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Na^+-K^+-ATPase, a new class of plasma membrane receptors

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Aperia A, Akkuratov EE, Fontana JM, Brismar H. Na^+-K^+-ATPase, a new class of plasma membrane receptors. *Am J Physiol Cell Physiol* 310: C491–C495, 2016. doi:10.1152/ajpcell.00359.2015.—The Na^+-K^+-ATPase (NKA) differs from most other ion transporters, not only in its capacity to maintain a steep electrochemical gradient across the plasma membrane, but also as a receptor for a family of cardiotonic steroids, to which ouabain belongs. Studies from many groups, performed during the last 15 years, have demonstrated that ouabain, a member of the cardiotonic steroid family, can activate a network of signaling molecules, and that NKA will also serve as a signal transducer that can provide a feedback loop between NKA and the mitochondria. This brief review summarizes the current knowledge and controversies with regard to the understanding of NKA signaling.

ouabain; Na^+-K^+-ATPase; receptor; signaling; apoptosis

THE NA^+-K^+-ATPASE (NKA) IS well known for its role as an ion pump, transporting three Na^+ ions out of the cell and two K^+ ions into the cell at the cost of one ATP. This active energy-dependent transport provides the diffusion gradient for multiple other transporters that control electrolyte and fluid homeostasis and uptake of nutrients and prevents the waste of neurotransmitters in a highly energy-efficient manner (7, 29). The eukaryotic cell does not survive without NKA. There is now mounting evidence that NKA also plays a vital role as a signal transducer, controlling a number of vital cell functions (4, 20, 42, 57, 61). Here we will summarize the evidence for NKA signaling, discuss whether NKA represents a novel class of signal transducers, describe some of the physiological and medical effects and consequences of NKA signaling, and propose that this signal allows for a bidirectional communication between NKA and its energy supplier, the mitochondria.

Activation of NKA Signaling

Most studies of NKA signaling have used the cardiotonic steroid ouabain to activate the signaling pathway. The minimal NKA functional unit is a heterodimer consisting of a catalytic α-subunit and a β-subunit required for the proper insertion of NKA into the membrane. There are four mammalian isoforms of the α-subunit. The cardiotonic steroids are specific NKA ligands, which bind to the extracellular domain of all NKA α-subunit isoforms. The binding site for cardiotonic steroids is highly conserved between species (42). Saturating concentrations of ouabain completely inhibit the NKA pumping capacity, but subsaturating concentrations, which have little or no effect on intracellular sodium concentration, can activate a variety of signaling molecules. Circulating levels of the endogenous cardiotonic steroids ouabain, marinobufagenin, and digoxin have been detected with mass spectrometry, immunoassays, and nuclear magnetic resonance (6, 26, 36, 63). The concentrations are in the picomolar-nanomolar range (18), and there is a current debate whether mass spectrometry may be a sufficiently sensitive tool for determination of circulating ouabain (9, 38).

The binding and release of Na^+ and K^+ and the ATP hydrolysis during the reaction cycle of NKA is accompanied by a series of conformational changes of the α-subunit, classified either as an E1 Na^+ binding state or an E2 K^+ binding state. Ouabain changes the conformational equilibrium of NKA by binding to the E2 state (53). Ouabain is not the only cardiotonic steroid that can activate NKA signaling (10). Both digoxin and marinobufagenin have been shown to trigger calcium signals that are almost identical to those triggered by ouabain (20).

NKA Signaling Pathways

The first evidence that ouabain-bound NKA might act as a signal transducer came from studies by Askari and Xie, who reported that exposure of myocytes to ouabain resulted in a calcium-dependent activation of early response genes and mitogen-activated protein kinases (35, 56). Two years later, the same group reported that nonsaturating concentrations of ouabain stimulated Src kinase phosphorylation (25). Xie and colleagues have since then in a series of studies reported that NKA maintains Src in an inactivated state, and that ouabain-bound NKA will release and phosphorylate Src kinase (41, 57, 66). Other groups have confirmed that nonsaturating concentrations of ouabain phosphorylate Src kinase (24, 74), but the

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question whether Src kinase binds directly with high affinity to the NKA α-subunit has recently become an area of controversy (21, 70). This controversy may, however, be resolved by the recent finding that NKA and Src kinase can exist in multiprotein complexes.

