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Immunohistochemical Study of a Rat Membrane Protein which Induces a Selective Potassium Permeation: Its Localization in the Apical Membrane Portion of Epithelial Cells

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Summary. We previously reported a novel rat membrane protein that exhibits a voltage-dependent potassium channel activity on the basis of molecular cloning combined with an electrophysiological assay. This protein, termed I_{sK} protein, is small and different from the conventional potassium channel proteins but induces selective permeation of potassium ions on its expression in *Xenopus* oocytes. In this investigation, we examined cellular localization of rat I_{sK} protein by preparing three different types of antibody that specifically reacts with a distinct part of rat I_{sK} protein. Immunohistochemical analysis using these antibody preparations demonstrated that rat I_{sK} protein is confined to the apical membrane portion of epithelial cells in the proximal tubule of the kidney, the submandibular duct and the uterine endometrium. The observed tissue distribution of rat I_{sK} protein was consistent with that of the I_{sK} protein mRNA determined by blot hybridization analysis. In epithelial cells, the sodium, potassium-ATPase pump in the basolateral membrane generates a sodium gradient across the epithelial cell and allows sodium ions to enter the cell through the apical membrane. Thus, taking into account the cellular localization of the I_{sK} protein, together with its electrophysiological properties, we discussed a possible function of the I_{sK} protein, namely that this protein is involved in potassium permeation in the apical membrane of epithelial cells through the depolarizing effect of sodium entry.

Key Words potassium channel · molecular cloning · Na^+, K^+-ATPase pump · renal proximal tubule · salivary duct

Introduction

Potassium ion channels are essential to not only the control of excitability of nerve and muscle but also the optimal operation of many functions such as cell growth, cell volume and secretion [7, 11]. K^+ channels constitute a diverse and widely distributed set of membrane ion channels in eukaryotic cells [7, 11]. In a previous study [19], we investigated the molecular basis for the diversity of K^+ channels by combining molecular cloning in an RNA expression vector with an electrophysiological assay in *Xenopus* oocytes. Through this strategy, we identified a novel rat membrane protein that induces selective permeation of K^+ ions by membrane polarization. This protein consists of 130 amino acids with a single putative membrane domain and differs from other known K^+ channel proteins. The K^+ current induced by this protein is unusually slow in activation and deactivation after electrical polarization. The unique structural and electrophysiological properties of this novel membrane protein strongly suggest that it serves as a discrete K^+-conducting ion channel, although it may represent a modulatory protein involved in K^+ permeation [19] (for convenience, we hereafter refer to it as I_{sK} protein).

The mRNA for rat I_{sK} protein is expressed in some particular tissues comprising glandular epithelial cells such as kidney and submandibular gland, but not in the brain or liver [19]. In these epithelial cells, K^+ ions permeate into both the lumen and the interstitial space, when the Na^+, K^+-ATPase pump generates a Na^+ gradient across the epithelial cell and creates a high intracellular K^+ concentration [17]. This investigation concerns the cellular localization of rat I_{sK} protein to examine a possible involvement of I_{sK} protein in K^+ permeation in epithelial cells. We raised antisera against several oligopeptides representing various parts of the I_{sK} protein sequence and investigated a detailed immunohistochemical localization of I_{sK} protein in the kidney, submandibular gland and uterus. The results demonstrate that I_{sK} protein is distinctly localized in the apical membrane portion of particular epithelial cells, supporting the view that I_{sK} protein plays a specific role in epithelial K^+ transport.

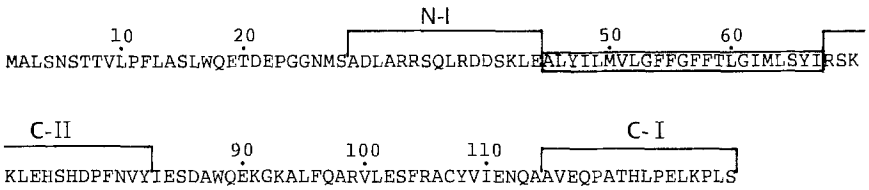

Fig. 1. Amino acid sequence of rat I_{sK} protein. The three oligopeptides (N-I, C-I, C-II) used for the antibody production are indicated above the sequences. The putative transmembrane domain is enclosed with a solid line

Materials and Methods

Materials

Materials were obtained from the following sources: keyhole limpet hemocyanin (KLH) from Hoechst; reticulocyte lysate translation kit from Du Pont; Protein A-Sepharose from Pharmacia; biotinylated goat anti-rabbit IgG and avidin-biotinylated horseradish peroxidase from Vector; and nylon membrane from Pall Ultrafine Filtration.

Preparation of Antisera

Three oligopeptides which correspond to different parts of the I_{sK} protein sequence shown in Fig. 1 were chemically synthesized [16]. N-I peptide was conjugated to KLH by the method of Sutcliffe et al. [18], while C-I and C-II peptides were conjugated to KLH by using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; according to Fig. 1, the three oligopeptides are referred to N-I, C-I and C-II, while the antibody preparations directed to these peptides are named anti-N-I, anti-C-I, and anti-C-II. The oligopeptide-KLH conjugate was emulsified with complete Freund's adjuvant and injected subcutaneously into 2–3 rabbits. The rabbits were given booster injections every three weeks, and antisera against the synthetic oligopeptide were obtained by bleeding after each booster. Antisera and control sera obtained from preimmunized rabbits were purified to an IgG fraction by passing through a column of Protein A-Sepharose as described [8].

