stored. The sections were treated with chloroform (5 min at room temperature), air dried, incubated with 5% pig serum in PBS, and then immunolabeled with MOM-Fluorescein Mouse-On-Mouse kit (Vector Laboratories), which includes biotin-conjugated anti-mouse antibodies and fluorescein-conjugated avidin. For double labeling the sections were incubated also with the primary rabbit antibodies and Alexa Fluor-594-conjugated anti-rabbit antibodies (Molecular Probes, Eugene, OR). Sections were mounted in VectaShield (Vector Laboratories). When labeling of nuclei was desirable, SYBR Gold (Molecular Probes) was added to the mounting medium at 100,000× dilution. This was superior to the use of ethidium bromide or propidium iodide because these dyes also labeled cytoplasm of the prostate epithelium. Images were collected with a Nikon Optiphot-2 fluorescent microscope equipped with a SPOT digital camera (Diagnostic Instruments, Sterling Heights, MI).

Two kinds of negative controls were used: first, the specific antibodies were applied together with an excess of the recombinant protein that was used as the antigen, and second, affinity-purified rabbit antibodies against a nonrelated His-tagged protein (human β_m; Ref. 45) were substituted for antibodies against α_{ng}. For a negative control of Na-K-ATPase staining, sections were incubated with mouse monoclonal antibodies against nonrelated proteins (sarcoplasmic reticulum Ca-ATPase and transhydrogenase).

RESULTS

Analysis of mouse ATP12A gene. We (46) previously determined the partial sequence of the cDNA encoding mouse α_{ng} and showed it to have a high level of sequence identity with the homologous rat protein (17). Using these and other sequence data as well as the known exon-intron structure of the human *ATP12A* gene (54), we have identified the corresponding mouse gene in genomic clone RP23–178B24 (Birren B, Linton L, Nusbaum C, and Lander E; GenBank accession no. AC021630). The deduced sequence of the mouse cDNA coding region exhibits 94.4% identity with that of the rat cDNA. The encoded protein consists of 1,035 amino acid residues and has a molecular mass of 114.7 kDa (Fig. 1). Mouse and rat protein sequences exhibit 95.9% identity (only 9 of 39 replacements are not isofunctional). The level of sequence homology between the mouse and related proteins from guinea pig, rabbit, and human is slightly lower, being 88%, 86.9%, and 86.3%, respectively.

It should be noted that structural diversity between rodent and human α_{ng} is higher than for other X-K-ATPase α-subunits (25, 27, 54). The human and rat homologous Na-K-ATPase isoforms and gastric H-K-ATPases exhibit 96.7–99.1% sequence identity. Most of the structural dissimilarities between human and rodent proteins are concentrated in the NH_2-terminal regions encoded by the first two exons in both human and mouse genes. In this region, human α_{ng} exhibits only 49.2% and 53.4% identity with the mouse and rat sequences, respectively. The greater degree of structural diversity may indicate that sequence requirements for the human α_{ng} and its rodent counterparts were not as rigid during evolution as in the extremely well-conserved H-K- and Na-K-ATPases. It also may indicate that the functional role of the NH_2-terminal domain is not as significant in these new enzymes as in Na-K-ATPases.

Production of fragments of nongastric H-K-ATPase and generation of specific antibodies. We previously described (31) the preparation of poly- and monoclonal antibodies specific to the NH_2-terminal fragment of human α_{ng}. This fragment was chosen because its sequence is the least homologous to other isoforms of X-K-ATPase including the ubiquitous α_1. Indeed, these antibodies showed no reactivity with Na-K-ATPase purified from kidney (results not shown). However, the advantage of the sequence dissimilarity of the NH_2 terminus became a drawback when we attempted to