At the time when the first NKA signaling studies were published from the Askari/Xie laboratory, we made the serendipitous observation that ouabain can trigger a calcium oscillatory signal (2). In a study of the dose-dependent effect of ouabain on cytosolic calcium in rat epithelial cells, we found that subsaturating concentrations of ouabain triggered calcium oscillations with a consistent periodicity that ranged between 4 and 5 min. The ouabain-triggered calcium oscillations are activated via direct interaction between the NH_2-terminus tail of the NKA α-subunit and the NH_2-terminus of the inositol 1,4,5-trisphosphate receptor (IP_3R) (73). Ankyrin-B binds the NH_2-terminus tail of the α-subunit and the NH_2-terminus of the IP_3R, and deletion of ankyrin-B modifies the calcium signal (44). Activation of the IP_3R is independent of activation of phospholipase C and release of inositol 1,4,5-trisphosphate (48). Recurrent calcium release via the IP_3R generally requires the activation of plasma membrane calcium transporters, and both voltage-gated calcium channels and the Na^+/Ca^{2+} exchanger play a role for the sustainability of the ouabain-triggered calcium oscillations (19, 20). An oscillatory calcium signal has a high level of specificity, as cells can decode differences in intracellular calcium oscillation frequency (14). In renal epithelial cells grown to confluence, ouabain-triggered calcium oscillations will spread to surrounding cells. A recent study by the Cereijido group demonstrated that ouabain signaling can facilitate the spread of calcium signals to surrounding cells by increasing gap junction communication (59). Cytosolic oscillatory calcium signals can be transferred to the mitochondria via specific calcium transporters.

Exposure of cells to subsaturating concentrations of ouabain has also been reported to activate the protein kinases phosphatidylinositide 3-kinase and Akt (known to play a role for proliferation and protection against apoptosis) (43). This signaling cascade is not Src kinase dependent (71). The ouabain-triggered calcium oscillatory signal is abolished in the presence of Src kinase inhibitors (20). These observations suggest that the ouabain activated signaling pathways are interconnected, and they also illustrate that our present understanding of the NKA signaling networks is still only fragmentary.

NKA, a Novel Class of Cell Surface Receptors

NKA may represent a novel class of cell-surface receptors that interacts with its first generation of signaling molecules via allosterism. NKA is not a G protein-coupled receptor or a ligand-gated ion channel. NKA activates IP_3R via protein-protein interaction (48). Saturating concentrations of ouabain inhibit ion transport, but signaling occurs with nonsaturating ouabain concentrations, and the read-outs do not depend on changes in intracellular ion concentration. Is NKA a catalytic receptor? Ligand-bound NKA phosphorylates Src kinase, but definite proof that the NKA α-subunit binds Src kinase with high affinity is lacking. An alternative explanation may be that NKA and Src kinase are members of a signaling complex, and that ouabain-bound NKA will via allosterism activate Src kinase. The NKA α-subunit is well suited to serve as an allosteric modifier, since it undergoes large conformational changes during its reaction cycle (52, 61). Both ATP and sodium have been classified as allosteric modifiers of NKA (22, 30), and a recent study by Weigand et al. (70) suggested that the ATP/ADP ratio can lead to Src kinase activation. NKA is often described as a member of multiprotein complex, and a large number of interacting partners for the NKA α-subunit have been identified (61).

Physiological and Medical Implications of NKA Signaling

Numerous studies have shown that NKA signaling protects cells from irreversible damage and death. Cell protection has been observed in brain, heart, and kidney (15, 40, 50, 65, 72). In mice that have undergone closed head injury, chronic treatment with low dose of ouabain significantly improves recovery and functional outcome (16). Ouabain/NKA signaling protects cardiac cells against ischemia-reperfusion injury (55). The effect is more pronounced if the cells have first been preconditioned with nonsaturating concentrations of ouabain (49, 58). The cardioprotective effect in cardiac cells is mediated via protection of mitochondrial function (23, 60, 67).

Developing organs must be precisely patterned, and, to ensure a robust pattern formation, apoptosis is used to remove cells in aberrant positions. Fetal malnutrition is associated with excessive apoptosis and adverse developmental programming. The embryonic kidney is particularly sensitive to malnutrition. Studies from our group have demonstrated that ouabain can protect from apoptosis. When we exposed explanted rat embryonic kidneys to serum starvation to mimic malnutrition, apoptosis was increased, and nephron formation retarded. Exposure of the malnourished kidneys to 10 nM ouabain was found to completely protect from excessive apoptosis and retarded nephron development. Proof of principle that ouabain rescues development of malnourished embryonic kidneys was obtained from studies on pregnant rats given a low-protein diet and treated with ouabain or vehicle throughout pregnancy (39). Circulating levels of ouabain are reported to be increased during pregnancy (27), and it was recently reported from the Lichtstein group that offspring of pregnant mice, which have been treated with anti-ouabain antibodies, have a lower birth weight and retardation of kidney and liver development (17). The mechanism by which ouabain protects from apoptosis is as yet not fully understood, but studies of shigatoxin-triggered apoptosis in primary rat proximal tubule cells have indicated that ouabain exerts its protective effect by interfering with the onset of the mitochondrial apoptotic pathway (11). Shigatoxin-triggered apoptotic pathway is initiated by a downregulation of the antiapoptotic factor Bcl-xL and an upregulation of the apoptotic factor Bax, which is recruited to the mitochondria, where it forms oligo- and heterodimers, which permeabilize the outer mitochondrial membrane. Ouabain protects from shigatoxin-triggered apoptosis by preventing the upregulation and mitochondrial recruitment of Bax and the downregulation of Bcl-xL. Interestingly, it has been reported that the NKA α-subunit can bind to Bcl-xL (37).

