

# P2C-Type ATPases and Their Regulation

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**Abstract** P2C-type ATPases are a subfamily of P-type ATPases comprising  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{H}^+/\text{K}^+$ -ATPase.  $\text{Na}^+/\text{K}^+$ -ATPase is ubiquitously expressed and has been implicated in several neurological diseases, whereas  $\text{H}^+/\text{K}^+$ -ATPase is found principally in the colon, stomach, and kidney. Both ATPases have two subunits,  $\alpha$  and  $\beta$ , but  $\text{Na}^+/\text{K}^+$ -ATPase also has a regulatory subunit called FXYD, which has an important role in cancer. The most important functions of these ATPases are homeostasis, potassium regulation, and maintaining a gradient in different cell types, like epithelial cells.  $\text{Na}^+/\text{K}^+$ -ATPase has become a center of attention ever since it was proposed that it might play a crucial role in neurological disorders such as bipolar disorder, mania, depression, familial hemiplegic migraine, rapid-onset dystonia parkinsonism,

chronic stress, epileptogenesis, and Alzheimer's disease. On the other hand, it has been reported that lithium could have a neuroprotective effect against ouabain, which is the best known  $\text{Na}^+/\text{K}^+$ -ATPase inhibitor, but and high concentrations of lithium could affect negatively  $\text{H}^+/\text{K}^+$ -ATPase activity, that has a key role in regulating acidosis and potassium deficiencies. Finally, potassium homeostasis regulation is composed of two main mechanisms, extrarenal and renal. Extrarenal mechanism controls plasma levels, shifting potassium from the extracellular to the intracellular, whereas renal mechanism concerns with body balance and is influenced by potassium intake and its urinary excretion. In this article, we discuss the functions, isoforms, and localization of P2C-type ATPases, describe some of their modulators, and discuss their implications in some diseases.

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## Introduction

P2-type constitute a family of ATPases consisting of  $\text{Ca}^{2+}$ -ATPases (P2A) from sarcoplasmic reticulum (SERCA) and plasma membrane; calmodulin-binding  $\text{Ca}^{2+}$ -ATPase (P2B);  $\text{H}^+/\text{K}^+$ -ATPases from stomach, colon, and kidney; and  $\text{Na}^+/\text{K}^+$ -ATPase (P2C). The family name is related to a transient phosphorylation of an aspartyl residue during cations' transport [1], such as  $\text{Cu}^{2+}$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{H}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cd}^{2+}$ , and  $\text{Na}^+$ , for which these proteins are responsible of. The P-type family members have the following five functional and structural domains: an actuator domain (A), a nucleotide binding domain (N), a phosphorylation domain (P), a transport domain (T), and a class-specific binding domain (S). The first three domains are

cytoplasmic, and the other two are membrane-embedded domains. It has been reported that  $\text{Na}^+/\text{K}^+$ -ATPase and SERCA sequence structures are 30 % identical and 65 % similar [2], this is the reason why SERCA model was used for  $\text{Na}^+/\text{K}^+$ -ATPase studies until it was crystalized. Also, both proteins have an  $\alpha$  subunit, with 10 transmembrane spans, but  $\text{Na}^+/\text{K}^+$ -ATPase differs in having also  $\beta$  and  $\gamma$  isoforms, with 12 transmembrane spans, so two subunits should affect the  $\alpha$  subunit conformation of  $\text{Na}^+/\text{K}^+$ -ATPase [3, 4]. In this review, we concentrate only in P2C-ATPases:  $\text{H}^+/\text{K}^+$ -ATPases and  $\text{Na}^+/\text{K}^+$ -ATPase. The carboxy-terminal domain is essential for the correct assembly and function of both  $\text{H}^+/\text{K}^+$ -ATPases and  $\text{Na}^+/\text{K}^+$ -ATPase; if it is not present, the affinity for  $\text{Na}^+$  and  $\text{H}^+$  is significantly affected [5, 6]. These enzymes are composed of  $\alpha$  and  $\beta$  subunits, but  $\text{Na}^+/\text{K}^+$ -ATPase has a third subunit called FXYD too [7]. These proteins have different structure since  $\text{Na}^+/\text{K}^+$ -ATPase is a monomeric heterodimer, which is essential for its catalytic activity and cation translocation, whereas  $\text{H}^+/\text{K}^+$ -ATPase is a dimeric heterodimer [8].

$\text{Na}^+/\text{K}^+$ -ATPase and  $\text{H}^+/\text{K}^+$ -ATPase are proteins that actively transport either three molecules of  $\text{Na}^+$  and two molecules of  $\text{K}^+$  or two molecules of  $\text{H}^+$  and two molecules of  $\text{K}^+$ , respectively, against a concentration gradient. This transport maintains ionic cellular homeostasis, keeps the extracellular concentrations of  $\text{Na}^+$  and  $\text{H}^+$  low and the intracellular concentration of  $\text{K}^+$  high.

$\text{Na}^+/\text{K}^+$ -ATPase uses almost 30 % of the ATP available to the cell and has important roles that include the following: (1) maintaining ionic balance, (2) providing energy through the coupled transport of nutrients, (3) re-establishing the ionic gradient after an action potential in neurons, (4) helping to maintain a gradient in epithelial cells, and (5) activating lymphocytes [9].

The  $\text{H}^+/\text{K}^+$ -ATPase also uses ATP, but its regulator role is related to gastric processes and maintaining a normal  $\text{K}^+$  concentration in organs such as the stomach, colon, and kidney. In the stomach, it regulates the HCl concentration, but in altered states, it can function as a  $\text{Na}^+/\text{K}^+$ -ATPase and as a  $\text{Ca}^{2+}$ -ATPase in parietal cells [10]; in the kidney,  $\text{H}^+/\text{K}^+$ -ATPase has a key role in states of altered  $\text{K}^+$  balance, such as hypokalemia, and in the colon, it is also involved in regulating  $\text{H}^+$  concentration, and it functions in  $\text{K}^+$  absorption [11]. Gastric  $\text{H}^+/\text{K}^+$ -ATPase, it can be stimulated through to the histamine H2 receptor [12].

