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.

Contents

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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 (α1-4), which are differentially expressed in different tissues throughout the development of the organism: α1 is widely expressed in various tissues; α2 is mainly expressed in the brain, heart and muscle; α3 is mainly distributed in the brain, retina and heart; and α4 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

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 (β1-3); among these, β1 is distributed throughout all tissues, β2 is concentrated in nervous tissue, heart, cartilage and erythrocytes, whereas β3 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^+ levels through Na^+-Ca^+ 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 αl and α2 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 ($KK^{18}SKK$) 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

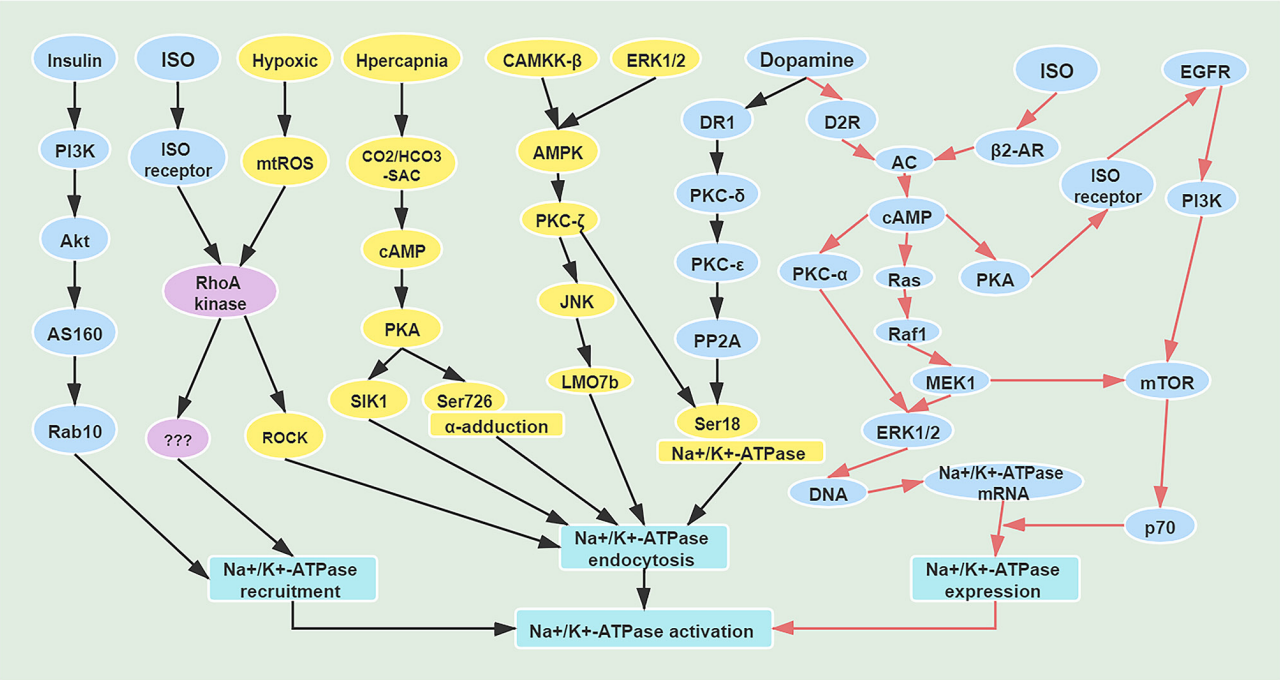

Figure 1. Mechanisms of short-term and long-term regulation of Na^+/K^+-ATPase. Black arrows indicate mechanisms of short-term regulation of Na^+/K^+-ATPase; red arrows indicate mechanisms of long-term regulation of Na^+/K^+-ATPase. AS160, AKT substrate protein of 160 kDa; Rab10, Ras-related GTP-binding 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.

