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The other functions of the sodium pump

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ABSTRACT

Na/K-ATPase (the sodium pump) was discovered in the 1950s as the plasma membrane enzyme that carries out the coupled active transports of Na^+ and K^+ across the membranes of nearly all eukaryotic cells. It was not until the 1990s when it was shown that besides pumping ions, Na/K-ATPase is also capable of stimulus-induced interactions with neighboring proteins that lead to activations of signal transduction pathways causing cell growth. This article is an attempt to review the progress of the research on these signaling functions of sodium pump during the past 2–3 decades. The covered topics include (a) the controversial digitalis-induced growth activations through the epidermal growth factor receptor and Src kinase in cardiac myocytes and several other cell types; (b) the extensive findings on digitalis-induced growth activations in cardiac myocytes and other cell types through phosphatidylinositol 3-kinases; and (c) a number of interesting but insufficiently studied signaling functions of the sodium pump.

1. Introduction

The sodium pump (Na/K-ATPase) is the energy transducing enzyme of the plasma membrane that transports Na^+ and K^+ against their physiological gradients in nearly all eukaryotic cells. This enzyme was discovered by Skou in the late 1950s [1,2]; and it was established soon thereafter that Na/K-ATPase was indeed the digitalis-sensitive sodium pump that had been sought after for many years [3]. During a few decades after the discovery of Na/K-ATPase, a number of isolated studies [reviewed in 4] suggested that Na/K-ATPase communicates with the cell nucleus to regulate genes by mechanisms distinct from its ion pumping function. These studies, however, were neglected by the field until the 1990s, when my laboratory [5] and that of Aperia [6] presented independent evidence indicating that digitalis interaction with Na/K-ATPase of cardiac myocytes or epithelial cells activate growth stimulatory pathways independent of digitalis inhibition of the pumping function of the enzyme. These early studies which have been reviewed by the two laboratories [4,7], have led to numerous subsequent studies on the signal transducing functions of Na/K-ATPase. This article is my attempt to review the work that has been done in this research area during the past 2–3 decades. Needless to say, this is not the first such review. As we know, however, reviews are always written from the different perspectives of the reviewers. Here, I have tried to make a distinction between those aspects of the signaling function of the pump that have been firmly established, those that have been strongly advocated but have been shown by others to be based on irreproducible results, and those that have been suggested by limited data but have been insufficiently studied.

2. Digitalis-induced signaling by the sodium pump

The most firmly established fact about the signaling function of the pump is that digitalis drugs which are inhibitors of the pumping function Na/K-ATPase are activators of its signaling function. These activating effects of digitalis were noted in the early work both on the hypertrophic growth of the terminally differentiated cardiac myocytes [5], and on the proliferative growth of the epithelia and smooth muscle cells [6]. It is also well established that both types of growth-stimulatory effects of digitalis drugs are due to induced protein-protein interactions between the digitalis-complexed Na/K-ATPase and neighboring proteins of the plasma membrane or those of the soluble intracellular proteins that are recruited to the plasma membrane [4,7,8]. What else is well established? A convenient way of answering this question is to see what has been found since the original observations [5] showing ouabain-induced stimulation of cardiac myocyte growth, and those [6] showing ouabain-induced calcium oscillations causing transcriptional regulation of genes in epithelial cells. Further progress of the work on calcium oscillations is being reviewed in the same issue by Aperia [9]; hence, I shall leave that in her capable hands. Below, I shall focus on what has followed the early observations of ouabain-induced growth of cardiac myocytes [5].

