GLUCOSE, GLUTAMINE, AND GLUCOSAMINE are metabolites that can alter cardioprotection (1, 3, 8, 9, 13–15). Numerous studies suggest that one molecular mechanism by which these metabolites protect cells is through their conversion to uridine diphasphate-N-acetylgalcosamine (UDP-GlcNAc) via the hexosamine biosynthetic pathway (Fig. 1) and the subsequent modification of nuclear and cytoplasmic proteins with O-linked β-N-acetylglucosamine (O-GlcNAc, a novel posttranslational modification) (1, 3, 7, 9, 10, 13–15). Inhibiting the conversion of glucose, glutamine, and glucosamine to UDP-GlcNAc or adding O-GlcNAc to the protein backbone blocks the cardioprotective effects of these metabolites (1, 3, 7, 9, 10). Moreover, elevating the O-GlcNAc protein modification is protective in numerous models of cellular injury (1, 3, 6, 7, 9–11, 16, 17, 19, 20). These and other studies have introduced the idea of O-GlcNAc-mediated cellular protection (Fig. 2, left). In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Liu et al. (8) show that specifically raising O-GlcNAc protein modification levels during reperfusion is sufficient for cardioprotection, thus identifying glucosamine and O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate (PUGNAc, an inhibitor of O-GlcNAc removal) as potentially useful cardioprotective agents.

Over 600 proteins in the nucleus and cytoplasm of metazoans are modified by O-GlcNAc (4). Deletion of the UDP-GlcNAc polypeptide O-β-N-acetylgalcosaminyltransferase (OGT), the enzyme that adds O-GlcNAc, is lethal, highlighting the importance of this simple posttranslational modification in cellular regulation (12). O-GlcNAc is thought to act as a modulator of protein function, analogous to protein phosphorylation; the addition of O-GlcNAc to the protein backbone is dynamic, responding to morphogens, the cell cycle, changes in glucose metabolism, and cellular stress. O-GlcNAc occurs at sites on the protein backbone similar to those modified by protein kinases and is reciprocal with phosphorylation on some well-studied proteins, such as RNA Pol II, estrogen receptor-β, SV-40 large T-antigen, endothelial nitric oxide synthase, and the c-Myc protooncogene product. These data suggest that one mechanism by which O-GlcNAc modulates cellular function is by competing with phosphorylation (Fig. 1, bottom). Numerous cellular pathways within the cell are modulated by O-GlcNAc, including transcription, translation, signal transduction, and protein degradation (4). Both in vitro and in vivo data support a model where increased UDP-GlcNAc levels, due to hyperglycemia, result in increased O-GlcNAc levels, leading to insulin resistance, a hallmark of type II diabetes. It has been suggested that elevated O-GlcNAc protein modification may be associated with many of the complications associated with type II diabetes (4).

In response to multiple forms of cellular stress, levels of the O-GlcNAc protein modification are elevated rapidly and dynamically on myriad nucleocytoplasmic proteins (19). Several studies demonstrate that elevation of O-GlcNAc preceding heat stress (6, 16, 19), trauma hemorrhage (11, 17, 20), and ischemia-reperfusion injury (1, 3, 7–10, 13–15) is protective, suggesting that increased O-GlcNAc in response to stress is a survival response of cells. The exact molecular mechanism(s) by which O-GlcNAc regulates protein function leading to cellular protection have not been identified. However, O-GlcNAc has been shown to regulate the following pathways in a manner consistent with stress tolerance: 1) heat shock protein levels (19), 2) protein solubility (6, 16), 3) p53 stability (18), 4) cytosolic Ca2+ influx (9, 10, 13–15), 5) calcineurin activation and NFAT (nuclear factor of activated T cells) translocation (1), 6) p38 MAP kinase phosphorylation (3), and 7) circulating IL-6 and TNF-α levels.