Immunoprecipitation of Cell-Free Translation Product and Gel Electrophoresis

The mRNA for the rat I_{sK} protein was synthesized in vitro from the cloned pKI27 cDNA by SP6 RNA polymerase in the presence of the capping nucleotide [15, 19]. The mRNA (0.2 μg) was translated in a cell-free protein-synthesizing system with the reticulocyte lysate translation kit (50 μl) in the presence of ^{35}S-methionine (40 μCi) [19]. The translation product was diluted by the addition of immunoprecipitation buffer (1 ml; 10 mM Tris · Cl pH 7.5, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40 and 1 mg/ml bovine serum albumin) and incubated at room temperature for 30 min after addition of Protein A-Sepharose (67 μg). The supernatant was collected by centrifugation at 100,000 × g for 1 hr. For immunoprecipitation, antioligopeptide IgG (20 μg) was added to a portion of the supernatant (85 μl) and was allowed to stand at room temperature overnight. In addition, either antioligopeptide IgG (20 μg) which was incubated with the corresponding oligopeptide (1 μg) for 1 hr at room temperature or control IgG was added to the supernatant under the same conditions. Protein A-Sepharose (33 μg) was then added and incubated at room temperature for 1 hr. The precipitate was collected by centrifugation at 15,000 × g for 5 min and washed three times with the immunoprecipitation buffer. The antibody-reactive translation product was eluted with the elution buffer (64 mM Tris · Cl pH 6.8 and 2% SDS) after 10 min incubation at 60°C. The eluted product was analyzed by SDS-PAGE as described by Laemmli [13]. Fluorography was performed as described by Chamberlain [3].

Immunohistochemical Analysis

Male Wistar rats (body weight, 180–250 g) were deeply anesthetized with an intraperitoneal overdose of sodium pentobarbital (50 mg/kg body weight) and then perfused through the ascending aorta with 0.9% NaCl, followed by 0.1 M sodium phosphate buffer (pH 7.3) containing 1% picric acid and 2% paraformaldehyde; female rats were similarly treated for the preparation of uterine sections. Immediately after perfusion, various tissues were removed and placed in the above buffer containing 25% sucrose for at least 2 days at 4°C. Each tissue was cut into sections of 4- or 10-μm thickness on a cryostat. The sections were mounted on gelatin-coated glass slides and then immunostained according to the avidin-biotinylated peroxidase method of Hsu, Raine and Fanger [9]. The IgG fraction of antibody was used as a primary antibody after incubation with 0.1% KLH. Slide-mounted tissue sections were pretreated with 0.1 M sodium phosphate buffer (pH 7.3) containing 0.9% NaCl and 2% nonimmunized goat serum for 2 hr and then incubated overnight with the IgG fraction at a concentration of 15 μg/ml at 4°C. Subsequently, biotinylated goat antirabbit IgG antibody was conjugated to the primary antibody at room temperature for 3 hr, and then avidin-biotinylated peroxidase complex was added. The reaction was made at room temperature for 3 hr. Immunolabeled peroxidase was visualized by placing tissue sections in an incubation medium containing 50 mM Tris · Cl (pH 7.3), 0.015% diaminobenzidine tetrahydrochloride and 0.003% hydrogen peroxide for 10 to 20 min at room temperature. Adjacent sections were stained with 0.1% cresylviolet or used for immunostaining with control IgG and antibody IgG pretreated with an excess of the antigen oligopeptide.

RNA Blot Hybridization Analysis

Total RNA and poly(A)$^+$ RNA were isolated from various tissues of male rats except for the preparation of the uterine RNA [19]. For the preparation of uterine poly(A)$^+$ RNA, 17α-ethynylestradiol (0.5 mg/kg body weight) was intracutaneously injected into each female rat every 24 hr for 3 days, and the rats were sacrificed 15 hr after the last estrogen administration. Uteri were isolated and the endometrial portions were dissected by using a razor, and were used as the source of endometrium-enriched uterus poly(A)$^+$ RNA. The remaining portions of uteri were used as the source of myometrium-enriched uterus poly(A)$^+$ RNA.

The yields of poly(A)$^+$ RNA/total RNA (shown in Fig. 8) were 3.4% (kidney), 4.2% (endometrium), 3.6% (myometrium) and 3.1% (submandibular gland). RNA blot hybridization analysis (20 μg of poly(A)$^+$ RNA each) was carried out by using a nylon membrane as described [14]. The 552-base pair *Bam*HI-*Kpn*I cDNA fragment was excised from clone pKI27 [19] and used as a probe. The size markers used were rat rRNAs and the *Hind*III-digested λDNA.

