H-K-ATPase type 2: relevance for renal physiology and beyond

Gilles Crambert
UPMC Université Paris 6/INSERM/CNRS U1138, Equipe 3 Laboratoire de Génomique, Physiologie et Physiopathologie Rénales ERL 8228, Paris, France

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MAINTAINING A LOW K⁺ CONCENTRATION in the external milieu and high intracellular K⁺ levels is required for conserving both the electrical properties of tissues and cell volume. Therefore, mechanisms contributing to the regulation of K⁺ homeostasis are crucial in any physiological situation. Many regulatory systems work in coordination for this purpose. Some of them consist of processes of storage/release of K⁺ from/to internal stores (internal balance), allowing for rapid exchanges between extracellular and intracellular spaces. Others, in parallel, involve excretion or retention of K⁺ (external balance) by the colon and the kidney. Both systems are finely tuned in a time-dependent manner to cope with K⁺ intake and to precisely adjust the outputs to the inputs. The kidney excretes 90% of the K⁺ intake. The daily filtered load of K⁺ is much higher than the K⁺ intake, indicating that the kidney has a massive capacity to secrete K⁺; however, in the case of large K⁺ ingestion, the kidney is also able to secrete K⁺. To achieve these functions (reabsorption and secretion), all segments of the nephron are mobilized. The segments of the proximal tubule, in the cortex, reabsorb 30–70% of the filtered K⁺ (57) through both passive (88) and active mechanisms. In the medullary part of the proximal tubule and Henle’s loop, epithelial K⁺ transport does not affect net K⁺ excretion because it forms a recycling loop (45). The cortical thick ascending limb (cTAL) contributes modestly to K⁺ reabsorption. Indeed, most of the luminal K⁺ that enters the cells through Na-K-2Cl cotransporter (NKCC2) is recycled and returns to the lumen through ROMK channels. In the distal part of the nephron, from the distal convoluted tubule to the collecting duct, the transepithelial transport of K⁺ is highly regulated and mediates either reabsorption or secretion depending on the physiological context (87).

Many transporters contribute to renal K⁺ reabsorption or secretion along the nephron. Among them, H-K-ATPase type 2 (HK2A), also known as the “nongastric” or “colonic” H-K-ATPase, is broadly expressed, and its presence in the kidney has puzzled experts in the field of renal ion transport systems for many years. One of the most important and robust characteristics of this transporter is that it is strongly stimulated after dietary K⁺ restriction. This result prompted many investigators to propose that it should play a role in allowing the kidney to efficiently retain K⁺ under K⁺ depletion. However, the apparent absence of a clear renal phenotype in HKA2-null mice has led to the idea that this transporter is an epiphenomenon. This review summarizes past and recent findings regarding the functional, structural and physiological characteristics of H-K-ATPase type 2. The findings discussed in this review suggest that, as in the famous story, the ugly duckling of the X-K-ATPase family is actually a swan.

In the early 1960s, Malnic et al. (57) showed that the distal tubules of rats fed a low-K⁺ diet retained K⁺. Giebisch’s group (82) observed that the direct injection of ouabain into the renal artery of rats fed a low-K⁺ diet increased renal K⁺ excretion by inhibiting luminal K⁺ reabsorption in the distal tubules. This result was supported by further experiments in isolated rat kidneys perfused with ouabain (41). These observations suggested that a ouabain-sensitive, potassium-absorptive pump is present on the luminal side of the distal nephron of K⁺-depleted rats. Later, this ability to retain K⁺ was associated with H-K-ATPase activity (28, 65). Identification of HKA2 as the H-K-ATPase isoform that is upregulated during K⁺ depletion (28, 65). Identification of HKA2 as the H-K-ATPase isoform that is upregulated during K⁺ depletion and putatively responsible for K⁺ reabsorption was achieved by measuring an increase in its mRNA and/or protein level under this specific condition (2, 49, 58). These results prompted many investigators to propose that this transporter should play a role in allowing the kidney to efficiently retain K⁺ under conditions of K⁺ depletion. In 1998, Meneton and collaborators (59) published a detailed analysis of the first HKA2 knockout mouse model. These mice, under basal or K⁺-depleted conditions, exhibited a higher colonic loss of K⁺ compared with wild-type littermates and an aggravated K⁺
depletion. However, the renal handling of K⁺ was not affected by the absence of this transporter either in normal conditions or during K⁺ restriction. This work led to a lack of interest regarding the role of HKA2 in the kidney, a situation reinforced by the difficulty in studying this transporter and the ambiguity regarding its intrinsic properties (see below). Despite these obstacles, a few groups have continued to accumulate data and have described interesting features regarding the kinetic, pharmacological, and physiological characteristics of HKA2 as well as the regulatory pathways controlling its expression and activity. In addition to the kidney and colon, this intriguing transporter is also found in other organs where its function is now being investigated. The aim of this review is to summarize past and recent findings regarding HKA2 to highlight its physiological relevance in the kidney and elsewhere.

