

Regulatory effect of insulin on the structure, function and metabolism of Na⁺/K⁺-ATPase (Review)

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Abstract. Na⁺/K⁺-ATPase is an ancient enzyme, the role of which is to maintain Na⁺ and K⁺ gradients across cell membranes, thus preserving intracellular ion homeostasis. The regulation of Na⁺/K⁺-ATPase is affected by several regulatory factors through a number of pathways, with hormones serving important short-term and long-term regulatory functions. Na⁺/K⁺-ATPase can also be degraded through activation of the ubiquitin proteasome and autophagy-lysosomal pathways, thereby affecting its abundance and enzymatic activity. As regards the regulatory effect of insulin, it has been found to upregulate the relative abundance of Na⁺/K⁺-ATPase and restore the transport efficiency in multiple *in vitro* and *in vivo* experiments. Therefore, elucidating the role of insulin in the regulation Na⁺/K⁺-ATPase may help uncover new drug targets for the treatment of related diseases. The aim of the present study was to review the structure and function of Na⁺/K⁺-ATPase and to discuss the possible mechanisms through which it may be regulated by insulin, in order to investigate the possibility of designing new therapies for related diseases.

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1. Introduction

Sodium/potassium-dependent ATPase (Na⁺/K⁺-ATPase) is a ubiquitous protein embedded between the phospholipid layers of the cell membrane (1). When the levels of intracellular Na⁺ or extracellular K⁺ increase, Na⁺/K⁺-ATPase becomes activated, and its main function is to regulate the transmembrane transport of Na⁺ and K⁺. In each cycle, the energy released by hydrolyzing ATP can pump three Na⁺ out of and two K⁺ into the cell. This transport across membranes is essential for maintaining cellular resting potential and adjusting the excitability of neurons (2,3).

In recent years, there has been ongoing research on the functions of the Na⁺/K⁺-ATPase (4-6), and the regulation of this enzyme appears to be influenced by multiple factors through several pathways, among which hormones play an important role in both short-term and long-term regulation (Fig. 1). Although there have been several previous studies on the regulatory effect of hormones on Na⁺/K⁺-ATPase (7-10), the mechanism underlying the regulatory effect of insulin on Na⁺/K⁺-ATPase in various organs and the related metabolic pathways have been attracting increasing attention. The aim of the present study was to review the structure, biochemical characteristics, function and metabolism of Na⁺/K⁺-ATPase and, subsequently, to discuss in depth all the possible mechanisms through which insulin may regulate Na⁺/K⁺-ATPase and investigate the possibility of new therapies for related diseases.

2. Structure and biochemistry of Na⁺/K⁺-ATPase

Na⁺/K⁺-ATPase is a ubiquitous enzyme consisting of three subunits, namely α -, β - and γ -subunits (Fig. 2). The α -subunit is a catalytic subunit composed of 10 transmembrane helices (m1-10) with a total molecular mass of 110 kD, which has binding sites for Na⁺ and K⁺ (11). Its main function is to transfer Na⁺ out of the cells and K⁺ into the cells, and it functions as an ATPase by hydrolyzing ATP (12). The α -subunit has four isoforms (α 1-4), which are differentially expressed in different tissues throughout the development of the organism: α 1 is widely expressed in various tissues; α 2 is mainly expressed in the brain, heart and muscle; α 3 is mainly distributed in the brain, retina and heart; and α 4 is mainly expressed in the testes (11,13,14).

The β -subunit consists of a transmembrane fragment and a highly glycosylated extracellular domain with a molecular

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mass of 55 kD, the role of which is regulatory, enabling the α -subunit to accurately fold and translocate from the endoplasmic reticulum (ER) to the plasma membrane (4), stabilizing the protein configuration and regulating its activity on the plasma membrane (4,7,15). The β -subunit has three isoforms ($\beta 1$ -3); among these, $\beta 1$ is distributed throughout all tissues, $\beta 2$ is concentrated in nervous tissue, heart, cartilage and erythrocytes, whereas $\beta 3$ is found predominantly in nervous tissue, as well as in skeletal muscle and the lung (7,15).

The γ subunit has only one transmembrane domain with a molecular mass of 15 kD, and it is a member of the FXYD protein family, which has seven isoforms (FXYD1-7) (5,13). Its function appears to be associated with the modulation of the enzyme affinity for different ligands, with a direct and positive effect on the maximum rate of ATP hydrolysis; thus, the γ -subunit is also considered to be regulatory in addition to the β -subunit (8,13,15-17). Furthermore, relevant clinical trials have demonstrated that the expression of FXYD1, 3 and 5 is upregulated in lung epithelial cells of patients with acute respiratory distress syndrome (ARDS), and FXYD5 is the key mediator (9), whereas FXYD1, 6 and 7 are mainly expressed in brain tissue, where they regulate the affinity between Na^+/K^+ -ATPase and substrate and the maximum response rate (13).

