



# Asian Journal of **Biochemistry**

ISSN 1815-9923



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## Sodium Pump Adaptability to Tissue-Specific Regulation: A Review

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### ABSTRACT

The sodium pump or Na<sup>+</sup>/K<sup>+</sup> ATPase is a membrane-bound enzyme that maintains the Na<sup>+</sup> and K<sup>+</sup> gradients across the plasma membrane of animal cells. Many basic and specialized cellular functions depend on the activity of this enzyme, hence, its adaptability to changing cellular and physiological stimuli. This review presents an overview of the many adaptations or mechanisms that regulate sodium pump activity in a tissue-specific manner. These mechanisms include regulations by substrates, membrane associated components such as cytoskeletal elements and the  $\gamma$ -subunits and circulating endogenous inhibitors as well as a variety of hormones, including corticosteroids, peptide-hormones and catecholamine. In addition, this review considers the effects of a range of specific intracellular signaling pathways involved in the regulation of sodium pump activity.

**Key words:** Na<sup>+</sup>/K<sup>+</sup> ATPase, membrane, inhibitors, cAMP, ATP

### INTRODUCTION

The Na<sup>+</sup>/K<sup>+</sup> adenosine triphosphatase (ATPase) is an enzyme and a glycoprotein composed of 2  $\alpha$  and 2  $\beta$  chains. Its activity depends on the presence of Na<sup>+</sup> and K<sup>+</sup> and requires adenosine triphosphate (ATP) and Mg<sup>2+</sup> as cofactors. The enzyme hydrolyzes a high energy phosphate bond of ATP and uses the energy thus released to transport 3 Na<sup>+</sup> outside and simultaneously 2 K<sup>+</sup> inside across the cell membrane (Therien and Blostein, 2000).

Sodium pump is also called Na<sup>+</sup>/K<sup>+</sup> ATPase, located at basal and lateral plasma membrane of intestinal and renal cells. It actively transports sodium ion (Na<sup>+</sup>) required for its activity. The Na<sup>+</sup>/K<sup>+</sup> ATPase maintain both magnitude and direction of transmembrane concentration gradients of Na<sup>+</sup> and K<sup>+</sup> (Aperia *et al.*, 1992; Anderson *et al.*, 1998). The Na<sup>+</sup>/K<sup>+</sup> ATPase is very active in those cells where activities depends largely on transmembrane Na<sup>+</sup> fluxes such as in the nervous system, muscle fibres, renal tubule cells and intestinal mucosal cells (Clausen, 1996; Aperia *et al.*, 1996). Na<sup>+</sup>/K<sup>+</sup> ATPase exist in two isoforms; E<sub>1</sub> and E<sub>2</sub>. The E<sub>1</sub> presents its ion binding and phosphate binding sites on the cytoplasmic surface of the membrane to which three Na<sup>+</sup> from the cytoplasm bind leading to phosphorylation of aspartate residue of E<sub>1</sub> (Fig. 1). The process is ATP and Mg<sup>2+</sup> dependent. This results in conformational change of E<sub>1</sub>→E<sub>2</sub>. The E<sub>2</sub> exposes both ion binding and phosphate binding sites on the extracellular surface of the membrane, thus lowering the affinity of ATPase for Na<sup>+</sup> and releases it into the extracellular fluid in exchange for K<sup>+</sup> extracellular fluid (ECF) causing dephosphorylation. Dephosphorylation again changes the conformation of E<sub>2</sub>→E<sub>1</sub> thereby lowering its affinity for K<sup>+</sup> from the ATPase into the cell (Therien and Blostein, 2000).

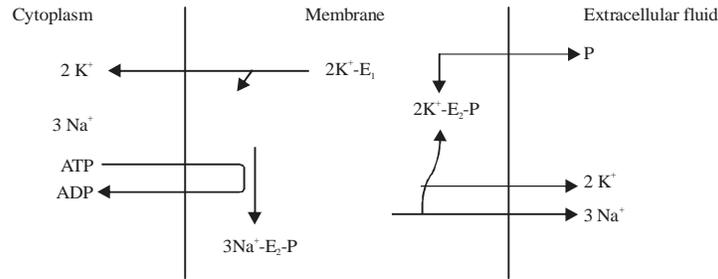


Fig. 1: Mechanism of action of sodium pump (source: Anderson *et al.*, 1998)

Thus, the Na<sup>+</sup> transported actively by sodium pump diffuses into the cell across its luminal or microvillus membrane from the lumen. Active absorption of Na<sup>+</sup> is coupled with glucose absorption or amino acid absorption (Aperia *et al.*, 1992).

