Glucosamine inhibits angiotensin II-induced cytoplasmic Ca$^{2+}$ elevation in neonatal cardiomyocytes via protein-associated O-linked N-acetylglucosamine

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Nagy, Tamas, Voraratt Champattanachai, Richard B. Marchase, and John C. Chatham. Glucosamine inhibits angiotensin II-induced cytoplasmic Ca$^{2+}$ elevation in neonatal cardiomyocytes via protein-associated O-linked N-acetylglucosamine. Am J Physiol Cell Physiol 290: C57–C65, 2006. First published August 17, 2005; doi:10.1152/ajpcell.00263.2005.—We previously reported that glucosamine, which inhibits the hexosamine biosynthesis pathway (HBP) and decreased Ca$^{2+}$, blocks ANG II-induced hypertrophy in NRVMs (15). However, a direct link between HBP and intracellular Ca$^{2+}$ homeostasis has not been established. Therefore, using neonatal rat ventricular myocytes, we investigated the relationship between glucosamine treatment; the concentration of UDP-N-acetylglucosamine (UDP-GlcNAc), an end product of the HBP; and the level of protein O-linked N-acetylglucosamine (O-GlcNAc) on ANG II-mediated changes in intracellular free Ca$^{2+}$ concentration ([Ca$^{2+}$]). We found that glucosamine blocked ANG II-induced [Ca$^{2+}$]$_i$ increase and that this phenomenon was associated with a significant increase in UDP-GlcNAc and O-GlcNAc levels. O-(2-acetamido-2-deoxy-p-glucopyranosylidine)-amino-N-phenylcarbazate, an inhibitor of O-GlcNAcase that increased O-GlcNAc levels without changing UDP-GlcNAc concentrations, mimicked the effect of glucosamine on the ANG II-induced increase in [Ca$^{2+}$]. An inhibitor of O-GlcNAc-transferase, alloxan, prevented the glucosamine-induced increase in O-GlcNAc but not the increase in UDP-GlcNAc; however, alloxan abrogated the inhibition of ANG II-induced increase in [Ca$^{2+}$]. These data support the notion that changes in O-GlcNAc levels mediated via increased HBP flux may be involved in the regulation of [Ca$^{2+}$]$_i$. Homeostasis in the heart.

ang II is a vasoactive peptide that has a direct effect on cardiomyocytes and plays a critical role in the response of cardiomyocytes to increased hemodynamic stress. ANG II is a potent stimulator of cardiomyocyte hypertrophy; however, sustained exposure is also associated with increased apoptosis (12). The effect of ANG II on cardiomyocytes is mediated via the ANG II type 1 receptor (AT$_1$R) and the ANG II type 2 receptor. The AT$_1$R receptor has been shown to mediate many of the physiological actions of ANG II (44). AT$_1$R couples to the Go$_q$ protein to stimulate PLC, generating two secondary messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP$_3$) (4, 17, 39). The major target of DAG is PKC, whereas IP$_3$ triggers an increase in cytosolic Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) through IP$_3$ receptors located in the sarcoplasmic reticulum (SR). A number of studies have demonstrated that ANG II treatment leads to an increase in basal (i.e., diastolic) [Ca$^{2+}$]$_i$ in both adult and neonatal cardiomyocytes (19, 43, 46). In addition to participating in excitation-contraction coupling (5), changes in [Ca$^{2+}$]$_i$ also influence acute inotropic responses (29, 48a) as well as modulate a number of different signaling pathways regulating myocyte hypertrophy and apoptosis (1, 3, 15, 38). The intracellular targets of [Ca$^{2+}$]$_i$, are numerous and include, e.g., CaM-regulated kinases (56), calcineurin (38, 41), PKC, MAPK (27), cytosolic phospholipase A$_2$, and proteases (3, 33).