3. Function of Na^+/K^+ -ATPase

Through pumping Na^+ into and K^+ out of the cells, Na^+/K^+ -ATPase plays an important role in maintaining cells in a resting state by preserving the balance of electrolytes and fluids, regulating the active transport of carbohydrates, amino acids, bile acids, neurotransmitters and ions, and regulating membrane potential, cell volume, energy metabolism and signal transmission (3). Therefore, the abnormal regulation and dysfunction of Na^+/K^+ -ATPase may lead to serious pathophysiological changes, and maintaining its stability is crucial.

The mechanism through which cardiac glycosides increase myocardial contractility is increasing intracellular Na^+ levels by inhibiting Na^+/K^+ -ATPase, then increasing intracellular Ca^{2+} levels through $\text{Na}^+/\text{Ca}^{2+}$ exchange, ultimately enhancing myocardial contractility (10,11). Consequently, Na^+/K^+ -ATPase activity appears to be closely associated with myocardial contractility.

In order to achieve pulmonary edema clearance in ARDS, Na^+ in the alveolar space enters the cells through the epithelial Na^+ channel in the apical membrane of alveolar type I and alveolar type II (ATII) epithelial cells, and then enters the pulmonary interstitium through the Na^+/K^+ -ATPase on the basal side of the cells, forming a local osmotic pressure gradient, thus driving fluid clearance from the alveolar space (18). Over a decade ago, Na^+/K^+ -ATPase downregulation was reported in several acute lung injury models (19-22). Current research is mainly focusing on the role of Na^+/K^+ -ATPase in promoting pulmonary edema clearance. Disruption in the function of Na^+/K^+ -ATPase is likely to aggravate the formation of pulmonary edema, which may be caused by limited Na^+ transport as well as disrupted alveolar barrier function (19). Therefore, regulating the function of Na^+/K^+ -ATPase in the alveolar epithelium is crucial for relieving pulmonary edema.

4. Metabolic pathway of Na^+/K^+ -ATPase

Ubiquitin proteasome pathway (UPP) of Na^+/K^+ -ATPase. The UPP is a highly efficient protein decomposition pathway, which has a wide range of biological functions and is closely associated with several diseases. Ubiquitin must bind to the relevant substrate proteins in the form of polyubiquitin chains to label the substrate proteins for further degradation (23). The UPP system comprises ubiquitin, E1 ubiquitin-activating enzyme, E2 ubiquitin-binding enzyme, E3 ubiquitin ligase, deubiquitinases (DUBs) and proteasome (24). During ubiquitin modification, ubiquitin is first activated by E1 ubiquitin activator, and then the activated ubiquitin binds to the E2 ubiquitin-binding enzyme. Then, under the action of E3 ubiquitin ligase, ubiquitin is transferred to the target protein to complete the process of ubiquitination (25). E3 ubiquitin ligase plays an important role in this process, which determines the timing and specificity of ubiquitination.

In 1997, Coppi and Guidotti (26) first proposed the ubiquitination of Na^+/K^+ -ATPase as a possible regulatory mechanism. It was reported that the $\alpha 1$ and $\alpha 2$ isoforms of the Na^+/K^+ -ATPase α subunit are modified by the covalent attachment of ubiquitin polymers in COS-7 cells (an African green monkey kidney fibroblast-like cell line), and polyubiquitination of the Na^+/K^+ -ATPase α subunit may play a role in regulating its degradation, namely by promoting the ER-associated degradation of unassembled and misfolded α subunits, and by participating in the internalization and subsequent degradation of cell surface Na^+/K^+ -ATPase molecules (26). Moreover, the physiological effect of ubiquitination on Na^+/K^+ -ATPase was first demonstrated in alveolar epithelial cells during hypoxia (27). Severe short-term hypoxia resulted in the endocytosis and degradation of Na^+/K^+ -ATPase in alveolar epithelial cells, whereas the phosphorylation of the protein kinase C (PKC) ζ -dependent- Na^+/K^+ -ATPase catalytic subunit Ser-18 triggered the ubiquitination and endocytosis of plasma membrane Na^+/K^+ -ATPase (27). Furthermore, long-term exposure of alveolar epithelial cells to hypoxia may lead to a decrease in total Na^+/K^+ -ATPase levels. Further studies demonstrated that, if the four lysine residues on Ser-18 (KK¹⁸SKK) side are immediately mutated to arginine, hypoxia-induced ubiquitination and endocytosis may be prevented (22,27). Hypoxia-induced Na^+/K^+ -ATPase degradation may be prevented by inhibiting its ubiquitination on the plasma membrane and treatment with lysosome inhibitors, which indicates that Na^+/K^+ -ATPase is ubiquitinated on the plasma membrane, but its degradation occurs in the lysosome (28). Accordingly, Na^+/K^+ -ATPase may serve as a vector for ubiquitin-dependent intracellular transport.