**Fig. 1.** Two to five percent of glucose imported into the cells is converted to uridine diphasphate-N-acetylgalcosamine (UDP-GlcNAc) through the hexosamine biosynthetic pathway (HBP). The HBP can be blocked by inhibiting the conversion of fructose-6-phosphate to glucosamine-6-phosphate by glutamine:fructose-6-phosphate amidotransferase (GFAT). UDP-GlcNAc is used by OGT in the biosynthesis of O-linked β-N-acetylglucosamine (O-GlcNAc)-modified proteins. Given that O-GlcNAc and O-phosphate have been mapped to the same Ser/Thr residue on a subset of proteins, this suggests that O-GlcNAc and O-phosphate may be reciprocal. DON, 6-diazo-5-oxo-norleucine.
20). Thus there is growing evidence to suggest that O-GlcNAc is a novel endogenously recruitable protective agent that modulates numerous proteins and cellular pathways to affect cellular survival and that O-GlcNAc-modulated cellular protection is relevant to diverse models of cellular injury.

In the studies discussed above, O-GlcNAc protein modification levels were raised before cellular injury was initiated. Although it would be possible to treat high-risk patients with prophylactic glucosamine, analogous to aspirin treatment, it would be ideal if O-GlcNAc levels could be raised postinjury and still result in cellular protection. In their report, Liu et al. (8) tested this scenario by raising O-GlcNAc levels postsischemia in hearts during reperfusion with either glucosamine or PUGNAc (an inhibitor of O-GlcNAcase removal; Fig. 1, bottom). With the use of a hanging heart model, both glucosamine and PUGNAc treatments increased O-GlcNAc levels during reperfusion and increased cellular ATP levels. Moreover, increased O-GlcNAc during reperfusion was associated with reduced cardiac troponin I release, suggesting improved cardiac recovery. Previously, these authors demonstrated that O-GlcNAc attenuated Ca\(^{2+}\) influx into cells during reperfusion, and consistent with these observations, both glucosamine and PUGNAc were shown to attenuate \(\alpha\)-fodrin proteolysis, which is presumably mediated by calpain. Moreover, increased glycosylation levels were associated with lower end-diastolic pressure (EDP), which is consistent with decreased Ca\(^{2+}\) upon reperfusion. Interestingly, PUGNAc was shown to increase the total levels of Ca\(^{2+}/\)calmodulin-dependent protein kinase II (CaMKII), resulting in an altered ratio of unphosphorylated to phosphorylated CaMKII. Together, these data suggest that raising O-GlcNAc levels postinjury will result in increased survival of heart tissue and decreased mortality rates (8). However, this concept is yet to be fully tested in whole animal models of ischemia-reperfusion injury. The next step is to understand how O-GlcNAc is regulating proteins in the heart, resulting in cellular survival. Presumably, this will require a comprehensive proteomic analysis of O-GlcNAc-modified cardiac proteins, with a focus on identifying and studying those proteins that display altered O-GlcNAc protein modification levels during cardiac injury or disease.

In contrast to the data discussed above, O-GlcNAc has been shown to negatively affect cardiac function in diabetic models (Fig. 2, right). Hyperglycemia, or overexpression of OGT, is associated with prolonged Ca\(^{2+}\) transients and reduced protein and mRNA expression of the sarcomplasmic reticulum Ca\(^{2+}\)-ATPase 2A (SERCA2A) protein. In myocytes or perfused hearts isolated from streptozotocin-induced diabetic mice, overexpression of O-GlcNAcase restored SERCA2A protein levels and calcium handling (2, 5). Currently, our understanding of the mechanisms dictating whether O-GlcNAc is protective or sensitizes cells to injury is in its infancy. Only further research identifying the molecular mechanisms by which O-GlcNAc alters cellular response to injury, the pathways that regulate O-GlcNAc biosynthesis in response to injury, and how these proteins/pathways are differentially regulated in the diabetic heart will reconcile the apparent paradox that exists between the role of O-GlcNAc in regulating protein function in nondiabetic and diabetic hearts.

Fig. 2. In both cell culture and animal models that are not diabetic, O-GlcNAc levels are rapidly elevated in response to stress. Raising levels before stress is protective (left). Paradoxically, in diabetic models, the reverse is true. Lowering glycosylation levels before stress improves heart function (right).

REFERENCES

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