In most cell types, the IP$_3$-generated [Ca$^{2+}$]$_i$ elevation is a consequence of an initial release from the endoplasmic reticulum followed by a subsequent influx of extracellular Ca$^{2+}$ into the cytoplasm. This latter process is termed store-operated or capacitative Ca$^{2+}$ entry (CCE) (35). Although CCE was first described in nonexcitable cells, studies have recently shown that CCE also coexists with L-type channels in smooth and skeletal muscle cells (25, 47). Furthermore, we have shown that CCE is present in neonatal and adult cardiomyocytes (15, 16) and that it appears to mediate the inotropic response of the intact heart to α-adrenergic agonists such as phenylephrine (31).

We have found that hyperglycemia blunts the ANG II-induced hypertrophy in neonatal rat ventricular myocytes (NRVMs) (31, 32). Interestingly, the inhibition of ANG II-induced hypertrophy by hyperglycemia was blocked by azaserine, which inhibits glucose entry into the hexosamine biosynthesis pathway (HBP). Furthermore, glucosamine, which enters cells via the glucose transporter system and selectively increases HBP flux, blocks CCE (48) and also inhibits ANG II-induced hypertrophy in NRVMs (15). However, a direct link between glucosamine or the HBP to [Ca$^{2+}$], homeostasis has yet to be established. The end product of the HBP, UDP-N-acetylglucosamine (UDP-GlcNAc) is the substrate for O- and N-glycosylation of proteins (50, 51). The addition of O-linked N-acetylglucosamine (O-GlcNAc) to nuclear and cytoplasmic proteins, which is catalyzed by O-GlcNAc-transferase (OGT), is a dynamic and abundant posttranslational modification that has increasingly been recognized as an important regulatory mechanism in signal transduction (14, 49, 52, 53) and that also may be especially important in mediating the cellular stress response (21, 55).

Therefore, the goal of the present study was to test the hypothesis that the impact of the HBP on the response of neonatal cardiomyocytes to the IP$_3$-generating agonist ANG II is mediated by an increase in protein O-GlcNAcylation that...
alters the regulation of $[\text{Ca}^{2+}]_i$. We found that glucosamine increased HBP flux in isolated neonatal cardiomyocytes, resulting in increased O-GlcNAc modification of proteins and attenuated ANG II-induced $[\text{Ca}^{2+}]_i$ elevation and thapsigargin-evoked CCE. We also have demonstrated that independent of the HBP, up- or downregulation of protein O-GlcNAcylation directly influenced $[\text{Ca}^{2+}]_i$. These data demonstrate for the first time a direct link between protein O-GlcNAcylation and cardiomyocyte $\text{Ca}^{2+}$ homeostasis, which may represent a novel mechanism for the regulation of cardiomyocyte function under normal and stress conditions.

**MATERIALS AND METHODS**

**Preparation of NRVMs.** All animal experiments were approved by the University of Alabama Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication no. 85-23, 1996). Primary culture of NRVMs was obtained from 2- to 5-day-old neonatal Sprague-Dawley rats and cultured as described previously (8, 37). Briefly, after isolation using collagenase digestion, cells were resuspended in PBS and maintained at 37°C in a 95% air-5% CO2 atmosphere. The cell suspensions from each digestion step were combined and centrifuged at 500 g for 5 min. The pellets were resuspended in DMEM and medium-199 (M-199) at a 4:1 ratio; supplemented with 15% FBS, penicillin (100 U/ml), streptomycin (100 μg/ml), and arabinose C (10 μM); and preplated for 10 min on collagen-coated, 100-mm culture dishes to deplete fibroblasts. Finally, NRVMs were plated densely on six-well plates (2 × 10^6 cells/well) or on four-chamber coverslips (0.3 × 10^6 cells/chamber). After 24 h, the medium was replaced with serum-free DMEM-M-199 supplemented with 2% Nutridoma (Roche) and antibiotics. Primary cultures were maintained for 1 wk, with the medium replaced every second day.