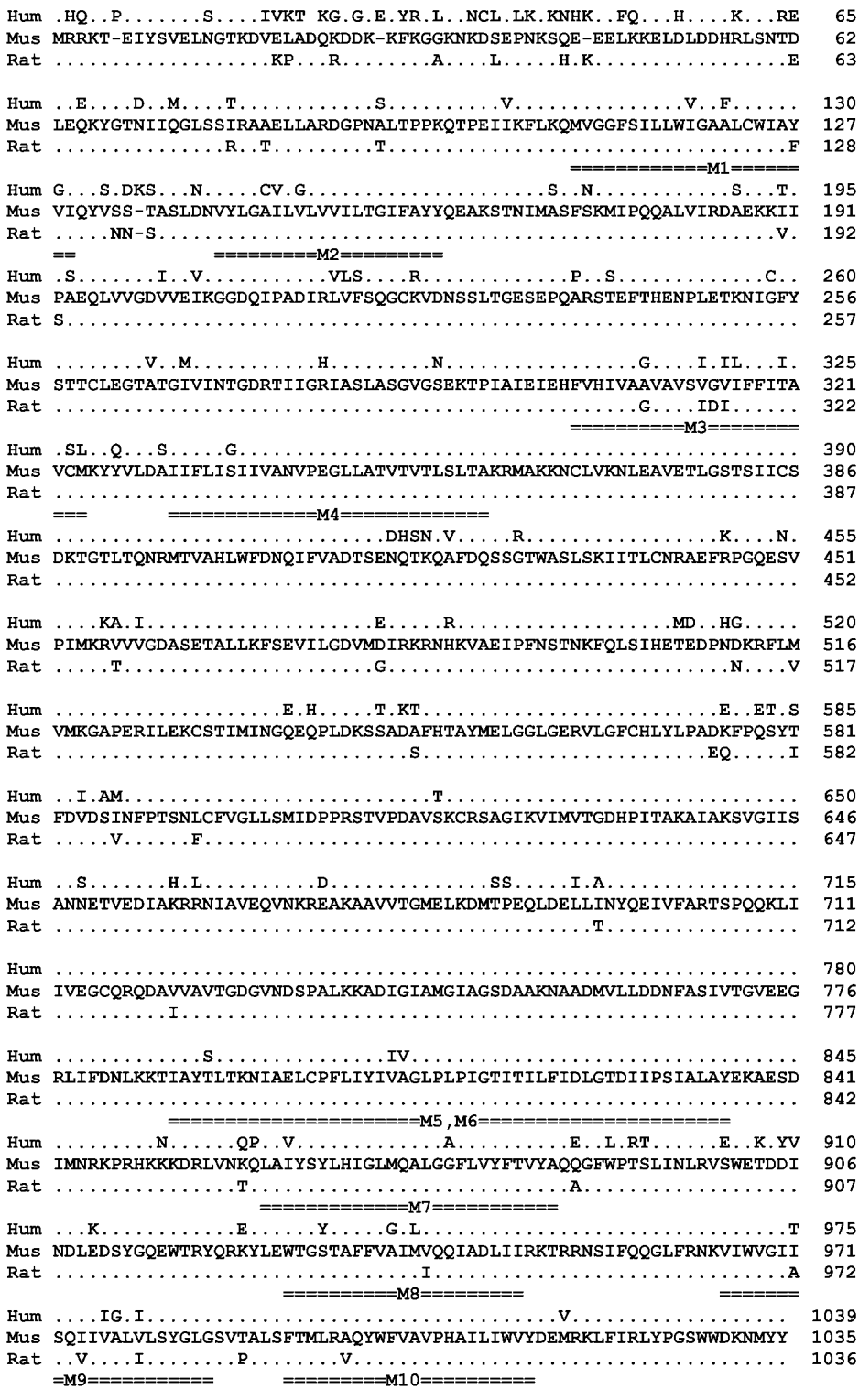

Fig. 1. Comparison of the amino acid sequences of mouse (Mus), human (Hum) and rat (Rat) nongastric H-K-ATPase catalytic α-subunits. Gaps (represented by dashes) were introduced to maintain the alignment. Dots indicate identity to the corresponding residues of the mouse protein. Potential transmembrane domains are underlined and labeled. The amino acids are numbered on *right*.

detect animal nongastric H-K-ATPases. No reliable immunodetection was achieved in Western blotting of rat or rabbit distal colon microsomes with these antibodies (data not shown). To circumvent this problem, we raised antibodies against the rat NH_2 terminus. The cDNA fragment coding for the rat NH_2-terminal fragment (coordinates 13–102) was cloned into an expression vector with the NH_2-terminal hexahistidine tag using RT-PCR from rat distal colon cDNA.