**General Aspects and Open Debates on HKA2 Properties**

Na-K-ATPase isoforms and HKA1 and HKA2 consist of two necessary subunits. The 100-kDa α-subunit bears all the functional properties of the transporters. Sequence comparison and phylogenetic analysis (Fig. 1) between the different αNKA and αHKA isoforms of human, rat, mouse, rabbit, pig, and bovine species show that αHKA2 is slightly closer to αHKA1 than to αNKA. Interestingly, the identity between αHKA2 from different species is very low (71%) compared with 95% for the αHKA1 and 95–98% for αNKA isoforms. This divergence in amino acid sequences could be at the core of some discrepancies regarding HKA2 function in a comparison between different species (see below). Crystal structure and molecular models confirm that the αNKA, αHKA1, and αHKA2 share a similar secondary structure consisting of 10 transmembrane domains, N- and C-terminal intracellular extremities, and a big intracellular loop (39, 79, 89). Moreover, all three α-subunits must be associated with a heavily glycosylated β-subunit, with one transmembrane domain, to be functional (6).

Despite these general similarities, NKA and HKA1 clearly diverge in the type of partnering β-subunit and exhibit strong differences in their pharmacological and functional properties. HKA2 shares some characteristics with both NKA and HKA1; this has rendered its investigation more difficult and generated numerous debates.

**Identity of the HKA2 β-subunit.** Three β-subunit isoforms have been described for NKA (β₁,NKA, β₂,NKA, β₃,NKA), and the association with one or another of these isoforms may modify the kinetic properties of the α-subunit (20). A specific HKA1 β-subunit has also been identified (βHKA). In the *Xenopus* oocyte expression system, the artificial combination of αNKA subunits and βHKA leads to a mature αβ complex with strongly altered kinetic properties (34, 44). The reverse combination (αHKA1/βNKA) does not allow the αβ complex to be efficiently stabilized and therefore leads to its degradation (34). The α-subunit of H-K-ATPase type 2 (αHKA2) is degraded when expressed in the absence of β-subunits, indicating that, like αNKA and αHKA1, it requires a partner for correct maturation. However, despite many efforts, no specific β-subunit has been identified for HKA2. In heterologous expression systems, several β-subunits may support the functional expression of HKA2, such as βHKA (1, 12, 37, 43, 47, 60) or βNKA (4, 12, 16, 18, 60). In mammalian systems (Madin-Darby canine kidney, LLC-PK, HEK293, and COS cells), the cotransfection of a βHKA subunit along with αHKA2 is required to promote its efficient maturation, indicating the inability of endogenous βNKA subunits to serve as partners (4, 37, 73). As opposed to αNKA or αHKA1, the association of the human HKA2 with one or another X-K-ATPase β-subunit coexpressed in *Xenopus* oocytes does not alter the kinetic properties of the resulting HKA2 complexes (21).

**Fig. 1. Representation of amino acid sequence divergence among the Na-K-ATPase (NKA) isoforms (ATP1A1, ATP1A2, and ATP1A3) and HKA1 (ATP4A) and HKA2 (ATP12A) of different species. The different sequences were taken from Uniprot and aligned using their alignment tool. The cladogram was drawn using the "one click" analysis of Phylogeny.fr web service (25).**

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associate tightly with and to support the correct folding of the αHKA2 subunit by measuring resistance of the α-β complex to detergent and to controlled proteolysis. Based on these criteria, the “real” endogenous β-subunit associated with the αHKA2 appeared to exhibit the characteristics of a β2NKA-like subunit. The authors therefore concluded that a yet unknown β-subunit having β2NKA characteristics was the authentic partner of αHKA2 in vivo. The development of genomic and proteomic tools has recently provided the opportunity to explore in silico the entire genome of many different species. Using this strategy, Modyanov’s group (69) identified the latest X-K-ATPase β-subunit (encoded by the ATP1B4 gene and named βm), but it turns out that βm is not able to associate with αHKA2 (19).