Results

Characterization of Antibody Against I_{sK} Protein

We previously reported the complete amino acid sequence of rat I_{sK} protein by molecular cloning and sequence analysis of its cDNA [19]. Based on this sequence, we chemically synthesized three oligopeptides corresponding to different parts of the I_{sK} protein sequence (N-I, C-I and C-II shown in Fig. 1) and raised antibody against the respective oligopeptide. The specific immunoreactivity of the antibody obtained was characterized by examining its reactivity with the I_{sK} protein synthesized in the cell-free translation system. The mRNA was first synthesized in vitro from the rat I_{sK} protein cDNA by SP6 RNA polymerase and translated in the reticulocyte lysate protein-synthesizing system. The translation product was then reacted with antibody with or without prior treatment of an excess of the corresponding oligopeptide, and the resultant immunoreactive product was analyzed by electrophoresis on SDS-polyacrylamide gel. The results representing the analysis of the three antibody preparations directed to the amino-terminal (N-I) and carboxyl-terminal (C-I and C-II) portions of I_{sK} protein are presented in Fig. 2. The three antibody preparations showed intense radioactive bands representing the in vitro synthesized rat I_{sK} protein, although the reactivity of anti-C-II antibody was lower than those of the two other antibody preparations. The control IgG obtained from preimmunized rabbits showed no such radioactive band. Furthermore, the radioactive bands observed for these different antibody preparations were completely abolished by the pretreatment of each antibody preparation with an excess of the corresponding oligopeptide. The results described here indicate that the three antibody preparations react with rat I_{sK} protein by specifically recognizing a different part of the I_{sK} protein sequence.

Immunohistochemical Localization of I_{sK} Protein

The above characterization of the antibody did not necessarily exclude the possibility that each antibody may cross-react with some other proteins by sharing a common epitope. However, it is also very unlikely that some unrelated proteins share all three different epitopes with the I_{sK} protein. Therefore, for the following immunohistochemical experiments, we prepared serial sections of tissues and examined immunostaining of these sections with the three different antibody preparations. These experiments showed that each antibody preparation gave rise to mutually consistent immunostaining patterns in all tissues described below (*see*, for example, Fig. 4). We also proved that the observed immunostaining indeed reflects the specific reaction with the antibody by confirming the negativity in the reaction with the control IgG or the antibody preadsorbed with an excess of the antigen oligopeptide. For the examination of cellular localization of I_{sK} protein, we chose three particular tissues—kidney, submandibular gland and uterus—because (*i*) I_{sK} protein was originally identified by molecular cloning of cDNA clones prepared from kidney mRNAs [19], (*ii*) the I_{sK} protein mRNA relative to the total mRNAs was found to be highest in the submandibular gland among all tissues analyzed, and (*iii*) it was reported that the uterine mRNA expressed in *Xenopus* oocytes showed K^+ channel activity [1, 2], which is very similar to the activity of I_{sK} protein [19].

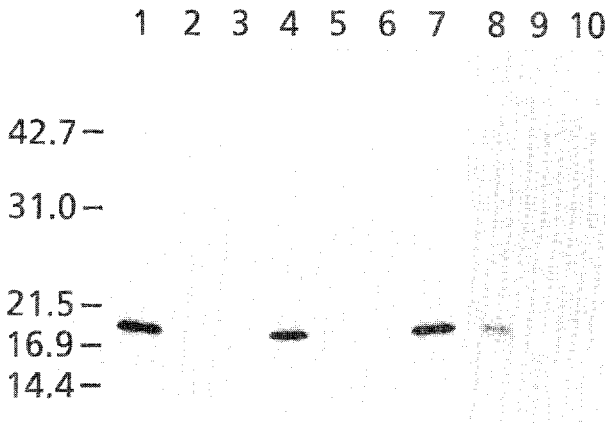

Fig. 2. Fluorography of SDS-PAGE of immunoprecipitates formed with different antibody preparations. Experimental details are described under Materials and Methods. The immunoprecipitation reactions were carried out with the following components: *1*, anti-N-I; *2*, anti-N-I plus N-I; *3*, control IgG; *4*, anti-C-I; *5*, anti-C-I plus C-I; *6*, control IgG; *8*, anti-C-II; *9*, anti-C-II plus C-II; *10*, control IgG. Lane *7* shows total cell-free translation products. The marker polypeptides used were hen egg white ovalbumin (42.7 kD), bovine carbonic anhydrase (31.0 kD), soybean trypsin inhibitor (21.5 kD), equine myoglobin (16.9 kD) and hen egg white lysozyme (14.4 kD)

Kidney

Figure 3 shows an immunostained section, at a low magnification covering the cortical (*co*), outer med-