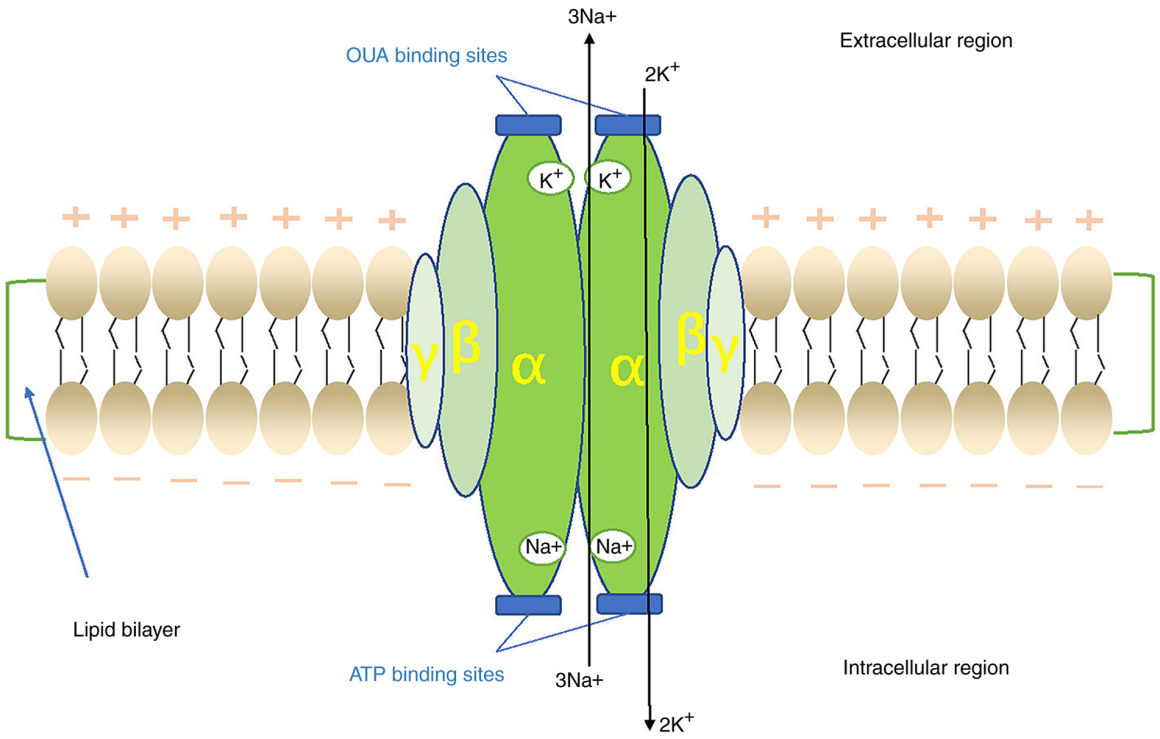

Figure 2. Structure and ionic transport of Na^+/K^+-ATPase. OUA, ouabain.

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 α1 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 α1 subunit (34). Recently, it was also reported that Na^+/K^+-ATPase α1 and AMP-dependent protein kinase may represent the 'on' and 'off' states of the autophagic pathway, respectively (34). Na^+/K^+-ATPase α1 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 α1 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 α1 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 α1 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 Na^+/Ca^{2+} exchanger 1. In the β-cell, the Na^+/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 α3 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 α1 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 α1 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.