$\text{Na}^+/\text{K}^+$ -ATPase and  $\text{H}^+/\text{K}^+$ -ATPase bound ATP, but there are alternatives: one is through extracellular signal-regulated kinase (ERK) [13], protein kinase C (PKC), or protein kinase A (PKA) [14–16] and another, which is not well understood, is through the insulin receptor [17]. Through PKC and PKA pathways, the phosphorylation site can stimulate or diminish  $\text{Na}^+/\text{K}^+$ -ATPase or  $\text{H}^+/\text{K}^+$ -ATPase activity [14, 16], and ERK activity could be activated by insulin stimulating  $\text{Na}^+/\text{K}^+$ -ATPase activity [13].

$\text{Na}^+/\text{K}^+$ -ATPase can function as a signal transducer, through calcium signaling or Src signaling pathways [18]. The Src pathway is present in the kidney and has an important role in renal function; a decline in the protein expression of  $\text{Na}^+/\text{K}^+$ -ATPase is sufficient to raise Src activity, and this interaction may depend on the conformational state of the ATPase [19].

$\text{Na}^+/\text{K}^+$ -ATPase has endogenous modulators whose expression and activity are regulated by hormones including steroids, thyroid hormones, peptide hormones, and catecholamines. These have different effects; some regulate its gene expression, and others regulate its activity [20]. Exogenous modulators include cardiotonic steroids, such as ouabain and bufadienolides [21], as well as lithium, which has different effects on the protein.

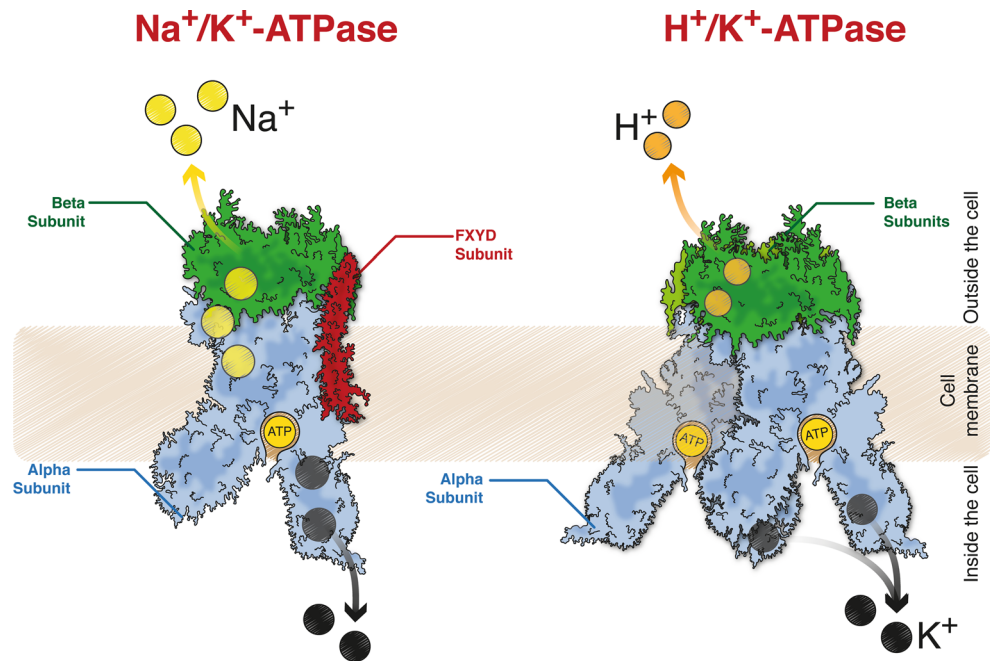
$\text{H}^+/\text{K}^+$ -ATPase also has inhibitors, such as benzimidazoles, that are commercially known as esomeprazole and proton pump inhibitors like rabeprazole, omeprazole, lansoprazole, and pantoprazole. Other  $\text{H}^+/\text{K}^+$ -ATPase inhibitors are  $\text{K}^+$ -competitive reagents, named either acid pump antagonist (APA) or potassium competitive acid blocker (P-CAB) class, which are imidazo-pyridines that are known to react on the outside surface of the pump in competition with potassium ions [22]. Lithium is a cation reported to activate  $\text{Na}^+/\text{K}^+$ -ATPase [23], that could inhibit  $\text{H}^+/\text{K}^+$ -ATPase in the cortical collecting tubule in the kidney, which can trigger a severe metabolic acidosis [24].

### Isoforms and Localization

P2C-type ATPases are conformed by  $\alpha$  and  $\beta$  subunits, and  $\text{Na}^+/\text{K}^+$ -ATPase has a third subunit called  $\gamma$  or FXYD.  $\alpha$  subunit contains the catalytic site, and in  $\text{Na}^+/\text{K}^+$ -ATPase and one type of  $\text{H}^+/\text{K}^+$ -ATPase, is the site of ouabain binding. Four isoforms for the  $\alpha$  subunit of  $\text{Na}^+/\text{K}^+$ -ATPase have been reported:  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 4$ . These isoforms occur in different tissues with the fourth found only in sperm.

$\text{H}^+/\text{K}^+$ -ATPase has only two isoforms,  $\alpha 1$  and  $\alpha 2$ , and one gastric and the other non-gastric. The  $\alpha$  subunits of  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{H}^+/\text{K}^+$ -ATPase have close to 60 % similarity [25].  $\beta$  subunit is a minor subunit that contributes to the  $\text{K}^+$  affinity in its binding site and may contribute to the trafficking and delivery of  $\text{Na}^+/\text{K}^+$ -ATPase to the cell membrane [26]. The  $\beta$  subunit also contributes to the function of the  $\alpha$  subunit.  $\text{Na}^+/\text{K}^+$ -ATPase has three  $\beta$  isoforms,  $\beta 1$ ,  $\beta 2$ , and  $\beta 3$ , whereas only one  $\beta$  subunit, called  $\beta\text{HK}$ , has been reported for  $\text{H}^+/\text{K}^+$ -ATPase. The third subunit of  $\text{Na}^+/\text{K}^+$ -ATPase, named FXYD, is also a regulatory unit and contributes to the affinity of  $\text{Na}^+$  to its binding site; there are seven different isoforms, FXYD1 to FXYD7 [27]. Figure 1 depicts representations of  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{H}^+/\text{K}^+$ -ATPase.

