FEBS Letters 584 (2010) 2589–2595

FEBS Letters
journal homepage: www.FEBSLetters.org

Review

Phosphorylation of the Na^+,K^+-ATPase and the H^+,K^+-ATPase

Hanne Poulsen [a,*], Preben Morth [a], Jan Egebjerg [b], Poul Nissen [a]

[a] Danish National Research Foundation, Centre for Membrane Pumps in Cells and Disease – PUMPKIN, Aarhus University, Dept. of Molecular Biology, Denmark
[b] H. Lundbeck A/S, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 9 April 2010
Accepted 13 April 2010
Available online 22 April 2010

Edited by Jan Rydström

Keywords:
Phosphorylation
Na^+,K^+-ATPase
H^+,K^+-ATPase
cAMP activated protein kinase
Protein kinase C

ABSTRACT

Phosphorylation is a widely used, reversible means of regulating enzymatic activity. Among the important phosphorylation targets are the Na^+,K^+- and H^+,K^+-ATPases that pump ions against their chemical gradients to uphold ionic concentration differences over the plasma membrane. The two pumps are very homologous, and at least one of the phosphorylation sites is conserved, namely a cAMP activated protein kinase (PKA) site, which is important for regulating pumping activity, either by changing the cellular distribution of the ATPases or by directly altering the kinetic properties as supported by electrophysiological results presented here. We further review the other proposed pump phosphorylations.

1. Introduction

The mammalian Na^+,K^+-ATPase (NKA) and H^+,K^+-ATPase (HKA) maintain key ionic imbalances between cells and their surroundings by exporting sodium ions and protons, respectively, in exchange for potassium ions. These gradients provide the driving force for numerous cellular functions, e.g. for establishing the membrane potential from potassium gradients, for sodium dependent secondary transport and neuronal signaling, and for stomach acidification. The fundamental roles of the ATPases make dysregulation of their activities potentially pathophysiological, and several human conditions are associated with the pumps; NKA mutations can cause the neurological diseases Rapid-onset Dystonia Parkinsonism and Familial Hemiplegic Migraine 2 [1], and HKA inhibitors are among the most sold drugs world-wide for treatment of duodenal ulcers and heartburn.

In addition to the pump function, it is becoming increasingly evident that the NKA is also a receptor for cardiotonic steroids, which are endogenously produced compounds that in high concentrations inhibit NKA function, but in low concentrations function as signaling molecules that via the NKA as a receptor activate associated protein kinases such as Src, phosphoinositol 3 kinase (PI3K) and ankyrin to modulate, e.g. apoptosis, cell adhesion and growth [2].

NKA and HKA pump function is fuelled by ATP via formation and break-down of a phosphoenzyme intermediate. ATP hydrolysis and ion transport are accomplished by the α subunit, but the pumps also depend on a β subunit for folding and membrane targeting. Besides a phosphorylation domain displaying a conserved aspartate as the phosphorylation site, α subunits contain domains for protein kinase, protein phosphatase and transmembrane ion transport activities. There are several different genes encoding both the α and β subunits: in mammals there are four NKA α subunits (NKAα1–4), two HKAα subunits (HKAα1 and 2), and four β subunits. Additionally, the NKA αβ complex associates with members of the FXYD proteins. The use of different subunits performing basically similar tasks offers the opportunity to regulate the functions in a time- and tissue-specific manner [3].

A further means of pump control is offered by phosphorylation, and this review will focus on how, why and where HKA and NKA are phosphorylated. The best studied pump kinases are the cAMP activated protein kinase, PKA, and protein kinase C, PKC, which both target serine and threonine residues. The kinases can be stimulated to phosphorylate the pump by activation of a cellular receptor or by change in an intracellular component, and the pump activity is thereby altered directly or because of endocytosis or plasma recruitment.

2. The pump structure

X-ray crystal structures of NKA from pig kidney [4] and from shark rectal gland [5] have revealed the overall structure of the ternary complex including the α subunit with 10 transmembrane (TM) helices, the heavily glycosylated single membrane spanning β subunit and the single membrane spanning γ subunit FXYD2

* Corresponding author. Fax: +45 8612 3178.
E-mail address: pn@mb.au.dk (H. Poulsen).

