Cardioprotective role of insulin on long QT-interval via recoveries in $\mathbf{K^+}$-channel currents in advanced age

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International consensus recognizes the impact of the aging process on the development of left ventricular hypertrophy in the heart. Both experimental and clinical data emphasize the intersection between aging and cardiovascular disease, which include age-associated co-morbidities such as metabolic syndrome, obesity, and diabetes that enhance the risk of developing cardiovascular disease. Indeed, cardiometabolic disturbances, including insulin resistance, contribute to aging-associated cardiac insufficiency and/or dysfunction. Cardiac aging is an intrinsic process accompanied by molecular and cellular changes (cardiac electrophysiological remodeling) mainly characterized by a long QT-interval, increased heart rate, and depressed cardiac output, as well as prolonged action potentials, altered sarcolemmal ionic currents and intracellular $\mathbf{Ca^{2+}}$-regulation at the cellular levels. Previous studies emphasize the benefits of insulin regulation as a critical component of pharmacotherapy after myocardial injury. Given the relationship between impaired insulin signaling and depression of various voltage-dependent $\mathbf{K^+}$-channels, which in turn contribute to prolonged action potentials and long-QT in the heart, insulin treatment to restore proper cellular signaling may become an emerging new field for treating age-related heart dysfunction. This review-article focuses on the molecular mechanisms linking insulin resistance, heart dysfunction, and advanced age in mammals, including recent experimental results associated with the possible contribution of a right-shift of the voltage-dependency of tetrodotoxin-sensitive $\mathbf{Na^+}$-currents in ventricular cardiomyocytes from aged rat hearts, as well as the beneficial effects of insulin treatment in aged-rats on their cardiovascular function. Hopefully, this document will encourage scientists and clinicians in this field to design new and more effective mechanism-based insulin-like agents to improve myocardial performance in aged humans.

Keywords: Insulin, Aging, Electrical remodeling, Heart dysfunction, Mitochondria

Introduction
Changes in individual cells in all organs of the body lead to progressive deterioration in their functions and structures that underlie the process of human aging. Aging is a major risk factor for cardiovascular diseases, the leading cause of death worldwide (Lakatta et al., 2001). The most common change in the cardiovascular system (CVS) is stiffening of the arteries and other blood vessels, causing the heart to work harder, which leads to further remodeling of the heart muscles to adjust to the increased workload (Waring et al., 2014). After such changes, the heart rate at rest will stay about the same, but will fail to increase during activities as it once did. These changes increase the risk of high blood pressure (hypertension) and other cardiovascular alterations.

Cardiovascular diseases are the leading cause of death in most countries. Although it has received the least public attention, aging is by far the dominant risk factor for the development of cardiovascular diseases, as the prevalence of cardiovascular diseases increases dramatically with increasing age (Lakatta et al., 2001). Aging hearts, in general, show a progressive decline in their structure, function, and metabolism (Ruiz-Meana et al., 2020; Strait and Lakatta, 2012). In large part this is due to impairment in electrical
Cardiometabolic disturbances are mainly characterized by a high level of oxidative stress, cell loss and heterogeneous properties of cardiomyocytes, as well as the changes in the conduction of electrical signals among myocytes. In addition, aged hearts show impairments in redox status, metabolic flexibility, and organelle dynamics (Lesnefsky et al., 2016). Most clinical observations point out the parallelism between the occurrence of inevitable functional decline with time in the heart and induction of insulin resistance (Chason et al., 2018). Both clinical and experimental data mentioned that cardiac aging is mainly characterized by a long QT-interval in electrocardiograms (ECGs), a reduction in the maximum heart rate, and a decrease in the contractile activity, at most, through cardiometabolic disturbances (Lakatta et al., 2001). Cardiometabolic disturbances are mainly characterized by insulin resistance and the appearance of glucose intolerance, which are factors that underlie the impairment in electrophysiological activities, and myocardial cell death (Boudina, 2013; Olgar et al. 2018; Olgar et al., 2020a; Olgar et al., 2020b; van Noord et al., 2010). Experimental animal studies, with either cardiac-specific insulin receptor deletion or insulin application, have shown the important role of insulin on the conduction velocity of electrical signaling and ventricular repolarization in the heart. Correspondingly, insulin application provided significant cardioprotection in the rat heart with not only metabolic syndrome but also advanced age (Olgar et al., 2021). Most importantly, these cardioprotective effects of insulin are due to its benefits on the depressed expression and function of voltage-dependent K+-channels in left ventricular cardiomyocytes (Bertrand et al., 2008; Lopez-Izquierdo et al., 2014; Olgar et al., 2020a; Olgar et al., 2020b).