Immunoprecipitation and immunodetection experiments using rat kidney (11, 50) and colon samples (11) have established that β2NKA associated in vivo with αHKA2. Moreover, the colocalization of αHKA2 and β2NKA has been observed at the apical side of rat distal colon cells (53). More recently, Sangan et al. (77) have identified a β-subunit present in both the apical and basolateral membranes of the rat distal colon, the expression of which is upregulated by a low-κ⁺ diet and which is associated with αHKA2. It turns out that this protein is identical to the β2NKA subunit. In the rat prostate gland, β2NKA is present at the apical side of the cells associated with αHKA2 (67). Finally, it has been recently demonstrated that the colonic but not the gastric κ⁺-ATPase activity is preserved in βHKA-null mice, indicating that αHKA2 may function without a βHKA subunit (78). The nature of the β-subunit associated with αHKA2 was not examined in this study, however.

Taken together, these results indicate that the existence of a specific β-subunit that associates only with αHKA2 and promotes its functional maturation is highly unlikely. Is αHKA2 an opportunistic protein, able to associate with any κ⁺-ATPase β-subunits? Is the composition of the αHKA2 subunit complex dependent on tissue or physiological status? Is the αHKA2/β subunit association a regulated process? To our knowledge, these questions remain unresolved.

Pharmacological properties. Sensitivity to inhibitors can serve to distinguish between different isoforms with similar activities. Regarding the X-K-ATPase family, if all members are inhibited by orthovanadate, there is a clear pharmacological profile that distinguishes NKA from HKA1. The first one is sensitive (to a degree that depends on the isoform and species) to compounds related to cardiac glycosides (ouabain, for instance) or digitalis (digoxin, etc.). HKA1 is sensitive to substituted benzimidazole compounds (omeprazole) that bind covalently to a specific cysteine present in the αHKA1 subunit (for a review, see Ref. 42). It also is sensitive to imidazopyridines, such as Sch28080, that compete with κ⁺ binding on αHKA1 (42). As for HKA2, it is clear that its pharmacological profile is highly dependent on the species and the cellular/tissue context. Here, we only summarize data for rat, human, and mouse species. In Xenopus oocytes, rat HKA2-mediated 86Rb⁺ flux is insensitive to Sch28080 (administered as a unique dose of 500 μM) but is inhibited by ouabain with Kᵢ values that depend on extracellular κ⁺ concentration (12, 18). These results are in good agreement with the K-ATPase activity present in the rat distal colon that is, indeed, inhibited by ouabain but insensitive to Sch28080 (24, 84). However, these findings differ from those obtained when rat HKA2 is expressed in Sf9 insect cells (52). Human αHKA2, when expressed in HEK cells (37), insect Sf21 cells (1), and Xenopus oocytes (21, 60), exhibits both ouabain (with Kᵢ values in the tens of μM)- and Sch28080 (Kᵢ ~ 100 μM)-sensitive ATPase activity or 86Rb⁺ flux.

Mouse HKA2 exhibits a pharmacological profile that clearly depends on the tissue context. Thus in the cortical (CCD) and outer medullary (OMCD) collecting duct of mice under a low-κ⁺ diet, Dherbecourt et al. (26) showed that HKA2 was sensitive to both ouabain and Sch28080 by measuring K⁺-ATPase activity. This result was confirmed by measurements of intracellular pH variations on micropерfused CCD of mice under a normal diet (55). However, the K-ATPase activity present in the colon of mice and attributable to HKA2 was insensitive to both Sch28080 and ouabain (78).

It therefore appears that the response to pharmacological inhibitors varies not only with the species but also with the cellular environment. This last aspect is a specificity of HKA2, and determining its origin would require more investigation regarding, for example, its possible protein partners in one tissue or another.