0014-5793/$36.00
doi:10.1016/j.febslet.2010.04.035

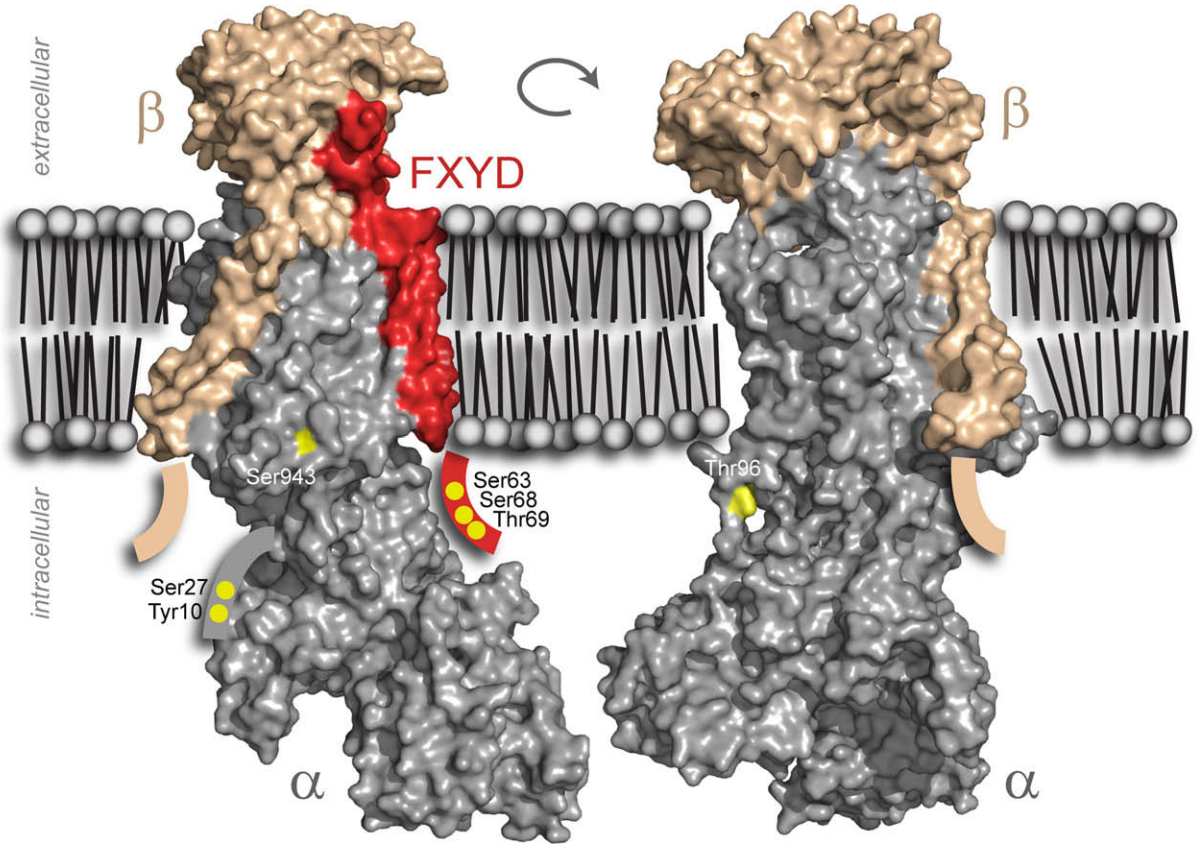

Fig. 1. Structural overview of XKA phosphorylation sites. The structure shows two views of the NKAα subunit in grey, the β subunit in wheat, the FXYD in red and possible phosphorylation sites in yellow. The protein parts not resolved by X-ray crystallography are indicated with thick lines in corresponding colors. The HKA structure will be similar, except that there are no indications of other subunits than α and β. The numbering in the α subunit is counting from the first methionine in human NKAα1 or (for Ser27) HKα2. The numbering in the FXYD subunit is excluding the signal peptide. The structures were made with PyMOL (www.pymol.org) using the shark NKA, PDB ID 2ZXE.

(Fig. 1). The HKAα's have primary structures very similar to the NKAα's (>60% identity), and electron microscopy support that they share similar three dimensional structures [6]. Unfortunately, the majority of the proposed phosphorylation sites are not resolved in the crystal structures, since they lie in the disordered N-terminal part of the α subunit, or in the C-terminal part of FXYD1 that, from homology to the subunit in the crystal forms, corresponds to disordered parts of the crystal structures (Fig. 1).

3. PKA and Ser943

The only well-characterized phosphorylation target in the structure is a serine (Ser943 in NKAα1) in a short helical segment between TM 8 and 9 (Fig. 1), which is a supposed PKA site. The consensus recognition sequence for PKA is Arg-Arg-X-Ser/Thr-Phe, where X is any residue and Phe is a hydrophobic residue. A sequence alignment of NKA and HKAα subunits shows that Ser943 is in an ideal PKA consensus motif in all of the pumps (Fig. 2). In early studies, PKA was shown to phosphorylate the residue, but only in the presence of detergent [7–10]. The expected localization of the residue close to the cytoplasmic membrane interface and the detergent dependence led to skepticism of whether the observed phosphorylation is physiologically relevant – will PKA ever be able to reach the site within an intact cell? Several studies in intact cells and even whole animals indicate that the answer is indeed yes.

Phosphorylation of NKA expressed in COS-7 cells was found to be enhanced by treatment with the PKA activator forskolin [11] or by stimulation of various G-protein coupled receptors (GPCRs [12]), unless the PKA site was mutated. Both adrenergic, cholinergic and dopaminergic receptors enhanced NKA phosphorylation, suggesting that the activated GPCRs stimulate the phosphorylation in live cells.

The dopaminergic signaling has been studied indirectly in mice treated with morphine [13]. Depending on the duration of the treatment, the PKA and NKA activities in the striatum were altered in opposite directions; short term morphine administration stimulated NKA and inhibited PKA activity, primarily via the dopamine 2 receptor (D2), while long term treatment upregulated PKA and downregulated NKA by a dopamine 1 receptor (D1) dependent pathway. The Ser943 phosphorylation status was not determined directly, but the PKA activity and the overall amount of NKA phosphorylation correlated, and the phosphorylation increase was sensitive to H89, a specific PKA inhibitor [14]. The differential phosphorylation effects were specific for NKAα3, no effects of morphine treatment were observed for NKAα1 [13]. In contrast, dopamine was found to inhibit the surface content of NKAα2 specifically in rat neostriatal neurons, while no change was observed for NKAα1 and NKAα3 [15].

Another group of GPCRs regulating NKA are the prostaglandin receptors. Prostaglandin E (PGE) modulates neuronal excitability, and the levels rise after, e.g. brain injury and seizure. In a neuroblastoma cell line, PGE_1 treatment resulted in reduced NKA activity [16], and PGE_2 inhibited NKA in rat hippocampal slices [17]. The PGE effects were abrogated by PKA inhibition, and PGE_2 led to increased Ser943 phosphorylation as shown with a specific antibody [17]. Prostaglandin treatment also caused hippocampal NKA inhibition in vivo [17], further supporting that the Ser943 phosphorylation is physiologically relevant.

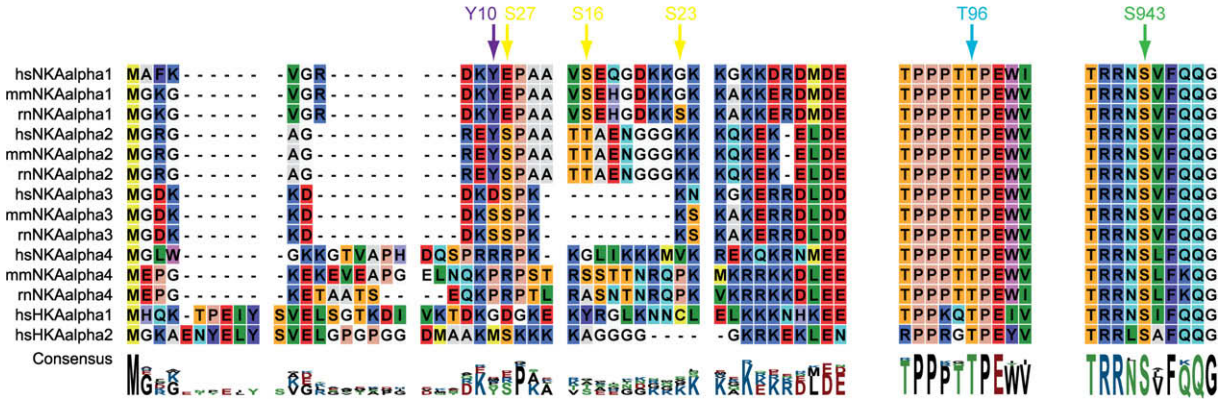

Fig. 2. Alignment of XKA phosphorylation sites. The sequences of the proposed phosphorylation sites in the α subunits from *Homo sapiens* (hs), *Mus musculus* (mm) and *Rattus norvegius* (rn) were aligned with CLCbio. The numbering in the α subunit is counting from the first methionine in human NKAα1 or for Ser27 HKAα2. Above the alignment, arrows indicate the residues that may be targeted by tyrosine phosphorylation by an unidentified kinase (purple), PKC phosphorylation (yellow), ERK phosphorylation (light blue) and PKA phosphorylation (green).