Ouabain, a cardiotonic steroidal glycoside obtained from digitalis inhibits the Na<sup>+</sup>/K<sup>+</sup> ATPase by blocking dephosphorylation of E<sub>2</sub> conformer (Hamlyn *et al.*, 1996), whereas, vanadate inhibits the pump in a similar manner when present inside the cell (Anderson *et al.*, 1998).

The basic function of Na<sup>+</sup>/K<sup>+</sup> ATPase or sodium pump is to maintain Na<sup>+</sup> and K<sup>+</sup> gradient across the plasma membrane of animal cells. The sodium pump is important in regulating action potential, cell volume, cytoplasmic pH and Ca<sup>2+</sup> levels through the Na<sup>+</sup>/H<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> exchangers, respectively and in driving a variety of secondary transport processes such as Na<sup>+</sup>-dependent glucose and amino acid transport.

In nerve transmission, transient propagated reversal of charge on the axonal membrane referred to as nerve action potential arriving pre-synaptic terminal cause the release of excitatory or inhibitory neurotransmitter (Rang *et al.*, 2011; Stephanie, 2015). A combination of excitatory transmitter with pre-synaptic receptors produces a localized depolarization resulting in excitatory postsynaptic potential through an increase in permeability of Na<sup>+</sup>. On the other hand, inhibitory transmitter causes selective permeability to K<sup>+</sup> or Cl<sup>-</sup> resulting in hyperpolarisation, hence, inhibitory postsynaptic potential (Rang *et al.*, 2011; Harvey *et al.*, 2012).

In skeletal muscles, released transmitter (Acetylcholine) at motor end-plate binds to nicotinic acetylcholine receptors causing increase in Na<sup>+</sup> and K<sup>+</sup> conductance in end-plate membrane with consequent generation of action potential in muscle fibres (Beebe, 1994; Rang *et al.*, 2011; Stephanie, 2015). Action potential in muscle fibres lead to inward spread of depolarization along T-tubules, which in turn open L-type Ca<sup>2+</sup> channels. Mechanical coupling of L-type Ca<sup>2+</sup> channel with Ryanodine receptor activates the release of Ca<sup>2+</sup> from sarcoplasmic reticulum and diffusion from thick and thin filaments. The Ca<sup>2+</sup> activates troponin C resulting in the formation of cross-linkages between actin and myosin and sliding of thin and thick filaments produce contraction (Rang *et al.*, 2011; Stephanie, 2015).

On the contrary, skeletal muscle relaxation occurs most importantly as a result of sequestration of Ca<sup>2+</sup> in sarcoplasmic reticulum. ATP-dependent Ca<sup>2+</sup> pump, pumps Ca<sup>2+</sup> against concentration gradient into sarcoplasmic reticulum which then bind to calsequestrin and calreticulin (Rang *et al.*, 2011). Another mechanism is by extrusion of Ca<sup>2+</sup> across plasma membrane via Na<sup>+</sup>-Ca<sup>2+</sup> exchanger and plasma membrane Ca<sup>2+</sup> pump (Doucet, 1988; Harvey *et al.*, 2012). These processes result in detachment of cross-bridges from the thin filament, restoration of troponin-tropomyosin regulated inhibition of actin and myosin interaction, followed by skeletal muscle relaxation.

In smooth muscles, contraction occurs when hormone, neurotransmitter or depolarization activates the release of  $\text{Ca}^{2+}$  from sarcoplasmic reticulum, ligand gated  $\text{Ca}^{2+}$  channels or voltaged gated  $\text{Ca}^{2+}$  channels. Released  $\text{Ca}^{2+}$  bind to calmodulin causing increase in cyclic adenosine triphosphate, which phosphorylates myosin light chain to form myosin ATPase a process catalysed by myosin light chain kinase. Phosphorylated myosin bind to actin forming cross-bridge producing contraction (Wang *et al.*, 1995; Meister and Aperia, 1993; Rang *et al.*, 2011).