Ion specificity and electrogenicity of HKA2. The major difference that distinguishes NKA from HKA is obviously the nature of the ions transported during their catalytic cycle. The first descriptions of NKA kinetic properties by Skou (80) clearly established that both Na⁺ and K⁺ were required to activate NKA. When searching for the nature of the proton pump present in hog stomach responsible for gastric acid secretion, Sachs et al. (74) demonstrated that this function was borne by an electroneutral K-ATPase independently of Na⁺, defined as an H-K-ATPase. That the activity of one is Na⁺ dependent whereas that of the other is Na⁻ independent creates a clear frontier between NKA and HKA. As for HKA2, Caplan’s group (37) was the first to report that the ability of human αHKA2 (expressed in HEK293 along with rabbit βHKA) to transport protons was more efficient in the presence of Na⁺. In parallel, they showed HKA2-mediated Rb⁺ uptake was stimulated by an electroneutral K-ATPase independently of Na⁺, taking into account all of these findings, they calculated that the proton-to-K⁺ exchange ratio was not equal to 1, indicating that another ion could be transported. In a follow-up study, this group demonstrated the ability of HKA2 to transport Na⁺ by measuring the rate of Na⁺ efflux in transfected and nontransfected HEK293 cells (38). This characteristic was also reported the same year by Coughon et al. (17), who expressed rat αHKA2 and rabbit βHKA in Xenopus oocytes. This observation was confirmed later (human αHKA2/rabbit βHKA in Xenopus oocytes) and extended with the calculation of the intracellular apparent Na⁺ affinity (Kᵢ/Na = 9 mM) for HKA2-mediated Rb⁺ uptake (21). Despite these results, obtained in heterologous expression systems, the activity responsible for the apical reabsorption of K⁺ in the colon was described as a Na-independent, Sch28080-sensitive K-ATPase (7, 40, 46). The latter experiments were performed in purified membrane vesicles, and we cannot exclude the possibility that accessibility to internal Na⁺ was reduced. Moreover, in the recent study by Belisario et al. (7) the colonic K-ATPase activity was not tested in the presence of both K⁺ and Na⁺. To our knowledge, the effects of intracellular Na⁺ on the transport activity of renal HKA2 have not been described. Finally, as is the case with many K⁺ transporters, NH₄⁺ could be a surrogate for K⁺, as
shown for rat HKA2 expressed in the heterologous system or present in colonic apical membranes (15, 16, 83).

Electrogenicity is another characteristic that allows us to discriminate between NKA and HKA. Since NKA moves three Na\(^+\) ions out of the cell in exchange for two K\(^+\) ions into the cell, it generates an electrical current through the membrane (3). Conversely, HKA1 exchanges two H\(^+\) ions for two K\(^+\) ions and is electroneutral (72). An analysis of the electrogenic properties of Bufo marinus HKA2 (8), performed in Xenopus oocytes, indicated that this transporter is not intrinsically electrogenic; i.e., it moves an equal amount of charge into and out of the cell. This absence of electrogenic activity was then shown to depend on the presence of a lysine in the fifth transmembrane domain of both HKA1 and HKA2 (9). Interestingly, the replacement of this lysine by an alanine renders the HKA2 electrogenic.

Together, these data suggest a strong flexibility in terms of ion specificity that could result in four different transporters, ranging from a pure H\(^+\)/K\(^+\)-ATPase to a putative electroneutral Na\(^+\)/K\(^+\)-ATPase, via unusual possibilities such as a H\(^+\)/NH\(_4\)^+-ATPase and a Na\(^+\)/NH\(_4\)^+-ATPase. It remains to be seen whether these different transport activities mediated by one transporter are physiologically relevant and, if so, whether the passage from one transport mode to another can be the target of a regulation pathway.

Relevance of HKA2 in Renal Physiology

As mentioned at the beginning of this review, the physiological relevance of HKA2 in the kidney has been challenged by the phenotype of HKA2-null mice published in 1998. In the following sections, we discuss past and recent data that suggest a role for this transporter in different physiological situations and describe regulatory pathways regulating its activity (Fig. 2).

Localization of HKA2 along the nephron. The characterization of HKA2 localization along the nephron has been performed at three different levels: activity, mRNA, and protein expression. In 1996, the Doucet group (58) localized HKA2 along the rat nephron by using PCR on microdissected tubules. They showed that under normal conditions, HKA2 mRNA was absent from proximal tubules and medullary thick ascending limb but present in cTAL, CCD, and to a lesser extent in OMCD. Feeding rats a low-K\(^+\) diet did not modify this localization profile. However, under these dietary conditions, the level of HKA2 expression was dramatically increased in CCD whereas it remained constant in cTAL. The final identification of the different H-K-ATPase activities in the CCD and OMCD was performed using HKA1 and HKA2 knockout mice (26). This study revealed that H-K-ATPase activity in these segments under normal conditions could be fully ascribed to HKA1. Conversely, after 1 wk of K\(^+\) restriction, HKA2 accounted for the total H-K-ATPase activity, thus revealing a complete shift from HKA1 to HKA2 under K\(^+\)-depletion conditions.