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3. Digitalis-induced signaling through EGFR/Src

After the first report of the ouabain-induced growth of cardiac myocytes [5], my colleagues and I reported extensive studies within a short time [10–13] showing that ouabain activates the Ras-Raf-MEK-ERK1/2 signal cascade in cardiac myocytes, and suggesting the involvement of this cascade in ouabain-induced growth. Because at the time the upstream connection of this cascade to classical growth factor receptors of the cell membrane was well-established [14], we set out to see if ouabain-induced hypertrophy of cardiac myocytes was indeed related to activation of a growth factor. These studies published in 2000 [15] clearly showed that (a) ouabain activation of EGFR and its coactivator Src occurred proximal to the activations of Ras-Raf-MEK-ERK1/2 ; and (b) ouabain activations of these signal pathways was not limited to cardiac myocytes, and was a common feature of signal transducing functions of Na/K-ATPase in many cell types. Even a casual reading of this publication would show that at the time we had an open mind on numerous possible mechanisms for the interactions of ouabain-complexed pump molecules with EGFR and Src, and the initiation of the growth-stimulating pathways. This changed dramatically soon thereafter upon the publication of a paper [16] from Xie's laboratory which was now independent of my group, but at the same institution. The presented results [16] made a strong case for the constitutive binding of Na/K-ATPase to Src causing Src kinase inhibition, and ouabain-induced release and activation of this Src which would then activate EGFR and set in motion the signaling pathway leading to activation of Ras-Raf-MEK-ERK1/2 cascade in both cardiac myocytes and epithelial cells. Though this was presented as a working hypothesis which was promised to be tested, I believe this publication started the serious advocacy of the direct Src/sodium pump interaction which has continued to be advocated by Xie and his collaborators to this day. This publication was soon followed by another from the same laboratory [17] proposing that the direct Src binding to Na/K-ATPase and subsequent ouabain-induced signaling events occur only in caveolar microdomains of the epithelial cells. These seminal publications [16,17] also signaled the separation of the group that started this research into two groups with different views on the issue of direct interaction of Src with Na/K-ATPase. The promised further support for direst Src/sodium pump interaction was published by Xie's group in 2006 [18]. This impressive paper reporting the results of a large number of experiments on intact cells and cell-free mixtures of purified Na/K-ATPase and Src, firmly concluded the establishment of binding of Src to Na/K-ATPase to form a signaling complex. I remained skeptical about the strength of this conclusion for two reasons. First, because several of their cell-free experiments on mixtures of Na/K-ATPase and Src were done in the presence of detergents, and many years before a respected laboratory had reported [19] that Src reacted with Na/K-ATPase only when the latter was denatured. Though I had pointed this out to Xie and his group while they were conducting their work, there was no evidence in their publication [18] that the old findings [19] had been taken into account. And the second reason for my skepticism about my colleagues' conclusions was their proposal that ouabain-induced signaling occurred only in caveolae [17,18]. Though I had also suggested [20] the occurrence of signaling by the pump in cardiac caveolae, I had not advocated that such signaling did not occur else-where in the plasma membrane. Nevertheless, in spite of these disagreements, Xie's laboratory and mine continued to collaborate when possible. Complete agreement is seldom seen among collaborating scientists.

My first publicly expressed disagreement with Xie' views appeared in the literature in 2011 [21]. In this work which focused on the evaluation of the suggested differences between the signaling functions of the pump in caveolar and noncaveolar membrane domains, we reported a new way of making large-scale preparations of caveolar and noncaveolar kidney membranes, and found that Na/K-ATPases of both have identical signaling functions. Using these preparations, we also presented results [21] that did not support direct Src binding to Na/K-ATPase. Though these findings which seriously challenged the conclusions of Xie's laboratory were known to these colleagues, we received no response from them either before or after publication. Interestingly, this publication also seemed to have little immediate impact in the field. I suppose that others assumed that disagreements between closely collaborating laboratories cannot be that serious.