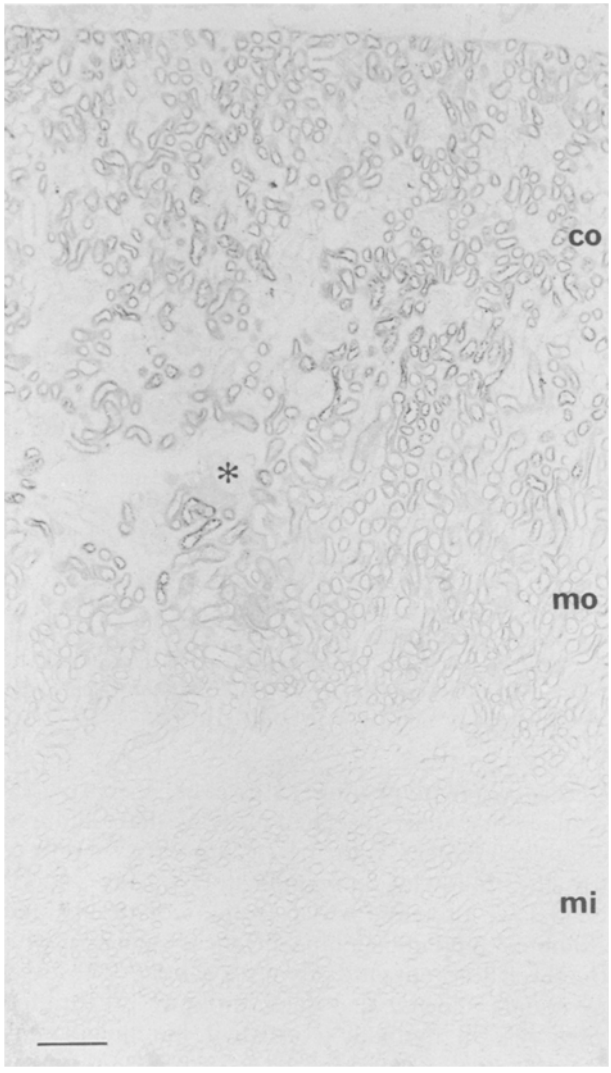

Fig. 3. A photomicrograph of immunostaining of a section of the kidney with anti-C-I antibody. *co*, the cortex; *mo*, the outer region of the medulla; *mi*, the inner region of the medulla; *, a glomerulus. The immunoreactive material is seen densely in the outer and juxtamedullary cortical regions and the subcortical medullary region. Scale bar: 200 μm

ullary (*mo*) and inner medullary (*mi*) regions of the kidney. The immunoreactive material was observed to be heavily localized in the tubular cells located in both outer and juxtamedullary cortical regions as well as the subcortical medullary region. In contrast, neither the glomeruli nor the blood vessels were stained with the antibody. Furthermore, the tubular cells in the deeper outer medullary region were not immunostained, nor were those in the inner medullary and papillary regions. Thus, I_{sK} protein is present in neither thin descending and ascending limbs of the loop of Henle nor collecting tubules.

The tubular cells in the immunostained regions constitute epithelial cells forming the proximal and distal tubules of the nephron. Figure 4*a-c* shows a more magnified view of the immunostaining in the juxtamedullary cortical region. The immunoreactive material was distinctly observed only in the cells of the tubules with thick walls but not in those of the thin-walled tubules, indicating that it is localized in the epithelial cells of the proximal tubules but not in those of the distal tubules. Furthermore, in both transverse (Fig. 4*a*) and longitudinal sections (Fig. 4*b*), the immunoreactive material was selectively observed in the apical (luminal) membrane portion of the epithelial cells. The photomicrographs in Fig. 4*d* and *e* show two adjacent sections which were immunostained with two other antibody preparations. These pictures clearly indicated that the immunoreactive material observed by the two preparations was present in the epithelial cells forming an identical proximal tubule. Thus, from the results presented in Fig. 4, we conclude that the immunoreactive material observed indeed represents I_{sK} protein, and that this protein is localized in the apical membrane portion of the epithelial cells of the proximal tubule.

The distribution of I_{sK} protein in the proximal tubule was investigated in more detail in the experiments shown in Fig. 5. In this investigation, we visualized a single nephrotubular system by staining several serial sections of the kidney with cresyl-violet. We then determined which segment of the proximal tubule contains I_{sK} protein by immunostaining the section adjacent to the serial sections described above. As shown in Fig. 5*a*, a single nephrotubular system starts with a glomerulus (marked with an asterisk) and passes through the proximal convoluted tubule (marked with numbers *1–4*) to the proximal straight tubule (marked with number *5*). Immunostaining gradually increases along this tubule and is maximized at the proximal portion (Fig. 5*b*); the immunoreactive material is totally absent in the glomerulus, sparsely present in the beginning part of the glomerular outflow (number *1*) and then is condensed at the proximal convoluted and early straight tubules (numbers *2–5*), then disappears at the following portion of the proximal straight tubule (*data not shown*). It was also found in this picture that the macula densa of the distal tubule observed as a tubule attached to the glomerulus was free from the immunoreactive material. Thus, the results presented in Fig. 5 demonstrate that I_{sK} protein is distinctly expressed in the proximal convoluted tubule and the early proximal straight tubule.