The corresponding recombinant protein was synthesized in *E. coli* and obtained in a highly purified form with the help of metal chelate affinity chromatography under denaturing conditions. The protein remained soluble after dialysis from a 6 M urea solution against

PBS. Subsequently, immune rabbit sera were obtained on immunization with the protein. The polyclonal antibody preparation showed no cross-reactivity with Na-K-ATPase from pig kidney by ELISA (results not shown). None of the four monoclonal antibodies directed against the human NH_2 terminus (31) reacted with the corresponding rat fragment, and the polyclonal sera showed little cross-reactivity (results not shown). Sequence homology in the antigen region between rat and mouse is 87%, whereas homology between the rat and human sequences is 67%. Although high specificity of these antibodies was confirmed by immunoenzyme assays (data not shown), the preparations used in subsequent immunochemical experiments were obtained by affinity purification on immobilized recombinant proteins to increase reliability of the detection.

Immunodetection of nongastric H-K-ATPase protein in membranes from various rat tissues. Using these specific antibodies we analyzed several rat tissues, chosen according to the previously described RT-PCR screening (46), for the presence of α_{ng}. Immunoblotting analyses of crude microsome proteins from these rat tissues are represented in Fig. 2. A strong band corresponding to an apparent molecular mass of ~108 kDa was observed in the distal colon (Fig. 2), in accordance with the results of other laboratories (20, 35, 47).

Examination of the coagulating and preputial glands revealed proteins with the same electrophoretic mobility as in distal colon (Fig. 2). The band intensities, however, were significantly different. Coagulating gland membranes produced a much stronger signal, in fact, significantly stronger than in the distal colon. Densitometry of Western blots indicated that the relative abundance of α_{ng} in crude membranes from the coagulating gland is ~2.5-fold higher than in crude membranes from the distal colon. The content of the

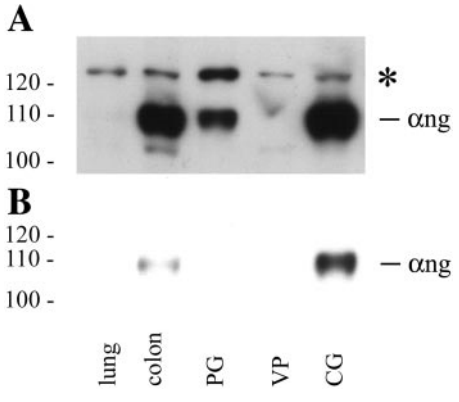

Fig. 2. Immunoblotting of membrane proteins from several rat tissues with antibodies against NH_2-terminal portion of the nongastric H-K-ATPase α-subunit (α_{ng}). *A*: a high-sensitivity blot that was obtained with biotinylated secondary antibodies and peroxidase-labeled streptavidin with a long exposure. *B*: a lower-sensitivity blot obtained with peroxidase-labeled secondary antibodies and a short exposure. Positions of molecular mass markers are shown on *left*. * Nonspecific band that can be produced by peroxidase-labeled streptavidin alone. PG, preputial gland; VP, ventral prostate; CG, coagulating gland.

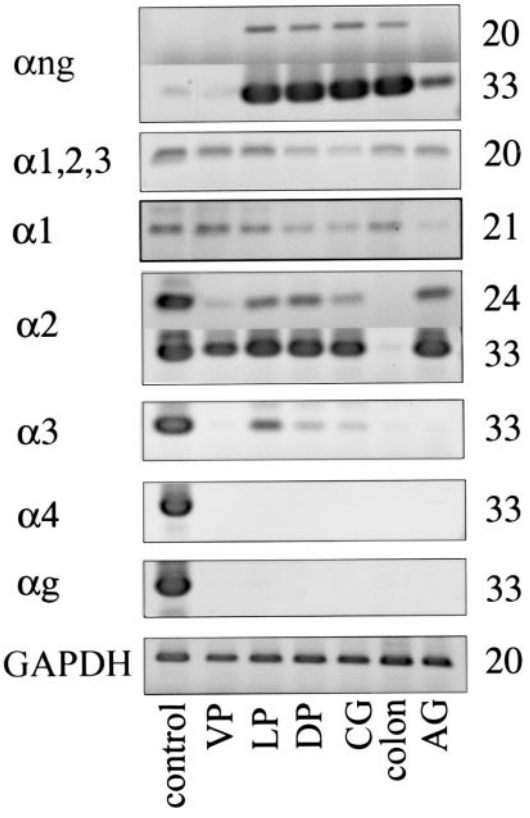

Fig. 3. RT-PCR analysis of lobe-specific expression of X-K-ATPase α-subunit isoforms in rat prostate. α_{ng}, Amplification products of α_{ng} mRNA; α_1, α_2, α_3, α_4, amplification products of mRNAs of Na-K-ATPase α_1, α_2, α_3, and α_4 isoforms, respectively; $\alpha_{1,2,3}$, amplification with primers that are universal to Na-K-ATPase α_1, α_2, and α_3 isoforms; α_g, amplification products of gastric H-K-ATPase α-subunit mRNA. Nos. of cycles used are indicated on *right*. Control, rat brain cDNA for α_1, α_2, α_3, and α_{ng}, rat testis cDNA for α_4, and rat stomach cDNA for α_g; DP, dorsal prostate; LP, lateral prostate; AG, ampullary gland; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

ATPase in the preputial gland (Fig. 2) is much lower than that in the colon or in the coagulating gland.