**Fig. 1** Conformation of P2C-type ATPase.  $\text{Na}^+/\text{K}^+$ -ATPase is composed by three subunits,  $\alpha$ ,  $\beta$ , and FXYP. Only  $\alpha$  subunit is the catalytic one and is the place where ion exchange occurs, in this case is three intracellular  $\text{Na}^+$  for two extracellular  $\text{K}^+$ .  $\text{H}^+/\text{K}^+$ -ATPase is a dimer composed of two subunits,  $\alpha$  and  $\beta$ , and  $\alpha$  is the catalytic subunit too; this enzyme exchanges one intracellular  $\text{H}^+$  for one extracellular  $\text{H}^+$ , but how it works as a dimer, the exchange is  $2\text{H}^+$  by  $2\text{K}^+$ .  $\beta$  and FXYP subunits are to stabilize the ion binding and the activity of the enzyme



### $\text{Na}^+/\text{K}^+$ -ATPase

The  $\alpha$  subunit for  $\text{Na}^+/\text{K}^+$ -ATPase, molecular weight of  $\approx 110$  kDa, contains the catalytic site and is where  $\text{Na}^+$  and  $\text{K}^+$  are exchanged. This exchange occurs against the concentration gradient. The first step occurs when three intracellular  $\text{Na}^+$  ions bound to the enzyme (E1) and ATP phosphorylates the  $\alpha$  subunit to E1P, first releasing one sodium ion into the extracellular space, then releasing the other two (E2P). In this state, the enzyme is phosphorylated and, when no ion is present, can bound ouabain. In the absence of ouabain, two extracellular  $\text{K}^+$  ions bound to the  $\alpha$  subunit (E2P), the phosphorus is released (E2), and finally, potassium enters to the cytoplasm (E1), and the protein can start the cycle again [28].

Only three of the four  $\alpha$  isoforms were originally identified [29]. The  $\alpha 4$  isoform [30], which is found only in male gametes [31], controls  $\text{Na}^+$  gradients and plays a role in  $\text{Ca}^{2+}$  and  $\text{H}^+$  homeostasis in cells. Sperm motility is dependent on pH balance and membrane excitement; therefore, regulation by  $\alpha 4$  may contribute to the motility of the sperm [32, 33]. The other isoforms are widely found in different tissues; for example, the  $\alpha 1$  isoform occurs in every tissue but principally in the kidney, which is considered a model for this isoform. The  $\alpha 2$  isoform is found in the brain, heart, and in skeletal muscle, and  $\alpha 3$  is also expressed in the heart and brain. An important role for the  $\alpha$  subunit in memory and mood is proposed; haploinsufficiency of  $\alpha 2$  and  $\alpha 3$  generate behavioral alterations [34], and a missense mutation in the  $\alpha 3$  isoform was found in a maniac-like mouse model [35].

The different affinities of these isoforms for ouabain, a  $\text{Na}^+/\text{K}^+$ -ATPase inhibitor, are also interesting. The  $\alpha 1$  subunit has a low affinity, whereas  $\alpha 2$  and  $\alpha 3$  have high affinities:

This is an important research subject because the  $\alpha 1$  isoform is present in  $\alpha$ -motoneurons and  $\alpha 3$  is present in  $\gamma$ -motoneurons [36]. The  $\alpha 2$  isoform has a more prominent role in regulating  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum of myocytes [37].

The  $\beta$  subunit for  $\text{Na}^+/\text{K}^+$ -ATPase, molecular weight of  $\approx 55$  kDa, is a glycoprotein that lacks a catalytic site and does not participate in ion exchange. Instead, it has regulatory activity that, in MDCK cells, is crucial for the correct maturation of the enzyme [38].  $\text{Na}^+/\text{K}^+$ -ATPase is resistant to non-ionic detergents when  $\alpha$  and  $\beta$  subunits are joined [39]. Inhibiting the glycosylation of the  $\beta$  subunit reduces the cellular abundance of both subunits, which implies a role in the expression of  $\text{Na}^+/\text{K}^+$ -ATPase on the cell [40].

There are three  $\beta$  isoforms of  $\beta$  subunit,  $\beta 1$ ,  $\beta 2$ , and  $\beta 3$ , where  $\beta 1$  is more expressed in the kidney (where it is part of the model for the  $\alpha 1$   $\beta 1$  isoform) and in organs related to homeostasis [26]. Normal glycosylation of this isoform is needed for the correct stability of adherent and tight junctions in mature epithelia [41]. The reported reduction of the  $\beta 2$  isoform in glioblastomas is interesting because this could be the factor that initiates invasion by these cells [42]. Inactivation of the  $\beta 3$  isoform can inactivate T and B lymphocytes, which is important for immune regulation [9].

Isoforms  $\alpha 1$ ,  $\alpha 2$ , and  $\alpha 3$  are present in the brain, where they have different expression profiles:  $\alpha 1$  is ubiquitous,  $\alpha 2$  is predominant in astrocytes, and  $\alpha 3$  is principally present in neurons. The  $\beta 1$  and  $\beta 2$  isoforms occur in the brain, with astrocytes expressing  $\alpha 1$ ,  $\alpha 2$ , and  $\beta 2$  and neurons expressing  $\alpha 1$ ,  $\alpha 3$  and  $\beta 1$  and  $\beta 2$  [43].

In one study, inhibition of  $\alpha 3$  nearly eliminated the ability of hippocampal dendrites to restore the ionic concentration of

$\text{Na}^+$  [44], and animals with  $\alpha 3$  haploinsufficiency had difficulties finding the platform in the Morris Water Maze test, even when it was removed, indicating an impaired short- and long-term memory [34]. In  $\alpha 3$  haploinsufficient mice, the NR1 subunit of the NMDA receptor is downregulated [45].

The additional regulatory protein, FXYD, is present in seven isoforms: FXYD1 or phospholemman; FXYD2 or  $\gamma$  subunit; FXYD3 or mammary tumor marker Mat-8; FXYD4 or corticosteroid hormone-induced factor CHIF; FXYD5 or related to ion channel RIC or dysadherin; FXYD6 or phosphohippolin; and FXYD7 [46].  $\text{Na}^+/\text{K}^+$ -ATPase might be a target for anticancer treatment because FXYD protein, e.g., FXYD3, is a cancer-related protein [47].