A more serious trouble for the Src/sodium pump interaction appeared in the literature in 2012, when two collaborating European laboratories reported that some critical experiments of the Xie laboratory in support of Src/pump interaction could not be repeated [22]. Because at the time Xie and I had a shared NIH grant (a Program Project Grant) on which I was the Principal Investigator,and since the questioned studies of Xie [18] were partially supported by the shared grant, I was legally obligated to look into the claimed irreproducibility of the data. Xie saw no need to repeat the experiments; hence, I proceeded to do that with the help of other colleagues supported by the shared grant. The results of these studies [23] showed the irreproducibilities of several of Xie's experiments, in agreement with the previous report [22]. Subsequently, other laboratories also reported that some other experiments of Xie's group in support of direct Src/pump interaction were not reproducible [24,25]; and the strong case against the existence of such a direct contact was aptly summarized [8]. Based on this record, and the well-known concerns about the alarming lack of reproducibility in published preclinical research [26–28], I suggest that we should be quite skeptical, at the very least, about the existence of direct binding of Src to Na/K-ATPase. Perhaps more important than the above is the fact that while the confusing issues about Src/ pump interaction were cluttering the literature, work on other aspects of the signaling function of the pump was ongoing and indicating that ouabain-induced activation of EGFR-Src-Ras-Raf-MEK-ERK1/2 pathway was indeed irrelevant to the growth-inducing actions of digitalis.

4. Digitalis-induced signaling through PI3K

When we first reported that exposure of neonatal cardiac myocytes to ouabain induces hypertrophic growth [5], we commented on the apparent paradox that digitalis seemed to be causing hypertrophy while its long-established use was in the treatment of the hypertrophied failing heart. This led us to look into the vast literature of the field that dealt with the cell signaling pathways regulating physiological and pathological cardiac hypertrophy [reviewed in 29,30]. Thus, we realized that in order to clarify the puzzle of ouabain effects on cardiac myocyte growth, we needed to study the drug's effect on phosphatidylinositol 3-kinases (PI3Ks). At the time it was well established [29,30] that physiological cardiac hypertrophy was associated with the activation of PI3K 1A, and that pathological cardiac hypertrophy resulted from the activation PI3K1B. The results of our initial studies in this direction [31] clearly showed that in the neonatal and adult rat cardiac myocytes, and in the intact rat heart, ouabain indeed activated PI3K1A, but had no effect on PI3K1B. While these studies established the linkage of PI3K1A -Akt-mTOR pathway to Na/K-ATPase, and the essential role of this linkage to ouabain-induced hypertrophy, they only hinted at the irrelevance of EGFR-Src-Ras-Raf-MEK-ERK1/2 pathway. This uncertainty was resolved by our subsequent work [32] showing that ouabain-induced activation of PI3K1A-Akt-mTOR pathway and the resulting proliferative growth was obtained both in normal fibroblasts and in fibroblasts devoid of Src. The irrelevance of EGFR-Src pathway was also reinforced by our studies [33] on mice with ventricular-specific knockout of NCX1, showing that ouabain-induced activation of PI3K1A and the resulting cardiac hypertrophy are independent of ouabain's positive inotropic effect. In this publication [33] we summarized our conclusions about the two ouabain-induced signaling pathways of the mouse heart in a figure. This is reproduced here as Fig. 1. I suggest that it is a fair summary, based on cumulative evidence, of what has been covered above. The above findings, coupled with the extensive work of others [29,30] suggested that ouabain-induced

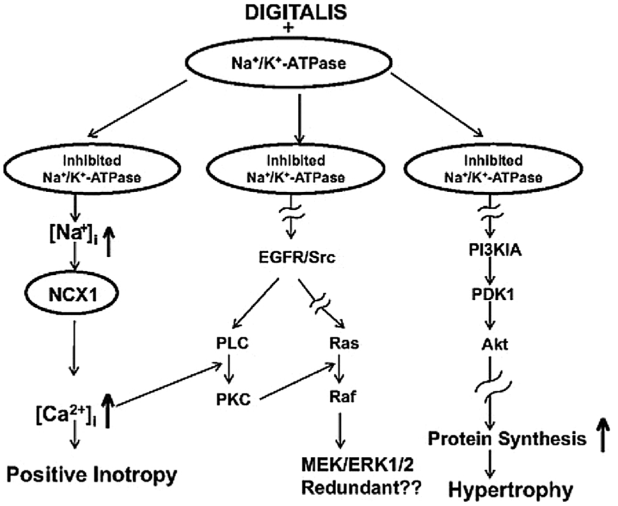

Fig. 1. Schematic presentation of the two parallel cell signaling cascades that are linked to Na/K-ATPase of cardiac myocytes, and their relations to digitalis-induced cardiac hypertrophy and positive inotropy. See Section 4 for the cumulative evidence indicating that the pathway through PI3K1A leads to hypertrophy, and suggests the redundance of the pathway through EGFR/Src.