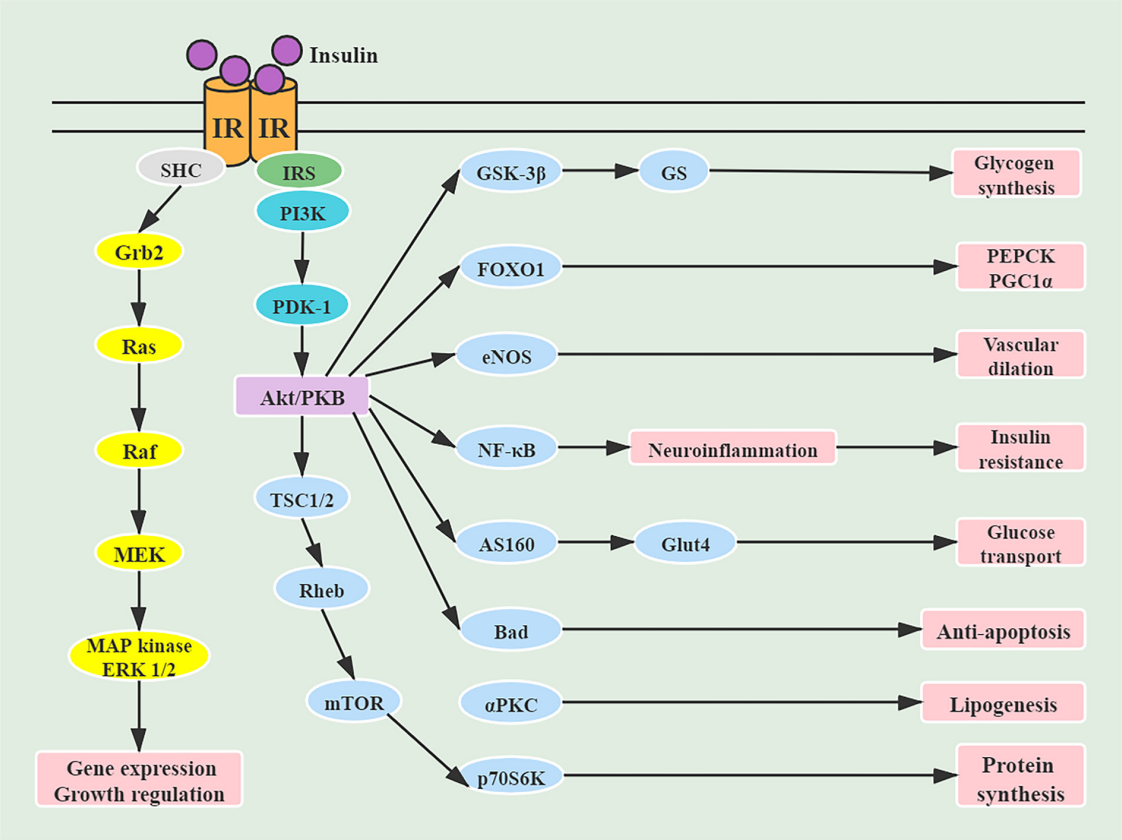

Figure 3. Signaling pathways activated by insulin. IR, insulin receptor; IRS, insulin receptor substrate; SHC, Src homology collagen protein; Grb2, growth factor receptor-bound protein 2; PDK-1, protein 3-phosphoinositide-dependent protein kinase-1; TSC, tuberous sclerosis complex; Rheb, Ras homolog 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, PPARγ-coactivator 1.

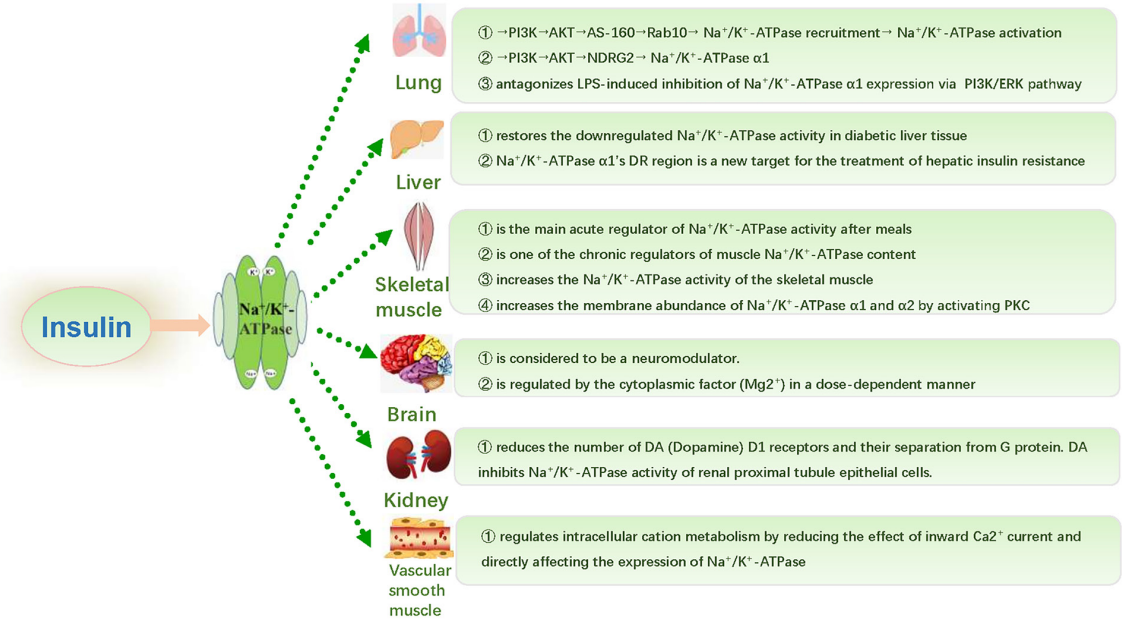

Figure 4. Regulatory mechanism of insulin on Na^+/K^+-ATPase in different organs. AS-160, 160 KD substrate protein downstream of AKT; Rab10, Member RAS oncogene family; NDRG2, N-Myc downstream-regulated gene 2 protein; PKC, protein kinase C.