### $\text{H}^+/\text{K}^+$ -ATPase

The  $\alpha$  subunit of  $\text{H}^+/\text{K}^+$ -ATPase, that has a molecular weight close to 100 kDa, is conformed by 10 transmembrane domains and has two isoforms [25]. It contains the catalytic domain of the protein to which  $\text{H}^+$  binds, but, interestingly, this ATPase also needs a molecule of  $\text{Mg}^{2+}$  for an entire  $\text{H}^+/\text{K}^+$  exchange cycle. In the presence of  $\text{Mg}^{2+}$ , the enzyme binds ATP, then one  $\text{H}^+$  is added and ADP detaches, leaving a phosphate bound to the enzyme. Next, with the anion still in the ATPase,  $\text{H}^+$  is exchanged for  $\text{K}^+$ ; later, the anion is liberated, and  $\text{K}^+$  can then be dissociated. Finally, the state in which  $\text{H}^+/\text{K}^+$ -ATPase is bound to  $\text{Mg}^{2+}$  is restored and the cycle can start again [11, 12, 28]. High  $\text{K}^+$  concentrations are needed for some functions of  $\text{H}^+/\text{K}^+$ -ATPase; for example, during fetal development, a  $\text{HK}\alpha_2$ -null mice may have problems with placental perfusion or even partial abortion. These may result from failure of compartment volume adjustment, most likely because of a high plasma  $\text{K}^+$  concentration [48]. Both benign prostate hyperplasia and prostatic tumor tissue had increased non-gastric  $\text{H}^+/\text{K}^+$ -ATPase compared with normal prostate cells, not only in the basal cells and membrane but also along the epithelium and cytosol [49]. The colonic  $\text{H}^+/\text{K}^+$ -ATPase acts as an  $\text{Na}^+/\text{K}^+$ -ATPase in *Xenopus leavis* oocytes because its function is devoted to  $\text{K}^+$  transport [50], and it can also transport  $\text{NH}_4^+$  in rat distal colon [51]. Most recently, it was reported to function as a  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{Ca}^{2+}$ -ATPase in parietal cells during altered pH states [10].

Two isoforms of  $\alpha$  subunits exist: the gastric or  $\text{HK}\alpha_1$   $\text{H}^+/\text{K}^+$ -ATPase and the non-gastric or colonic  $\text{H}^+/\text{K}^+$ -ATPase or  $\text{HK}\alpha_2$   $\text{H}^+/\text{K}^+$ -ATPase [52]. The  $\text{HK}\alpha_2$  gene, however, produces three alternative transcripts.  $\text{HK}\alpha_{2(a)}$  is found in the colonic  $\text{H}^+/\text{K}^+$ -ATPase,  $\text{HK}\alpha_{2(b)}$  is reported in the rat and has been observed in kidney, and  $\text{HK}\alpha_{2(c)}$  has been reported in rabbit and rat but may not be found in humans [53]. Omeprazole is a specific modulator of  $\text{H}^+/\text{K}^+$ -ATPase [54], and it binds in the  $\alpha$  subunit during acid transporter conditions [12]. Ouabain can bind to the  $\text{HK}\alpha_2$   $\text{H}^+/\text{K}^+$ -ATPase, but not

the  $\text{HK}\alpha_1$   $\text{H}^+/\text{K}^+$ -ATPase, of guinea pig colon [55]; this is an interesting finding because ouabain is a specific modulator of  $\text{Na}^+/\text{K}^+$ -ATPase.

Four isoforms of  $\beta$  subunit,  $\text{HK}\beta$ ,  $\text{NaK}\beta_1$ ,  $\text{NaK}\beta_2$ , and  $\text{NaK}\beta_3$ , with molecular weights of  $\approx 35$ – $55$  kDa [52], can pair with the  $\text{HK}\alpha$  subunits.  $\text{HK}\alpha_1$  is known to pair with  $\text{HK}\beta$  [53], but apparently, the  $\beta_1$  subunit of  $\text{Na}^+/\text{K}^+$ -ATPase pairs with  $\text{HK}\alpha_2$  [56].  $\beta$  subunit lacks of a catalytic site, but it is crucial for  $\text{H}^+/\text{K}^+$ -ATPase function, localization on the plasma membrane, and for  $\text{K}^+$  binding to the  $\alpha$  subunit, which is similar to  $\text{Na}^+/\text{K}^+$ -ATPase.

### $\text{X}^+/\text{K}^+$ -ATPases and Their Modulation

#### $\text{H}^+/\text{K}^+$ -ATPases

$\text{H}^+/\text{K}^+$ -ATPases have specific inhibitors that are principally divided into two groups: the cardiac glycosides and the non-cardiac glycosides which are included substituted benzimidazoles, potassium-competitive acid blockers, and vanadate [22] (Table 1). The most common substituted benzimidazole known is omeprazole, and it inhibits proton exchange, thereby reducing stomach acidity and the risk for gastric ulcer. Omeprazole derivatives that share the same action but differ chemically include timoprazole, esomeprazole, rabeprazole, pantoprazole, and lansoprazole [57]. Omeprazole sulfide is a metabolite of omeprazole found in plasma that is eliminated in the urine and feces; it competes with the activated form of omeprazole to reduce its inhibitory effect. This metabolite was used to locate the site of omeprazole binding, and the sulfur moiety has a nucleophilic attraction to Cys813 in the transmembrane (TM) helices TM1 and TM2 [58].

In the potassium-competitive acid blocker class, SCH28080 inhibits ATPase function when potassium is not in the enzyme; other inhibitors in this family are TAK.438, AZD0865, and revaprazan [22]. Vanadate binds to all P-type ATPases in the phosphate binding site and irreversibly inhibits them. The binding affinity of vanadate is higher in the E2 of

**Table 1** Examples of some modulators for  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{H}^+/\text{K}^+$ -ATPase

Modulators	$\text{Na}^+/\text{K}^+$ -ATPase	$\text{H}^+/\text{K}^+$ -ATPase
Most known	Ouabain	Omeprazole
Competitor (drug)	Chlorpromazine, chloroquine	SCH28080, TAK.438, AZD0865 and revaprazan
Irreversible inhibitor (drug)	Vanadate	Vanadate
Endogenous	Bufadienolides, ouabain	Ouabain, aldosterone, histamine
Others	Lithium	Lithium