The inverse correlation between the NKA and PKA activities has been found in various cell types, where the plasma membrane levels of NKA do not appear to be affected, implying that the phosphorylated serine inhibits pump function directly, at least at adequate Ca^{2+} concentrations [13,17–20].

PKA phosphorylation of NKA can also alter the amount of NKA at the cell surface, probably by cAMP independent PKA activation. A rise in intracellular Na^+ concentration increased NKA at the plasma membrane of kidney collecting duct cells, and though cAMP levels appeared unaltered, the PKA activity was elevated and the surface recruitment was sensitive to PKA specific inhibitors [21]. That elevated sodium levels promote NKA surface recruitment may also provide a simple explanation for the observation that glutamate treatment of neurons increases the plasma membrane abundance of NKAα1 and NKAα2 [15].

PKA phosphorylation of HKA was similarly shown to promote its expression and cell surface localization [22]. Inhibition of PKA or mutation of the PKA site, Ser955, to alanine (which cannot be phosphorylated) reduced activity and plasma membrane levels, while mutation to aspartate (mimicking constitutive phosphorylation) gave protein levels and activity comparable to wild-type [22].

We have also found that mutation of the PKA site in NKAα2 to glutamate affected its functional properties when expressed in *Xenopus laevis* oocytes. In particular, the mutant gave rise to leak currents at low membrane potentials, i.e. an inward current sensitive to the NKA specific drug ouabain (Fig. 3a). In the wild-type pump, leak currents have been observed, but not at physiological levels of extracellular sodium. Intriguingly, leak currents are also characteristic of C-terminally truncated NKA mutants [23,24], and the structure of the pump shows that the introduction of a negative charge at the PKA site is likely to affect the intricate structure of the C-terminus, which is coordinated by positively charged arginine residues (Fig. 3b). The C-terminal region has only been recognized as a possible regulatory element after the elucidation of the NKA structure, but there is accumulating evidence to suggest that the highly conserved C-terminal tyrosines are essential for pump function and several of the disease-causing mutations map to the region [1,25]. It will be interesting to follow the future characterizations of how PKA phosphorylation can influence pump function via the C-terminus.

The NKA activity is usually determined as uptake of radioactively labeled Rb^+ into living cells or as ATPase activity in purified preparations. The exact mechanism of inhibition remains undetermined, but the direct effect of phosphorylation on the enzyme activity is likely to arise from an altered structure caused by phosphorylation of the serine residue. In one study, enzyme purified from forskolin treated cells were found to have lower Na affinity, but similar V_{max} [8], while another study reported that both Na

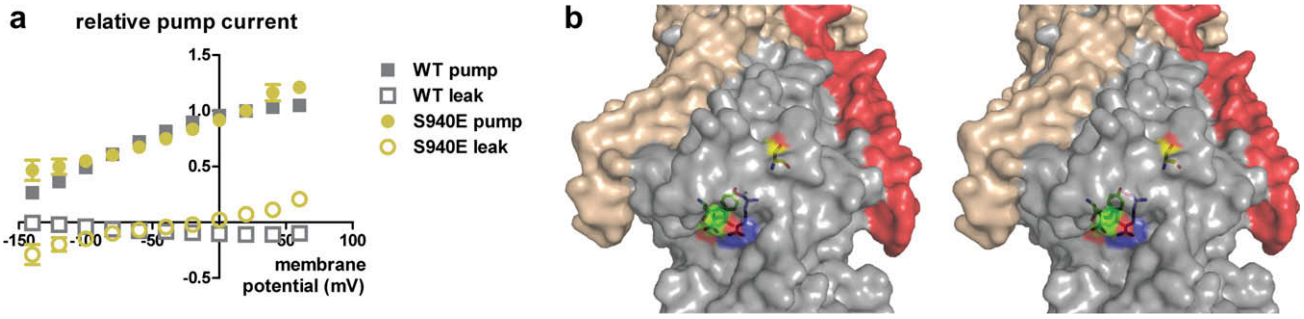

Fig. 3. The electrophysiological effect of mutating the PKA site Ser940 in NKAα2 to glutamate. (a) The voltage dependence of steady-state currents. The pump current is calculated as the ouabain-sensitive current in the presence of 15 mM potassium, the leak current is determined as the ouabain-sensitive current in the absence of potassium. Both pump and leak currents are normalized to the value of pump current at 20 mV. The pumps were expressed in oocytes from *X. laevis* by co-injecting cRNAs encoding ouabain insensitive hsNKAα2 and hsNKAβ1. After 1–3 days at 19 °C, oocytes were loaded with sodium in 95 mM Na, 90 mM sulfamic acid, 5 mM HEPES, 10 mM TEACl, 0.1 mM EGTA, pH 7.6. Two-electrode voltage-clamping was performed in 115 mM Na, 110 mM sulfamic acid, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 5 mM $BaCl_2$, 10 mM HEPES, 1 μM ouabain, pH 7.4. To determine steady-state currents, 15 mM K replaced 15 mM Na, a series of 200 ms voltage steps was run and the 10 mM ouabain background was subtracted. (b) Stereo-view showing the proximity of the PKA site and the C-terminus. The α subunit is in grey, the β subunit in wheat and the FXYD in red. The C-terminal tyrosines are highlighted with green sticks, an arginine (Arg937 in NKAα2) coordinating them in a cation–π interaction with blue sticks and the PKA target with yellow sticks. The structures were made with PyMOL (www.pymol.org) from the shark NKA, PDB ID 2ZXE.

affinity and V_{max} were lower in forskolin treated cells, unless the intracellular concentration of Ca^{2+} was raised, and in that case, there was no difference [18]. The molecular mechanism for the effect of PKA phosphorylation thus remains to be determined.