Most experimental findings support the hypothesis that lack of insulin signaling can provide cardioprotection in a reshape action potential of left ventricular cardiomyocytes which further leads to abnormal ventricular repolarization in the advanced heart. Therefore, this review focuses particularly on the molecular mechanisms linking insulin resistance, heart dysfunction and advanced age in mammals, including recent experimental results showing a possible contribution of a right-shift of the voltage-dependency of tetrodotoxin (TTX)-sensitive Na+-currents in ventricular cardiomyocytes from aged rat hearts. The beneficial effects of insulin treatment on cardiovascular function in aged-rats will also be discussed, including original in vivo and in vitro work examining the actions of insulin in the aged rat hearts will be Therefore, this review will open the door to design new and more effective mechanism-based insulin-like agents to improve myocardial performance in elderly humans.

Cardiac ventricular electrophysiological remodeling in the elderly mammalian heart

Biological aging is the greatest risk factor for cardiovascular-related morbidity and mortality (Mozaffarian et al., 2016). Normal physiological remodeling in healthy aging hearts mainly includes changes in electrical conduction among tissues, valve function, large and small coronary vessels, and contractile activity. These age-related physiological alterations can in turn be exaggerated stimuli for tissue remodeling during pathophysiological states. There are marked structural remodeling in aging hearts, including atrial and ventricular fibrosis, which can further promote progression to ventricular arrhythmia, heart failure, and sudden cardiac death in aged rabbit heart (Lin et al., 2018; Stuart et al., 2018). Similar structural changes have been determined in the aged rat heart together with marked fragmentation in mitochondria in isolated ventricular cardiomyocytes (Olgar et al., 2020a; Olgar et al., 2021). Among these changes, passive ventricular remodeling is also defined by the process of molecular remodeling of gap junctions, that can affect cell-to-cell propagation of the electrical impulse, inducing re-modification in the excitability (Kessler et al., 2014; Xie et al., 2013).

Cardiomyocytes isolated from the left ventricular part of the aged animal heart showed marked myocyte modification characterized by a high level of oxidative stress, cell loss and hypertrophy, apoptosis, and autophagy (Sheydina et al., 2011;
Aging also prolongs the duration of action potentials (APs) of cardiomyocytes isolated from the left ventricle, through alterations in the electrophysiological characteristics of the ionic currents (Olgar et al., 2020a; Olgar et al., 2020b). Ventricular cardiomyocytes undergoing age-associated electrophysiological remodeling typically exhibit a prolonged AP duration that has been attributed to a decrease in voltage-dependent outward K$^{+}$-channel currents (Janczewski, Spurgeon, & Lakatta, 2002) together with significant increases in the Na$^{+}$/K$^{+}$-pump current, Na$^{+}$/Ca$^{2+}$-exchanger current, and intracellular levels of Na$^{+}$, Ca$^{2+}$ and H$^{+}$, parallel to long QT-interval in the surface ECGs (Olgar et al., 2020a; Olgar et al., 2020b). Although there were no significant changes in the peak amplitude of the voltage-dependent Na$^{+}$-channel currents in the left ventricular cardiomyocytes from the aged rat hearts, the characteristic of the frequency distribution of APs seems to be different in the aged rats (24-mo-old) compared to the adult rats (6-mo-old), including a marked shift with the positive potentials (Fig. 1). This right-side shift of the histograms provides strong evidence of an increase in late Na$^{+}$-channel currents in the ventricular myocytes. Besides this preliminary observation, other studies demonstrated that neither the density nor the expression level of voltage-dependent Na$^{+}$-channels is affected by aging (Baba et al., 2006), whereas a significant increase is observed in the late Na$^{+}$-channel currents in the aged ventricular cardiomyocytes (Signore et al., 2015).

Moreover, it has been demonstrated that aging was associated with depression of the sarcoplasmic reticulum (SR) Ca$^{2+}$-level and intracellular Ca$^{2+}$ changes under electrical stimulation in cardiomyocytes from not only aged humans but also other aged animals (Herraiz-Martínez et al., 2015; Olgar et al., 2020a; Olgar et al., 2020b). These alterations are also closely associated with a progressive decline in contractile function with aging even under physiological conditions.