hypertrophy may be akin to physiological hypertrophy. And since there was also substantial evidence to indicate that selective activation of PI3K1A not only causes physiological hypertrophy but may also antagonize the effects of stimuli that cause pathological hypertrophy [29,30], we set out to determine if in the mouse ouabain could antagonize the induction of pathological hypertrophy resulting from transverse aortic constriction. The results of these studies [34] showed that low and safe doses of ouabain that did not activate the EGFR-Src-Ras-Raf-MEK-ERK1/2 pathway, but activated PI3K1A, indeed prevented or delayed cardiac dysfunction and failure caused by pressure overload on the heart. These findings also clearly suggested that such uses of ouabain and related digitalis drugs for prevention of heart failure in man should be given serious consideration. To the best of my knowledge there has been no follow-up work to these promising studies. I have closed my laboratory due to necessary retirement, and the colleagues involved in the studies [31–34] are pursuing other goals. Other important work on digitalis-induced signaling through PI3K are a series of elegant studies initiated in Garlid's laboratory [35,36] showing that low and safe doses of digitalis protect the hearts of experimental animals from ischemia-reperfusion injury. Subsequently, it was shown [37,38] that these effects of ouabain or digoxin were obtained through selective activations of PI3K1A. I am not aware if these promising studies are being continued.

5. Activators of the signaling function of the sodium pump

The early studies that brought attention to the signaling functions of Na/K-ATPase [5,6] showed that cardiac glycosides, the well-established inhibitors of the pump, activated its signaling functions. At the time, many respected investigators of the field were advocating the hypothesis that one or several of these drugs are indeed hormones [e.g., 39,40]; hence, the signaling function of the pump was proposed to be hormonally controlled [6]. However, as I have pointed out elsewhere [41], whether or not any of the cardiac glycosides are hormones is still a matter of intensive debates. With the safe assumption that these drugs are simply inhibitors of Na/K-ATPase and its ion pumping function, we may ask if other inhibitors of this enzyme also affect its signaling function? In Section 3 of this review I discussed how the proposed direct binding of Src to Na/K-ATPase was partly based on the claim that while ouabain activated signaling by the pump, vanadate which is another inhibitor of Na/K-ATPase did not [18]. Later, however, these findings could not be repeated, and vanadate was found to activate signaling just like ouabain [22,23]. Also in relation to the Src/pump controversy, oligomycin which is another well-characterized inhibitor of Na/K-ATPase and its pumping function, was shown to be the an activator of the enzyme's signaling function [23]. Interestingly, these activating effects of ouabain and oligomycin were shown to be additive [23] because oligomycin is known to be a partial inhibitor of the Na/K-ATPase [23, and references therein].

Since all the known inhibitors of the sodium pump seem to be activators of its signaling function, may we assume that newly discovered pump inhibitors will also activate its signaling function? I think yes. In this regard it is important to note that recent observations [42,43] clearly indicate that fluorescent calcium indicators that have been used by most of us for decades are indeed good inhibitors of Na/K-ATPase. Though I expect them to be activators of the pump's signaling function, because conjecture is not a good substitute for data, experiments on the effects of a few of these newly found pump inhibitors on the pump's signaling function are needed.

6. Insufficiently studied signaling functions of the sodium pump

Soon after the early studies revealing the signaling functions of the pump [5,6], it became abundantly clear [4] that while Na/K-ATPase pumps ions by the same basic mechanism in all cells, there are significant differences in the mechanisms and consequences of its signaling functions in different cell types, and that a major reason for these differences is the diversity of the neighboring plasma membrane proteins that interact with the pump in various cell types. Many such diverse interactions have been identified, but not sufficiently studied for unknown reasons. I want to close by mentioning a few that I find intriguing, hoping that current or future active investigators of the field will do the necessary follow-up studies.