Ouabain in low concentrations has also been reported to stimulate proliferation of nonmalignant cells (3, 33, 68). Stud-

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ies on vascular smooth muscle cells, Sertoli cells, and mammalian retinal cells indicate that this downstream effect of ouabain is of great physiological importance (1, 5, 46, 62).

The antiapoptotic and proliferative effects are observed in cells exposed to 5–10 nM ouabain. These concentrations are much lower than the ouabain concentrations required to inhibit the enzyme, indicating that the NKA-triggered signal will be amplified to a similar extent as signals triggered from G protein-coupled receptors. Ouabain 5–10 nM has no effect on intracellular sodium concentration (39). A question that sometimes comes up is whether inhibition of isolated ouabain-bound NKA molecules may mediate its downstream effects via local increases in sodium concentration. We find this unlikely, taking into account that diffusion of ions in the cytoplasm is a transport process that is orders of magnitude faster than can be achieved by the slow turnover of isolated NKA. The NKA has a turnover on the millisecond scale, where the diffusion equilibration radius for cytoplasmic ions is on the micrometer scale (13).

Ouabain has an opposite effect on the apoptotic process in nonmalignant and malignant cells and has been used in clinical trials as an antitumor drug (12, 47). The reason for this discrepancy is not understood, but should be a high priority question to address.

Adverse Effects of NKA Signaling

In certain disease conditions, the cell might misinterpret a plasma membrane signal. For example, signals from the angiotensin receptors can contribute to the pathology of cardiovascular and kidney disorder (64). The same may be true for the signals from NKA. Cardiomyopathy is a common complication in end-stage renal failure, and, in studies on rats with $<25\%$ remaining renal tissue, it was found that high circulating levels of the cardiotonic steroid marinobufagenin significantly contributed to the cardiac pathology (31, 34). Hypercalcemia might have been the cause of the digoxin toxicity (69). An elegant study from the Lingrel laboratory on mice expressing the ouabain-resistant NKA-α_2 isoform showed that ouabain contributes to ACTH-dependent hypertension (45). However, it was also reported that the blood pressure elevating effect of ouabain was beneficial during pregnancy (54). Studies from Blanco's laboratory have demonstrated that ouabain signaling can contribute to the pathology of cystic kidney disease (51). Ouabain in nanomolar concentrations was found to enhance cAMP-dependent fluid secretion and cyst growth of human cyst epithelial cells that express the cystic fibrosis transmembrane conductance regulator (CFTR), but not in cells lacking CFTR (8, 28).

Perspectives

The NKA pumping and NKA signaling functions are strongly related. As a salt pump, NKA provides the cell with a maximally efficient use of energy and is a prerequisite for life. As a signal transducer, NKA provides a feedback loop between NKA and its energy supplier, which serves to protect life. This important feedback loop is just beginning to be understood.

NKA should be considered as a novel class of plasma membrane receptors that act via allosteric modification of the target molecule or of other signaling molecules, such as Src kinase and the IP_3R. The series of conformational changes that NKA undergoes during its reaction cycle makes it extraordinarily well suited for the role as an allosteric signal transducer. NKA may act as an allosteric activator in a multiprotein complex. The identification and cell-specific effects of NKA signaling in such complexes should be explored.

The role of NKA as a signal transducer is just beginning to be understood, and the contributions to this field have so far come from a rather limited number of research groups. Much more work needs to be done in this field to expand our knowledge about energy control and the protective role of the NKA signal in health and disease. More work in this field is also needed to define the border between the beneficial and adverse effects of NKA signaling. The role and possibly aberrant signaling pathways of NKA in cancer cells is another important and urgent topic for future studies.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: A.A. drafted manuscript; A.A., E.E.A., J.M.F., and H.B. edited and revised manuscript; A.A., E.E.A., J.M.F., and H.B. approved final version of manuscript.

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