The other means of regulating pump function is to alter which proteins it interacts with. The increased plasma localization of pumps phosphorylated by PKA [21,22] indicates that interaction with other cellular partners is determined by the phosphorylation status, though the protein promoting plasma recruitment in response to PKA activity is as yet unknown, and it has also been suggested that PKA phosphorylation of the interaction partners rather than of the NKA can regulate its distribution [26].

4. PKC and the αN-terminus

The NKAα1N-termini from various species are phosphorylated by PKC [11,27], although there is no obvious conserved PKC site, i.e. a serine or threonine, which on both sides has at least one basic residue maximally two residues away (Fig. 2). The best studied PKC site is Ser23 in NKAα1 from rat [28–34], but this residue is unique to rat (Fig. 2), making its general regulatory role less obvious.

The only serine/threonine conserved in the N-termini of NKAα1 subunits is Ser16, which has been identified as a PKC target although there is just a single basic residue in its vicinity, namely a downstream histidine and only in the rodent sequences (Fig. 2). Mutating Ser16 to an alanine reduces or abolishes PKC phosphorylation [11,35,36], and mutation to an aspartate can mimic the consequences of phosphorylation [35]. However, Ser16 phosphorylation was reported to be abolished if the downstream histidine was mutated to glutamine [27], which is the residue found at the analogous position in the human sequence (Fig. 2). Accordingly, in vitro phosphorylation with PKC was undetectable for the N-terminus of hsNKAα1 [27] and low or undetectable for the full length protein [37], making it further questionable if the physiological effects reported of N-terminal PKC phosphorylation would also apply to human cells. The fairly large sequence variation in the α subunit N-termini therefore makes it difficult to generalize studies of PKC regulation of rodent NKAα1, especially if it is not shown that the effects are independent of Ser16 and Ser23 phosphorylation [36,38–44].

There are no apparent PKC sites in the N-terminus of HKAα1, but Ser27 in HKAα2 is within a consensus sequence (Fig. 2) and can be efficiently phosphorylated by PKC in vitro [9], which increases V_{max} at saturating ATP conditions. In kidney cells, PKC activation by phorbol esters leads to endocytosis of HKAα2, though it is not shown directly where (or if) PKC phosphorylates the pump [45].

In chick embryos, thyroid hormones were shown to inhibit NKA activity by a process depending on PI3K and PKA as well as PKC [46], while thyroid hormones in rat alveolar epithelial cells increased NKA activity, again depending on PI3K [47]. There is clearly a tight interplay between various signaling pathways, and slight variations during development and between different tissues can lead to opposite effects of the same treatment [36,46,47]. Future experiments are required to determine if direct phosphorylation of the NKA is important, or if regulatory proteins are the main targets.

5. Alternative phosphorylation sites in α subunits, ERK phosphorylation and insulin

The NKA has also been suggested to be phosphorylated directly by the extracellular signal-regulated kinases (ERK). Using human skeletal muscle, insulin was shown to stimulate NKA activity, possibly due to the increased cell surface abundance observed for both NKAα1 and NKAα2 [48], and the plasma membrane pump levels were similarly not to increase upon insulin treatment of rats, especially for NKAα2 [49]. In vitro, insulin generally stimulated phosphorylation of Ser, Thr and Tyr residues [48,50], and ERK1/2 could phosphorylate the pump [37]. The most obvious ERK recognition sequence in the NKAs is Thr96, which is in the optimal context, Pro-X-X-Thr-Pro, and the site is conserved between the NKAs, but not in the HKAs (Fig. 2). In the structure, this threonine is cytoplasmic though close to the membrane (Fig. 1), and its accessibility may change during the catalytic cycle. The study suggests that insulin leads to Thr96 phosphorylation and thereby promotes insertion of pumps into the plasma membrane [48]. The insulin-induced surface recruitment of NKAα1 was found to depend on activation of the kinase Akt and the downstream activation of the GTPase Rab10 [51]. Rab10 colocalized with the sodium pump in the perinuclear region and was proposed to mediate its increase at the plasma membrane [51], but it remains to be investigated whether the pump phosphorylations play a direct role in the exocytosis process.

The increased tyrosine phosphorylation [37] is in agreement with an earlier study of the insulin-induced NKA stimulation, where Tyr10 was identified as a phosphorylation target in rat NKAα1 (Fig. 2), and mutation of the tyrosine abolished the effect of insulin [52]. It would be intriguing to see if, e.g. the suspected interaction with Rab10 is dependent upon the phosphorylation status of Tyr10.

Tyrosine phosphorylation may, however, also serve to downregulate NKAα1 activity, since tyrosine phosphatase inhibitors dose-dependently inhibit the ATPase activity, correlating with increased overall tyrosine phosphorylation [53]. Whether Tyr10 is involved is unknown, and again it is important to distinguish between direct effects on the enzymatic kinetics and effects on regulatory protein–protein interactions.

6. FXYD1

The seven mammalian members of the FXYD family are small, single membrane spanning proteins that share a consensus Pro-Phe-X-Tyr-Asp sequence in their extracellular N-termini. Oligomers of the FXYDs may form ion channels [54,55], but they have mainly been studied for their interactions with NKA. The transmembrane domain associates closely with TM9 of the α subunit (Fig. 1), and the interaction modulates NKA activity by altering the apparent affinities for sodium, potassium and ATP [3].

In heart and skeletal muscle, the primary membrane protein target for PKA and PKC is FXYD1 [56], as indicated by its alternative name, phospholemman. The main phosphorylation sites are Ser63, Ser68 and Thr69 in the very C-terminal end of the protein, a part that was relatively disordered in the crystals, so its structure remains unknown. PKC promotes phosphorylation of all three residues, while PKA only targets Ser68 [57]. PKA phosphorylation of FXYD1 increases NKA activity in mouse cardiac myocytes, unless FXYD1 has been knocked out. This fits well with the suggestion that the unphosphorylated FXYD1 lowers pump affinity for sodium and potassium, while phosphorylation relieves the inhibitory effect [58] and even stimulates the pump [57,59].

In ventricular myocytes, just the intracellular part of FXYD1 gave increased NKA currents when phosphorylated by PKA, while the unphosphorylated peptide was inhibitory [59]. The peptide was coimmunoprecipitated with NKA regardless of its phosphorylation status [59,60], although fluorescence resonance energy transfer has indicated that phosphorylation significantly reduces energy transfer, i.e. the phosphorylation status of FXYD1 strongly influences its relative orientation to the α subunit [61].