The separation of the basolateral membrane revealed the presence of Na^+/K^+ -ATPase/ubiquitin conjugates, suggesting that ubiquitination occurs on the plasma membrane. However, when Ser-18 was mutated to alanine, ubiquitination was inhibited, suggesting that phosphorylation may be a prerequisite for ubiquitination. Therefore, Na^+/K^+ -ATPase appears to be regulated by a mechanism involving phosphorylation, ubiquitination, recognition, endocytosis and degradation (29). Phosphorylation, as a signal that triggers ubiquitination, provides a 'pass check' for endocytosis

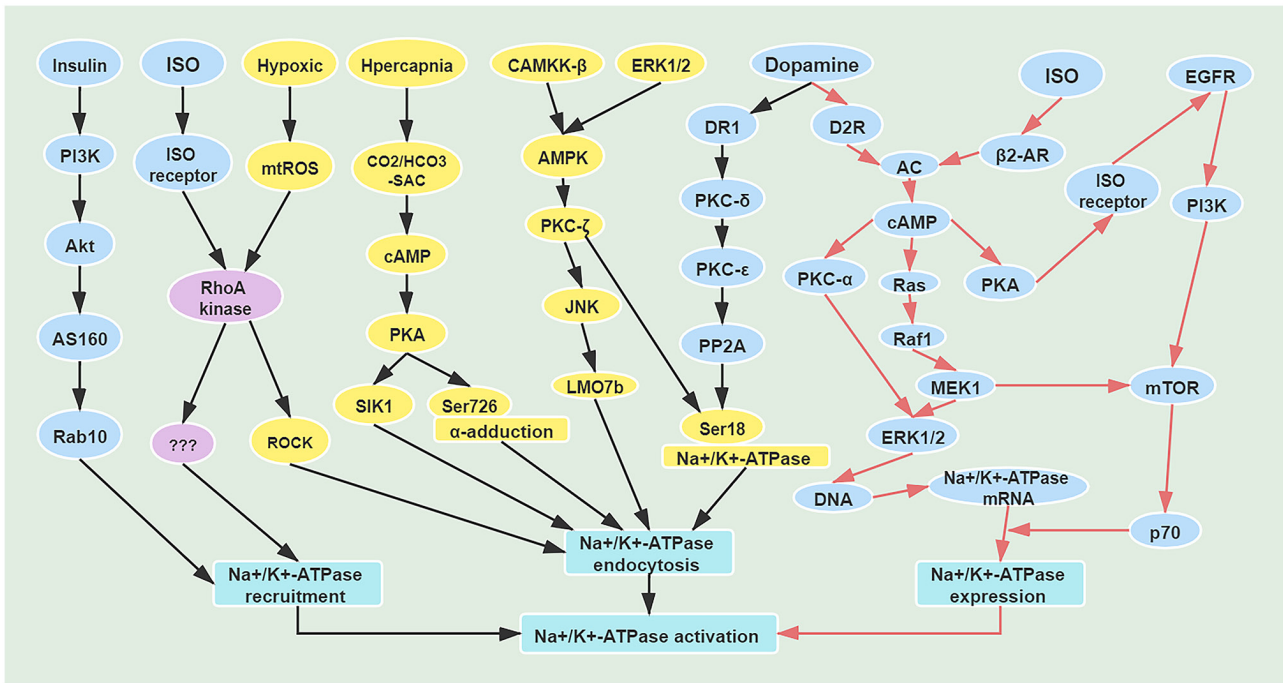


Figure 1. Mechanisms of short-term and long-term regulation of Na^+/K^+ -ATPase. Black arrows indicate mechanisms of short-term regulation of Na^+/K^+ -ATPase; red arrows indicate mechanisms of long-term regulation of Na^+/K^+ -ATPase. AS160, AKT substrate protein of 160 kDa; Rab10, Ras-related GTP-binding protein; ISO, isoprenaline; RhoA, Ras homolog family member A; mtROS, mitochondrial reactive oxygen species; ROCK, Rho-associated kinase; PKA, protein kinase A; SIK1, salt-inducible kinase 1; CAMKK- β , Ca^{2+} /calmodulin-dependent protein kinase β ; AMPK, AMP-activated protein kinase; JNK, c-Jun N-terminal kinase; LMO7b, LIM domain only protein 7-like; DR1, dopamine D1 receptor; D2R, dopamine D2 receptor; PP2A, protein phosphatase 2A; AC, adenylate cyclase; Raf1, Raf-1 proto-oncogene; MEK1, mitogen-activated protein kinase kinase 1.