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 α1 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 α1 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 α1 mRNA level and enzymatic activity (55). In our previous research, insulin was found to antagonize LPS-induced inhibition of Na^+/K^+-ATPase α1 expression via the PI3K/ERK pathway. LPS induced a decrease in Na^+/K^+-ATPase α1 protein levels in ATII cells, while insulin significantly increased the protein levels of Na^+/K^+-ATPase α1 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 α1 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 α1 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 α1 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 α1 and α2 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 α1, β1 and β3 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 β3 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-IR 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.

References

1. Liu J, Lilly MN and Shapiro JI: Targeting Na/K-ATPase signaling: A new approach to control oxidative stress. Curr Pharm Des 24: 359-364, 2018.
2. Kaplan JH: Biochemistry of Na,K-ATPase. Annu Rev Biochem 71: 511-535, 2002.
3. Suhail M: Na, K-ATPase: Ubiquitous multifunctional transmembrane protein and its relevance to various pathophysiological conditions. J Clin Med Res 2: 1-17, 2010.
4. Shrivastava AN, Triller A and Melki R: Cell biology and dynamics of Neuronal Na^+/K^+-ATPase in health and diseases. Neuropharmacology 169: 107461, 2020.
5. Yang P, Cartwright C, Efuet E, Hamilton SR, Wistuba II, Menter D, Addington C, Shureiqi I and Newman RA: Cellular location and expression of Na^+, K^+ -ATPase α subunits affect the anti-proliferative activity of oleandrin. Mol Carcinog 53: 253-263, 2014.
6. Clausen MV, Hilbers F and Poulsen H: The structure and function of the Na,K-ATPase isoforms in health and disease. Front Physiol 8: 371, 2017.
7. Tidow H, Aperia A and Nissen P: How are ion pumps and agrin signaling integrated? Trends Biochem Sci 35: 653-659, 2010.
8. Li Z and Langhans SA: Transcriptional regulators of Na,K-ATPase subunits. Front Cell Dev Biol 3: 66, 2015.
9. Brazee PL, Soni PN, Tokhtaeva E, Magnani N, Yemelyanov A, Perlman HR, Ridge KM, Sznajder JI, Vagin O and Dada LA: FXYD5 Is an essential mediator of the inflammatory response during lung injury. Front Immunol 8: 623, 2017.
10. Marck PV and Pierre SV: Na/K-ATPase signaling and cardiac pre/postconditioning with cardiotonic steroids. Int J Mol Sci 19: 2336, 2018.
11. Lingrel JB: The physiological significance of the cardiotonic steroid/ouabain-binding site of the Na,K-ATPase. Annu Rev Physiol 72: 395-412, 2010.
12. Johnson MD, Widdicombe JH, Allen L, Barbry P and Dobbs LG: Alveolar epithelial type I cells contain transport proteins and transport sodium, supporting an active role for type I cells in regulation of lung liquid homeostasis. Proc Natl Acad Sci USA 99: 1966-1971, 2002.
13. Blanco G: Na,K-ATPase subunit heterogeneity as a mechanism for tissue-specific ion regulation. Semin Nephrol 25: 292-303, 2005.
14. Wang HY and O'doherty GA: Modulators of Na/K-ATPase: A patent review. Expert Opin Ther Pat 22: 587-605, 2012.
15. Geering K: Functional roles of Na,K-ATPase subunits. Curr Opin Nephrol Hypertens 17: 526-532, 2008.
16. Toyoshima C, Kanai R and Cornelius F: First crystal structures of Na^+,K^+-ATPase: New light on the oldest ion pump. Structure 19: 1732-1738, 2011.
17. Mijatovic T, Dufrasne F and Kiss R: Na^+/K^+-ATPase and cancer. Pharm Pat Anal 1: 91-106, 2012.
18. Herold S, Gabrielli NM and Vadász I: Novel concepts of acute lung injury and alveolar-capillary barrier dysfunction. Am J Physiol Lung Cell Mol Physiol 305: L665-L681, 2013.
19. Laffey JG and Matthay MA: Fifty years of research in ARDS. Cell-based therapy for acute respiratory distress syndrome. Biology and potential therapeutic value. Am J Respir Crit Care Med 196: 266-273, 2017.
20. Vadász I, Raviv S and Sznajder JI: Alveolar epithelium and Na,K-ATPase in acute lung injury. Intensive Care Med 33: 1243-1251, 2007.
21. Mutlu GM and Sznajder JI: Mechanisms of pulmonary edema clearance. Am J Physiol Lung Cell Mol Physiol 289: L685-L695, 2005.
22. Lecuona E, Trejo HE and Sznajder JI: Regulation of Na,K-ATPase during acute lung injury. J Bioenerg Biomembr 39: 391-395, 2007.
23. Hunter T: The age of crosstalk: Phosphorylation, ubiquitination, and beyond. Mol Cell 28: 730-738, 2007.
24. Calistri A, Munegato D, Carli I, Parolin C and Palù G: The ubiquitin-conjugating system: Multiple roles in viral replication and infection. Cells 3: 386-417, 2014.
25. Heaton SM, Borg NA and Dixit VM: Ubiquitin in the activation and attenuation of innate antiviral immunity. J Exp Med 213: 1-13, 2016.
26. Coppi MV and Guidotti G: Ubiquitination of Na,K-ATPase alpha1 and alpha2 subunits. FEBS Lett 405: 281-284, 1997.
27. Dada LA, Welch LC, Zhou G, Ben-Saadon R, Ciechanover A and Sznajder JI: Phosphorylation and ubiquitination are necessary for Na,K-ATPase endocytosis during hypoxia. Cell Signal 19: 1893-1898, 2007.