The sodium pump is the target of multiple regulatory mechanisms activated in response to changing cellular requirements. The requirement for modulators of the  $\text{Na}^+/\text{K}^+$  ATPase is likely to be greatest in tissues in which perturbations of the intracellular alkali cation content underlie their specialized functions, such as; nerve impulse propagation, exercise and changes in diet (Skou, 1957; Beebe, 1994).

One primary need for sodium pump adaptation comes from changes in dietary  $\text{Na}^+$  and  $\text{K}^+$ . The mediators of natriuresis and diuresis; hormones that control the volume and ionic composition of blood and urine, often act directly on the sodium pump of the nephron and intestine. The function of the pump in absorption or reabsorption of  $\text{Na}^+$  and  $\text{K}^+$  and secondarily, other solutes requires tight regulation of the enzyme to maintain normal levels of  $\text{Na}^+$  and  $\text{K}^+$  during altered salt intake (Blanco and Mercer, 1998). The  $\text{Na}^+$  transport across epithelia is linked with water reabsorption. Thus, impairment of the sodium pump in kidney and small intestine is associated with the pathophysiology of hypertension and chronic diarrhea, respectively (Bonvalet, 1998; Blaustein, 1996).

In excitable tissues such as neurons, skeletal muscle cells and pace maker fibres of the heart, the sodium pump must reestablish the electrical potential across the plasma membrane following excitation-induced depolarization (Clausen, 1996; Hernandez, 1992). In skeletal muscle, continuous stimulation of muscle fibre during exercise leads to dissipation of the cation gradient necessary for muscle contraction. To offset excessive release of  $\text{K}^+$  from the muscle cells during exercise, rapid activation of  $\text{Na}^+/\text{K}^+$  ATPase is essential to cause delay in the onset of muscular fatigue and reducing potentially toxic levels of plasma  $\text{K}^+$  (Anderson *et al.*, 1998). The  $\text{Na}^+/\text{K}^+$  ATPase regulation in cardiac muscle is particularly critical to the myocardium, where the enzyme indirectly regulate contraction (Clausen, 1996; Blaustein, 1996). Thus, the sodium pump controls the steady-state cytoplasmic  $\text{Na}^+$  concentration which then determines  $\text{Ca}^{2+}$  concentration via the  $\text{Na}^+/\text{Ca}^{2+}$  exchange. The  $\text{Ca}^{2+}$ , in turn, is pumped into the Sarcoplasmic Reticulum (SR) by the Sarco-Endoplasmic Reticulum Calcium (SERCA) pumps (Doucet, 1988; Clausen, 1996).

Regulation of the sodium pump in these tissues is paramount for determination of the “set point” for cardiac muscle contraction and the steady-state contraction of vascular smooth muscle (Nielsen and Clausen, 1996). Physiological regulation that act in a manner analogous to that ascribed to cardiac glycoside inhibition of the  $\text{Na}^+/\text{K}^+$  ATPase are likely to be critical for normal heart contraction. Thus, the force of contraction is increased via increasing cell  $\text{Na}^+$  and may be considered to be the basis of digitalis therapy for cardiac insufficiency (Hernandez, 1992; Gloor, 1997).

This study therefore, focuses on adaptations to tissue-specific regulation of the sodium pump with emphasis on two mechanisms involving pathways that result in modulation in sodium pump activity and the interaction of the pump complex with other membrane components, causing further modulation via other signaling cascades.

**Role of substrate concentration in sodium pump activity:** The simplest and most straightforward determinants of sodium pump activity are the concentrations of substrates. The sodium pump is activated by  $\text{Na}^+$  at extracellular sites. Thus, small changes in the cytoplasmic  $\text{Na}^+$  concentration secondary to activation of either various  $\text{Na}^+$  dependent transporters or  $\text{Na}^+$  channels can have dramatic effect on sodium pump activity (Blaustein, 1996; Skou, 1957).