Analysis of lobe-specific expression of nongastric H-K-ATPase and Na-K-ATPase α-subunit in rat prostate complex. The dramatic difference in the expression of α_{ng} in coagulating gland and ventral prostate (Fig. 2) persuaded us to investigate the lobe specificity of α_{ng} expression in more detail as well as to obtain some information on the lobe specificity of expression of other known X-K-ATPase isoforms. Additionally, ampullary gland was also included in some experiments because this organ had not been studied before.

The lobe-specific expression of the nongastric H-K-ATPase gene has been analyzed at both the mRNA and protein levels. For mRNA analysis, RT-PCR reaction with primers complementary to the 3′ end of the coding part (46) has been used and conditions adjusted to allow an estimation of the expression level with a minimum number of cycles. As shown in Fig. 3, the gene is expressed in a lobe-specific manner. A strong RT-PCR signal could be detected in coagulating gland and lateral and dorsal lobes but no signal in the ventral

prostate. This is in sharp contrast with the observations on Na-K-ATPase α_1-subunit: the signal intensities are comparable, being stronger in ventral prostate and weaker in coagulating gland. Isoform-specific analysis of Na-K-ATPase α-subunit expression indicates that α_2 transcripts are present in rat prostate (Fig. 3), although at a low level (compare signal intensities with that of the rat brain as well as the number of cycles used for this isoform). The α_2 isoform has a more pronounced expression in the lateral and dorsal lobes, and in the coagulating gland, than in the ventral lobe. The α_3 isoform is expressed in prostate lobes at trace levels (Fig. 3). No signal was observed with primers specific for gastric H-K-ATPase α-subunit and Na-K-ATPase α_4-subunit in any of the tissues tested. Thus the Na-K-ATPase α_1 subunit is present in all rat prostate lobes and also in the ampullary gland. Nongastric H-K-ATPase expression is highly lobe specific, and there is a trace level in the ampullary gland.

Figure 4 illustrates that nongastric H-K-ATPase is also expressed in mouse coagulating gland, at a higher level than in distal colon. The Na-K-ATPase, however, is just the reverse: the α-subunit transcripts are lower in the mouse coagulating gland than in the colon.

Immunoblotting of the membrane proteins from the rat prostate lobes (Fig. 5) demonstrates that the abundance of α_{ng} is highest in the coagulating gland, lower in the dorsal prostate, even lower in the lateral lobe, and absent from the ventral part. In contrast, the Na-K-ATPase was detected in all parts but at a higher level in the ventral prostate and at similar levels in other lobes. In general, the contents of mRNA correlate with those of protein, with the exception of the lateral lobe, which has somewhat higher levels of α_{ng} mRNA but a relatively low level of the protein. Putatively, the contents of α_{ng} in dorsal and especially in lateral lobes are lower than in the coagulating gland because of a higher rate of protein processing/degradation. Indeed, in Fig. 5 one can see a weak band at ~66 kDa in the lateral lobe. Very likely, this band is a proteolytic degradation product. Occasionally, this band was also seen in the coagulating gland (results not shown).

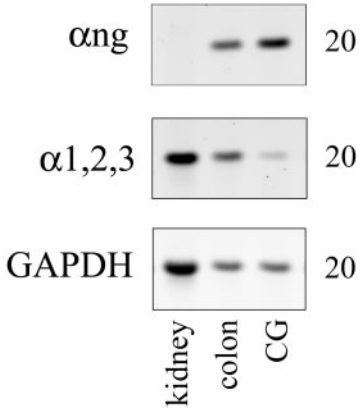

Fig. 4. RT-PCR detection of transcripts of α_{ng} and Na-K-ATPase α-subunits in mouse coagulating gland. Nos. of cycles used are indicated on *right*.