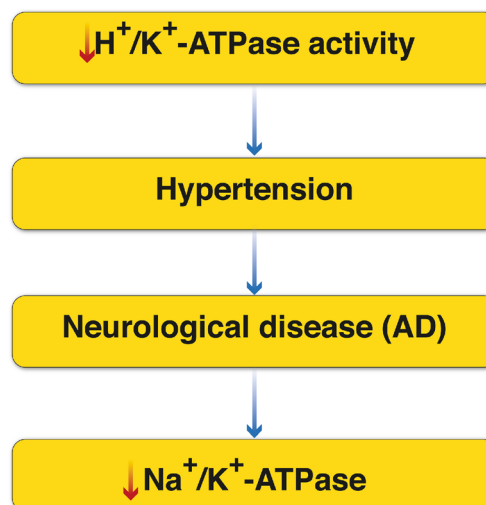
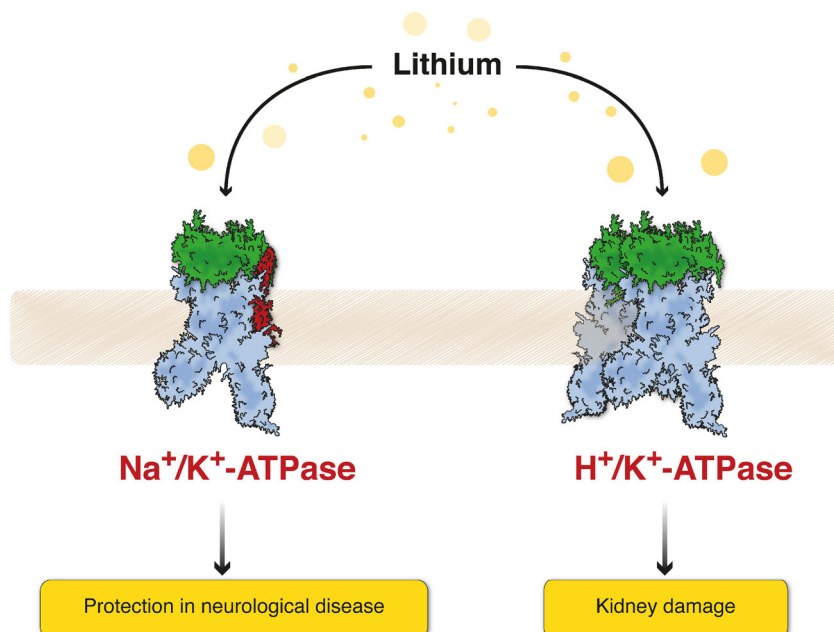
conformation, that is, after ATP hydrolysis [53]. Finally, of the cardiac glycosides, the best known is ouabain, an inhibitor of  $\text{Na}^+/\text{K}^+$ -ATPase. Its function in  $\text{H}^+/\text{K}^+$ -ATPase is controversial because the degree of enzyme inhibition is variable, depending on the cell type and expression system used. For example,  $\text{HK}\alpha_2$  is insensitive to ouabain in HEK-293 cells [59] but sensitive when it was expressed on *Xenopus oocytes* [60]. Lithium, a well-characterized cation that is used to treat psychiatric conditions such as bipolar disorder, is known to cause kidney failure at high concentrations. Significantly, lithium can decrease  $\text{H}^+/\text{K}^+$ -ATPase activity in cortical collecting tubules but not in medullary collecting tubules [24].

### $\text{Na}^+/\text{K}^+$ -ATPase

$\text{Na}^+/\text{K}^+$ -ATPase has several endogenous modulators and non-endogenous modulators. Endogenous modulators include the following: steroidal hormones, endogenous ouabain, non-steroidal peptide hormones, and catecholamines. Non-endogenous modulators include the following: digitalis (ouabain-like cardiotoxic steroid modulators), bufadienolides [21], competitors such as chlorpromazine and chloroquine [61], and lithium (Table 1). From all these, we focused on ouabain, which is the most common inhibitor used, and on lithium, which is a very important monovalent cation that is used for treatment of bipolar disorder. In particular, lithium has been observed to have protective effects against ouabain, which is really interesting to understand how lithium could modulate  $\text{Na}^+/\text{K}^+$ -ATPase (Fig. 2 and 3).

Ouabain is an endogenous cardiac glycoside that inhibit  $\text{Na}^+/\text{K}^+$ -ATPase, is thought that is able to bind the  $\alpha$  subunit. It is interesting to note that, in cardiotoxic steroids, ouabain's

**Fig. 2** Lithium and its role in P2C-type ATPase. Lithium is a well-known cation that is used for neurological diseases like *bipolar disease* and *depression*, and lately, it has been reported a possible role in *Alzheimer's disease*, and it has been seen that it stimulates  $\text{Na}^+/\text{K}^+$ -ATPase activity at the same time that at this concentrations, it affects  $\text{H}^+/\text{K}^+$ -ATPase activity in kidney and can produce other illness. Now would be really interesting to find the correct lithium concentration that could enhance  $\text{Na}^+/\text{K}^+$ -ATPase and not affect  $\text{H}^+/\text{K}^+$ -ATPase to be able to use it to prevent or stabilize aging affections as Alzheimer's disease



**Fig. 3** Possible relation between  $\text{Na}^+/\text{K}^+$ -ATPase and  $\text{H}^+/\text{K}^+$ -ATPase. Since  $\text{H}^+/\text{K}^+$ -ATPase has a role in potassium excretion, if this enzyme fails could be possible to have a role in hypertension, an illness that is well-known to be a risk factor in neurological diseases as *dementia* and *Alzheimer's disease (AD)* and is acknowledged that  $\text{Na}^+/\text{K}^+$ -ATPase activity is reduced in these diseases

hydroxyl group position is very important because it has been reported that this position might be involved in the inhibitory activity with the enzyme [62]. As previously mentioned, inhibition could take place when  $\text{Na}^+/\text{K}^+$ -ATPase is in E2P state, after the first  $\text{Na}^+$  release, which could allow ouabain binding. In addition, it has been observed that inhibition site ouabain might be in the TM helices TM1, TM2, TM4, and TM6 of  $\text{Na}^+/\text{K}^+$ -ATPase [63]. However, in another report, results showed that Gln111 and Asn 122 in TM1 and TM2 are key amino acids that may be involved in the cardiac steroid-enzyme interaction [58]. Bonding can occur in two steps:

First, the junction bonding occurs, and then, a sugar residue interaction occurs at a slower rate [62].