The high affinity of the enzyme for  $\text{K}^+$  at activating sites generally precludes the effects of variations in extracellular  $\text{K}^+$  concentrations which have been shown to act as a competitive inhibitor of  $\text{Na}^+$  binding at cytoplasmic sites (Garay and Garrahan, 1973). The variation in cytoplasmic  $\text{K}^+$  concentration, or, more likely, in the affinity of the enzyme for  $\text{K}^+$  as an antagonist at cytoplasmic  $\text{Na}^+$  activating binding sites, is a plausible mechanism for determining the set point for the physiological concentration at half maximal activation ( $k = 0.05$ ) for cytoplasmic  $\text{Na}^+$  activation. Hence, the use of  $\text{K}^+$  channels activators as antiarrhythmic agents. Half-maximal activation of the enzyme by intracellular  $\text{Na}^+$  occurs at concentrations of -10 to 40 mM, which, depending on the tissue, are often at or above the steady state  $\text{Na}^+$  concentration (Blaustein, 1996). Because the physiological concentration at half maximal activation of the  $\text{Na}^+/\text{K}^+$  ATPase for ATP is between 300 and 800 mM (Blaustein, 1996), the ATP concentration in most cells is saturating for the enzyme. However, in some tissues and under certain conditions, ATP levels may fall to sub-saturating levels. For example, cells of the kidney medulla are known to function under near anoxic conditions (Brezis and Rosen, 1995) and such conditions could lead to dramatic drop in ATP levels (Soltoff and Mandel, 1984). Thus, variations in ATP concentration or in the affinity of the sodium pump for ATP may be a physiologically relevant mechanism of pump regulation in this tissue.

**Role of membrane-associated components in sodium pump activity:** The  $\text{Na}^+/\text{K}^+$  ATPase is a membrane-embedded protein, hence, the nature of the membrane components might be important determinants of enzyme function. Little is known on the nature and mechanistic basis of sodium pump modulation by specific membrane components (the role of membrane lipids). The main effects of lipids on the sodium pump are related to membrane fluidity and thickness (Paller, 1994). Membrane lipids promote bilayer formation of physiological thickness and increased fluidity which tend to promote optimal  $\text{Na}^+/\text{K}^+$  ATPase activity as do negatively charged lipids such as phosphatidylserine and phosphatidylglycerol (Paller, 1994; Kimelberg and Papahadjopoulos, 1972). The effects of cholesterol on enzyme activity are often related to membrane fluidity free fatty acids present in the membrane or as the products of phospholipase  $\text{A}_2$  ( $\text{PLA}_2$ ) dependent regulatory pathway tend to inhibit the  $\text{Na}^+/\text{K}^+$  ATPase (Oguchi *et al.*, 1993).

**Interactions of sodium pump with components of the cytoskeleton of cells:** Components of the cytoskeleton are proteins include spectrin, actin, adducin, pascin and ankyrin (Cantiello, 1995, 1997). The main consequence of interactions between the  $\text{Na}^+/\text{K}^+$  ATPase and the cytoskeleton is proper processing and targeting of sodium pump to the appropriate membrane-compartment. Example, disruptions in the cellular distribution of  $\text{Na}^+/\text{K}^+$  ATPase induced either by ATP depletion or hypoxia, are linked to alterations in cytoskeleton proteins and a spectrin-ankyrin complex is required for transport of pumps from the endoplasmic reticulum to the Golgi apparatus (Paller, 1994). Recently, actin has been shown to activate the sodium pump by a mechanism mediated by cAMP-dependent protein kinase (Skou, 1957).

Thus, the role of cytoskeleton proteins in regulating sodium pump is suggestive of a mechanism, which appears to involve acceleration of the rate of the conformational change  $E_2(K^+) \rightarrow E_1(Na^+)$  or  $E_2(K^+) ATP \rightarrow E_1(Na^+) ATP$ .

**Role of gamma ( $\gamma$ )-subunit in sodium pump activity:** The  $\gamma$ -subunit is a small transmembrane protein that specifically associate with the  $Na^+/K^+$  ATPase in a tissue-specific manner and thus mediate increase in the affinity of the enzyme for ATP (Arystarkhova *et al.*, 1999), which may however, lead to a secondary decrease in its apparent affinity for  $K^+$  (Ewart and Klip, 1995). The  $\gamma$ -subunit is expressed in the presence of  $Na^+/K^+$  ATPase (Arystarkhova *et al.*, 1999; Ewart and Klip, 1995). The likely change in ATP affinity for  $K^+$  that is perhaps of physiological importance. In most cells, ATP levels are sufficient to saturate the  $Na^+/K^+$  ATPase and therefore, a modest shift in ATP affinity should not have dramatic effects (Ewart and Klip, 1995).