In 2006, collaborators from several U.S. and Russian institutions, and led by the capable itinerant scientist F. Mandel, reported [44] on the interaction of Na/K-ATPase with nicotinic acetylcholine receptor in skeletal muscle and in Torpedo electric organ. Showing that functional interaction between these neighboring receptors were stimulated by ouabain or by acetylcholine, as possible interaction mechanisms of the two receptors, they suggested the involvement of the cell signaling pathways that had been revealed by the earlier work [5,6]. A few years later, another publication by the same group [45] extended their earlier studies, establishing that the nicotinic receptor interaction is with the α2- isoform of Na/K-ATPase, and further identified the functional consequences of stimulus-induced interactions between these two receptor at the neuromuscular junction. I know of no follow-ups to these fascinating observations. Similar to the above case, limited studies of S.P. Soltoff [46,47] have clearly shown functional interactions between Na/K-ATPase and muscarinic acetylcholine receptors in parotid acinar cells, and implicated the activations of ERK1/2 and several other pathways by ouabain and carbachol in the regulation of saliva production. I have no knowledge of follow-up work to these interesting studies. Another intriguing but meagerly studied signaling function of Na/K-ATPase was brought to my attention by a clever colleague. This is based on the work of the late D. C. Gadsby, a true icon of the modern field of research on the sodium pump. A longtime advocate of the proposal that when deprived of extracellular K^+ and Na^+, the pump can transport protons, in a 2014 paper [48], Gadsby's laboratory presented solid data to show that even under physiological conditions when Na/K-ATPase is catalyzing the uphill transports of three Na^+ and two K^+, it may also carry out the downhill transports of one or more protons. Speculating on the potential pathophysiological implications of this finding [48–50], it was suggested that when extracellular pH is low (e. g., in the ischemic heart), the inward move-ment of protons through the pump may have significant consequences. Considering that prior work ofa respected laboratory [51] had shown that decreased intracellular pH activates the proto-oncogene Src, one may speculate on the possible existence of novel gene regulatory signaling events through the pump, but without the involvement of growth factors and digitalis drugs as discussed in the previous sections of this review. It remains to be seen if present or future active laboratories will do the necessary follow-up studies to test this possibility. Other insufficiently studied and interesting observations on the signaling functions of the pump resulting from its interaction with neighboring proteins include reports on pump interaction with (a) dopamine receptors [52]; (b) cofilin [53]; (c) NMDA receptors [54]; and (c) the volume-regulated anion channels [55].

7. Concluding remarks

Since the wide-spread realization of the other functions of the sodium pump about three decades ago, a good deal of progress has been made. It seems to me, however, that what remains unknown far outweighs what has been clarified. I suggest that those working on Na/K-ATPase should not have been surprised that this membrane enzyme can affect a multitude of cell functions through protein-protein interactions. Soon after the discovery of Na/K-ATPase by Skou [1,2], it was realized by two pioneers of the field that selected soluble enzymes of the glycolytic cycle were recruited to the plasma membrane to interact with the sodium pump and regulate the glycolitic rate [56,57]. We should have heeded the messages of these wise colleagues (S.L. Schrier and J.F. Hoffman) much sooner than we did.

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References

[1] J.C. Skou, The influence of some cations on an adenosine triphosphatase from peripheral nerves, Biochim. Biophys. Acta 23 (1957) 394–401.

[2] J.C. Skou, Further investigations of Mg^{++} + Na^{+}-activated adenosine triphosphatase, possibly related to active, linked transport of Na^{+} and K^{+} across the nerve membrane, Biochim. Biophys. Acta 42 (1960) 6-233.

[3] J.D. Robinson, Moving questions: a history of membrane transport and bioenergetics, Am. Physiol. Soc. Oxford University Press, 1997, pp. 103–146.

[4] Z. Xie, A. Askari, Na(+)/K(+)-ATPase as a signal transducer, Eur. J. Biochem. 269 (2002) 2434–2439.

[5] M. Peng, L. Huang, Z. Xie, W.H. Huang, A. Askari, Partial inhibition of Na^{+}/K^{+}-ATPase by ouabain induces Ca^{++}-dependent expressions of early-response genes in cardiac myocytes, J. Biol. Chem. 271 (1996) 10372–10378.