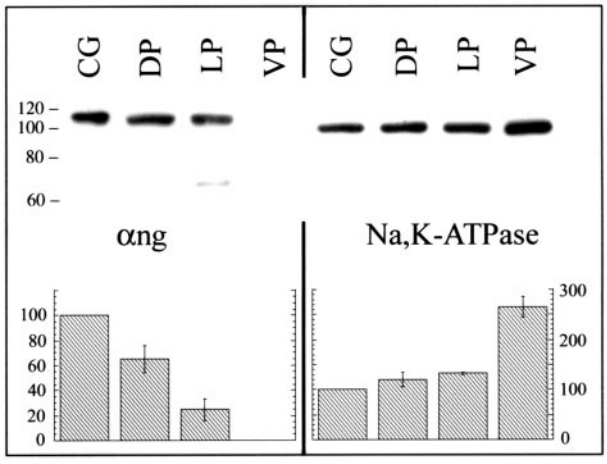

Fig. 5. Immunoblotting of membrane proteins from different lobes of rat prostate with antibodies against α_{ng} and Na-K-ATPase α-subunits. Affinity-purified antibodies against NH_2-terminal portion of α_{ng} and COOH-terminal peptide KETYY of the Na-K-ATPase α-subunit were used. Samples were run in parallel. Positions of molecular mass markers are shown on *left*. The densitometry data are expressed as mean ± SE % of those found in coagulating gland; $n = 3$.

Immunohistochemical localization of nongastric H-K-ATPase protein in rodent coagulating gland. Immunohistochemical localization of α_{ng} protein is demonstrated in Fig. 6 with both immunoperoxidase (Fig. 6*A*) and immunofluorescence (Fig. 6*B*) techniques. Affinity-purified antibodies against the NH_2-terminal fragment of rat α_{ng} produce a strong labeling, which is confined to the luminal surface of epithelial cells. Antibodies directed against a nonrelated hexahistidine-tagged recombinant protein (β_m; Ref. 45) did not produce any significant labeling, and neutralization of the primary antibodies with the original antigen eliminates the specific labeling (results not shown). The fact that the labeling was strictly confined to the lumen-exposed surface suggests that the nongastric H-K-ATPase is localized to the apical membranes of the epithelial cells and is absent from their basolateral membranes.

Labeling with a monoclonal anti-α_1 antibody (Fig. 6*C*) shows that the Na-K-ATPase is present in lateral and basal membranes; no labeling was observed on the luminal surface of the coagulating gland epithelium. A weak labeling of stromal elements can also be observed but is hard to distinguish from nonspecific labeling produced by anti-mouse secondary antibodies (results not shown).

Double labeling of rat (Fig. 6, *C* and *D*) and mouse (Fig. 6*E*) coagulating glands with both the anti-α_{ng} and anti-α_1 antibodies illustrates that the ATPases are differentially polarized in epithelial cells of the rodent coagulating gland: nongastric H-K-ATPase and Na-K-ATPase are specific components of, respectively, apical and basolateral membranes.

Additionally, immunofluorescent labeling with anti-α_{ng} and anti-Na-K-ATPase of different rat prostate lobes demonstrates their lobe-specific expression (Fig. 7). As in the coagulating gland, anti-α_{ng} labeling of dorsal and lateral lobes corresponds to apical mem-