The presence of HKA2 mRNA and protein has been confirmed in the connecting tubules (CNT), CCD, and OMCD by many studies in different species (32, 48, 85, 90). Among them, Verlander et al. (85) showed the abundant presence of HKA2 in rabbit intercalated cells and to a lower extent in principal cells. By measuring ouabain-sensitive proton fluxes on microdissected mouse collecting ducts, Lynch et al. (55) demonstrated that HKA2 was present in both A- and B-type intercalated cells. Moreover, this study showed that under normal dietary conditions, HKA2 activity is measurable when one is specifically focusing on B-type intercalated cells.

Adaptation to alterations in the potassium balance. One of the most robust features of renal HKA2 is its stimulation by dietary K\(^+\) restriction (2, 49, 58). More precisely, Greenlee et al. (35) recently showed, using a minralocorticoid-induced hypokalemia strategy, that this stimulation is initiated by a decrease in plasma K\(^+\) levels. However, the hormonal and cellular mechanisms involved in this stimulation have remained obscure for a long time. Some recent studies have started to answer this question. The group of Wang et al. (86, 87) published a series of papers describing how K\(^+\) depletion leads to the production of reactive oxygen species (ROS). These compounds activate different regulatory pathways, leading to phosphorylation of ROMK channels and inhibition of K\(^+\) secretion (for a review, see Ref. 86). A possible link between this production of ROS and the stimulation of HKA2 is suggested by the finding that Nrf2, a ROS-induced transcription factor, is stimulated by K\(^+\) depletion (51). Nrf2 is an antioxidant molecule that triggers protective pathways against oxidative stress. In their study, Lee et al. (51) showed that overexpression of Nrf2 in HEK293 and CV-1 cells enhances the expression of HKA2. Pretreatment of these cells with a low-K\(^+\) culture medium increased the endogenous expression of Nrf2 and HKA2. Transfection of a dominant negative Nrf2 abolished this low-K\(^+\)-mediated HKA2 expression. This system has not yet been validated in vivo, but these results provide a hint that should be investigated further.

Another regulatory pathway has been recently identified. Under chronic dietary K\(^+\) restriction, adrenal steroidogenesis is modified, leading to a decrease in plasma aldosterone as expected, but more surprisingly to an increase in plasma progesterone (31). This result, obtained in male mice, fits with the presence of different types of progesterone receptors along the nephron of male mice (36). Moreover, using plasma samples from men (healthy volunteers), it was shown that a rather low daily consumption of potassium was correlated with a high
concentration of plasma progesterone. It was further demonstrated that an increase in plasma progesterone concentration in male mice is positively related to the ability of the kidney to retain K⁺ and to stimulate the expression of HKA2. Inhibition of progesterone action using a progesterone receptor antagonist (RU486) elicited an increase in urinary K⁺ excretion and prevented HKA2 stimulation.

As opposed to potassium depletion, an elevated potassium load should lower K⁺ retention and inhibit HKA2 function. It is precisely what El Moghrabi et al. (30) observed; their results suggest that after an acute K⁺ load, tissue kallikrein level is increased and inhibits HKA2. In microperfused CCD of tissue kallikrein-null mice, transepithelial K⁺ reabsorption is stimulated and is correlated with the enhanced expression and activity of HKA2. The inability to reduce HKA2 activity after a K⁺ load via the kallikrein pathway leads to a transient increase in the plasma K⁺ level.

To face variable K⁺ intakes and to defend against the risk of hypo- or hyperkalemia, the organism brings into play many different regulatory pathways. The data mentioned above outline the participation of HKA2 in these processes.