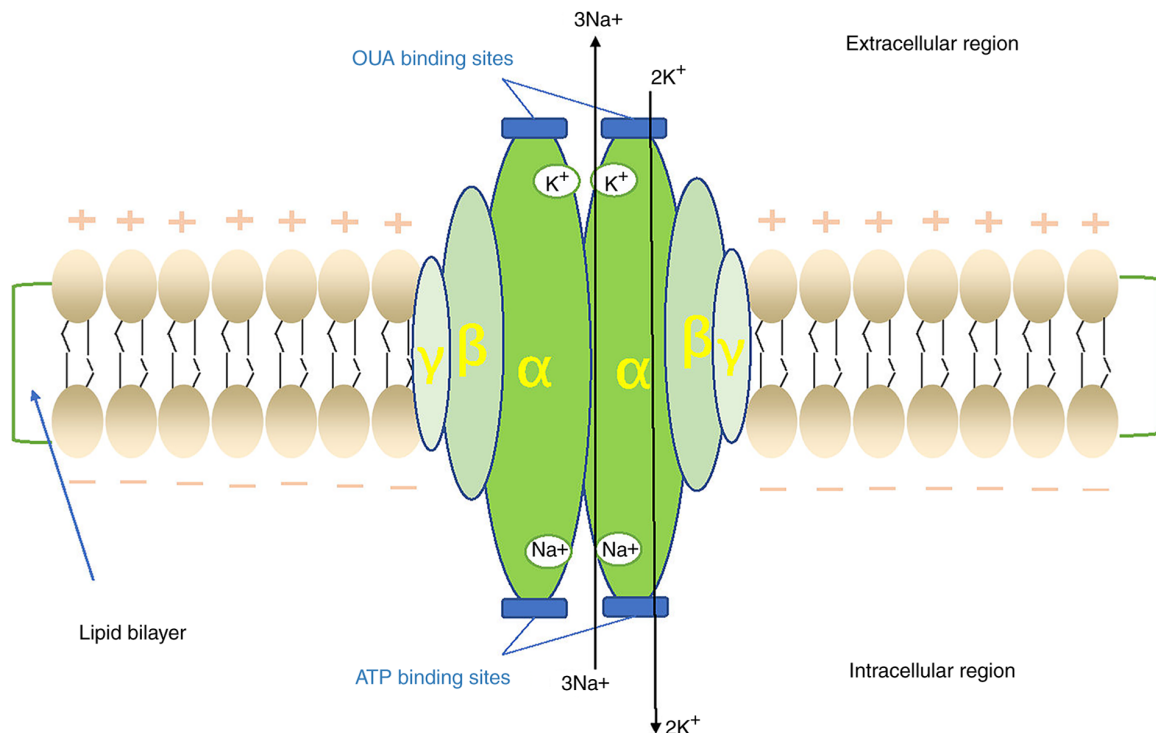


Figure 2. Structure and ionic transport of Na^+/K^+ -ATPase. OUA, ouabain.

and degradation. Hoxhaj *et al* (30) confirmed that the E3 ubiquitin ligases ZNRF1 and ZNRF2 are new participants in the regulation of Na^+/K^+ -ATPase. In human 293

cells, insulin was shown to increase the phosphorylation of ZNRF1 and ZNRF2, thereby increasing its binding to 14-3-3 and reducing polyubiquitination modification of the

Na^+/K^+ -ATPase $\alpha 1$ protein and subsequent degradation by proteases (30). Phosphorylation may alter the subcellular localization of proteins, thus regulating the availability of interaction between the target protein and E3 ligase (23). In conclusion, the ubiquitination of Na^+/K^+ -ATPase is one of the metabolic pathways of this enzyme (i.e., one of the scavenging pathways). However, ubiquitinase or DUBs do not appear to regulate the degradation of Na^+/K^+ -ATPase itself or its related pathway proteins. Therefore, the exact function and underlying mechanism of DUBs in this field have yet to be fully elucidated.

Autophagy-lysosomal pathway of Na^+/K^+ -ATPase. The autophagy-lysosomal pathway is a process through which macromolecules, damaged organelles and residual metabolites are transported to the lysosomes for degradation. During this process, materials may be reused for biosynthesis or energy production (31). Abnormalities in the autophagic process may adversely affect the development of various organs in the body. Few previous studies have mentioned whether Na^+/K^+ -ATPase actively participates in autophagy or endosome cycling by altering the Na^+ and K^+ contents. However, Na^+ and K^+ are all located in the α -subunit, which is considered as the catalytic subunit (32,33). Therefore, the α subunit may be used to investigate whether Na^+/K^+ -ATPase is actively involved in autophagy or endosome cycling by altering the cell Na^+ and K^+ content. It has been demonstrated that the association between Na^+/K^+ -ATPase and the autophagy-lysosomal pathway requires the $\alpha 1$ subunit (34). Recently, it was also reported that Na^+/K^+ -ATPase $\alpha 1$ and AMP-dependent protein kinase may represent the 'on' and 'off' states of the autophagic pathway, respectively (34). Na^+/K^+ -ATPase $\alpha 1$ may serve as a new mechanism of signal transduction and autophagy in the process of ischemia/reperfusion, which may provide a new approach to therapeutic intervention in ischemic stroke (32,33).