[6] O. Aizman, P. Uhlen, M. Lal, H. Brismar, A. Aperia, Ouabain, a steroid hormone that signals with slow calcium oscillations, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 13420–13424.

[7] A. Aperia, E.E. Akkuratov, J.M. Fontana, H. Brismar, Na^{+}-K^{+}-ATPase, a new class of plasma membrane receptors, Am. J. Physiol., Cell Physiol. 310 (2016) C-491–C495.

[8] L. Reinhard, H. Tidow, M.J. Clausen, P. Nissen, Na^{+}, K^{+}-ATPase as a docking station: protein-protein complexes of the Na^{+}, K^{+}-ATPase, Cell. Mol. Life Sci. 70 (2013) 205–222.

[9] A. Aperia, Na, K-ATPase, The Calcium Pathway, This issue (2019).

[10] L. Huang, H. Li, Z. Xie, Ouabain-induced hypertrophy in cultured cardiac myocytes is accompanied by changes in expressions of several late response genes, J. Mol. Cell. Cardiol. 29 (1997) 429–437.

[11] L. Huang, P. Kometiani, Z. Xie, Differential regulation of Na/K-ATPase α-subunit isoform gene expressions in cardiac myocytes by ouabain and other hypertrophic stimuli, J. Mol. Cell Cardiol. 29 (1997) 315–3167.

[12] P. Kometiani, J. Li, L. Gnudi, B.B. Kahn, A. Askari, Z. Xie, Multiple signal transduction pathways link Na^{+}/ K^{+}-ATPase to growth-related genes in cardiac myocytes, J. Biol. Chem. 273 (1998) 15249–15256.

[13] Z. Xie, P. Kometiani, J. Liu, J. Li, J.I. Shapiro, A. Askari, Intracellular reactive oxygen species mediate the the linkage of Na^{+}/K^{+}-ATPase to hypertrophy and its marker genes in cardiac myocytes, J. Biol. Chem. 274 (1999) 19323–18328.

[14] T. Pawson, J.D. Scott, Signaling through scaffold, anchoring, and adaptor proteins, Science 278 (1997) 2075–2080.

[15] M. Haas, A. Askari, Z. Xie, Involvement of Src and epidermal growth factor receptor in the signal-transducing function of Na^{+}/K^{+}-ATPase, J. Biol. Chem. 275 (2000) 27832–27837.

[16] M. Haas, H. Wang, J. Tian, Z. Xie, Src-mediated inter-receptor cross-talk between the Na^{+}/K^{+}-ATPase and the epidermal growth factor receptor relays the signal from ouabain to mitogen-activated protein kinases, J. Biol. Chem. 277 (2002) 18694–18702.

[17] H. Wang, M. Hass, M. Liang, et al., Ouabain assembles signaling cascades through the caveolar Na^{+}/K^{+}- ATPase, J. Biol. Chem. 279 (2004) 17250–17259.

[18] J. Tian, T. Cai, Z. Yuan, et al., Binding of Src to Na^{+}/K^{+}-ATPase forms a functional signaling complex, Mol. Biol. Cell 17 (2006) 317–326.

[19] S. Nakamura, S. Braun, E. Racker, A tyrosine-specific protein kinase from Ehrlich ascites tumor cells, Arch. Biochem. Biophys. 252 (1987) 538–548.

[20] L. Liu, K. Mohammadi, B. Aynafashar, et al., Role of caveolae in signal transducing function of cardiac Na^{+}/K^{+}-ATPase, Am. J. Physiol. Cell Physiol. 284 (2003) C1550–C1560.

[21] L. Liu, A.V. Ivanov, M. Gable, et al., Comparative properties of caveolar and non-caveolar preparations of kidney Na^{+}/K^{+}-ATPase, Biochemistry 50 (2011) 8664–8673.

[22] K.M. Weigand, H.G. Swarts, N.U. Fedosova, F.G. Russel, J.B. Koenderink, Na,K-ATPase activity modulates Src activation: a role for ATP/ADP ratio, Biochim. Biophys. Acta 1818 (2012) 1269–1273.