Cardioprotective action of insulin in the aged mammalian heart

There is a close relationship between increased life span and the development of at least one chronic disease, which directly impacts the overall health of the elderly population. Although intrinsic cardiac aging is an independent risk factor for the development of heart insufficiency/dysfunction, its impact is confounded by various age-related risk factors, including insulin resistance (Lakatta, 2000). The involvement of the insulin-signaling pathway in life span extension is widely known, and as the most conserved signaling pathway, it also has an important impact on cardiac physiological aging (Inuzuka et al., 2009; Kenyon, 2005; Wessells et al., 2004). So, insulin may attenuate cardiac aging by affecting cardiac insulin signaling, although the underlying mechanisms are not well understood. A few studies have examined the impact of a reduction in insulin/IGF-signaling on aging-associated cardiac alterations and the role of its alleviation on the preservation of cardiac performance (Barzilai and Ferrucci, 2012; Yang et al., 2017; Yu et al., 2011). The early studies on the cardiovascular actions of insulin in humans have determined there is a correlation between insulin resistance and both reduction in cardiac index and stroke volume without a change in heart rate (Baron et al., 1990). The findings from these early studies strongly emphasized the in vivo role of insulin as an endocrine regulator of cardiovascular physiology through both glucose uptake and hemodynamic regulation.

Insulin is an anabolic peptide hormone that plays a vital role in the regulation of human metabolism during the lifespan.
as well as it actions on a specific cell membrane receptor. Although it is widely accepted to play a key role in glucose-homeostasis, it is now appreciated how insulin can target different signaling pathways and that it has a wide-range of pleiotropic roles in mammalian cells. Insulin is known to be a main energy-storage and metabolism regulator, and it has critical but very complex effects in basic organs such as liver, muscle, brain, and adipose tissue (Fig. 2, left). As can be seen in the proposed schema, insulin targets several systems through its receptor and affects multiple physiological processes in the brain. Also, insulin through its receptor plays critical roles in intracellular metabolic pathways directly (i.e. insulin signaling pathways) or indirectly (via other metabolic pathways). As can be seen in Fig. 2 (right), intracellular pathways can be summarized as increases in glucose uptake, synthesis of glucose, protein, and DNA, and attenuation of gene expression and lipogenesis with decreases mainly in mitochondrial dysfunction and related facors, glucogenesis, apoptosis, and autophagy.

Although previous experimental studies examined the contribution of the regulatory roles of insulin/IGF-signaling in aging heart dysfunction (Inuzuka et al., 2009; Kenyon, 2005; Wessells et al., 2004), the undelying mechanism is not well understood yet. Wessells et al. (2004) studied the regulatory role of insulin on heart function in aging fruit flies and demonstrated that insulin-IGF signaling influences age-dependent organ physiology and senescence directly and autonomously, in addition to its systemic effect on lifespan. In further review articles, the role of insulin/IGF signaling in preserving cardiac performance in the aged mammalian heart was discussed (Boudina, 2013; Lee and Kim, 2018). In addition, it is well known that the absence of insulin signaling in the heart induces changes in voltage-dependent K⁺-channel expression/function and prolongation in AP duration of isolated ventricular cells, parallel to long-QT in mammalian heart (Lopez-Izquierdo et al., 2014). Although these results support the above notation that the leading cause of insulin signaling disturbance in the heart is repolarization abnormalities, including long-QT, in animal models of diabetes as well as aged-mammals (Bertrand et al., 2008; Durak et al., 2018; Olgar et al., 2020a; Olgar et al., 2020b), it is not exactly known whether or not there is a direct insulin effect on cardiac performance in aged mammals. Some experimental studies have demonstrated the efficacy of insulin in preventing long-QT and the associated arrhythmias in diabetic animals through reduction of the rapid delayed rectifier K⁺-current (Zhang et al., 2006). These results support the hypothesis that lack of insulin signaling can produce abnormal repolarization in cardiomyocytes and the development of the arrhythmogenic potential, further leading to an increase in the incidence of sudden cardiac death. Supporting this hypothesis, recent, Olgar et al. (2021b), for the first time, demonstrated that insulin provided important benefits on long QT in insulin-resistant aged rats by accelerating the ventricular action potential-repolarization by reversing inhibited KCNQ1/KCNE channel-current (I_{Ks}) through the beta adrenergic receptor subtype 3 (β3-ARs) signaling. Indeed, the protective role of insulin therapy as an adjunct to reperfusion after ischemia in the heart has been demonstrated with insulin directly promoting myocardial cell survival independent of any effects on metabolic modulation (Sack and Yellon, 2003). Later studies showed that insulin is a key component of the GIK-cocktail (glucose-insulin-potassium) that modulates the PI3K-Akt-eNOS-dependent signal mechanism (Alburquerque-Béjar et al., 2015; Boucher et al., 2014; Lopez-Izquierdo et al., 2014; Yu et al., 2011). Taking into consideration the role of cAMP and PKA activation on I_{Ks} (Kanda et al., 2011), one can hypothesize their contribution to this current. However, previous studies
studies have shown an inhibitory role of tumor necrosis factor-alpha (TNF-alpha) on insulin induced increases on serine phosphorylation of IR substrate (IRS) proteins, thereby decreasing tyrosine phosphorylation of IRS proteins (Kanety, 1995). With cell level examinations, it has been reported that TNF-a can increase mitochondrial ROS production in tumor cells, while hyperglycemia increased mitochondrial ROS production in endothelial cells (Corda et al., 2001; Kukidome et al., 2006; Nishikawa et al., 2000). Supporting data also demonstrated that this TNF-a-induced ROS over production was suppressed by overexpression of manganese superoxide dismutase (MnSOD) (Imoto et al., 2006). In these regards, further experimental studies performed in insulin-resistant aged rat heart have demonstrated that there are significantly high levels of mitochondrial superoxide and Ca2+, which are major activators of mitochondrial permeability transition pore opening, and increases in the mRNA level of Bnip3, which has important roles in cell death, autophagy, mitophagy, myocardial stiffness, and SR and mitochondrial Ca2+-homeostasis (Chaanine et al., 2013). More importantly, incubation of ventricular cardiomyocytes isolated from aged rat heart with a mitochondria-targeting antioxidant significantly attenuated most of these alterations (Olgar et al., 2020a).