Ouabain can inhibit different  $\alpha$  isoforms depending on its concentration, because it has different affinities as mentioned above, where the  $\alpha 1$  subunit has low affinity to ouabain and  $\alpha 2$  and  $\alpha 3$  have high affinity. Ouabain also has different effects, including promotes apoptosis and necrosis at high concentrations [64], and it is involved in neurological diseases. In fact, it has been reported that ouabain is involved in mood disorders such as depression [65], and, interestingly, ouabain is used to make mania rat models, which is achieved with an intracerebroventricular administration of ouabain, and it was reported that lithium can prevent ouabain's effect [66].

Notably, because  $\text{Na}^+/\text{K}^+$ -ATPase could be a potential target for the treatment of neurological diseases, it is important to identify which isoforms are involved in different diseases because it has been observed that different haploinsufficient mice have different deficits [34]. Mice deficient in the  $\alpha 3$  isoform have more difficulties in the Morris water maze test, whereas the mice deficient in  $\alpha 2$  have less entries in an open field area and do not perform well in the elevated zero maze test. In this context, it is very important to remember that  $\alpha 3$  is an isoform found in neurons,  $\alpha 2$  in astrocytes, and  $\alpha 1$  is ubiquitous. Therefore,  $\alpha 3$  issues are related to memory deficits, and  $\alpha 2$  issues could be related to the homeostatic stabilization.

It has been reported that insulin promotes translocation of  $\text{Na}^+/\text{K}^+$ -ATPase [67], which is preventing digoxin toxicity [68], and it has also been observed that insulin reduces  $\text{Na}^+/\text{K}^+$ -ATPase's activity in diabetic liver [69]. This translocation induced by insulin is interesting because in diabetes, it has been observed a cognitive deficiency, as demonstrated by the Morris water maze test [70].

Lithium is a monovalent cation that it is known to replace  $\text{Na}^+$  in protein, but it protects  $\text{Na}^+/\text{K}^+$ -ATPase against inhibition by ouabain. However, the mechanisms of action are not understood. Lithium protects human neuroblastoma SH-SY5Y cells from ouabain-induced damage [71], and lithium delays ouabain-stimulated multiple spikes [72]. Lithium also enhances  $\text{Na}^+/\text{K}^+$ -ATPase's activity in animal models of bipolar disorder [73] and depression [74].

The main concern with lithium is that its concentration in the human body should be closely monitored because it can cause problems such as hypothyroidism, weight gain, an increased risk of reduced urinary concentrating ability, hyperparathyroidism, and, in some cases, a significant reduction in renal function [75]. Nephrotoxicity can be observed after 2 months of lithium treatment, and lithium can also lead to diabetes insipidus [76]. A recent review reported that a safe long-term treatment dose of lithium is up to 0.8 mmol/L [77], and it has also been reported that lithium treatment of 40 nmol in humans for 15 months did not present any problems in kidney nor thyroid dysfunction [78], which indicates that this

dose may be an appropriate limit for human treatment. This is an important point because lithium treatment has been shown to diminish Alzheimer's disease (AD) risk in patients with bipolar disease [79] and stabilized cognitive impairment in Alzheimer's disease [78].

### $\text{Na}^+/\text{K}^+$ ATPase and Neurological Diseases

Several studies have related  $\text{Na}^+/\text{K}^+$ -ATPase to neurological disorders. In this paper, we discuss several examples of this relationship, and then, we focus on mood disorders and Alzheimer's disease. A study has suggested that  $\text{Na}^+/\text{K}^+$ -ATPase and endogenous digitalis-like compounds are involved in mood disorders, similar to that reported in an animal model of depression [65]. For bipolar disorder, a genetic variation related to the  $\alpha$  isoform has been observed [80], and a mania-like behavior has been reported in *Myshkin* mice that are heterozygous for the  $\alpha 3$  isoform [35]. Familial hemiplegic migraine has been related to deficiencies in and mutations of the  $\alpha 2$  isoform [81, 82], and mice with the same isoform deficiency have shown similar behavior [82]. In rapid-onset dystonic parkinsonism (RPD), six missense mutations have been found in the  $\alpha 3$  isoform of  $\text{Na}^+/\text{K}^+$ -ATPase (ATP1A3) [81, 83] in seven unrelated families [84]. The same mutation in the  $\alpha 3$  subunit has also been reported in two cases of RPD [85], and it has also been proposed that a possible mutation in D923N could be involved in this illness [86]. In other illnesses, such as alternating hemiplegia of childhood, patients have shown *de novo* mutations in ATP1A3, which do not affect the protein expression but do cause a reduction in the ATPase activity [87]. In Alzheimer's disease that is principally related with aging,  $\text{Na}^+/\text{K}^+$ -ATPase activity is lower [88]. It is thought that lithium, a treatment for bipolar disorders, could be acting through  $\text{Na}^+/\text{K}^+$ -ATPase to repair neurological damage because an improvement in the activity of  $\text{Na}^+/\text{K}^+$ -ATPase has been observed in patients with Alzheimer's disease [78].

By contrast, studies have shown a relationship between neurological diseases and a reduction in intracellular  $\text{Na}^+$  that could be associated with a decreased affinity of  $\text{Na}^+$  to this ATPase. This is particularly important because  $[\text{Na}^+]_i$  is reduced when  $\text{Na}^+$  affinity increases, and  $[\text{Na}^+]_i$  rises when  $\text{Na}^+$  affinity is reduced, suggesting that affinity itself could be regulating  $[\text{Na}^+]_i$  [89].

### $\text{Na}^+/\text{K}^+$ -ATPase and Mood States

$\text{Na}^+/\text{K}^+$  ATPase is altered in mood-related illnesses such as depression and bipolar disease. It has also been shown that early life experiences, e.g., neonatal handling in rats, have significant effects on  $\text{Na}^+/\text{K}^+$ -ATPase activity, which in rats results in a model of depression during adulthood [90]. In

bipolar disease, there is evidence that  $\text{Na}^+/\text{K}^+$ -ATPase activity is significantly reduced together with an increase in lipid peroxidation [73], and two single nucleotide polymorphisms (SNPs) are significantly associated with this illness. Both SNPs are related to the  $\alpha 3$  isoform, and one SNP is related to the  $\alpha 1$  isoform [80].