Importantly, Na^+/K^+ -ATPase can be degraded by the UPP and the autophagy-lysosomal pathway. Sequestosome 1 (SQSTM1)/p62, is an autophagic protein (35), and defects in autophagy may lead to the accumulation of SQSTM1 and may induce cell stress and disease states. SQSTM1 regulates multiple signaling pathways by binding to different proteins to form an important cellular signaling hub (36). Thus, SQSTM1 is involved in the UPP and autophagy-lysosomal degradation processes, and appears to be an important regulatory molecule connecting ubiquitinated proteins to the autophagy mechanism (37). Hancock *et al* (38) demonstrated that insulin significantly reduced SQSTM1 mRNA expression. Proteomic analysis demonstrated that Na^+/K^+ -ATPase $\alpha 1$ was able to bind with SQSTM1, which was verified by endogenous protein interaction analysis. Therefore, the decreased expression of SQSTM1 mRNA is likely to reduce the transport of the polyubiquitinated Na^+/K^+ -ATPase $\alpha 1$ protein to the autophagy-lysosomal system for degradation.

In conclusion, few studies on the degradation of Na^+/K^+ -ATPase through these two pathways have been conducted to date, and the relationship between the two may require further investigation in the future to fully elucidate the role of Na^+/K^+ -ATPase $\alpha 1$ abundance and enzymatic activity.

5. Insulin-mediated regulation of ATPase pumps

Insulin is a key hormonal factor that has been extensively investigated in the context of various metabolic disorders, such as obesity and non-insulin-dependent diabetes mellitus, as well as cardiovascular disorders, such as essential hypertension and atherosclerosis (39). The signaling pathways that are activated by insulin are summarized in Fig. 3. Understanding the biochemical and cellular properties of insulin receptor signalling constitutes a priority in biomedical research (40). Borge *et al* (41) reported that the existence of a novel positive-feedback pathway in which insulin may regulate insulin secretion in the β -cells of the pancreas by interaction between the insulin receptor substrate 1 (IRS-1) protein and sarco(endo)plasmic reticulum Ca^{2+} -ATPase (SERCA). IRS-1 is present in the ER and can directly bind to the β -cell isoforms of SERCA, specifically SERCA3b. Insulin stimulation results in increased binding of IRS-1 to SERCA3b, which inhibits the Ca^{2+} -ATPase, increases cytosolic Ca^{2+} , and augments fractional insulin secretion. The rat pancreatic β -cell expresses 6 splice variants of the plasma membrane Ca^{2+} -ATPase and two splice variants of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger 1. In the β -cell, the $\text{Na}^+/\text{Ca}^{2+}$ exchanger displays a high capacity, contributing to both Ca^{2+} outflow and influx, and participating in the control of insulin release (42,43). In addition, vacuolar-type ATPase (V-ATPase) is present in unique organelles, such as insulin secretory granules, neural synaptic vesicles and acrosomes of spermatozoa. Futai *et al* (44) reported that the V-ATPase $\alpha 3$ isoform was found to be highly expressed in pancreatic Langerhans islets, and ~80% of the isoform was shown to be localized to insulin-containing secretory granules of pancreatic islet β -cells. This evidence indicates that insulin may serve an important role in ATPases.

6. Regulatory effect of insulin on Na^+/K^+ -ATPase

It is well known that Na^+ regulates Na^+/K^+ -ATPase in most mammalian cells (45). Under insulin stimulation, increasing Na^+ influx may stimulate Na^+/K^+ -ATPase. There are tissue-specific differences in this mechanism, which may be a receptor-mediated process (46). As regards the mechanism through which insulin regulates Na^+/K^+ -ATPase, it was found to promote a relative abundance of Na^+/K^+ -ATPase and restore the transport efficiency in multiple *in vitro* and *vivo* experiments (47-51). However, in most studies, the effect of insulin on Na^+/K^+ -ATPase mainly manifests as short-term regulation, i.e., only Na^+/K^+ -ATPase $\alpha 1$ is translocated to the plasma membrane and internalized into the cytoplasm, and insulin changes its molecular conformation and regulates the transport efficiency (47). Moreover, Na^+/K^+ -ATPase activity may also be subject to long-term regulation, i.e., insulin may promote its transcription and translation, or reduce degradation of the $\alpha 1$ subunit. The mechanism underlying the regulation of Na^+/K^+ -ATPase by insulin in the human body is summarized in Fig. 4.

Lung. Insulin can prevent or reduce lipopolysaccharide (LPS)-induced acute lung injury in rats (48) and can also reduce the in-hospital mortality of patients with ARDS (49). Insulin is considered to be of potential therapeutic value in ARDS.