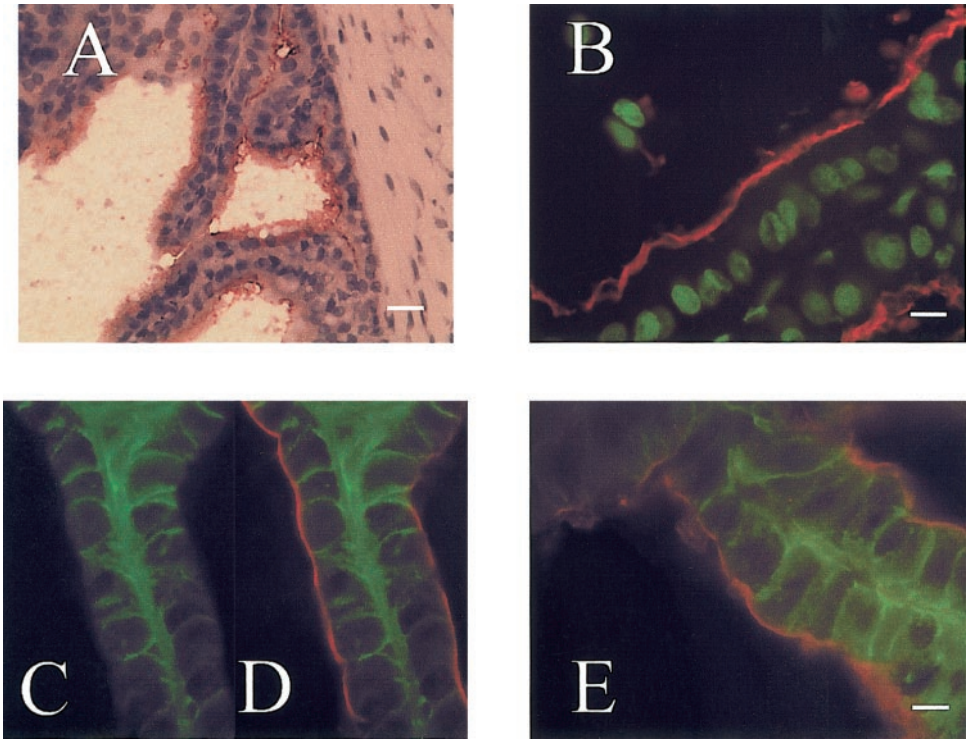

Fig. 6. Immunohistochemical detection of α-subunits of nongastric H-K-ATPase and Na-K-ATPase in rodent coagulating gland. *A–D*: rat coagulating gland; *E*: mouse coagulating gland. *A*: immunoperoxidase labeling of a formaldehyde-fixed section with antibodies against α_{ng} visualized with NovaRED peroxidase substrate counterstained with hematoxylin; bar, 50 μm. *B–E*: immunofluorescence labeling of sections fixed with methanol-acetone; bar, 10 μm. *B*: red fluorescence represents anti-α_{ng} antibodies; nuclei stained in green with SYBR Gold. *C–E*: double labeling with antibodies against α_{ng} (Alexa Fluor-594, red fluorescence) and α_1 (fluorescein, green fluorescence). *C*: α_1 labeling. *D*: merged images of α_1 and α_{ng} labeling.

branes and the labeling intensity is decreasing in the following order: coagulating gland > dorsal prostate > lateral prostate (Fig. 7*A*). No specific labeling can be observed in the ventral lobe (Fig. 7*A)*. In contrast, anti-α_1 labeling is significantly stronger in the ventral prostate than in other lobes (Fig. 7*B*). The relative intensities of both anti-α_{ng} and anti-α_1 immunofluorescent labeling of rat prostate lobes are in accordance with the Western blotting results (Fig. 5).

DISCUSSION

Expression of novel genes in tissues like small specialized secretory glands are not usually well studied.

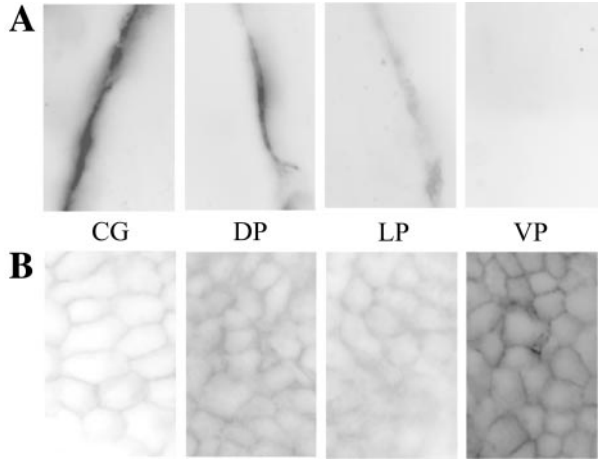

Fig. 7. Immunohistochemical detection of α-subunits of nongastric H-K-ATPase and Na-K-ATPase in different lobes of the rat prostate. *A*: immunofluorescence labeling with anti-α_{ng} antibodies. *B*: labeling with anti-α_1 monoclonal antibody. Labeling conditions and exposures were identical between the lobes.

However, the physiological significance of paralogous genes and protein isoforms cannot be fully understood without exhaustive knowledge of their tissue-specific expression. The present work illustrates this by the finding that rat α_{ng} is expressed in several lobes of the prostate at a higher level than in distal colon, the tissue that provided one of the names of H-K-ATPase.