**Role of circulating endogenous inhibitors in sodium pump activity:** Circulating endogenous cardiac glycosides such as ouabain inhibit  $Na^+/K^+$  ATPase (Hamlyn *et al.*, 1996) and is synthesized in the adrenal gland. Structurally, endogenous cardiac glycosides are homologous to ouabain, consisting of a cholesterol core conjugated to either a lactone or pyrone ring and containing various combinations of hydroxylsulfate or carbohydrate groups (Blaustein, 1977; Rossi *et al.*, 1995). The main physiological role of endogenous cardiac glycosides appears to be in regulating blood pressure. Thus, hypertension has been linked to increased levels of plasma endogenous cardiac glycosides and can result from long term treatment with cardiac glycosides (Rossi *et al.*, 1995). The mechanism by which endogenous cardiac glycosides mediate increased blood pressure is linked to the transmembrane equilibrium between  $Na^+$  and  $Ca^{2+}$  via the  $Na^+/Ca^{2+}$  exchanger (Blaustein, 1977). Thus, inhibition of the sodium pump in vascular smooth muscle cells and myocytes by endogenous cardiac glycosides leads to an increase in the cytoplasmic  $Na^+$  concentration, causing  $Ca^{2+}$  to enter the cell and be sequestered in the sarcoplasmic reticulum. Increased  $Ca^{2+}$  in the sarcoplasmic reticulum causes greater and sustained contraction of the vascular and heart muscle fibers, directly increasing blood pressure. Such a mechanism is believed to be the basis for the partial reversal of cardiac insufficiency following treatment with cardiac glycosides (Thomas *et al.*, 1990). However, renal dysfunction appears to be more implicated in hypertension rather than changes in peripheral tension caused by endogenous cardiac glycosides.

**Role of peptide hormone and catecholamine on activity sodium pump:** The  $Na^+/K^+$  ATPase is subjected to both short and long term regulation by a variety of hormones. Some hormones appear to alter sodium pump activity by changing its apparent affinity for  $Na^+$  (Wang *et al.*, 1995). Sodium ion has been shown to induce other mechanisms of up-regulation of the sodium pump (Whorwood and Stewart, 1995). The  $Na^+$  influx is thought to be the first signal leading to an increase in membrane  $Na^+$  in one kind of aldosterone-mediated short-term regulation (Geering *et al.*, 1982).

Short term regulation involves either direct effect on the kinetic behavior of the enzyme or translocation of sodium pump between the plasma membrane and intracellular stores. On the other hand, long term regulatory mechanisms generally affect *de novo*  $Na^+/K^+$  ATPase synthesis or degradation.

Catecholamine such as epinephrine, norepinephrine and dopamine affect  $\text{Na}^+/\text{K}^+$  ATPase activity in the kidney and as such regulate salt and fluid reabsorption (Meister and Aperia, 1993). Dopamine is a natriuretic factor synthesized in the kidney proximal tubule. Dopamine inhibits  $\text{Na}^+/\text{K}^+$  ATPase in the kidney and this represents a physiologically important mechanism for regulating salt reabsorption during high salt intake (Nathanson *et al.*, 1995). Dopamine-dependent inhibition of  $\text{Na}^+/\text{K}^+$  ATPase appears to be both age related and cell specific (Bertorello and Aperia, 1990).

Epinephrine is involved in stimulating  $\text{K}^+$  uptake by skeletal muscle after exercise induced hyperkalemia, whereas norepinephrine, acting as dopamine antagonist, appears to have a role in  $\text{Na}^+$  reabsorption in the nephron (Meister and Aperia, 1993). Several catecholamine, including norepinephrine, act as neurotransmitters in the central nervous system. Their likely importance in the activation of  $\text{Na}^+/\text{K}^+$  ATPase in neural tissue is to reestablish the electrochemical cation gradient across the cell membrane following transmission of electrical impulses (Hernandez, 1992).

Peptide hormones such as insulin regulate  $\text{Na}^+/\text{K}^+$  ATPase activity. Insulin, a metabolic hormone that regulates glycolytic storage plays an important role in  $\text{K}^+$  homeostasis. A well known effect of insulin is the stimulation of the  $\text{Na}^+/\text{K}^+$  ATPase which result in increased uptake of  $\text{K}^+$  by various tissues (Vaandrager and de Jonge, 1996). Insulin therefore, mediates cell function by binding to the insulin receptor, which leads to activation of a variety of intracellular signaling processes. In some diabetic conditions, insulin decreases  $\text{Na}^+/\text{K}^+$  ATPase activity (Vaandrager and de Jonge, 1996).