[23] M.E. Gable, S.L. Abdallah, S.M. Najjar, L. Liu, A. Askari, Digitalis-induced cell signaling by the sodium pump: on the relation of Src to Na(+)/K(+)-ATPase, Biochem. Biophys. Res. Commun. 446 (2014) 1151–1154.

[24] R.J. Clifford, J.H. Kaplan, Human breast tumor cells are more resistant to cardiac glycoside toxicity than non-tumorogenic breast cells, PLoS One 8 (2013) e84306.

[25] E. Yosef, A. Katz, Y. Preleg, T. Mehlman, S.J. Karlish, Do Src kinase and caveolin interact directly with Na,K-ATPase? J. Biol. Chem. 291 (2016) 11736–11750.

[26] L.P. Freedman, I.M. Cockburn, T.S. Simcoe, The economics of reproducibility in preclinical research, PLoS Biol. 13 (2015) e1002165.

[27] J. Brainard, Rethinking retractions, Science 362 (2018) 390–393.

[28] K.R. Sipido, Irreproducible results in preclinical cardiovascular research: opportunities in times of need, Cardiovasc. Res. 115 (2019) e34–e36.

[29] B.C. Bernardo, K.L. Weeks, L. Pretorius, J.R. McMullen, Molecular distinctions between physiological and pathological cardiac hypertrophy: experimental findings and therapeutic strategies, Pharmacol. Ther. 128 (2010) 191–227.

[30] Y.K. Tham, B.C. Bernardo, J.Y.Y. Ooi, K.L. Weeks, J.R. McMullen, Pathophysiology of cardiac hypertrophy and heart failure: signaling pathways and novel therapeutic targets, Arch. Toxicol. 89 (2015) 1401–1438.

[31] L. Liu, X. Zhao, S.V. Pierre, A. Askari, Association of PI3K-Akt signaling pathway with digitalis-induced hypertrophy of cardiac myocytes, Am. J. Physiol. Cell Physiol. 293 (2007) C1489–C1497.

[32] J. Wu, E.E. Akkuratov, Y. Bai, et al., Cell signaling associated with Na^{+}/K^{+}-ATPase: Activation of phosphatidylinositide 3-kinase 1A/Akt by ouabain is independent of Src, Biochemistry 52 (2013) 9059–9067.

[33] Y. Bai, E.E. Morgan, D.R. Giovannucci, et al., Different roles of the cardiac Na^{+}/Ca^{2+}-exchanger in ouabain-induced inotropy, cell signaling, and hypertrophy, Am. J. Physiol. Heart Circ. Physiol. 304 (2013) H427–H435.

[34] J. Wu, D. Li, L. Du, et al., Ouabain prevents pathological cardiac hypertrophy and heart failure through activation of phosphoinositide 3-kinase α in mouse, Cell Biosci. (2015) 5–64 10.1186/s13578-015-0053-7.

[35] P. Pasdois, C.L. Quinlan, A. Rissa, et al., Ouabain protects rat hearts against ischemia-reperfusion Injury via pathway involving src kinase, mitoKATP, and ROS, Am. J. Physiol. Heart Cir. Physiol. 292 (2007) H1470–H1478.

[36] K.D. Garlid, A.D.T. Costa, C.L. Quinlan, S.V. Pierre, P. Dos Santos, Cardioprotective signaling to mitochondria, J. Mol. Cell. Cardiol. 46 (2009) 858–866.

[37] Q. Duan, N.D. Madan, J. Wu, et al., Role of phosphoinositide 3-kinase 1A (PI3K1A) activation in cardioprotection induced by ouabain preconditioning, J. Mol. Cell. Cardiol. 80 (2015) 114–125.

[38] Q. Duan, Y. Xu, P.V. Mark, et al., Preconditioning and postconditioning by cardiac glycosides in the mouse heart, J. Cardiovasc. Pharmacol. 71 (2018) 95–103.

[39] J.M. Hamlyn, M.P. Blaustein, S. Bova, et al., Identification and characterization of a ouabain-like from From human plasma, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 6259–6263.