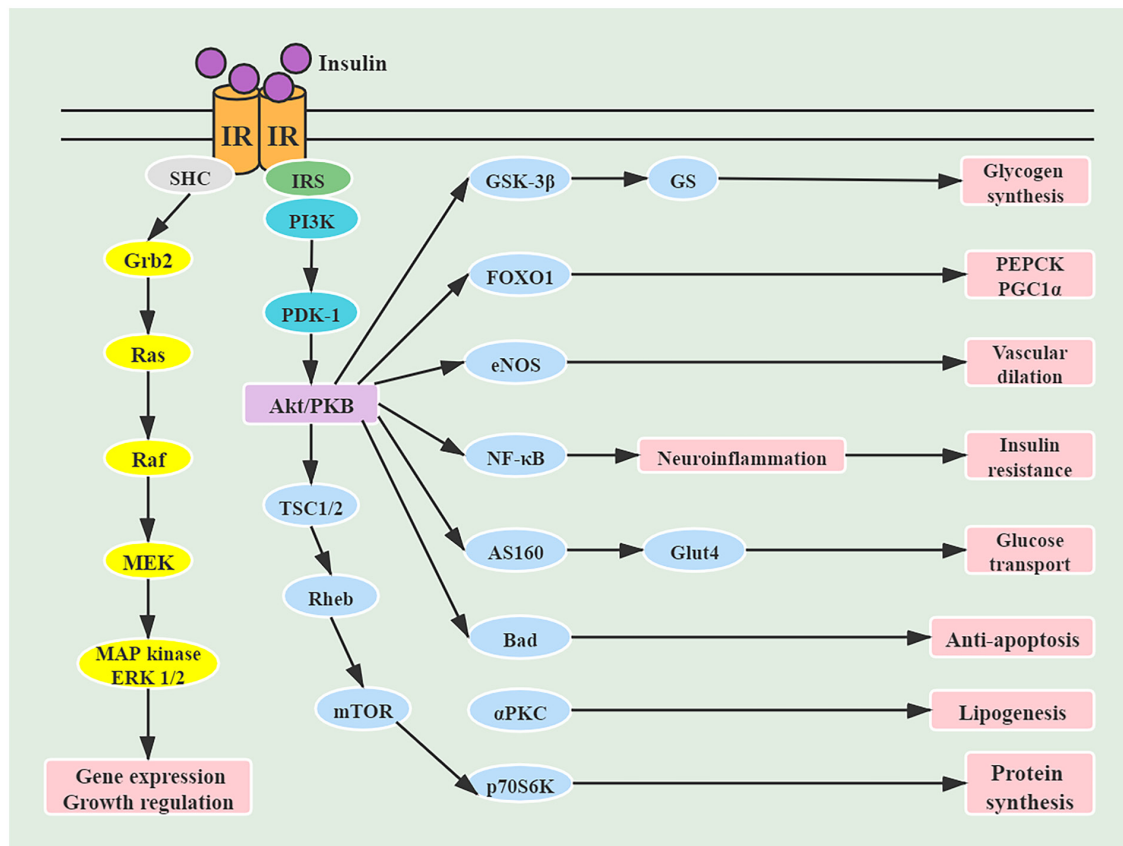


Figure 3. Signaling pathways activated by insulin. IR, insulin receptor; IRS, insulin receptor substrate; SHC, Src homology collagen protein; Grb2, growth factor receptor-bound protein 2; PDK-1, protein 3-phosphoinositide-dependent protein kinase-1; TSC, tuberous sclerosis complex; Rheb, Ras homolog enriched in brain; GSK, glycogen synthase kinase; FOXO1, Forkhead box protein O1; eNOS, endothelial nitric oxide synthase; AS160, AKT substrate protein of 160 kDa; PKC, protein kinase C; GS, glycogen synthase; Glut4, glucose transporter 4; PEPCK, phosphoenolpyruvate carboxykinase; PGC1, PPAR γ -coactivator 1.