An interesting fact is that the ATPase is expressed in the rat prostate in a lobe-specific manner, being virtually absent in the ventral part and present in the lateral and dorsal lobes and the coagulating gland (anterior prostate), an expression pattern that can be called anteriodorsolateral. This fact by itself is not unusual—other proteins are known to be expressed lobe-specifically, for example, β-microseminoprotein is specific for lateral prostate (33). However, to our knowledge this is the first report of the lobe-specific expression of a P-type ATPase.

Immunohistochemical localization of the ATPase in mouse and rat coagulating glands demonstrates that the ATPase is strictly confined to the lumen-exposed surface of the epithelium, and this means that the ATPase has apical polarization in rodent prostate epithelial cells. In contrast, α_1-subunit of Na-K-ATPase was detected only in basolateral membranes of the coagulating gland. This is in line with observations of localization of the ATPases in rat distal colon (35, 47) and strengthens the view that protein sorting machinery destines Na-K-ATPase to basolateral and H-K-ATPase (both gastric and nongastric) to apical membrane compartments. Basolateral polarization of Na-K-ATPase in rat prostate has already been observed (39, 44), unfortunately without indication of the lobe used. However, Mobasheri et al. (40) recently reported

that in human prostate epithelium, in contrast to the rat, Na-K-ATPase is present in both apical and lateral membranes. Although apical Na-K-ATPase was also found in some other epithelia, for example, in choroid plexus (37), the observation of species-specific differences in prostatic Na-K-ATPase polarization is intriguing.

The function of H-K-ATPase in rodent prostate is not easy to understand because the available data on ion composition on its secretion are rather limited (7). To the best of our knowledge, there are no data on the ion content in the secretions of the rat or mouse coagulating gland. However, Chow et al. (10) have extensively studied the secretions in these glands in the golden hamster. These studies showed that the ionic composition of the secretions from different parts of the prostate varies significantly. For example, the potassium concentration was 2.59 meq/l for the coagulating gland, 21.44 meq/l for the dorsolateral prostate, and 103.2 meq/l for the ventral prostate. Assuming that these values are also true in other rodent species and that the golden hamster has the same lobe-specific expression of nongastric H-K-ATPase, it would be reasonable to predict that the potassium concentration in the secretions is inversely related to the content of nongastric H-K-ATPase. This suggests that the function of prostatic H-K-ATPase is to take up potassium from the luminal fluids, thus maintaining the low potassium concentration. Because this enzyme performs H^+/K^+ exchange, its function could also result not only in the decrease of potassium in the extracellular fluid but also in its acidification. This luminal acidification may also be accomplished by V-type H^+-ATPase. Interestingly, the pattern of this enzyme's cellular expression was shown to be different in different lobes of rat prostate. It was located mostly intracellularly in coagulating gland and ventral prostate but in both apical and basolateral membranes of the lateral prostate (26). Clearly, every lobe of the prostate has a very specific set of ion transporters.

Nongastric H-K-ATPase is thought to be involved in maintenance of electrolyte homeostasis through K^+ absorption and proton secretion in kidney and colon, especially in disease processes including ionic and acid-base disorders. The physiological importance of the differences in potassium concentration in secretions from different prostate lobes (10) is currently unknown, and it is especially difficult to examine because during copulation these secretions are mixed together (also with semen and fluids from seminal vesicles and uterus). This mixing leads to the formation of the so-called copulatory plug for which secretions of the coagulating gland are especially important (9), being the major source of the protein-cross-linking enzyme transglutaminase. At present, it is hardly possible to suggest a satisfactory explanation for the differences in the potassium concentration in secretions of different lobes (10). However, we can suggest that either the activity of some enzymes is regulated by potassium (for example, they might be inactive in the low-potassium fluid of the coagulating gland and be activated after the mixing of the secretions during coitus) or the prostate epithelium may use the K^+ gradient for secondary transport processes. Whatever the exact relevance of the H^+/K^+ exchange in some parts of the rodent prostate may be, it is reasonable to speculate that the function of nongastric H-K-ATPase in these male accessory glands is important for normal formation of the copulatory plug.

In conclusion, we have found that α_{ng} is highly expressed in rodent prostate epithelium and is polarized to the apical membranes. Because the relative content of nongastric H-K-ATPase is higher in the coagulating gland than in distal colon (traditional tissues for the studies on the enzyme) whereas the content of Na-K-ATPase is lower, the coagulating gland may be a convenient source for isolation and further characterization of the structure and the function of nongastric H-K-ATPase.

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