[40] W. Schoner, Endogenous cardiotonic steroids, Cell. Mol. Biol. (Noisy-le-grand) 47 (2001) 273–280.

[41] A. Askari, The sodium pump and digitalis drugs: dogmas and fallacies, Pharmacol. Res. Perspect. 7 (2019) e00505, , https://doi.org/10.1002/prp2.505.

[42] N.A. Smith, B.T. Kress, Y. Lu, et al., Fluorescent Ca^{2+} indicators directly inhibit the Na,K-ATPase and disrupt cellular functions, Sci. Signal. 11 (2018), https://doi.org/10.1126/scisignal.aa1239.

[43] M.D. Bootman, S. Allman, K. Rietdorf, G. Bultynck, Deleterious effects of calcium indicators within cells; an inconvenient truth, Cell Calcium 73 (2018) 82–87.

[44] I.I. Krivoi, T.M. Drabkina, V.V. Kravtsova, et al., On the functional interaction between nicotinic acetylcholine receptor and Na+,K+-ATPase, Pflugers Arch. 452 (2006) 756–765.

[45] J.A. Heiny, V.V. Kravtsova, F. Mandel, et al., The nicotinic acetycholine receptor and the Na,K-ATPase alpha2 isoform interact to regulate membrane electrogenesis in skeletal muscle, J. Biol. Chem. 285 (2010) 28614–28624.

[46] S.P. Soltoff, L. Hedden, Regulation of ERK1/2 by ouabain and Na-K-ATPase-dependent energy utilization and AMPK activation in parotid acinar cells, Am. J. Physiol. Cell Physiol. 295 (2008) C590–C599.

[47] S.P. Soltoff, J.M. Ansara, L. Hedden, Regulation and identification of Na,K-ATPase α1 subunit phosphorylation in rat parotid acinar cells, J. Biol. Chem. 285 (2010) 36330–36338.

[48] N. Vedovato, D.C. Gadsby, Route, mechanism, and implications of proton import during Na+/K+ Exchange by native Na+/K+-ATPase pumps, J. Gen. Physiol. 143 (2014) 449–464.

[49] E.M. Adler, Of pumps, protons, chloride gradients, and microvesicles at the

immunological synapse, J. Gen. Physiol. 143 (2014) 417–418.
[50] D.W. Hilgemann, Fishing for holes in transporters: how protons breach the Na/K pump security gates, J. Gen. Physiol. 143 (2014) 437–441.
[51] Y. Yamaji, H. Tsuganezawa, O.W. Moe, R.J. Alpern, Intracellular acidosis activates c-Src, Am. J. Physiol. 272 (1997) C886–C893.
[52] L.A. Hazelwood, R.B. Free, D.M. Cabrera, M. Skinbjerg, D.R. Sibley, Reciprocal modulation of function between the D_1 and D_2 dopamine receptors and Na^+, K^+-ATPase, J. Biol. Chem. 283 (2008) 36441–36453.
[53] K. Lee, J. Jung, M. Kim, G. Guidotti, Interaction of the α subunit of Na,K-ATPase with cofilin, Biochem. J. 353 (2001) 377–385.
[54] E.E. Akkuratov, O.M. Lopacheva, M. Kruusmagi, et al., Functional interaction between Na/K-ATPase and NMDA receptor in cerebellar neurons, Mol. Neurobiol. 52 (2015) 1726–1734.
[55] T. Fujii, T. Shimizu, S. Yamamoto, et al., Crosstalk between Na^+,K^+-ATPase and a volume-regulated anion channel in membrane microdomains of human cancer cells, BBA – Mol. Basis Disease 1864 (2018) 3792–3804.
[56] S.L. Schrier, Organization of enzymes in human erythrocyte membranes, Am. J. Physiol. 210 (1966) 139–145.
[57] J.C. Parker, J.F. Hoffman, The role of membrane phosphoglycerate kinase in control of glycolytic rate by active cation transport in human red blood cells, J. Gen. Physiol. 50 (1967) 893–916.