**Adaptation to acidosis.** The contribution of HKA2 to H⁺ excretion in distal nephron segments (56) and the strong expression of this transporter in intercalated cells (85) suggest that HKA2 could be involved in the response to acidosis. However, in rats, chronic metabolic acidosis does not stimulate the mRNA expression of HKA2 (29). In rabbit CCD, after acute metabolic acidosis, HKA2 mRNA levels are even 60–70% lower than in control animals (33). In 2006, Cheval et al. (10) provided the global identification of gene expression modifications induced by short- and long-term metabolic acidosis in mice CCD and showed that mRNA HKA2 was increased two- to threefold. These discrepancies may be due to species specificity or the use of techniques with different sensitivity, but they at least indicate that metabolic acidosis does not modify HKA2 mRNA expression as strongly as K⁺ depletion does. A possible posttranslational regulatory pathway, suggesting a stimulation of HKA2 during acidosis, is suggested by recent studies. Dubose et al. (13) observed that HKA2 insertion at the plasma membrane of epithelial cultured cells (HEK293) is a PKA-dependent process that can be triggered by the acid activation of the pH-sensor GPR4 (14). It is tempting to speculate that a regulatory pathway that links a decrease in plasma pH to the activation of GPR4 and a more stable insertion of HKA2 at the plasma membrane is set in motion, in vivo, in response to metabolic acidosis.

Finally, to our knowledge, there is no description of the phenotype of HKA2-null mice under experimentally induced metabolic acidosis, which would be necessary to prove, or disprove, the involvement of this pump in this physiological situation.

**Adaptation to circadian alternation of rest and activity periods.** Urinary K⁺ excretion is known to follow circadian variations, with increased excretion during the period of activity independently of nutritional behavior (61, 62). These circadian variations persist in dark-dark condition and are partially abolished in clock-null mice, indicating that they depend on the internal clock system (63). This ability to anticipate the periods of high and low food intake, respectively, during periods of activity and rest and to adapt the level of excretion accordingly enables the organism to maintain a stable level of plasma K⁺ (71). The renal mechanisms involved in the circadian excretion of K⁺ have not been investigated in depth. The sensitivity of K⁺ excretion to amiloride (23) during the activity period suggests a role for ROMK channels, which need a functional epithelial Na channel to efficiently transport K⁺ in the tubular lumen.

In a recent report (75), we showed that the renal expression of HKA2 under normal dietary conditions displays a circadian profile with a higher expression during the rest phase of the cycle (acrophase at ZT11). These circadian variations are abolished when the circadian system is disrupted, as in clock-null mice. In mice lacking the HKA2 gene, a urinary K⁺ leak was observed during the rest period that correlates with a decrease in the plasma K⁺ value. We concluded that under a normal diet, HKA2 is involved in the conservation of K⁺ during the rest period when K⁺ intake is low.

Interestingly, dietary K⁺ restriction reversed the circadian expression profile of HKA2, inducing a higher level of HKA2 messenger and protein expression during the activity period (acrophase at ZT20). Under these dietary conditions, when K⁺ is absent from food, the inversion of the circadian profile of HKA2 expression may limit K⁺ losses during the activity period when muscle K⁺ release is maximal and not compensated by a normal ingestion of K⁺. Analysis of the circadian phenotype of HKA2-null mice fed a low-K⁺ diet showed a leak of K⁺ during the activity period, concomitantly with a decrease in the plasma K⁺ value (compared to the rest period).

All of these data suggest that renal HKA2 plays an important role in allowing the kidney to reabsorb K⁺ “on time” so as to adjust urinary excretion to food intake.

**Adaptation to gestation.** The finding that progesterone is a hormonal regulator of HKA2 expression led us to investigate the putative role of this transporter in the renal adaptation to gestation (76). Gestation has been shown to induce renal K⁺ retention, as needed to cope with fetal development (54), when many factors favor renal K⁺ loss instead (increase of glomerular filtration, stimulation of aldosterone production, etc.). We therefore examined whether HKA2 participates in the renal retention of potassium in the gravid female. In the kidney of gravid mice, HKA2 mRNA and protein abundance as well as HKA2 ATPase activity are indeed increased compared with nonpregnant mice. Compared with gravid wild-type mice, which retain K⁺ through their colon and kidney, gravid HKA2-deficient mice exhibit a loss of ~160 μmol of K⁺/24 h (day 16 postcoitus) in both their urine and feces. This leak of K⁺ is not trivial since it corresponds roughly to the K⁺ content of 2 g of muscle. In addition to this inability to retain K⁺ efficiently, HKA2-deficient mice display gestational defects such as a decrease in the fertility rate, an increase in maternal mortality, and a decrease in the number of pups per litter. Interestingly, increasing the daily K⁺ intake reverses all these defects. Although many questions remain to be resolved, these results demonstrate a new physiological role for HKA2 in the renal adaptation to gestation.