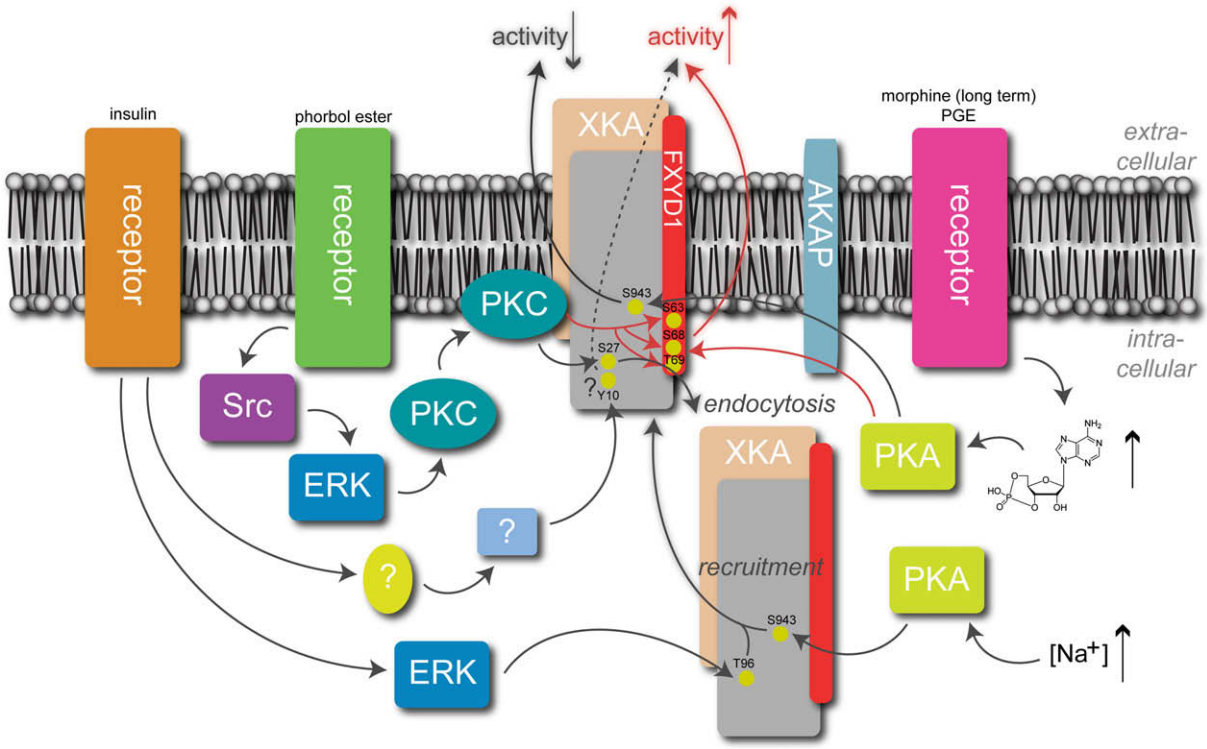

Fig. 4. Regulation of XKA by phosphorylation. Schematic overview of phosphorylation pathways described to modulate pump function. The XKA subunits are colored as in Fig. 1, red arrows indicate pathways specific for FXYD1, and numbering of the yellow phosphorylation sites is as in Fig. 1. The arrows from PKA crossing AKAP (A-kinase anchoring protein) signify that NKA phosphorylation by PKA may depend on initial membrane recruitment of the kinase [63]. Similarly, PKC phosphorylation probably depends on membrane attachment of the kinase [42,64]. Numerous other signaling molecules have been shown to activate receptors and thereby alter XKA activity, only the ones mentioned in the review are indicated. See text for further details.

7. Concluding remarks

Although an extensive amount of literature links various signaling pathways to regulation of NKA and HKA pump function, the underlying molecular mechanisms of phosphorylation remain largely unknown. We have summarized some of the many studies in Fig. 4.

Especially in the heart, it is evident that phosphorylation of FXYD1 is a means of promoting NKA activity. It also seems relatively clear that PKA phosphorylation can either lower pump function directly or increase it indirectly by promoting cell surface recruitment. The role of PKC phosphorylation at the α subunit, especially for human physiology, is less well characterized, but even though specific target residues have not been identified in the human pumps, many of the signaling pathways described for the rodent enzymes may apply generally, and there will probably in many or most cases be cross-talk between the different pathways regulating the enzymes, including the phosphorylations by ERK and by the yet unidentified tyrosine kinase.

One intriguing possibility that has not, to our knowledge, been examined is whether NKA phosphorylation is important for autoregulation. When the NKA acts as a ouabain receptor, it stimulates a number of kinases that are also known from the NKA phosphorylation pathways (e.g. PI3K and Src), and the ouabain activation leads to endocytosis of the NKA receptor [62]. It would be interesting to learn if NKA as the ouabain signal transducer is itself specifically regulated by phosphorylation.

We also hope that future experiments will further clarify the direct enzymatic consequences of phosphorylations. Apparently, PKA phosphorylation of the NKAα subunits promotes leak currents, an effect previously noted to characterize C-terminal deletion mutants [23,24], but the mechanism remains elusive. Further structural and functional studies will be important to understand the role of PKA on NKA and HKA.

Acknowledgements

We are grateful for valuable discussions on pump phosphorylations with Himanshu Khandelia and Anita Aperia.