28. Comellas AP, Dada LA, Lecuona E, Pesce LM, Chandel NS, Quesada N, Budinger GR, Strous GJ, Ciechanover A and Sznajder JI: Hypoxia-mediated degradation of Na,K-ATPase via mitochondrial reactive oxygen species and the ubiquitin-conjugating system. Circ Res 98: 1314-1322, 2006.
29. Helenius IT, Dada LA and Sznajder JI: Role of ubiquitination in Na,K-ATPase regulation during lung injury. Proc Am Thorac Soc 7: 65-70, 2010.
30. Hoxhaj G, Najafov A, Toth R, Campbell DG, Prescott AR and Mackintosh C: ZNRF2 is released from membranes by growth factors and, together with ZNRF1, regulates the Na^+/K^+ATPase. J Cell Sci 125: 4662-4675, 2012.
31. Ryter SW, Bhatia D and Choi ME: Autophagy: A lysosome-dependent process with implications in cellular redox homeostasis and human disease. Antioxid Redox Signal 30: 138-159, 2019.
32. Zhu M, Cao L, Xiong S, Sun H, Wu Z and Bian JS: Na^+/K^+-ATPase-dependent autophagy protects brain against ischemic injury. Signal Transduct Target Ther 5: 55, 2020.
33. Felippe Gonçalves-de-Albuquerque C, Ribeiro Silva A, Ignácio da Silva C, Caire Castro-Faria-Neto H and Burth P: Na/K-ATPase as a modulator of apoptosis and autophagy. Molecules 22: 578, 2017.
34. Liu Y, Shoji-Kawata S, Sumpter RM Jr, Wei Y, Ginet V, Zhang L, Posner B, Tran KA, Green DR, Xavier RJ, *et al*: Autosis is a Na^+,K^+-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia-ischemia. Proc Natl Acad Sci USA 110: 20364-20371, 2013.
35. Liu WJ, Ye L, Huang WF, Guo LJ, Xu ZG, Wu HL, Yang C and Liu HF: p62 links the autophagy pathway and the ubiqutin-proteasome system upon ubiquitinated protein degradation. Cell Mol Biol Lett 21: 29, 2016.
36. Lin X, Li S, Zhao Y, Ma X, Zhang K, He X and Wang Z: Interaction domains of p62: A bridge between p62 and selective autophagy. DNA Cell Biol 32: 220-227, 2013.
37. Wurzer B, Zaffagnini G, Fracchiolla D, Turco E, Abert C, Romanov J and Martens S: Oligomerization of p62 allows for selection of ubiquitinated cargo and isolation membrane during selective autophagy. Elife 4: e08941, 2015.
38. Hancock ML, Meyer RC, Mistry M, Khetani RS, Wagschal A, Shin T, Ho Sui SJ, Naar AM and Flanagan JG: Insulin receptor associates with promoters genome-wide and regulates gene expression. Cell 177: 722-736.e22, 2019.
39. Tokarz VL, MacDonald PE and Klip A: The cell biology of systemic insulin function. J Cell Biol 217: 2273-2289, 2018.
40. Haeusler RA, McGraw TE and Accili D: Biochemical and cellular properties of insulin receptor signalling. Nat Rev Mol Cell Biol 19: 31-44, 2018.
41. Borge PD, Moibi J, Greene SR, Trucco M, Young RA, Gao Z and Wolf BA: Insulin receptor signaling and sarco/endoplasmic reticulum calcium ATPase in beta-cells. Diabetes 51 (Suppl 3): S427-S433, 2002.
