A Novel Action of Insulin on Cardiac Membrane

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Insulin is an essential hormone for the control of blood glucose level and glucose metabolism. Additionally, insulin mediates a wide spectrum of biological responses including lipid metabolism, protein synthesis, activation of transcription of specific genes, modulation of cellular growth and differentiation, and functional regulation of ion channels and transporters in various types of cells. The action of insulin is mediated through the insulin receptor, a transmembrane glycoprotein with intrinsic protein tyrosine kinase activity. The insulin receptor is an α2/β2 tetramer with the α-subunit located on the extracellular face of the plasma membrane having the insulin binding site. The intracellular portion of the β-subunit contains the insulin-regulated tyrosine protein kinase. Insulin action is exerted through its binding to the α-subunit of the insulin receptor, resulting in regulation of the tyrosine protein kinase located in the β-subunit of the insulin receptor. This leads to the autophosphorylation of the receptor and other substrates including insulin receptor substrate-1 (IRS-1) and other related proteins. Diverse and complex intracellular signaling pathways downstream to the receptor activation are involved in different responses depending on the target proteins, and it is believed that intrinsic tyrosine kinase is critical for the signal transduction of insulin action.1-2

Insulin receptors are also present in cardiac membranes. Because tissue sensitivity to insulin is dictated by receptor abundance, it is relevant to note that the number of functional insulin receptors in the heart is comparable to that in other insulin-sensitive cell types (10 000 to 100 000 per cell).3 In the heart, insulin regulates various physiological and pathophysiological functions, including myocardial energy metabolism, contractility, protein expression, hypertrophy and cardiomyopathy in diabetes mellitus, and ion transport mechanisms.3 The actions of insulin on ionic channels and transporters of normal cardiac membrane have been considered to be unremarkable,4 but recent evidence has been accumulating to indicate the regulatory roles played by insulin on various channels and transporters, eg, stimulation of the L-type Ca\(^{2+}\) current5,6 and the Na\(^+\)-Ca\(^{2+}\) exchanger,7 and upregulation of K\(^+\) channels.8 Given that some of these results were obtained at unphysiologically high insulin levels, the actual contribution of each process to physiological function and signal transduction pathways needs careful consideration.

In the study reported in this issue of *Circulation Research,*9 Zhang and Hancox demonstrated that insulin at 1 nanomol to 1 μmol concentrations activated a novel voltage-dependent nonselective cation current (NSCC) in guinea pig ventricular myocytes, under conditions where other conductances were inhibited. The NSCCs so far reported in cardiac membrane are intracellular Ca\(^{2+}\)-activated and background type,10,11 and stretch-activated channels,12 which are voltage-independent with a linear I-V relation. Zhang and Hancox found that the ion selectivity of this current was equally permeable to Cs\(^+\), K\(^+\), Li\(^+\), and Na\(^+\), but not to NMDG. As to the pharmacological sensitivity, insulin-activated current (I_\text{insulin}) was blocked by NSCC blockers, Gd\(^{3+}\) and SKF96365, but not by flufenamic acid (FFA), exhibiting some but not complete similarity to NSCC in other cell types.13-15 Activation of I_\text{insulin} was abolished by pretreatment with insulin-receptor tyrosine kinase inhibitor, hydroxy-2-naphthalenyl-methyl phosphonic acid trisacetoxy-methyl ester (HNMPA-(AM)3). This study, therefore, appears to be the first report describing the involvement of tyrosine kinase phosphorylation in the activation of cardiac NSCC. A specific phospholipase C (PLC) inhibitor (U73122) significantly abbreviated the current activation, whereas an inactive analogue (U73433) did not affect the response, suggesting that a PLC-dependent pathway is involved in the current activation. Their data also suggest that the insulin-induced activation of NSCC is possibly mediated by a direct involvement by diacylglycerol (DAG) but not by activation of protein kinase C (PKC). This interpretation was based on their observations that membrane-permeable DAG analogue, 1-oleoyl-2-acetyl-sn-glycerol (OAG), mimicked the effect of insulin, but application of staurosporine to inhibit PKC did not affect the response.