References

[1] Morth, J.P., Poulsen, H., Toustrup-Jensen, M.S., Schack, V.R., Egebjerg, J., Andersen, J.P., Vilsen, B. and Nissen, P. (2009) The structure of the Na^+,K^+-ATPase and mapping of isoform differences and disease-related mutations. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 364, 217–227.
[2] Aperia, A. (2007) New roles for an old enzyme: Na,K-ATPase emerges as an interesting drug target. J. Intern. Med. 261, 44–52.
[3] Geering, K. (2008) Functional roles of Na,K-ATPase subunits. Curr. Opin. Nephrol. Hypertens. 17, 526–532.
[4] Morth, J.P., Pedersen, B.P., Toustrup-Jensen, M.S., Sorensen, T.L., Petersen, J., Andersen, J.P., Vilsen, B. and Nissen, P. (2007) Crystal structure of the sodium–potassium pump. Nature 450, 1043–1049.
[5] Shinoda, T., Ogawa, H., Cornelius, F. and Toyoshima, C. (2009) Crystal structure of the sodium-potassium pump at 2.4 Å resolution. Nature 459, 446–450.
[6] Abe, K., Tani, K., Nishizawa, T. and Fujiyoshi, Y. (2009) Inter-subunit interaction of gastric H^+,K^+-ATPase prevents reverse reaction of the transport cycle. EMBO J. 28, 1637–1643.
[7] Feschenko, M.S. and Sweadner, K.J. (1994) Conformation-dependent phosphorylation of Na,K-ATPase by protein kinase A and protein kinase C. J. Biol. Chem. 269, 30436–30444.
[8] Fisone, G., Cheng, S.X., Nairn, A.C., Czernik, A.J., Hemmings Jr., H.C., Hoog, J.O., Bertorello, A.M., Kaiser, R., Bergman, T. and Jornvall, H. (1994) Identification of the phosphorylation site for cAMP-dependent protein kinase on $Na^+,K(+)$-ATPase and effects of site-directed mutagenesis. J. Biol. Chem. 269, 9368–9373.
[9] Cornelius, F. and Mahmmoud, Y.A. (2003) Direct activation of gastric H,K-ATPase by N-terminal protein kinase C phosphorylation. Comparison of the acute regulation mechanisms of H,K-ATPase and Na,K-ATPase. Biophys. J. 84, 1690–1700.