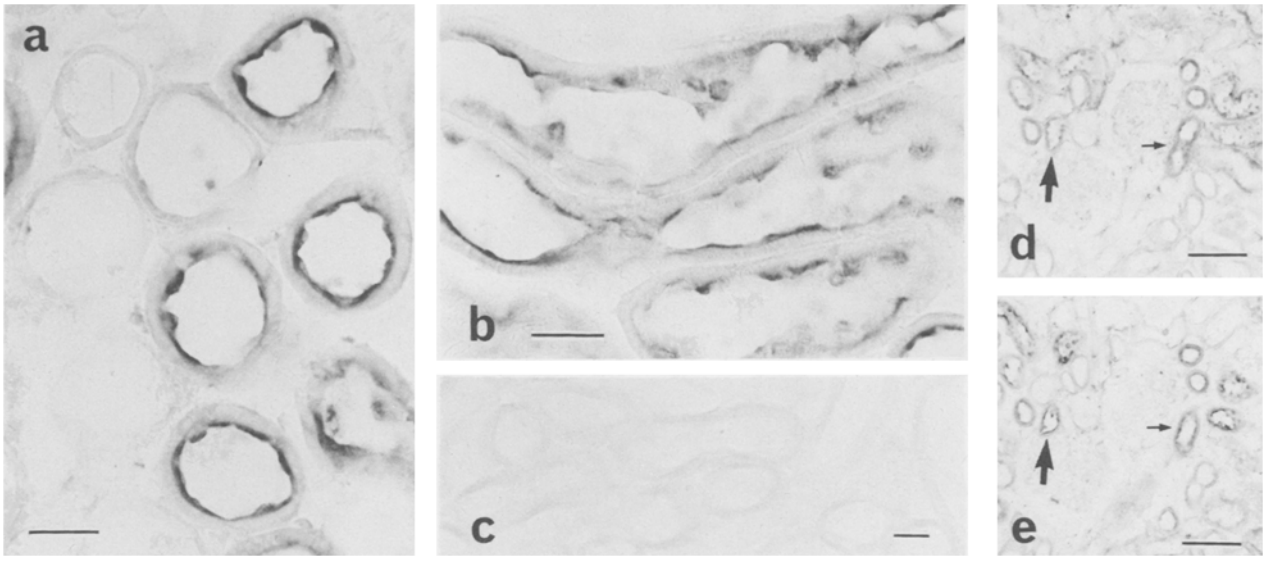

Fig. 4. Photomicrographs of immunostaining of juxtamedullary cortical regions with (*a*, *b*) anti-C-I antibody, (*c*) control IgG, (*d*) anti-N-I antibody and (*e*) anti-C-II antibody. (*a*) The epithelial cells of the thick-walled proximal convoluted tubules are positively stained, while those of the thin-walled distal tubules are not. (*b*) The epithelial cells of the proximal straight tubules are positively stained. (*d*) and (*e*) Identical tubules (e.g., those indicated with large and small arrows) are positively stained in two adjacent sections. Scale bars: (*a–c*) 20 μm and (*d*, *e*) 100 μm

Submandibular Gland

Figure 6 shows the photomicrographs of immunostaining of a submandibular gland. The submandibular gland constitutes a relatively simple architecture starting from the acinus and forming a duct system. The latter is divided into the intercalated, striated and small and large excretory ducts. Among these cell constituents, the immunoreactive material was selectively observed in epithelial cells of the striated duct and of the small excretory duct located between the striated and large excretory ducts (Fig. 6*a*). The epithelial cells in the striated and small excretory ducts were characterized by a common morphology in that they are cuboidal in shape and contain vertically oriented striated structures in the basal cytoplasm (Fig. 6*b*). The immunoreactive material was concentrated in the apical membrane portion encircling the salivary passage (Fig. 6*b*).

Uterus

The immunoreactive material was preferentially observed in the epithelial cells of the endometrium, which were located around the bicornicated uterine cavity (Fig. 7). Each epithelial cell exhibited a heavy immunostaining in the periluminal membrane portion. In contrast, the myometrium, the vascular system and the uterine covering were free of any immunoreactive material. In the uterus, the immunostained material was sometimes observed in the apical cytoplasmic domain underneath the membrane of epithelial cells. However, the large remaining stroma portion was virtually free of immunoreactive material.

Blot Hybridization Analysis of I_{sK} Protein mRNA

Since I_{sK} protein is confined to epithelial cells in the uterus, kidney and submandibular gland, the correlation of its localization with expression of the mRNA was investigated by RNA blot hybridization analysis. The uterus can be relatively easily dissected into the endometrial and myometrial portions, when endometrial cells are proliferated by the administration of estrogen to animals. We therefore separated uteri of estrogen-administered rats into the endometrium- and myometrium-enriched portions, and these separated tissues were used for the isolation of the poly(A)$^+$ RNA preparations. Figure 8 shows the result of blot hybridization analysis of the poly(A)$^+$ RNAs of the endometrium- and myometrium-enriched fractions of uteri, together with those isolated from the kidney and submandi-