Therefore, the study contains two novel findings: insulin activates voltage-dependent NSCC in cardiac cells, and the type of NSCC appears to represent a ligand-gated channel directly activated by DAG. This short report may shed light on a novel and important action of insulin on cardiac cell physiology and its function in several aspects. The insulin-activated NSCC carries cationic current flows, including Na\(^+\), in an outward-going rectification at positive voltages and inward direction at negative voltages. This current flow may, in turn, influence the activity of the Na\(^+\)-Ca\(^{2+}\) exchanger inducing increased Ca\(^{2+}\) influx into myocardial cells. This can explain, at least in part, the increased contractility produced by insulin in the heart.
Insulin induces pleiotropic effects on various cell types. The pleiotropic action of insulin is mediated by diverse signaling pathways, and the action has postulated the involvement of intracellular Ca\(^{2+}\). Actually, recent studies have shown that insulin affects cellular Ca\(^{2+}\) metabolism. So, further attention has to be paid to the mechanism of insulin-induced cellular responses in relation to increased Ca\(^{2+}\) influx into cardiac cells. Second, the molecular identity of NSCC has not been clarified in cardiac cells. Voltage-dependent NSCCs in several other cell types have differential sensitivity to pharmacological NSCC blockers, and the transient receptor potential protein homologue (TRIP) is a molecular candidate for NSCC. This may give us a clue to approach the molecular identity of cardiac voltage-dependent NSCC. Third, while the present results suggest that \(I_{\text{insulin}}\) may represent the ligand-gated channel activated by DAG, like NSCC in other cell types, the work identifies a new member of ligand-gated channels in the heart, in addition to K\(^+\) channel families such as G protein–gated K\(^+\) channels, ATP-sensitive K\(^+\) channels and others. Clarification of the activation mechanism will help us to understand the properties of cardiac NSCC channels.

The signaling pathway that the authors implicated as the background of this study is the activation of phospholipase C\(\gamma\) (PLC\(\gamma\)) with G protein, coupled in a manner to hydrolyze phosphatidylinositol 4,5-biphosphate leading to production of inositol 1,4,5-triphosphate (IP3) and DAG. Increased IP3 and DAG in cytoplasm function as second messengers to induce Ca\(^{2+}\) influx. PLC\(\gamma\) pathway has not convincingly been presented. If the authors could supply data showing the activation of this pathway by demonstrating the production or increased level of PLC\(\gamma\) after insulin application, the actual role of the PLC\(\gamma\) pathway in insulin response might be strengthened. Future studies are encouraged to pursue this issue. Furthermore, one of the earliest steps in the insulin signaling pathways is the activation of phosphatidylinositol 3-kinase (PI3-kinase), which catalyzes the phosphorylation of phosphoinositides, and a possible contribution through this pathway to activate PLC\(\gamma\) should be examined, as well as an involvement of other signaling pathways.

As to the functional significance for activation of \(I_{\text{insulin}}\) and OAG-activated current, Zhang and Hancox showed shortening of action potential duration in the presence of insulin plus staurosporine to block PKC. The shortening effects, however, may need qualification before reaching a simple conclusion of their functional role, since insulin activates other currents and transporters to influence repolarization. As the plateau of action potential is formed by overlapping inward and outward currents, and their delicate balance, \(I_{\text{insulin}}\) activation may provide complex effects on action potentials depending on the basal conditions and the experimental situation. According to their results, insulin-activated NSCC shows time-dependent activation and inactivation, the mechanism and functional significance of which are not known. Therefore, the study by Zhang and Hancox opens a new way to recognize important insulin action on cardiac membrane, but many unanswered questions remain. Further study is mandatory for the elucidation and understanding of the insulin action on the heart.

References


**Key Words:** insulin ■ voltage-dependent nonselective cation channel ■ signaling pathway ■ diacylglycerol
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