**Reinterpretation of the renal phenotype of HKA2-null mice.** When they published the phenotype of the first HKA2-null mouse model, Meneton et al. (59) demonstrated without ambiguity the role of this transporter in the response to hypokalemia. Those mice are, indeed, unable to appropriately retain K⁺ when placed under dietary K⁺ restriction and display a dramatic decrease in their body weight and a more pronounced
hypokalemia than their wild-type littermates. Under these conditions, HKA2-null mice lose two to five times more K⁺ in their feces than wild-type animals. As for urinary K⁺ excretion, it remains similar in both strains. From these data it has been concluded that the renal action of HKA2 is negligible. However, the kidney is expected to adapt to an intestinal leak of K⁺ by retaining more K⁺, to counterbalance fecal K⁺ losses. The absence of a renal phenotype in HKA2-null mice would have been characterized by a more important decrease in renal K⁺ excretion compared with wild-type mice, which do not exhibit this marked intestinal K⁺ loss. We propose that the inability of these mice to compensate for the intestinal K⁺ leak is actually the signature of the absence of HKA2. To prove this hypothesis, it would be necessary to investigate the phenotype of colon-specific HKA2-null mice. These mice should exhibit an intestinal K⁺ loss similar to that observed in the whole HKA2-null mice but should be able to compensate for this loss by stimulating renal HKA2 and significantly decreasing urinary K⁺ excretion.

**Beyond the Kidney**

HKA2 is broadly expressed, at least at the mRNA level, in many different tissues. In addition to the colon and kidney, HKA2 has been found in the brain (cortex, hypothalamus, choroids plexus), ovaries, uterus, vagina, placenta, epididymis, penis, lung, pancreas, skin (22, 70), and prostate (66). Among all these tissues, the role of HKA2 has been investigated only in the prostate and pancreas.

In rat and mouse prostate, HKA2 presents a lobe-specific expression. It is absent in the ventral part but highly expressed in the lateral and dorsal lobes and in the coagulating gland where it exhibits an apical localization (66). In normal human prostate, HKA2 is expressed at the apical side of the epithelial cells (81). In benign prostate hyperplasia and prostate cancer, the HKA2 protein level is increased compared with healthy tissue but displays an altered cellular localization. By studying the prostate phenotype of HKA2-null mice, Pestov et al. (68) established that HKA2 is responsible for the acidification of the anterior prostate secretion: in the absence of HKA2, its pH is 0.8 unit higher. This effect is not believed to impact the pH of the semen but could be related to the ability of rodent semen to build a plug after copulation.

The pancreas produces and secretes a bicarbonate-rich fluid that conveys digestive hormones toward the intestine. The production of such a fluid requires pancreatic duct cells to be equipped with H⁺ and HCO₃⁻ transporters. Recently, Novak et al. (64) identified both HKA (HKA1 and HKA2) in rat pancreatic ducts. An ammonium pulse in isolated pancreatic ducts, under conditions where most of the classic transporters (Na/H exchangers, Na-HCO₃ cotransporters, and anion exchangers 1) were inhibited, revealed Sch28080-sensitive intracellular pH recovery. In this study, HKA2 was localized at the apical and lateral sides of cells, which suggests that it may play a role in extruding protons generated by carbonic anhydrase activity, particularly in the serosa. HKA (1 or 2 or both) contributes significantly to fluid secretion in the pancreatic duct since HKA inhibitors drastically reduce the volume of secretin-stimulated fluid. Here again, further experiments are necessary to clearly delineate the contribution of HKA1 and HKA2 to these processes.

Unfortunately, despite consistent evidence of the presence of HKA2 in tissues such as skin or brain, its specific function there remains unknown.

**Conclusion**

A rapid glance at the number of reports published since 1995 on NKA (~13,000 papers), HKA1 (1,900 papers), and HKA2 (barely 200 papers) reveals without ambiguity that this last transporter has aroused far less interest than its two close cousins. The variability of its intrinsic characteristics, which depend on species and tissues, the lack of specific and widespread tools to measure its expression, and the difficulties in measuring its activity have probably contributed to this lack of interest. However, the findings summarized in this review demonstrate that HKA2 is a physiologically relevant ion transporter. We believe that much remains to be understood regarding HKA2 and that efforts to explore this “terra incognita” could potentially generate new surprises.

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