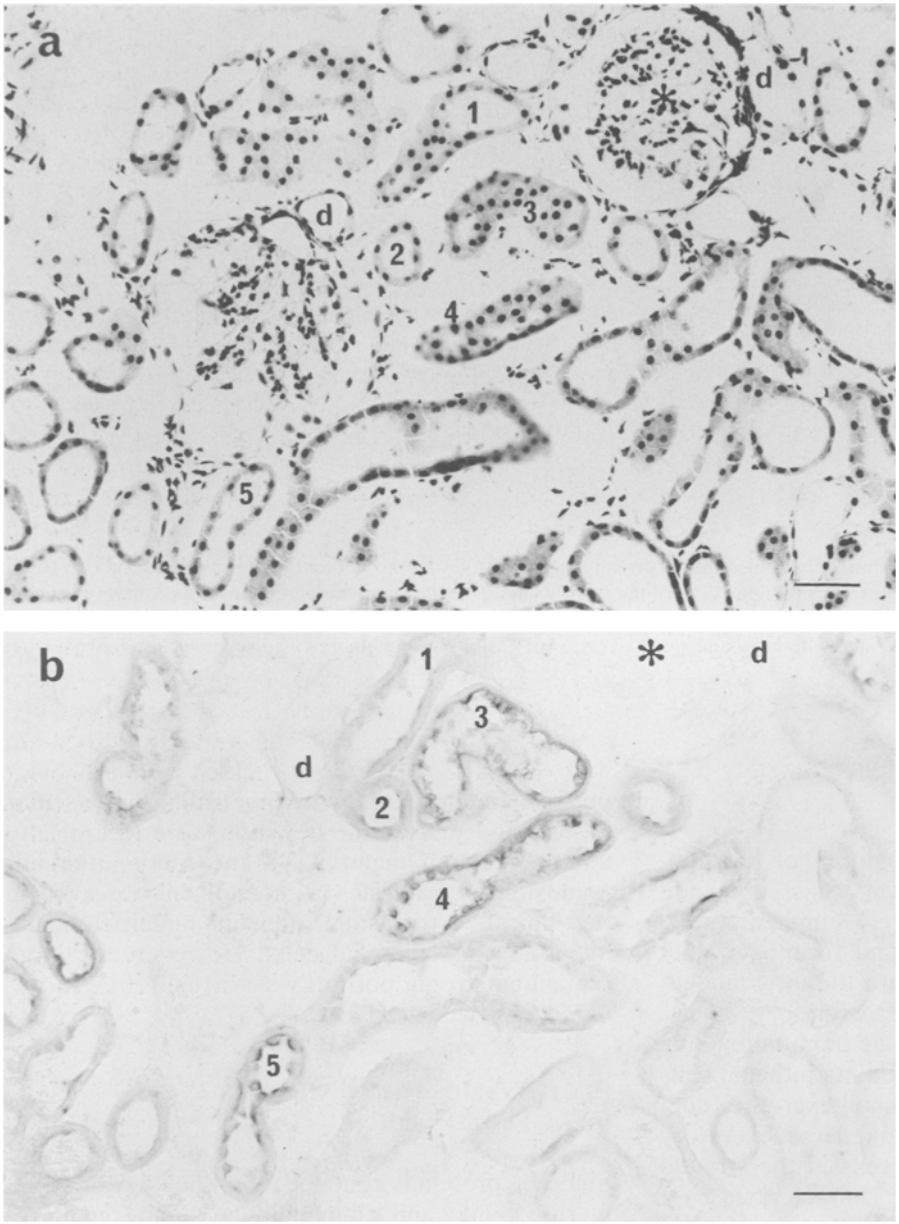

Fig. 5. Photomicrographs of (*a*) cresylviolet-staining and (*b*) immunostaining of two adjacent sections indicating a single nephrotubular system. For immunostaining, anti-C-I antibody was used. A profile of a single nephrotubular system is indicated by numbers. (*d*) A macula densa region of the distal tubule identified by its association with a glomerulus; *, a glomerulus. Scale bars: 40 μm

bular gland. It is clear from this analysis that the I_{sK} protein mRNA is principally expressed in the endometrium-rich portion but not significantly in the myometrium-rich portion. The blot hybridization analysis also showed that the submandibular gland expresses the highest amount of the mRNA relative to the total mRNA among all tissues analyzed, and the relative amount of the mRNA in the submandibular gland is 10–20 times higher than that of the kidney mRNA. This finding is also consistent with our immunohistochemical observation that sections of the submandibular gland are most densely stained with the three antibody preparations. Thus, the tissue distribution of the I_{sK} protein mRNA agrees with the immunohistochemical localization of I_{sK} protein and further supports our conclusion that I_{sK} protein is expressed in particular epithelial cells and functions as a specific apical membrane protein involved in selective permeation of K^+ ions.

The hybridization analysis also showed several radioactive bands corresponding to the mRNAs with estimated molecular sizes of approximately 5.2, 3.7 and 2.6 kilonucleotides. Molecular cloning of additional cDNAs encoding the I_{sK} protein as well as hybridization analyses with different DNA probes derived from the genomic clone indicated