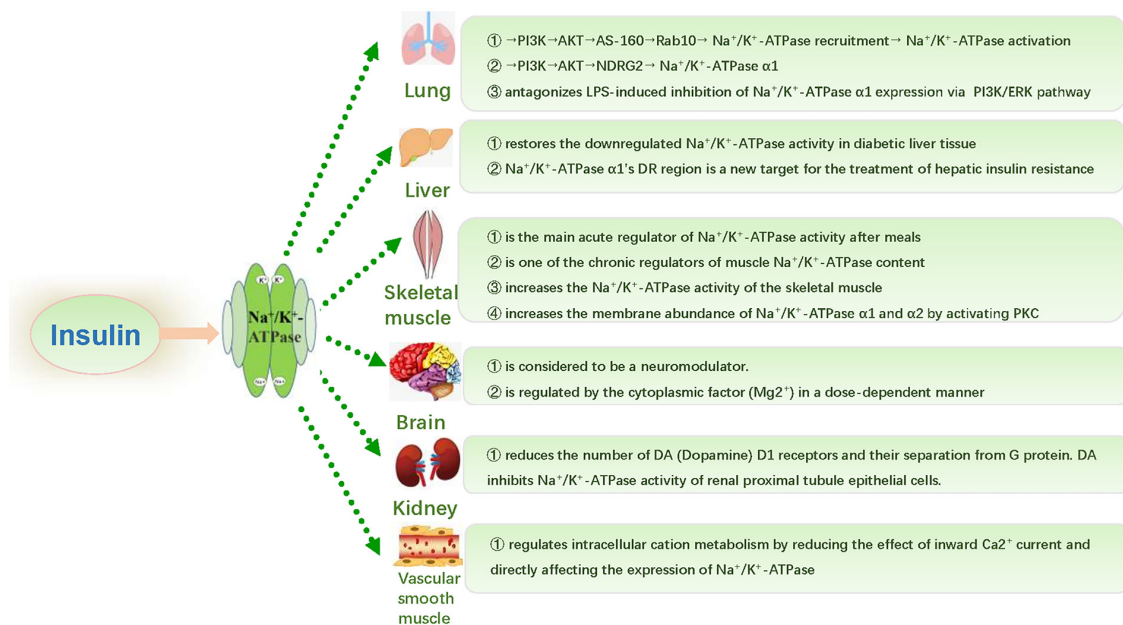


Figure 4. Regulatory mechanism of insulin on Na⁺/K⁺-ATPase in different organs. AS-160, 160 KD substrate protein downstream of AKT; Rab10, Member RAS oncogene family; NDRG2, N-Myc downstream-regulated gene 2 protein; PKC, protein kinase C.

However, the specific mechanism through which it regulates the activity and relative abundance of Na⁺/K⁺-ATPase on ATII cells remains unclear (49). A549 cells, which were isolated

from human lung adenocarcinoma, have been widely used as a model of ATII cells due to the presence of lamellar bodies and surfactant proteins. A study on ATII and A549 cells

reported that insulin increased Na^+/K^+ -ATPase activity and pulmonary edema clearance by recruiting Na^+/K^+ -ATPase to the cell membrane of ATII epithelial cells within 5 min (50). This short-term regulation was induced by phosphorylation of AKT substrate protein of 160 kDa (AS160). It should be noted that the promoting effect of insulin was found to be fully mediated via the PI3K/AKT pathway upstream of AS160 (50). AKT mediates insulin-induced glucose transporter type 4 exocytosis by phosphorylating AS160, which contains a Rab GTPase-activating protein (GAP) domain (51). In addition, Rab proteins switch between a GTP-bound active state and GDP-bound inactive state; the phosphorylation of AS160 by AKT inhibits GAP activity and stabilizes the Rab proteins in their GTP-bound form, which has been described to promote vesicle trafficking (52). Specifically, they described that i) insulin increases Na^+/K^+ -ATPase abundance at the plasma membrane; ii) the activation of AKT is both necessary and sufficient to recruit Na^+/K^+ -ATPase molecules to the cell surface; and iii) the effects of AKT on Na^+/K^+ -ATPase are mediated in part by the phosphorylation of AS160 and the activity of Rab10 (52). Thus, AS160 may be a new substrate of AKT.

Furthermore, insulin regulates the expression of Na^+/K^+ -ATPase $\alpha 1$ through the PI3K/AKT/N-Myc downstream-regulated gene 2 protein and PI3K/ERK pathways. It has been reported that insulin upregulates Na^+/K^+ -ATPase activity and $\alpha 1$ subunit protein abundance on cell membranes through the PI3K pathway, which can prevent the occurrence and development of ARDS and improve patient prognosis (53,54). In recent years, it was also reported that insulin increases Na^+/K^+ -ATPase $\alpha 1$ mRNA level and enzymatic activity (55). In our previous research, insulin was found to antagonize LPS-induced inhibition of Na^+/K^+ -ATPase $\alpha 1$ expression via the PI3K/ERK pathway. LPS induced a decrease in Na^+/K^+ -ATPase $\alpha 1$ protein levels in ATII cells, while insulin significantly increased the protein levels of Na^+/K^+ -ATPase $\alpha 1$ inhibited by LPS for at least 24 h (56). Therefore, insulin appears to promote pulmonary edema clearance through long-term regulation of Na^+/K^+ -ATPase. However, the specific mechanism remains to be further investigated.

Liver. Obesity is associated with hyperglycemia and hyperinsulinemia, which may inhibit or inactivate Na^+/K^+ -ATPase (57). In diabetic rats, the activity of Na^+/K^+ -ATPase in the liver was also found to be decreased, and antidiabetic compounds were able to restore the downregulated Na^+/K^+ -ATPase activity in diabetic liver tissue (58). It was also recently demonstrated that Na^+/K^+ -ATPase $\alpha 1$ is a physiological regulator of glucose homeostasis, and its DR region may represent a new target for the treatment of hepatic insulin resistance (59). Therefore, Na^+/K^+ -ATPase $\alpha 1$ may be a key gene involved in the homeostasis of glucose and lipid metabolism. However, the role and exact underlying mechanism of Na^+/K^+ -ATPase $\alpha 1$ in obesity and insulin resistance remain unclear.