**Materials.** All experiments were performed at 37°C in HBSS supplemented with 1.2 mM CaCl2 and 1.0 mM MgSO4 unless otherwise indicated. To elicit an increase in $[\text{Ca}^{2+}]_i$, 1 μM ANG II (Sigma) or 1 μM thapsigargin (Molecular Probes) was added directly to the coverslip chambers during image acquisition. O-(2-acetamido-2-deoxy-n-glucopyranosylidene)-α-mannose (UDP-Mannuronic acid) (36); consequently, the nucleotide sugars were detected at 262 nm using a 2 ml/min flow rate, a linear salt gradient from 5 to 750 mM (NH4)H2PO4, and a pH gradient from 2.8 to 7.3. This method cannot fully separate UDP-GlcNAc from UDP-N-acetyl galactosamine (UDP-GalNAc) (36); consequently, the results are presented as the sum of UDP-GlcNAc and UDP-GalNAc and referred to as UDP-HexNAc. In cardiomyocytes, the ratio of UDP-GlcNAc to UDP-GalNAc is ~3:1 (9).

**Data analysis.** Data are presented as means ± SE throughout. Comparisons were performed using Student’s t-test, and statistically significant differences between groups were defined as P < 0.05.

**RESULTS**

**ANG II-induced $[\text{Ca}^{2+}]_i$, elevation in NRVMs is inhibited by glucosamine.** Cultured NRVMs beat spontaneously; the addition of 1 μM ANG II increased the frequency of these spontaneous contractions and also provoked a rapid elevation in baseline $[\text{Ca}^{2+}]_i$ (i.e., diastolic), which was sustained throughout the subsequent recording period (Fig. 1A). In contrast to the effect on diastolic $[\text{Ca}^{2+}]_i$, ANG II did not significantly increase the observed peak $[\text{Ca}^{2+}]_i$ (i.e., systolic). The increase in baseline $[\text{Ca}^{2+}]_i$, observed with the addition of ANG II was not observed in the absence of extracellular $\text{Ca}^{2+}$ (data not shown). These effects of ANG II on NRVMs are consistent with the findings of earlier studies in which investigators examined the effect of ANG II on cardiomyocyte function (19, 20, 43, 46).

Preincubation of NRVMs for 10 min with 5 mM glucosamine almost completely blocked the ANG II-induced increase in diastolic $[\text{Ca}^{2+}]_i$ level (Fig. 1A); however, glu-
Glucosamine did not block the ANG II-induced increase in beating frequency and did not attenuate the peak \([\text{Ca}^{2+}]_{\text{i}}\) levels. Addition of the O-GlcNAcase inhibitor PUGNAc, which increases protein-O-GlcNAc levels (13), had an effect similar to that of glucosamine, almost completely abolishing the effect of ANG II on baseline \([\text{Ca}^{2+}]_{\text{i}}\) but without causing a significant effect on beating frequency. Pretreatment with alloxan, an inhibitor of OGT, for 45 min partially reversed the effect of glucosamine, whereas alloxan alone did not inhibit the ANG II-induced increase in basal \([\text{Ca}^{2+}]_{\text{i}}\). The effects of glucosamine, PUGNAc, and alloxan on the ANG II-mediated increase in basal \([\text{Ca}^{2+}]_{\text{i}}\) and the frequency of the spontaneous contractions averaged from 25 cells from at least five separate experiments are shown in Fig. 1, B and C.

**HBP inhibits thapsigargin-induced \([\text{Ca}^{2+}]_{\text{i}}\) elevation.** We previously showed that the \([\text{Ca}^{2+}]_{\text{i}}\) increase caused by ANG II in NRVMs is mediated at least in part via CCE (15, 16). Thapsigargin is an inhibitor of SR \(\text{Ca}^{2+}\)-ATPase (SERCA) that prevents uptake of \(\text{Ca}^{2+}\) into the SR and also leads to activation of CCE in cardiomyocytes (15, 16). Therefore, we examined the effect of glucosamine and PUGNAc on thapsigargin-mediated increase in \([\text{Ca}^{2+}]_{\text{i}}\). The addition of 1 \(\mu\text{M}\) thapsigargin increased diastolic \([\text{Ca}^{2+}]_{\text{i}}\), in a manner similar to that of ANG II, but it had no effect on beating frequency (Fig. 2). The addition of exogenous glucosamine significantly inhibited the thapsigargin-induced \([\text{Ca}^{2+}]_{\text{i}}\), and this phenomenon was mimicked by the addition of PUGNAc. Alloxan pretreatment significantly attenuated the effect of glucosamine, whereas alloxan alone did not inhibit the effect of thapsigargin.