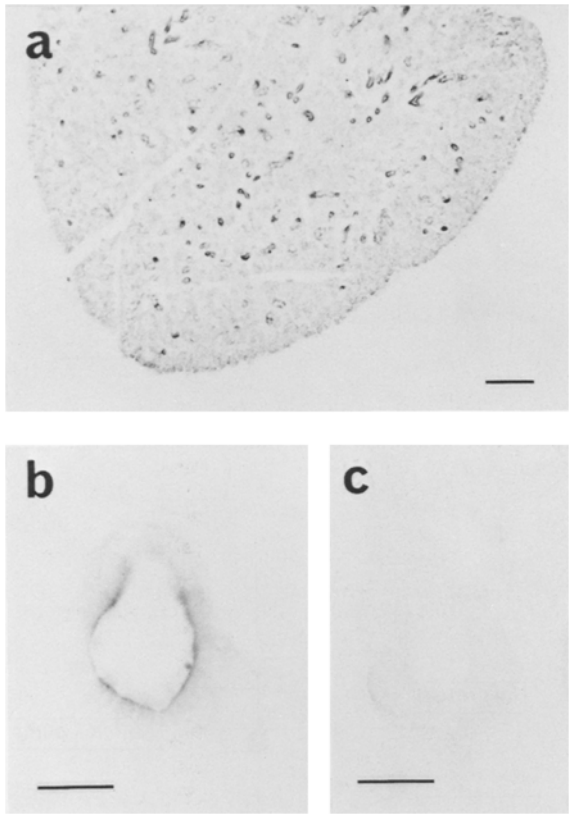

Fig. 6. Photomicrographs of immunostaining of sections of the submandibular gland. The sections in (*a*) and (*b*) were immunostained with anti-C-II antibody and anti-N-I antibody, respectively, while that in (*c*) was immunostained with control IgG. Highly magnified small excretory duct cells are shown in (*b*) and (*c*). Scale bars: (*a*) 200 μm and (*b*, *c*) 20 μm

that the above mRNAs are produced from a single I_{sK} protein gene as a result of alternative RNA processing (*in preparation*).

Discussion

I_{sK} protein is a small protein consisting of 130 amino acids and contains only a single putative transmembrane domain [19]. The K^+ current elicited by this protein on its expression in *Xenopus* oocytes is unusually slow in activation and deactivation after membrane polarization [19]. Furthermore, the I_{sK} protein mRNA is not expressed in excitable cells such as nerve cells [19]. Thus, I_{sK} protein differs from the conventional K^+ channel proteins in its structure, electrophysiology and distribution. However, this protein is capable of inducing selective permeation of K^+ ions in a voltage-dependent manner. Furthermore, our recent mutational analysis of rat I_{sK} protein indicated that replacements of particular charged amino acids immediately following the putative transmembrane domain markedly affected the K^+ channel activity of I_{sK} protein (*unpublished observation*). Therefore, although it remains possible that I_{sK} protein may subserve as a modulatory protein involved in K^+ permeation, the most likely explanation for the above observations is that it *per se* acts as a discrete K^+-conducting channel protein.

In the present investigation, we prepared three different types of antibody that is directed to a distinct part of rat I_{sK} protein. By using these antibody preparations, we immunohistochemically demonstrated that I_{sK} protein is restrictedly localized in the apical membrane portion of epithelial cells in the renal proximal tubule, the submandibular duct and the uterine endometrium. The renal proximal tubule and the submandibular duct are known to show a similar morphology and share many functional properties [12]. Both of these epithelial cells actively reabsorb Na^+ ions and amino acids from tubular fluid following ultrafiltration of these compositions from glomerular cells or acinar cells [6, 20]. Both of them also actively secrete urea into the lumen. In the submandibular duct, K^+ ions are permeated from epithelial cells to the lumen and K^+ concentrations increase along the duct [20]. Similarly, K^+ secretion into the lumen was found in proximal tubules, although its physiological role has not been considered significant in view of the substantially greater entry of K^+ ions in segments of the descending limb of the loop of Henle [6, 10]. All of these transports are thought to result from the asymmetrical distribution of the transport systems and to depend energetically on the electrochemical Na^+ gradient which is driven by the Na^+, K^+-ATPase pump located in the basolateral membrane of epithelial cells [6, 17, 20]. Therefore, taking into account the electrophysiological properties of the I_{sK} protein, together with the direction of the Na^+ transport across the epithelium, a model of the I_{sK} protein function is schematically illustrated in Fig. 9. The Na^+, K^+-ATPase pump generates a lower intracellular Na^+ concentration. The resultant Na^+ gradient across the apical membrane causes the entry of Na^+ ions into the cell from the lumen through the Na^+/sugar or amino acid cotransport system [17]. This flow of Na^+ ions induces depolarization of 10–15 mV over the stable apical membrane potential of the order of −70 mV [4, 5]. Under the magnitude of this depolarization, the I_{sK} protein is capable of inducing a slowly activating K^+ current [19]. Therefore, the depolarizing effect on the apical membrane can stimulate the K^+ channel activity of I_{sK} protein and results in permeations of K^+ ions from the epithelial cells to the lumen. This model of the function of the I_{sK} protein can now be tested by