Skeletal muscle. In skeletal muscle tissue, insulin is the main acute regulator of postprandial Na^+/K^+ -ATPase activity (60). In addition, it is one of the chronic regulators of muscle Na^+/K^+ -ATPase content. Insulin can significantly increase the Na^+/K^+ -ATPase activity of skeletal muscle, thus reducing

extracellular and increasing intracellular K^+ concentration. The mechanisms for increasing Na^+/K^+ -ATPase transport to the plasma membrane include insulin regulation and skeletal muscle contraction (60). In cultured human skeletal muscle cells, insulin treatment was found to increase the membrane abundance of the $\alpha 1$ and $\alpha 2$ subunits, which required activation of PKC (61).

Brain. Insulin is considered to function as a neuromodulator in the brain, and its inhibitory effect is regulated by the cytoplasmic factor Mg^{2+} in a dose-dependent manner (62).

Kidney. In kidney tissue, Banday *et al* (63) reported that long-term exposure to insulin can reduce the number of dopamine (DA) D1 receptors and their separation from G protein, which may be the mechanism through which DA inhibits Na^+/K^+ -ATPase activity in renal proximal tubule epithelial cells in hyperinsulinemic hypertensive rats.

Vascular smooth muscle. Vascular smooth muscle is a type of insulin-insensitive tissue. Insulin regulates intracellular cation metabolism by reducing the effect of inward Ca^{2+} current and directly affecting the expression of Na^+/K^+ -ATPase (64).

Carcinogenesis. Na^+/K^+ -ATPase has been proposed as a signal transducer involved in various pathobiological processes, including carcinogenesis (65). Lu *et al* (66) found upregulation of the mRNA expression of the Na^+/K^+ -ATPase $\alpha 1$, $\beta 1$ and $\beta 3$ subunits in hepatocellular carcinoma (HCC) using The Cancer Genome Atlas (<https://cancergenome.nih.gov/>), International Cancer Genome Consortium (<https://icgc.org/daco>) and Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/gds>) databases, and indicated that Na^+/K^+ -ATPase $\beta 3$ may serve as an oncogene; it was also demonstrated to be an independent prognostic factor and was associated with immune cell infiltration in HCC. In addition to its prominent metabolic action, insulin has a well-known mitogenic effect, promoting proliferation of both non-cancerous and malignant cells (67,68). It is a known fact that several types of cancer cells require insulin for optimal growth *in vitro*. Recent data (67,68) have demonstrated that: i) Insulin stimulates growth mainly through its own receptor rather than insulin-like growth factor-1 receptor (IGF-1R). By employing blocking monoclonal antibodies specific to both the insulin receptor (IR) and IGF-1R, it was demonstrated that the growth response of breast cancer cell lines to insulin could be specifically blocked by an anti-IR, but not by the anti-IGF-1R blocking antibody (67); and ii) several types of cancer cells overexpress the IR as shown using immunostaining; it was observed that overexpression of IR was not unique to breast cancer, but a common phenomenon across several types of human cancers. Increased IR levels were observed in colon, lung, ovarian and thyroid cancer (69,70). Furthermore, the a-genotype is more efficient than the b-genotype in promoting mitosis. When exposed to insulin, these characteristics confer a selective growth advantage to malignant cells. However, the evidence on the combined effect of insulin and Na^+/K^+ -ATPase on carcinogenesis is insufficient, and extensive further research is required to explore and confirm their relationship in the future.

7. Conclusions and perspectives

Na⁺/K⁺-ATPase has been implicated in the development of various diseases. In the present study, the structure, biochemical characteristics, function and metabolism of Na⁺/K⁺-ATPase were reviewed to further elucidate the mechanism underlying the regulatory effect of insulin on Na⁺/K⁺-ATPase in the whole body. The mRNA transcription and protein expression levels of Na⁺/K⁺-ATPase can be regulated by insulin (55). The Na⁺/K⁺-ATPase α1 subunit is regulated by the ERK1/mTORC1, PI3K/AKT/ZNRF and other pathways (30,54). Furthermore, it is known that Na⁺/K⁺-ATPase may be degraded by UPP and autophagy-lysosomal pathway (26,27,33,34). However, to the best of our knowledge, no in-depth research of these mechanisms has been conducted and there have been no breakthroughs to date.

Based on the present review, it appears that insulin may affect the abundance of Na⁺/K⁺-ATPase α1 through the UPP and autophagy-lysosomal system, and it may also affect the expression of Na⁺/K⁺-ATPase α1 through a variety of pathways. Therefore, it is crucial to study the dynamic process underlying the regulatory action of insulin on Na⁺/K⁺-ATPase. Although there remain several unresolved issues, more factors that may be implicated in the development of several diseases through regulating Na⁺/K⁺-ATPase must be discovered and explored, as Na⁺/K⁺-ATPase may represent a promising potential target for the clinical treatment of ARDS and other diseases.

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Availability of data and materials

Not applicable.

Authors' contributions

XPW was responsible for integrating all the information and wrote and edited this review. Supervisor QQW made substantial contributions to conception and design and revised the manuscript. All authors have read and approved the final version of the article. Data sharing is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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