**Glucosamine increases HBP flux and protein O-GlcNAc.** Glucosamine enters cells via the glucose transporter system and selectively increases HBP flux, which should increase both UDP-GlcNAc and protein O-GlcNAc levels. The mean basal level of UDP-HexNAc in controls was 14.5 \(\pm\) 1.1 nmol/mg of protein, and this level was increased significantly by glucosamine treatment (Fig. 3). However, PUGNAc and alloxan treatment had no significant effects on UDP-HexNAc levels.

To assess the effect of glucosamine and PUGNAc on protein O-GlcNAc levels, NRVMs were labeled with CTD110.6 MAb.
specific against protein O-GlcNAc, under conditions analogous to the Ca\(^{2+}\) imaging data (Fig. 4). The abundance of cytoplasmic O-GlcNAc was noticeable, and the labeling patterns displayed a punctuate, granulated structure rather than a homogeneous distribution. The density of the CTD110.6-labeled granules was highest at the perinuclear region, most likely the location of the SR. In contrast, the nuclei were almost completely excluded from labeling. This finding was somewhat unexpected, because other cell types have exhibited strong positive nuclear CTD110.6 staining (18, 24, 54). Using this method, we found that glucosamine treatment led to a modest but significant increase in intensity that was blocked by alloxan (Fig. 5B). Consistent with the immunofluorescence data, CTD110.6 staining was increased much more after PUGNAc treatment than after glucosamine treatment. The intensities of five selected bands from the Western blot analysis shown in Fig. 5A are summarized in Fig. 5C. Although no significant increase in intensity was observed in the glucosamine group, PUGNAc markedly increased the overall level of CTD110.6 fluorescence.

The changes in O-GlcNAc levels also were assessed using immunoblot analysis of cell extracts with CTD110.6 (Fig. 5A), and the resulting banding patterns were similar to those published in previous reports (21, 22, 54). Using this method, we found that glucosamine treatment led to a modest but significant increase in intensity of overall staining that was blocked by alloxan (Fig. 5B). Consistent with the immunofluorescence data, CTD110.6 staining was increased much more after PUGNAc treatment than after glucosamine treatment. The intensities of five selected bands from the Western blot analysis shown in Fig. 5A are summarized in Fig. 5C. Although
more pronounced for some protein bands than for others. due to glucosamine treatment; however, this phenomenon was not significant (Fig. 4). As anticipated, alloxan explained why the increase in overall CTD110.6 immunofluorescence was not due to glucosamine for bands of a particular molecular weight may suggest that this effect is mediated by the HBP (31, 32). In the present study, we used increases in baseline [Ca\textsuperscript{2+}], to measure acute ANG II stimulation of NRVMs. A similar response was reported in several other studies, in which researchers used primary rat cardiomyocytes (19, 20, 43, 46). We have shown not only that the effect of ANG II on baseline [Ca\textsuperscript{2+}], was blunted by glucosamine but also that this event was mimicked by PUGNAc. In other cell systems, PUGNAc increases protein O-GlcNAc levels independently of HBP flux by inhibiting O-GlcNAcase thereby preventing the recycling of O-GlcNAc (13). Our results in NRVMs are entirely consistent with these findings, because we found that PUGNAc markedly increased levels of O-GlcNAc but had no significant effect on UDP-HexNAc levels. Further support for the role of increased O-GlcNAc levels in blocking the ANG II-induced rise in baseline [Ca\textsuperscript{2+}], comes from the fact that alloxan, an inhibitor of OGT, prevented the effect of glucosamine treatment.

Furthermore, alloxan significantly reduced the glucosamine-induced increase in UDP-GlcNAc. However, a cause-and-effect relationship had not been established.