### **Effects of protein phosphorylation cascade in long term regulation of $\text{Na}^+/\text{K}^+$**

**ATPase by corticosteroids:** Steroid hormones have specific long and short term regulatory effects on the  $\text{Na}^+/\text{K}^+$  ATPase (Bertorello and Aperia, 1989). Long-term effects are generally, mediated by changes in mRNA/protein synthesis induced by direct interaction of receptor/corticosteroid complexes with nuclear DNA example, mineralocorticoid [aldosterone] and the glucocorticoid [dexamethasone] (Verrey *et al.*, 1996).

Endogenous corticosteroid such as aldosterone is synthesized in and released by the adrenal cortex. Aldosterone plays an important role in  $\text{Na}^+$  and  $\text{K}^+$  transport in epithelial tissues of the kidney. Its physiological role involves adaptation to decrease in  $\text{Na}^+$  or increase in  $\text{K}^+$  intake (O'Neil, 1990), which is a target site for aldosterone antagonist in the management of hypertension. It has been shown that the main effect of aldosterone and dexamethasone on the  $\text{Na}^+/\text{K}^+$  ATPase is to sustain a long term increase in expression of sodium pump observed directly or as an increase in ouabain binding in kidney and kidney derived cell lines, colon, heart, inner ear and liver cells, vascular smooth muscle cells and cardiocytes (Whorwood and Stewart, 1995). While, aldosterone increase sodium pump mRNA expression of the  $\alpha$ - and  $\beta$ -subunit genes via mineralocorticoid (type I) receptors, dexamethasone presumably bind to glucocorticoid (type II) receptors (Wang *et al.*, 1995).

Corticosteroid/receptor complexes mediate mRNA synthesis by interacting with regulatory elements of both the  $\alpha_1$  and  $\beta_1$ -subunits genes (Oguchi *et al.*, 1993). Corticosteroid-mediated increase in protein synthesis of sodium pump may be dependent on changes in cytoplasmic  $\text{Na}^+$  concentration by abrogation of the effects in the presence of blockers of  $\text{Na}^+$  transport. In mammals the effects of corticosteroid may be facilitated by the thyroid hormone triiodothyronine (Geering *et al.*, 1982). Interestingly, long-term stimulation of the sodium pump by aldosterone is

abrogated by inhibitors of the protein phosphatase calcineurin (Aperia *et al.*, 1996). There is evidence that cAMP-inducible factors have a role in mediating aldosterone-dependent increase in both  $\alpha$  and  $\beta$  subunit mRNA (Whorwood *et al.*, 1994). These findings may suggest the involvement of a protein phosphorylation cascade in long term regulation by corticosteroids.

**Effect of cyclic AMP activated protein kinases on the  $\text{Na}^+/\text{K}^+$  pump:** Cyclic AMP (cAMP) activated protein kinase A is activated by the intracellular accumulation of cAMP. The cAMP levels in the cell is regulated by adenylate cyclase which synthesizes it and cAMP phosphodiesterase, which degrades it. Therefore, signals that activate or inhibit these two enzymes affect cAMP levels and thus protein kinase A activation, which phosphorylates the sodium pump (Beebe, 1994).