Bipolar disease is known for episodes of mania and episodes of depression. It has been observed that intracerebroventricular administration of ouabain, which decreases  $\text{Na}^+/\text{K}^+$ -ATPase activity, can produce mania episodes, detectable by motoric hyperactivity [66]. A mania-like behavior can also be produced in mice heterozygous for the  $\alpha 3$  isoform of  $\text{Na}^+/\text{K}^+$ -ATPase, in *Myshkin* mice (*Atp1a3* *Myk*<sup>+/+</sup>; *Myk*<sup>+/+</sup>) [35]. In double transgenic mice that are heterozygous for the  $\alpha 3$  subunit of  $\text{Na}^+/\text{K}^+$ -ATPase (*Myk*<sup>+/+</sup>) and agrin (*Agrn*<sup>+/+</sup>), a reduced mania-like behavior has been observed, which could indicate an interesting relation between agrin and  $\text{Na}^+/\text{K}^+$ -ATPase that could function as a target for mood stabilizers [91].

#### Does $\text{Na}^+/\text{K}^+$ ATPase Have a Role in Alzheimer's Disease?

In different models of AD, the activity of  $\text{Na}^+/\text{K}^+$ -ATPase is affected by different inhibitors. First, a 40 % decrease in ouabain binding in the brains of patients with AD was described [92]; then, it was found that amyloid  $\beta$ -peptide impairs  $\text{Na}^+/\text{K}^+$ -ATPase [93] and that the activity of  $\text{Na}^+/\text{K}^+$ -ATPase was reduced in the brains of AD patients [88, 94]. A review also reported that  $\text{Na}^+/\text{K}^+$ -ATPase has a function in aging and energy balance [95]. In oocytes of *X. leavis*, an inhibition of  $\text{Na}^+/\text{K}^+$ -ATPase activity by  $\beta$ -amyloid was observed [96], and in the APP+PS1 double transgenic mice model of AD, a reduced activity of  $\text{Na}^+/\text{K}^+$ -ATPase was observed, particularly in the areas bordering the  $\beta$ -amyloid plaques [97]. Moreover, Zhang et al. proposed that  $\text{Na}^+/\text{K}^+$ -ATPase is an important factor to account for in the investigation of neuroprotection in AD [98], and, more recently, it was shown that microinjections of  $\beta$ -amyloid into the rat brain produces an impairment in  $\text{Na}^+/\text{K}^+$ -ATPase activity [99].

#### $\text{H}^+/\text{K}^+$ -ATPases, Kidney Function, and Regulation

In the kidney, there are three isoforms of  $\text{H}^+/\text{K}^+$ -ATPases:  $\text{HK}\alpha_1$ ,  $\text{HK}\alpha_2$ , and  $\text{HK}\alpha_{2b}$ . These three enzymes are expressed in the collecting duct, which deals with the renal regulation of pH and potassium secretion;  $\text{HK}\alpha_1$  has also been reported on the ascending limb of Henle. It is likely that  $\text{HK}\alpha_1$  and  $\text{HK}\alpha_2$  may have different functions; thus,  $\text{HK}\alpha_1$  and  $\text{HK}\alpha_2$  are both upregulated during acidosis and cellular metabolism, whereas during cellular respiration, only the  $\text{HK}\alpha_1$  is upregulated.  $\text{HK}\alpha_2$  has increased expression during chronic hypokalemia in the renal medulla [100] [101]. During metabolic acidosis, it has been described an increase in enzyme

activity but without change in gene expression. During alkalosis, there is no agreement whether it is increased or decreased since there are studies reporting both. Interestingly, vasopressin and aldosterone can modulate proton secretion of  $\text{H}^+/\text{K}^+$ -ATPase and  $\text{H}^+$ -ATPase through the mineralocorticoid receptor and V1aR, and both aldosterone and vasopressin can also stimulate proton secretion [102].

By contrast, it is well known that urinary  $\text{K}^+$  excretion has a circadian profile, although the pathway involved is not elucidated.  $\text{HK}\alpha_2$  also has a circadian profile regulated by Nrf2, so this could be the protein responsible for the excretion of  $\text{K}^+$  in a circadian rhythm [103]. Furthermore, in one study, serum hypokalemia was reported in two women taking omeprazole, and the hypokalemia was reversed by administration of potassium supplements and discontinuation of the  $\text{H}^+/\text{K}^+$ -ATPase inhibitor [104].

#### Importance of Dietary Potassium Intake in Health and Disease

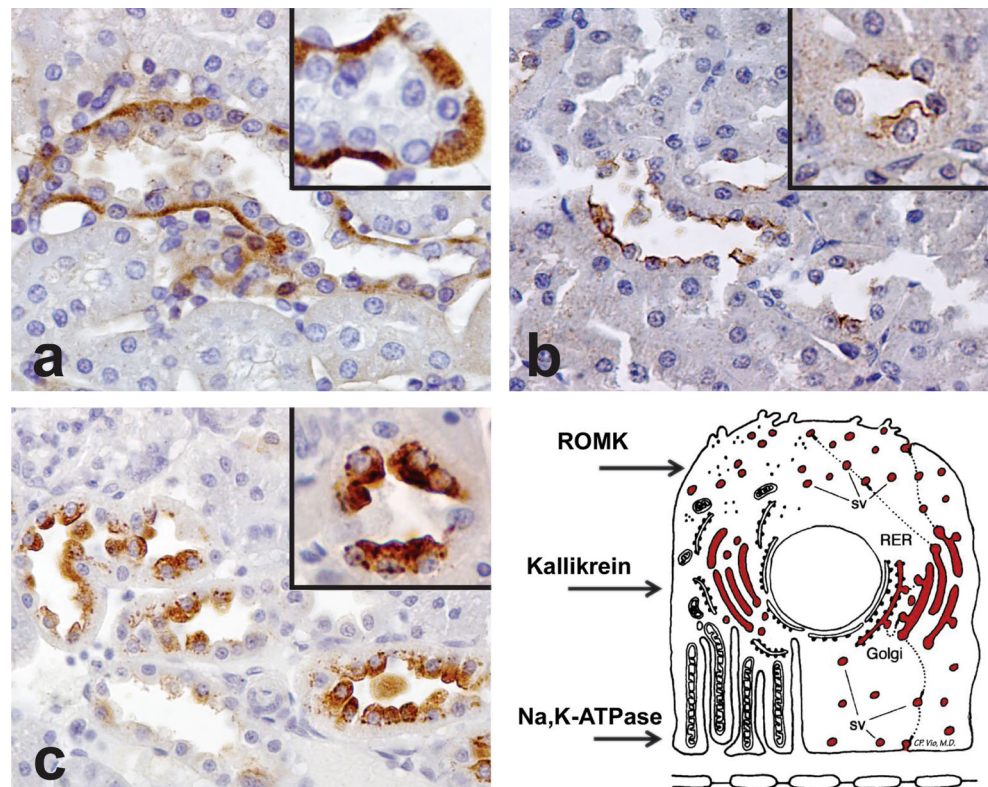
The regulation of potassium homeostasis is composed of two main mechanisms: extrarenal and renal. The extrarenal mechanism controls very tightly plasma levels shifting potassium from the extracellular to the intracellular, whereas the renal mechanism deals with the body balance and is largely influenced by the intake and urinary excretion.

