Overcoming an Energy Crisis? 
An Adaptive Role of Glycogen Synthase Kinase-3 Inhibition in Ischemia/Reperfusion

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A prolonged period of ischemia leads to death of cardiac myocyte (myocardial infarction). In theory, quicker restoration of blood supply to the ischemic myocardium should reduce the extent of myocardial injury. However, reperfusion itself has the potential to exacerbate myocardial damage, a phenomenon known as reperfusion injury. Mitochondrial dysfunction plays a central role in mediating myocardial cell death by ischemia/reperfusion (IR). In particular, opening of the mitochondria permeability transition pore (mPTP) has been attributed to irreversible cell damage.1,2 Studies on the effects of preconditioning and postconditioning have revealed a number of signaling cascades that afford cardioprotection involving the mitochondria. Among them, inhibition of glycogen synthase kinase (GSK)-3 has been causatively related by many investigators to the protective effects of ischemic preconditioning,3,4 pharmacological preconditioning,5 anesthetic postconditioning,6 ischemic postconditioning,7 and many chemical cardioprotective interventions.8–13 However, some controversies exist regarding the involvement of GSK-3 inhibition in the ischemic preconditioning and postconditioning effects observed in mice.14

GSK-3 is a serine/threonine kinase, the activity of which is inhibited by phosphorylation induced by activation of upstream kinases. The activity of GSK-3β is decreased by ischemia because of phosphorylation of serine 9 through a phosphatidylinositol 3-kinase–dependent mechanism.4 Inhibition of GSK-3 during IR appears to be an important mechanism of myocardial adaptation because a number of cardioprotective agents use inhibition (phosphorylation) of mitochondrial GSK-3β as the common downstream target.15 We speculate that GSK-3 inhibitors either mimic preconditioning or ensure greater levels of GSK-3 inhibition during IR. However, an important question remains: How does GSK-3 inhibition achieve cardioprotection against ischemia?

One proposed mechanism, endorsed by nearly all of the available literature in the field, is that GSK-3 inhibition may delay or suppress mPTP opening.3,6,11,12,15,16 However, a question remains as to how inhibition of GSK-3 results in the delay of mPTP opening. Addressing this issue is complicated by the uncertain identity of the mPTP. The mPTP has recently been viewed as a pore with unidentified composition and, thus, previously proposed components such as cyclophilin D, adenine nucleotide translocase (ANT), voltage-dependent anion channel (VDAC), benzodiazepine receptors, hexokinases, and creatine kinases may be regulatory but not structural.17 Because GSK-3 does exist in mitochondria18 and its content in mitochondria increases after IR,3 GSK-3 may modulate the function of these mitochondrial proteins through protein–protein interaction and/or phosphorylation (see Figure). Phosphorylated GSK-3β interacts with ANT and reduces the affinity of ANT to cyclophilin D19, which theoretically suppresses the opening of mPTP. Importantly, however, it remains obscure whether the delay of mPTP opening is the direct consequence of modulation in the putative mPTP components by GSK-3 inhibition or secondary to reduction in mitochondrial injury.

In this issue of Circulation Research, Das et al19 report that GSK-3 inhibitors induce dephosphorylation of VDAC, thereby reducing adenine nucleotide (such as ATP) transport across the outer mitochondrial membrane. The action of the GSK-3 inhibitors on VDAC not only preserves ATP by blocking mitochondrial consumption of ATP generated through anaerobic glycolysis but also prevents mitochondrial Ca2+ overloading and oxidative stress by reducing mitochondrial membrane potential. Interestingly, this effect of GSK-3 inhibition appears to be independent of mPTP opening.

The findings of Das et al19 suggest that inhibition of GSK-3 may facilitate hypoxic adaptation during ischemia through dephosphorylation of VDAC. Under hypoxic conditions, generation of ATP shifts from oxidative phosphorylation in the mitochondria to glycolysis in the cytoplasm, a phenomenon known as metabolic adaptation described by Louis Pasteur in the 19th century. This process is controlled by transcription factors, such as hypoxia-inducible factor (HIF)-1α. In response to hypoxia, HIF-1α is stabilized and induces expression of glucose transporters and glycolytic enzymes.20,21 As a result, anaerobic glycolysis is enhanced and ATP production is promoted. In addition, HIF-1α also decreases mitochondrial oxygen consumption,22,23 increases cellular ATP content,24 and attenuates mitochondrial reactive oxygen species generation.23,24 Because GSK-3 phosphorylates HIF-1α and increases its proteasomal degradation,25 inhibition of GSK-3 would stabilize HIF-1α. Beneficial effects of the inhibition of GSK-3 during ischemia related to...
improved anaerobic glycolysis have been reported. Taken together, these findings suggest that inhibition of GSK-3 may be involved in hypoxic adaptation through HIF-1α-mediated transcription. The finding by Das et al suggests that GSK-3 induces hypoxic adaptation also through posttranslational modification of VDAC, which would take place faster than the aforementioned transcriptional mechanisms.