The cascade of events that result in activation of protein kinase C is usually initiated by activation of the membrane bound phospholipase C, which cleaves phospholipids into two components; phosphatidylinositol triphosphate (which, in turn, increases cytosolic  $\text{Ca}^{2+}$ ) and diacylglycerol [DAG] (Lea *et al.*, 1994). The DAG allows the inactive, cytoplasmic form of protein kinase C to bind to the membrane and increases its affinity for  $\text{Ca}^{2+}$  and phospholipids, its final activators. Activated protein kinase C is a potent regulator of many enzymes, including the  $\text{Na}^+/\text{K}^+$  ATPase (Lea *et al.*, 1994), which involves phosphorylation of the  $\text{Na}^+/\text{K}^+$  ATPase activity. The phosphorylation of the sodium pump is facilitated by agents that stabilize the  $\text{E}_2$  conformation of the enzyme and that the  $\text{Na}^+/\text{K}^+$  ATPase itself can stimulate autophosphorylation (Borin, 1997). Cyclic guanylate monophosphate (GMP)-dependent protein kinase G is a kinase with specific effect on the  $\text{Na}^+/\text{K}^+$  ATPase. Protein kinase G is activated by the cytoplasmic concentration of cGMP which is regulated by guanylate cyclase and degraded by cGMP phosphodiesterase (Vaandrager and de Jonge, 1996). Increase in cGMP has been shown to inhibit the  $\text{Na}^+/\text{K}^+$  ATPase in colon, skeletal muscle, brain and alveolar cells (Clausen, 1996; Nielsen and Clausen, 1996). The mechanism of protein kinase G activation appears to involve activation of guanylate cyclase by Nitric Oxide (NO). The NO stimulates cGMP synthesis, which is mediated by the neurotransmitter acetylcholine and glutamate (McKee *et al.*, 1994; Nathanson *et al.*, 1995). The cGMP appears to stimulate the phosphorylation of the sodium pump indirectly by increasing  $\text{Na}^+$  influx via the  $\text{Na}^+/\text{K}^+$  ATPase co-transporter (O'Neil, 1990). Thus nitrates are used therapeutically to relieve angina pain as they release NO, which is a potent vasodilator and enhance myocardial blood flow.

**Effect of protein phosphatases on  $\text{Na}^+/\text{K}^+$  pump:** Many of the effects of protein kinase on the  $\text{Na}^+/\text{K}^+$  ATPase can be reversed by protein phosphatases. Majorly, protein phosphatase inhibitors and protein phosphatase-2B (PP-2B) are involved in protein phosphatase dependent modulation of the  $\text{Na}^+/\text{K}^+$  ATPase. Protein phosphatase inhibitors counteract the effects of protein kinases by pump inhibition through dopamine bound dopamine-receptor and isoproterenol via the  $\beta$ -adrenergic receptor. Such inhibition is mediated in part by the activation of the protein phosphatase inhibitors dopamine receptor protein phosphatase-32 (DAR PP-32) and inhibitor-I (Aperia *et al.*, 1992). Thus, it has been shown that increase in cAMP levels mediated by dopamine or isoproterenol in kidney and brain leads to phosphorylation of DAR PP-32, which, in turn, cause inhibition of protein phosphatase inhibitors (Cantiello, 1997). The physiological role of PP-2B, which is a  $\text{Ca}^{2+}$  and calmodulin-dependent enzyme upon activation by epinephrine and  $\alpha$ -adrenergic receptor agonists, activates the  $\text{Na}^+/\text{K}^+$  ATPase of most segments of the nephron (Lea *et al.*, 1994), although, its main effects are on the enzyme of proximal convoluted tubules (Aperia *et al.*, 1996).

The role of calcineurin in the kidney is to counter dopamine-induced inhibition of Na<sup>+</sup>/K<sup>+</sup> ATPase and occurs by dephosphorylating targets of dopamine-stimulated protein kinases (Aperia *et al.*, 1992; Lea *et al.*, 1994). Thus, it may suggest that calcineurin mediates its stimulatory effects at least in part by increasing the apparent affinity of the sodium pump for Na<sup>+</sup> (Lea *et al.*, 1994). In addition to its role in the kidney, calcineurin mediates ouabain-pumps and has a role, in sodium pump activation during glutamate toxicity in neurons (Aperia *et al.*, 1996).

Protein phosphatase 2A has been shown to modulate Na<sup>+</sup>/K<sup>+</sup> ATPase activity by increasing sodium pump plasma membrane expression in cortical collecting duct (Blot-Chabaud *et al.*, 1996) and antagonise protein kinase C-mediated inhibition of the Na<sup>+</sup>/K<sup>+</sup> ATPase (Blanco and Mercer, 1998). Paradoxically, inhibitors of PP 2A stimulate the pump in hepatocytes (Meister and Aperia, 1993).

## CONCLUSION

The need for the ubiquitous Na<sup>+</sup>/K<sup>+</sup> ATPase to adapt to the diverse needs of different tissues underscores the importance of mechanisms for regulating its activity. The signaling cascades involved in hormonal regulation, in particular, are varied and complex. Alterations in activity may be the result of post translational modification, such as, phosphorylation. In certain instances, alterations in Na<sup>+</sup>/K<sup>+</sup> ATPase activity and kinetic behavior result from specific interaction with other membrane components. These include proteins intrinsic to the plasma membrane as well as those of the cytoskeleton.

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