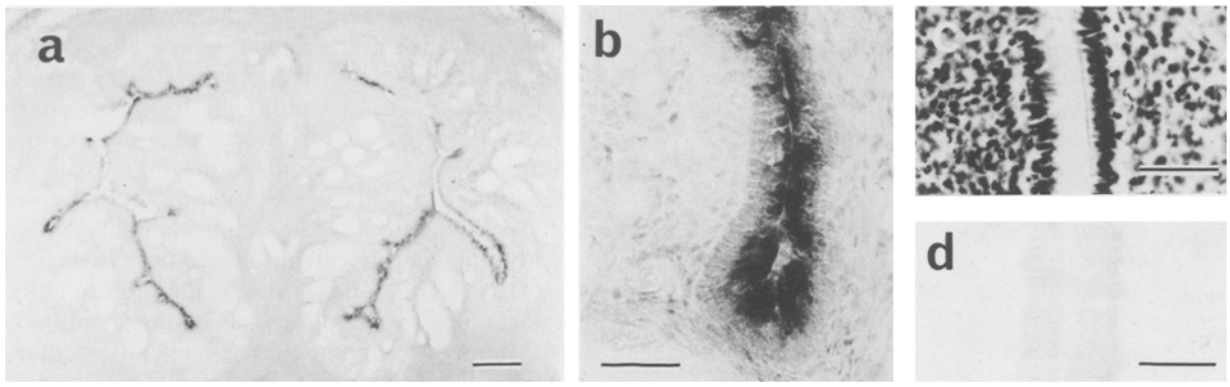

Fig. 7. Photomicrographs of immunostaining (*a*, *b*, *d*) and cresylviolet-staining (*c*) of sections of the uterus. For immunostaining, anti-C-I antibody was used (*a*, *b*). In two adjacent sections of the endometrium, one was stained with cresylviolet (*c*), while the other was immunostained with the preadsorbed antibody (*d*). Scale bars: (*a*) 200 μm and (*b–d*) 40 μm

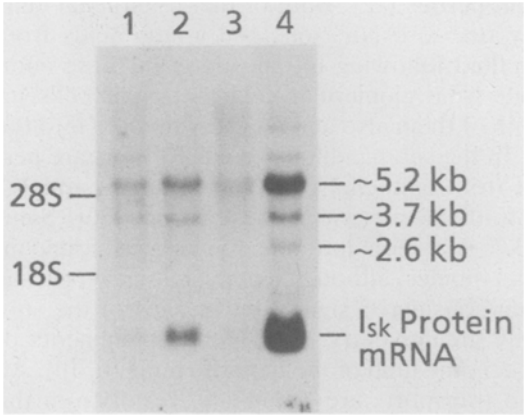

Fig. 8. Blot hybridization analysis of poly(A)$^+$ RNAs from various tissues. Experimental details were described under Materials and Methods. *1*, kidney; *2*, endometrium-enriched fraction of uterus; *3*, myometrium-enriched fraction of uterus; and *4*, submandibular gland

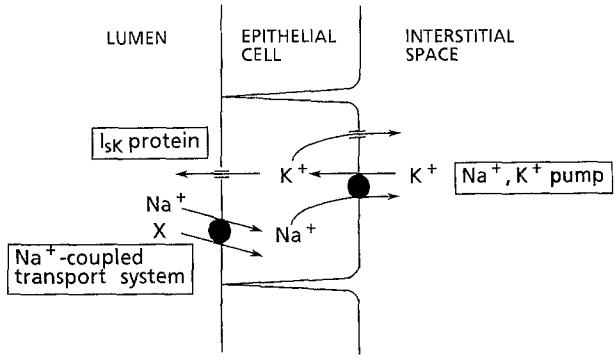

Fig. 9. A model of the function of I_{sK} protein. For detailed explanation, *see* the text. The Na^+ entry indicated at the apical membrane represents the Na^+/glucose or amino acid cotransport system

comparing the properties of the possible K^+ channel in the epithelial apical membrane with those of the I_{sK} protein, which have been well characterized in the oocyte expression system [19].

The restricted distribution of I_{sK} protein in the apical membrane portion of endometrial cells is also interesting. By using an oocyte expression system, Boyle et al. [1, 2] indicated that poly(A)$^+$ RNA of estrogen-treated rat uteri induces a slowly activating, voltage-dependent K^+ current. This current is very similar to that characterized for I_{sK} protein. However, this group discussed that the mRNA identified originated from myometrial cells, because the K^+ current was expressed to a similar degree whether or not the endometrium was removed by dissection before the RNA extraction [2]. The group also reported that the expression patterns of the mRNA correlated with changes in membrane potentials in the myometrium during the oestrous cycle and estrogen treatment and suggested that the expression of the slow voltage-dependent K^+ current plays an important role in controlling electrical excitability in the myometrium [2]. The question thus arises as to whether the observed difference in the distribution of the mRNAs responsible for induction of a similar K^+ channel activity is due to the presence of a different mRNA in the myometrium or the different RNA preparations used. In any case, the restricted distribution of I_{sK} protein suggests its specific function in the K^+ transport in the endometrium, and its physiological role will need further investigation.

Both proximal tubular cells and salivary duct cells share with other ion-transporting epithelial cells the asymmetrical distribution of active and passive transport systems [6]. We previously showed that the I_{sK} protein mRNA is expressed in

some other tissues comprising epithelial cells, such as the pancreas, stomach and duodenum [19]. It will thus be interesting to see the localization of the I_{sK} protein in these tissues. On the other hand, it is well known that the distal tubular cells of the kidney are involved in the active secretion of K^+ ions from the cells to the lumen [6]. However, the present immunohistochemical study did not show any immunoreactive material in the distal tubular cells of the kidney. This implies that the mechanisms underlying the K^+ permeation are diversified in the epithelial cells and poses a further interesting question for the molecular basis of the diversity of the K^+ transport systems.

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