[10] Sweadner, K.J. and Feschenko, M.S. (2001) Predicted location and limited accessibility of protein kinase A phosphorylation site on Na-K-ATPase. Am. J. Physiol. Cell. Physiol. 280, C1017–C1026.
[11] Beguin, P., Beggah, A.T., Chibalin, A.V., Burgener-Kairuz, P., Jaisser, F., Mathews, P.M., Rossier, B.C., Cotecchia, S. and Geering, K. (1994) Phosphorylation of the Na,K-ATPase alpha-subunit by protein kinase A and C in vitro and in intact cells. Identification of a novel motif for PKC-mediated phosphorylation. J. Biol. Chem. 269, 24437–24445.
[12] Beguin, P., Beggah, A., Cotecchia, S. and Geering, K. (1996) Adrenergic, dopaminergic, and muscarinic receptor stimulation leads to PKA phosphorylation of Na-K-ATPase. Am. J. Physiol. 270, C131–C137.
[13] Wu, Z.Q., Chen, J., Chi, Z.Q. and Liu, J.G. (2007) Involvement of dopamine system in regulation of Na^+,K^+-ATPase in the striatum upon activation of opioid receptors by morphine. Mol. Pharmacol. 71, 519–530.
[14] Pearce, L.R., Komander, D. and Alessi, D.R. (2010) The nuts and bolts of AGC protein kinases. Nat. Rev. Mol. Cell Biol. 11, 9–22.
[15] Teixeira, V.L., Katz, A.I., Pedemonte, C.H. and Bertorello, A.M. (2003) Isoform-specific regulation of Na^+,K^+-ATPase endocytosis and recruitment to the plasma membrane. Ann. N.Y. Acad. Sci. 986, 587–594.
[16] Woolcock, K. and Specht, S.C. (2006) Modulation of Na, K-ATPase activity by prostaglandin E1 and [D-Ala2,N-Me-Phe4,Gly5-ol]-enkephalin. Life Sci. 78, 1653–1661.
[17] Oliveira, M.S., Furian, A.F., Rambo, L.M., Ribeiro, L.R., Royes, L.F., Ferreira, J., Calixto, J.B., Otalora, L.F., Garrido-Sanabria, E.R. and Mello, C.F. (2009) Prostaglandin E2 modulates Na^+,K^+-ATPase activity in rat hippocampus: implications for neurological diseases. J. Neurochem. 109, 416–426.
[18] Cheng, S.X., Aizman, O., Nairn, A.C., Greengard, P. and Aperia, A. (1999) Ca2+]i determines the effects of protein kinases A and C on activity of rat renal Na^+,K^+-ATPase. J. Physiol. 518 (Pt 1), 37–46.
[19] Cheng, X.J., Fisone, G., Aizman, O., Aizman, R., Levenson, R., Greengard, P. and Aperia, A. (1997) PKA-mediated phosphorylation and inhibition of Na(+)-K(+)-ATPase in response to beta-adrenergic hormone. Am. J. Physiol. 273, C893–C901.
[20] Bertorello, A.M., Aperia, A., Walaas, S.I., Nairn, A.C. and Greengard, P. (1991) Phosphorylation of the catalytic subunit of Na^+,K(+)-ATPase inhibits the activity of the enzyme. Proc. Natl. Acad. Sci. USA 88, 11359–11362.
[21] Vinciguerra, M., Deschenes, G., Hasler, U., Mordasini, D., Rousselot, M., Doucet, A., Vandewalle, A., Martin, P.Y. and Feraille, E. (2003) Intracellular Na^+ controls cell surface expression of Na,K-ATPase via a cAMP-independent PKA pathway in mammalian kidney collecting duct cells. Mol. Biol. Cell 14, 2677–2688.
[22] Codina, J., Liu, J., Bleyer, A.J., Penn, R.B. and DuBose Jr., T.D. (2006) Phosphorylation of S955 at the protein kinase A consensus promotes maturation of the alpha subunit of the colonic H^+,K^+-ATPase. J. Am. Soc. Nephrol. 17, 1833–1840.
[23] Meier, S., Tavraz, N.N., Durr, K.L. and Friedrich, T. (2010) Hyperpolarization-activated inward leakage currents caused by deletion or mutation of carboxy-terminal tyrosines of the Na^+/K^+-ATPase {alpha} subunit. J. Gen. Physiol. 135, 115–134.
[24] Yaragatupalli, S., Olivera, J.F., Gatto, C. and Artigas, P. (2009) Altered Na^+ transport after an intracellular alpha-subunit deletion reveals strict external sequential release of Na^+ from the Na/K pump. Proc. Natl. Acad. Sci. USA 106, 15507–15512.
[25] Toustrup-Jensen, M.S., Holm, R., Einholm, A.P., Schack, V.R., Morth, J.P., Nissen, P., Andersen, J.P. and Vilsen, B. (2009) The C-terminus of Na^+,K^+-ATPase controls Na^+ affinity on both sides of the membrane through Arg935. J. Biol. Chem. 284 (28), 18715–18725.
[26] Mordasini, D., Bustamante, M., Rousselot, M., Martin, P.Y., Hasler, U. and Feraille, E. (2005) Stimulation of Na^+ transport by AVP is independent of PKA phosphorylation of the Na–K-ATPase in collecting duct principal cells. Am. J. Physiol. Renal Physiol. 289, F1031–F1039.
[27] Beguin, P., Peitsch, M.C. and Geering, K. (1996) alpha 1 but not alpha 2 or alpha 3 isoforms of Na,K-ATPase are efficiently phosphorylated in a novel protein kinase C motif. Biochemistry 35, 14098–14108.
[28] Efendiev, R., Yudowski, G.A., Zwiller, J., Leibiger, B., Katz, A.I., Berggren, P.O., Pedemonte, C.H., Leibiger, I.B. and Bertorello, A.M. (2002) Relevance of dopamine signals anchoring dynamin-2 to the plasma membrane during Na^+,K^+-ATPase endocytosis. J. Biol. Chem. 277, 44108–44114.
[29] Belusa, R., Wang, Z.M., Matsubara, T., Sahlgren, B., Dulubova, I., Nairn, A.C., Ruoslahti, E., Greengard, P. and Aperia, A. (1997) Mutation of the protein kinase C phosphorylation site on rat alpha1 Na^+,K^+-ATPase alters regulation of intracellular Na^+ and pH and influences cell shape and adhesiveness. J. Biol. Chem. 272, 20179–20184.
[30] Chibalin, A.V., Ogimoto, G., Pedemonte, C.H., Pressley, T.A., Katz, A.I., Feraille, E., Berggren, P.O. and Bertorello, A.M. (1999) Dopamine-induced endocytosis of Na^+,K^+-ATPase is initiated by phosphorylation of Ser-18 in the rat alpha subunit and Is responsible for the decreased activity in epithelial cells. J. Biol. Chem. 274, 1920–1927.
[31] Chibalin, A.V., Zierath, J.R., Katz, A.I., Berggren, P.O. and Bertorello, A.M. (1998) Phosphatidylinositol 3-kinase-mediated endocytosis of renal Na^+,K^+-ATPase alpha subunit in response to dopamine. Mol. Biol. Cell 9, 1209–1220.
[32] Dada, L.A., Chandel, N.S., Ridge, K.M., Pedemonte, C., Bertorello, A.M. and Sznajder, J.I. (2003) Hypoxia-induced endocytosis of Na,K-ATPase in alveolar epithelial cells is mediated by mitochondrial reactive oxygen species and PKC-zeta. J. Clin. Invest. 111, 1057–1064.
[33] Efendiev, R., Das-Panja, K., Cinelli, A.R., Bertorello, A.M. and Pedemonte, C.H. (2007) Localization of intracellular compartments that exchange Na,K-ATPase molecules with the plasma membrane in a hormone-dependent manner. Br. J. Pharmacol. 151, 1006–1013.
[34] Pedemonte, C.H., Pressley, T.A., Lokhandwala, M.F. and Cinelli, A.R. (1997) Regulation of Na,K-ATPase transport activity by protein kinase C. J. Membr. Biol. 155, 219–227.
[35] Feraille, E., Beguin, P., Carranza, M.L., Gonin, S., Rousselot, M., Martin, P.Y., Favre, H. and Geering, K. (2000) Is phosphorylation of the alpha1 subunit at Ser-16 involved in the control of Na,K-ATPase activity by phorbol ester-activated protein kinase C? Mol. Biol. Cell 11, 39–50.
[36] Khundmiri, S.J., Bertorello, A.M., Delamere, N.A. and Lederer, E.D. (2004) Clathrin-mediated endocytosis of Na^+,K^+-ATPase in response to parathyroid hormone requires ERK-dependent phosphorylation of Ser-11 within the alpha1-subunit. J. Biol. Chem. 279, 17418–17427.
[37] Al-Khalili, L., Kotova, O., Tsuchida, H., Ehren, I., Feraille, E., Krook, A. and Chibalin, A.V. (2004) ERK1/2 mediates insulin stimulation of Na(+),K(+)-ATPase by phosphorylation of the alpha-subunit in human skeletal muscle cells. J. Biol. Chem. 279, 25211–25218.
[38] Ridge, K.M., Dada, L., Lecuona, E., Bertorello, A.M., Katz, A.I., Mochly-Rosen, D. and Sznajder, J.I. (2002) Dopamine-induced exocytosis of Na,K-ATPase is dependent on activation of protein kinase C-epsilon and -delta. Mol. Biol. Cell 13, 1381–1389.
[39] Guerrero, C., Lecuona, E., Pesce, L., Ridge, K.M. and Sznajder, J.I. (2001) Dopamine regulates Na–K-ATPase in alveolar epithelial cells via MAPK-ERK-dependent mechanisms. Am. J. Physiol. Lung Cell. Mol. Physiol. 281, L79–L85.
[40] Efendiev, R., Budu, C.E., Cinelli, A.R., Bertorello, A.M. and Pedemonte, C.H. (2003) Intracellular Na^+ regulates dopamine and angiotensin II receptors availability at the plasma membrane and their cellular responses in renal epithelia. J. Biol. Chem. 278, 28719–28726.
[41] Efendiev, R., Bertorello, A.M., Pressley, T.A., Rousselot, M., Feraille, E. and Pedemonte, C.H. (2000) Simultaneous phosphorylation of Ser11 and Ser18 in the alpha-subunit promotes the recruitment of Na(+),K(+)-ATPase molecules to the plasma membrane. Biochemistry 39, 9884–9892.
[42] Khundmiri, S.J., Dean, W.L., McLeish, K.R. and Lederer, E.D. (2005) Parathyroid hormone-mediated regulation of Na^+-K^+-ATPase requires ERK-dependent translocation of protein kinase Calpha. J. Biol. Chem. 280, 8705–8713.
[43] Budu, C.E., Efendiev, R., Cinelli, A.M., Bertorello, A.M. and Pedemonte, C.H. (2002) Hormonal-dependent recruitment of Na^+,K^+-ATPase to the plasmalemma is mediated by PKC beta and modulated by $[Na^+]i$. Br. J. Pharmacol. 137, 1380–1386.
[44] Gusarova, G.A., Dada, L.A., Kelly, A.M., Brodie, C., Witters, L.A., Chandel, N.S. and Sznajder, J.I. (2009) Alpha1-AMP-activated protein kinase regulates hypoxia-induced Na,K-ATPase endocytosis via direct phosphorylation of protein kinase C zeta. Mol. Cell. Biol. 29, 3455–3464.
[45] Reinhardt, J., Kosch, M., Lerner, M., Bertram, H., Lemke, D. and Oberleithner, H. (2002) Stimulation of protein kinase C pathway mediates endocytosis of human nongastric H^+–K^+-ATPase, ATP1AL1. Am. J. Physiol. Renal Physiol. 283, F335–F343.
[46] Scapin, S., Leoni, S., Spagnuolo, S., Fiore, A.M. and Incerpi, S. (2009) Short-term effects of thyroid hormones on Na^+–K^+-ATPase activity of chick embryo hepatocytes during development: focus on signal transduction. Am. J. Physiol. Cell. Physiol. 296, C4–C12.
[47] Lei, J., Mariash, C.N., Bhargava, M., Wattenberg, E.V. and Ingbar, D.H. (2008) T3 increases Na–K-ATPase activity via a MAPK/ERK1/2-dependent pathway in rat adult alveolar epithelial cells. Am. J. Physiol. Lung Cell. Mol. Physiol. 294, L749–L754.
[48] Al-Khalili, L., Kotova, O., Tsuchida, H., Ehren, I., Feraille, E., Krook, A. and Chibalin, A.V. (2004) ERK1/2 mediates insulin stimulation of Na(+),K(+)-ATPase by phosphorylation of the alpha-subunit in human skeletal muscle cells. J. Biol. Chem. 279, 25211–25218.
[49] Rosta, K., Tulassay, E., Enzsoly, A., Ronai, K., Szantho, A., Pandics, T., Fekete, A., Mandl, P. and Ver, A. (2009) Insulin induced translocation of Na^+/K^+-ATPase is decreased in the heart of streptozotocin diabetic rats. Acta Pharmacol. Sin. 30, 1616–1624.
[50] Chibalin, A.V., Kovalenko, M.V., Ryder, J.W., Feraille, E., Wallberg-Henriksson, H. and Zierath, J.R. (2001) Insulin- and glucose-induced phosphorylation of the Na(+),K(+)-adenosine triphosphatase alpha-subunits in rat skeletal muscle. Endocrinology 142, 3474–3482.
[51] Comellas, A.P., Kelly, A.M., Trejo, H.E., Briva, A., Lee, J., Sznajder, J.I. and Dada, L.A. (2010) Insulin regulates alveolar epithelial function by inducing Na^+/K^+-ATPase translocation to the plasma membrane in a process mediated by the action of Akt. J. Cell. Sci. 123, 1343–1351.
[52] Feraille, E., Carranza, M.L., Gonin, S., Beguin, P., Pedemonte, C., Rousselot, M., Caverzasio, J., Geering, K., Martin, P.Y. and Favre, H. (1999) Insulin-induced stimulation of $Na^+,K(+)$-ATPase activity in kidney proximal tubule cells depends on phosphorylation of the alpha-subunit at Tyr-10. Mol. Biol. Cell 10, 2847–2859.
[53] El-Beialy, W., Galal, N., Deyama, Y., Yoshimura, Y., Suzuki, K., Tei, K. and Totsuka, Y. (2010) Effects of estrogen on PMCA 2 and 4 in human fibroblast-like synovial cells and mouse macrophage-like cells. Endocr. J. 57, 93–97.
[54] Beevers, A.J. and Kukol, A. (2006) Secondary structure, orientation, and oligomerization of phospholemman, a cardiac transmembrane protein. Protein Sci. 15, 1127–1132.
[55] Davis, C.E., Patel, M.K., Miller, J.R., John 3rd, J.E., Jones, L.R., Tucker, A.L., Mounsey, J.P. and Moorman, J.R. (2004) Effects of phospholemman expression on swelling-activated ion currents and volume regulation in embryonic kidney cells. Neurochem. Res. 29, 177–187.