Recently it has been demonstrated that insulin treatment can provide significant cardioprotection against insufficient-heart function in elderly mammals with myocardial insulin resistance by reversing long QT, particularly affecting dysfunctional KCNQ1/KCNE1-channels. Similar benefits were also obtained with a protein kinase G (PKG) inhibitor under both in vitro and in vivo applications. Furthermore, inhibited Ik, in β1-ARs-stimulated cells could be reversed with a PKG inhibitor, indicating the correlation between activated PKG and β1-ARs activation. These data further imply a strong relationship between the inhibited Ik, in aged-rat cardiomyocytes and activated β1-ARs, via the associated interaction between

Beneficial effects of insulin on mitochondria function in aged rat ventricular cardiomyocytes

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Figure 4. Representative confocal images to demonstrate the colocalization of KCNQ1 and KCNE1, and reactive oxygen species (ROS) and mitochondrial membrane potential (MMP) values in ventricular cells. (A) Colocalization of KCNQ1 and KCNE1 in adult and aged rat cardiomyocytes with and without insulin treatment (2 IU/kg/day, for two weeks) at room temperature, as described previously (Olgar et al., 2021). All cells were fixed and permeabilized and then incubated with specific primary antibodies and were followed by secondary antibodies. The cells were mounted in a medium containing DAPI (blue to stain nuclei). (B) The representative confocal images of the cells to demonstrate the levels of ROS imaging with DCFDA (10-µM for 60-min loading) in insulin-treated cells (100 nM for 24-h) with respect to untreated cells. To monitor the ROS level, the DCFDA loaded cells were calibrated with H2O2 (100-µM). MMP imaging with JC-1 (5-µM for 30-min loading) in D-Gal induced aging-mimicked H9c2 cell line with insulin-treated cells (100 nM for 24-h) with respect to untreated cells (Olgar et al., 2019). The probes were excited at 488 nm, and the red fluorescence image was detected at both 535 and 585 nm. To calibrate the changes in MMP, cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP, 5-µM) was used.
Consequently, the findings of experimental studies clearly reveal that IR/IGF-1R interaction with mitochondrial function in organs, including the heart is crucial for cellular energy homeostasis and proper energy metabolism. Thus, experiments examining insulin or insulin-like agents as a treatment modality in any aging animal model, will be a novel approach to targeting cardiovascular disease in the elderly (Olgar et al., 2020a; Olgar et al., 2021b).

Concluding remarks

Cardiac aging is a progressive and intrinsic decline in heart function characterized by a long QT together with reduced heart rate and depressed contractile activity. Recently, a relationship between impairment in insulin signaling and aging leading to heart failure and sudden cardiac arrest has been noted. With the continuously growing elderly population worldwide, there is a great need for interventions in cardiac aging. This article provides recent findings detailing some important targets for insulin in cardiac aging such as $I_{Ks}$ and mitochondria. An understanding of these parameters with further studies will present additional knowledge regarding the molecular mechanisms underlying these types of changes including channelopathies and organellopathies, and more novel advances in the development of interventions to delay or reverse cardiac aging.

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Conflict of interests

The author declares that there is no conflict of interest.

References


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References