42. Herchuelz A and Pachera N: The Na^+/Ca^{2+} exchanger and the Plasma Membrane Ca^{2+}-ATPase in β-cell function and diabetes. Neurosci Lett 663: 72-78, 2018.
43. Herchuelz A, Nguidjoe E, Jiang L and Pachera N: Na(+)/Ca (2+) exchange and the plasma membrane Ca(2+)-ATPase in β-cell function and diabetes. Adv Exp Med Biol 961: 385-394, 2013.
44. Futai M, Sun-Wada GH, Wada Y, Matsumoto N and Nakanishi-Matsui M: Vacuolar-type ATPase: A proton pump to lysosomal trafficking. Proc Jpn Acad Ser B Phys Biol Sci 95: 261-277, 2019.
45. Lichtstein D, Ilani A, Rosen H, Horesh N, Singh SV, Buzaglo N and Hodes A: Na^+, K^+-ATPase signaling and bipolar disorder. Int J Mol Sci 19: 2314, 2018.
46. Therien AG and Blostein R: Mechanisms of sodium pump regulation. Am J Physiol Cell Physiol 279: C541-C566, 2000.
47. Shahidullah M, Mandal A and Delamere NA: Src family kinase links insulin signaling to short term regulation of Na,K-ATPase in nonpigmented ciliary epithelium. J Cell Physiol 232: 1489-1500, 2017.
48. Chen HI, Yeh DY, Liou HL and Kao SJ: Insulin attenuates endotoxin-induced acute lung injury in conscious rats. Crit Care Med 34: 758-764, 2006.
49. Brunkhorst FM, Engel C, Bloos F, Meier-Hellmann A, Ragaller M, Weiler N, Moerer O, Gruendling M, Oppert M, Grond S, *et al*: Intensive insulin therapy and pentastarch resuscitation in severe sepsis. N Engl J Med 358: 125-139, 2008.
50. Comellas AP, Kelly AM, Trejo HE, Briva A, Lee J, Sznajder JI and Dada LA: Insulin regulates alveolar epithelial function by inducing Na^+/K^+-ATPase translocation to the plasma membrane in a process mediated by the action of Akt. J Cell Sci 123: 1343-1351, 2010.
51. Bruss MD, Arias EB, Lienhard GE and Cartee GD: Increased phosphorylation of Akt substrate of 160 kDa (AS160) in rat skeletal muscle in response to insulin or contractile activity. Diabetes 54: 41-50, 2005.
52. Ishikura S, Bilan PJ and Klip A: Rabs 8A and 14 are targets of the insulin-regulated Rab-GAP AS160 regulating GLUT4 traffic in muscle cells. Biochem Biophys Res Commun 353: 1074-1079, 2007.
53. He J, Qi D, Wang DX, Deng W, Ye Y, Feng LH, Zhu T, Zhao Y and Zhang CR: Insulin upregulates the expression of epithelial sodium channel in vitro and in a mouse model of acute lung injury: Role of mTORC2/SGK1 pathway. Exp Cell Res 331: 164-175, 2015.
54. Deng W, Li CY, Tong J, He J, Zhao Y and Wang DX: Insulin ameliorates pulmonary edema through the upregulation of epithelial sodium channel via the PI3K/SGK1 pathway in mice with lipopolysaccharide-induced lung injury. Mol Med Rep 19: 1665-1677, 2019.
55. Bashir SO: Concomitant administration of resveratrol and insulin protects against diabetes mellitus type-1-induced renal damage and impaired function via an antioxidant-mediated mechanism and up-regulation of Na^+/K^+-ATPase. Arch Physiol Biochem 125: 104-113, 2019.