In the kidney, the distal nephron is a main site for the regulation of potassium handling; in particular, the connecting tubule cells (CNTc) highly specialized on potassium secretion [105]. The CNTc contains  $\text{Na,K-ATPase}$  in the basolateral side and a potassium channel (ROMK) in the apical side, whereas the neighboring intercalated cells (Ic) display  $\text{H,K-ATPase}$  in the apical side (Figs. 4 and 5).

The CNTc is the site of origin of renal kallikrein [106]; this enzyme is the main component of the renal kallikrein-kinin system, a vasoactive system with vasodilatory and sodium excretory function, which is stimulated by increased potassium intake [105]. In response to a diet high in potassium, the CNTc cell hypertrophy increases the basolateral membrane and the  $\text{Na,K-ATPase}$  as a compensatory mechanism to secrete increased potassium intake. This mechanism is known as potassium adaptation and participates in the potassium balance. Along with cell hypertrophy, CNTc increased renal kallikrein synthesis and kallikrein system stimulation by high potassium diet increases the excretion of sodium and water with a net effect on decreasing high blood pressure [105]. This points to the kallikrein system as a protective vasoactive system against hypertension, renal diseases, and stroke. Moreover, previous studies from our group have demonstrated in hypertensive human potassium supplementation in the diet



**Fig. 4** Localization of Na,K-ATPase, H,K-ATPase, and kallikrein in the kidney. In the panel is shown the localization of Na,K-ATPase in the basolateral side of distal tubule cell (a), the localization of H,K-ATPase in the apical or luminal side of Intercalated cells (b), and the localization of kallikrein in the connecting tubule cells (c). The insets in each photograph display at higher magnification their subcellular distribution. A cartoon showing the distribution polarized distribution of Na,K-ATPase and ROMK in the CNTc is a key regulator of potassium and the site of origin of kallikrein

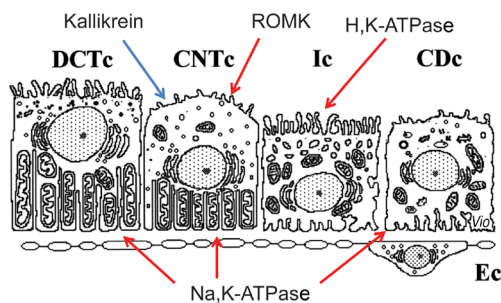


significantly increased kallikrein, urinary sodium excretion and lower blood pressure [107].

On the contrary, we have demonstrated that during hypokalemia, there is a reduction of renal kallikrein, and despite of the mechanism underlying the decreased kallikrein, an impaired kallikrein system contributed to salt sensitivity, hypertension, and renal damage [108].

The importance placed on the relevance of dietary potassium on health and disease [109, 110] is underscored compared with that placed on dietary sodium. Much effort has been placed on dietary sodium reduction on the control of several

diseases, but much less have been placed on the adequate dietary potassium intake. Despite that natural food contains 10–15 times more potassium than sodium, however, our intake has  $\approx 3$  times more sodium (186 mmol/day) than potassium (65 mmol/day) due to the main component of processed food in our diet. Moreover, although it is known for decades that adequate potassium diet is beneficial to health, particularly for the control of blood pressure, stroke, and kidney disease [109] which has recently been confirmed, there are few studies on biomedical bases explaining this beneficial effect, and the cellular and molecular mechanisms responsible for the beneficial effects of increased dietary potassium remain unknown [111], and they are subject on active research by our group.



**Fig. 5** Cell types of the distal nephron, a main site for regulation of potassium by the kidney. The distal nephron is composed of four different cell types: the distal convoluted tubule cell (*DCTc*), the connecting tubule cell (*CNTc*), the intercalated cell (*Ic*), and the collecting duct cell (*CDc*). Na,K-ATPase is located in the basolateral side of *DCTc*, *CNTc*, and *CDc*, whereas H,K-ATPase is located in the apical or luminal side of *Ic*. The *CNTc* contains Na,K-ATPase and ROMK and is a key regulator of potassium by the kidney and the site of origin of kallikrein

## Concluding Remarks

P2C-type ATPases have different but important functions and expression. Na<sup>+</sup>/K<sup>+</sup>-ATPase has an important function in potassium regulation in neurons and is able to re-establish the membrane potential of neurons to produce regenerative action potentials and restore neuronal communication. H<sup>+</sup>/K<sup>+</sup>-ATPase is focused on the kidney, stomach, and colon; it is crucial for potassium serum concentrations in the kidney and for re-establishing acid–base homeostasis in the stomach and colon. It would be very interesting to evaluate the impact that H<sup>+</sup>/K<sup>+</sup>-ATPase could have in hypertension because Na<sup>+</sup> and K<sup>+</sup> homeostasis are key factors in this disease. Moreover, Na<sup>+</sup>



also influences urinary  $K^+$  secretion [112]. Because hypertension is a very common illness induced by an unhealthy diet, altogether with aging are risk factors for Alzheimer's disease, another disease that might be influenced by a  $Na^+/K^+$ -ATPase deficient activity (Fig. 3). Finally, these two enzymes are more closely related than previously supposed, not only because of homology. It is possible that  $H^+/K^+$ -ATPase dysfunction in the maintenance of potassium homeostasis could affect neuronal activity and survival, as well as affect  $Na^+/K^+$ -ATPase activity.

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