Das et al speculate that reducing ATP consumption by modulating VDAC reduces mitochondrial membrane potential through suppression of F1F0-ATPase, which in turn provides protection against IR injury by decreasing Ca2+ overload and oxidative stress. However, knocking out VDAC fails to decrease and may even enhance Ca2+- and oxidative stress-induced mPTP opening and cell death, suggesting that suppression of VDAC may not be sufficient for preventing Ca2+ overloading or oxidative stress. Thus, the effect of GSK-3 inhibition on Ca2+ overloading and oxidative stress and the involvement of VDAC in this process need to be tested experimentally.

Several consensus sites for GSK-3 phosphorylation exist in rat mitochondrial VDAC. This raises the possibility that GSK-3 may directly phosphorylate VDAC. The possibility is supported by the finding that GSK-3β interacts with VDAC in cardiac tissue and phosphorylates it at least in vitro. VDAC is a transmembrane protein, composed of several β-strands that span the outer mitochondrial membrane. It would be interesting to identify which of these consensus sites are exposed and phosphorylated by GSK-3. Alternatively, GSK-3 could phosphorylate another kinase, which, in turn, phosphorylates mitochondrial VDAC. Indeed, the phosphorylation level of VDAC is reportedly increased by cAMP-dependent protein kinase A, protein kinase Ce, p38 mitogen-activated protein kinase, and hexokinases. GSK-3 usually inhibits its substrates under basal conditions, and inhibition of GSK-3 removes this inhibitory effect toward the substrates. If another kinase is the substrate that phosphorylates VDAC, inhibition of GSK-3 would increase the phosphorylation of VDAC. This makes it unlikely, if not impossible, that GSK-3 influences the phosphorylation of VDAC through another kinase. Another possibility is that GSK-3 regulates a phosphatase that modulates the phosphorylation of VDAC. GSK-3β associates with type 1 phosphatase (PP1)/inhibitor-2 complex, phosphorylates inhibitor-2 and thereby regulates the activity of PP1. However, this phosphorylation of inhibitor-2 by GSK-3β activates PP1, which would then dephosphorylate substrate proteins. This means that VDAC may not be a direct substrate of PP1 or that GSK-3 inactivates another phosphatase, for example PP2A, either directly or indirectly. If other kinases and phosphatases, besides GSK-3, are involved in the VDAC phosphorylation, they could be other targets of drugs for cardioprotection.

GSK exists as 2 ubiquitously expressed isoforms, GSK-3α and GSK-3β, which have different structures and functions in addition to their similarities. Knockdown of GSK-3β, rather than GSK-3α, is associated with increasing the threshold of reactive oxygen species–induced mPTP in cardiac myocytes, indicating an isomorphism-specific role in reactive oxygen species–mediated injury. Recent evidence obtained from GSK-3α knockout mice suggest that GSK-3α inhibition is
critical for glucose utilization and glycogen deposition. Given that glycolysis is an important pathway of energy production under anaerobic conditions, it will be interesting to determine whether ATP content in the ischemic myocardium is increased when GSK-3 is inhibited or knocked out in an isoform-specific manner and whether glucose utilization is enhanced under these same conditions. If proven to be true, this will provide another possible mechanism of GSK-3 inhibition on improving energy availability to maintain cellular integrity during IR.

There is a growing interest to study GSK-3 inhibition as a cardioprotective strategy. Many small molecule inhibitors of GSK-3 have been developed. The utilization of GSK-3 inhibitors as therapeutic agents is promising but cannot be adopted unless critical issues regarding the targeting molecules of GSK-3, the confined cellular compartment of action, and cell type-, organ-, or system-specific effects are solved, because GSK-3 is ubiquitously expressed and is involved in and cell type–, organ-, or system-specific effects are solved, because GSK-3 is ubiquitously expressed and is involved in
cellular integrity during IR.

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Disclosures
None.

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