56. Liu H, Chen Z, Wu D, Huang X and Wan Q: Insulin attenuates the inhibition effect of lipopolysaccharide on Na^+ -K^+ -ATPase α1 via PI3 K/AKT and PI3 K/ERK pathway. Chin J Clin Pharm Therap 24: 896-902, 2019.
57. Obradovic M, Bjelogrlic P, Rizzo M, Katsiki N, Haidara M, Stewart AJ, Jovanovic A and Isenovic ER: Effects of obesity and estradiol on Na^+/K^+-ATPase and their relevance to cardiovascular diseases. J Endocrinol 218: R13-R23, 2013.
58. Siddiqui MR, Moorthy K, Taha A, Hussain ME and Baquer NZ: Low doses of vanadate and trigonella synergistically regulate Na^+/K^+ -ATPase activity and GLUT4 translocation in alloxan-diabetic rats. Mol Cell Biochem 285: 17-27, 2006.
59. Sun HJ, Cao L, Zhu MY, Wu ZY, Shen CY, Nie XW and Bian JS: DR-region of Na^+/K^+-ATPase is a target to ameliorate hepatic insulin resistance in obese diabetic mice. Theranostics 10: 6149-6166, 2020.
60. Pirkmajer S and Chibalin AV: Na,K-ATPase regulation in skeletal muscle. Am J Physiol Endocrinol Metab 311: E1-E31, 2016.
61. Pirkmajer S and Chibalin AV: Hormonal regulation of Na^+-K^+-ATPase from the evolutionary perspective. Curr Top Membr 83: 315-351, 2019.
62. Catalán RE, Martínez AM, Aragonés MD, Fernández I and Miguel BG: Inhibitory effect of insulin and cytoplasmic factor(s) on brain (Na(+) + K+) ATPase. Neurosci Res 13: 139-145, 1992.
63. Banday AA, Asghar M, Hussain T and Lokhandwala MF: Dopamine-mediated inhibition of renal Na,K-ATPase is reduced by insulin. Hypertension 41: 1353-1358, 2003.
64. Sowers JR: Effects of insulin and IGF-I on vascular smooth muscle glucose and cation metabolism. Diabetes 45 (Suppl 3): S47-S51, 1996.
65. Silva CID, Gonçalves-De-Albuquerque CF, Moraes BPT, Garcia DG and Burth P: Na/K-ATPase: Their role in cell adhesion and migration in cancer. Biochimie 185: 1-8, 2021.
66. Lu S, Cai S, Peng X, Cheng R and Zhang Y: Integrative transcriptomic, proteomic and functional analysis reveals ATP1B3 as a diagnostic and potential therapeutic target in hepatocellular carcinoma. Front Immunol 12: 636614, 2021.
67. Vigneri R, Goldfine ID and Frittitta L: Insulin, insulin receptors, and cancer. J Endocrinol Invest 39: 1365-1376, 2016.
68. Vigneri R, Sciacca L and Vigneri P: Rethinking the relationship between insulin and cancer. Trends Endocrinol Metab 31: 551-560, 2020.
69. Belfiore A, Frasca F, Pandini G, Sciacca L and Vigneri R: Insulin receptor isoforms and insulin receptor/insulin-like growth factor receptor hybrids in physiology and disease. Endocr Rev 30: 586-623, 2009.
70. Vella V, Sciacca L, Pandini G, Mineo R, Squatrito S, Vigneri R and Belfiore A: The IGF system in thyroid cancer: New concepts. Mol Pathol 54: 121-124, 2001.