[56] Palmer, C.J., Scott, B.T. and Jones, L.R. (1991) Purification and complete sequence determination of the major plasma membrane substrate for cAMP-dependent protein kinase and protein kinase C in myocardium. J. Biol. Chem. 266, 11126–11130.
[57] Fuller, W., Howie, J., McLatchie, L.M., Weber, R.J., Hastie, C.J., Burness, K., Pavlovic, D. and Shattock, M.J. (2009) FXYD1 phosphorylation in vitro and in adult rat cardiac myocytes: threonine 69 is a novel substrate for protein kinase C. Am. J. Physiol. Cell. Physiol. 296, C1346–C1355.
[58] Despa, S., Bossuyt, J., Han, F., Ginsburg, K.S., Jia, L.G., Kutchai, H., Tucker, A.L. and Bers, D.M. (2005) Phospholemman-phosphorylation mediates the beta-adrenergic effects on Na/K pump function in cardiac myocytes. Circ. Res. 97, 252–259.
[59] Pavlovic, D., Fuller, W. and Shattock, M.J. (2007) The intracellular region of FXYD1 is sufficient to regulate cardiac Na/K ATPase. FASEB J. 21, 1539–1546.
[60] Bibert, S., Roy, S., Schaer, D., Horisberger, J.D. and Geering, K. (2008) Phosphorylation of phospholemman (FXYD1) by protein kinases A and C modulates distinct Na,K-ATPase isozymes. J. Biol. Chem. 283, 476–486.
[61] Bossuyt, J., Despa, S., Martin, J.L. and Bers, D.M. (2006) Phospholemman phosphorylation alters its fluorescence resonance energy transfer with the Na/K-ATPase pump. J. Biol. Chem. 281, 32765–32773.
[62] Liu, J. and Shapiro, J.I. (2007) Regulation of sodium pump endocytosis by cardiotonic steroids: molecular mechanisms and physiological implications. Pathophysiology 14, 171–181.
[63] Kurihara, K., Nakanishi, N., Amano, O., Yamamoto, M. and Iseki, S. (2003) Specific expression of an A-kinase anchoring protein subtype, AKAP-150, and specific regulatory mechanism for Na(+),K(+)-ATPase via protein kinase A in the parotid gland among the three major salivary glands of the rat. Biochem. Pharmacol. 66, 239–250.
[64] Khundmiri, S.J., Ameen, M., Delamere, N.A. and Lederer, E.D. (2008) PTH-mediated regulation of Na^+–K^+-ATPase requires Src kinase-dependent ERK phosphorylation. Am. J. Physiol. Renal Physiol. 295, F426–F437.