In the present study, we used increases in baseline [Ca\textsuperscript{2+}], to measure acute ANG II stimulation of NRVMs. A similar response was reported in several other studies, in which researchers used primary rat cardiomyocytes (19, 20, 43, 46). We have shown not only that the effect of ANG II on baseline [Ca\textsuperscript{2+}], was blunted by glucosamine but also that this event was mimicked by PUGNAc. In other cell systems, PUGNAc increases protein O-GlcNAc levels independently of HBP flux by inhibiting O-GlcNAcase thereby preventing the recycling of O-GlcNAc (13). Our results in NRVMs are entirely consistent with these findings, because we found that PUGNAc markedly increased levels of O-GlcNAc but had no significant effect on UDP-HexNAc levels. Further support for the role of increased O-GlcNAc levels in blocking the ANG II-induced rise in baseline [Ca\textsuperscript{2+}], comes from the fact that alloxan, an inhibitor of OGT, prevented the effect of glucosamine treatment. Furthermore, alloxan significantly reduced the glucosamine-induced increase in O-GlcNAc (Fig. 5B) but had no effect on UDP-HexNAc levels, which remained significantly increased compared with controls. These data support the notion that the attenuation of ANG II-induced elevation of baseline [Ca\textsuperscript{2+}], in NRVMs by glucosamine is due at least in part to increased levels of O-GlcNAc and suggest that cardiomyocyte Ca\textsuperscript{2+} homeostasis may be regulated by O-GlcNAcylation.

One limitation of these studies is that unlike PUGNAc, which is a high-affinity inhibitor of O-GlcNAcase and is effective at fairly low concentrations, relatively high concentrations of alloxan were required to block the effect of glucosamine. Thus, while alloxan is known to inhibit OGT (23), given the relatively high concentrations needed, we cannot exclude the possibility that alloxan may have some effects on NRVMs independent of its effects on OGT. Unfortunately, alloxan is the only known inhibitor of OGT (23), and because ablation of the OGT gene is embryonically lethal (42), a more specific demonstration of the role of OGT in regulating cardiomyocyte [Ca\textsuperscript{2+}], would require the development of tissue-specific conditional knockout mice or the use of small interfering RNA approaches to reduce OGT expression in cardiomyocytes.

Despite the potential limitations of alloxan, it should be noted that incubation of NRVMs for 20 h with a slightly higher (3 mM) concentration of alloxan had no adverse effects on cell

**Fig. 3.** UDP-HexNAc [sum of UDP-N-acetylglucosamine (UDP-GlcNAc) and UDP-N-acetylgalactosamine (UDP-GalNAc)] levels from NRVMs were pre-treated with control (HBSS), 5 mM glucosamine for 10 min, 2.5 mM alloxan for 45 min, 2.5 mM alloxan for 45 min with glucosamine (5 mM) added for 10 min, and 100 μM PUGNAc for 45 min. UDP-HexNAc levels were measured using HPLC and normalized according to total ADP levels (UDP-HexNAc/ATP+ADP+AMP). Data are means from at least 5 independent experiments. *P < 0.05 vs. control.

**Discussion**

We previously reported that the increase in [Ca\textsuperscript{2+}], induced by ANG II occurs at least in part by CCE, both in NRVMs and in adult cardiomyocytes (15, 16). We also previously showed that the physiological responses to ANG II, such as cardiomyocyte hypertrophy, were attenuated by hyperglycemia and that this response could be mimicked by glucosamine, suggesting that this effect is mediated by the HBP (31, 32). In the present study, we have demonstrated for the first time that the attenuation of both ANG II- and thapsigargin-induced increases in [Ca\textsuperscript{2+}], by glucosamine not only is mediated by increased flux through the HBP but also is dependent on the subsequent formation of O-GlcNAc. Furthermore, we have demonstrated that PUGNAc, which increases protein O-GlcNAc levels independently of flux through the HBP, also attenuated ANG II- and thapsigargin-induced increase in [Ca\textsuperscript{2+}],. These results suggest that protein O-GlcNAc levels play a role in regulating Ca\textsuperscript{2+} homeostasis in NRVMs, which may have important implications for the understanding of the role of hyperglycemia in modulating cardiomyocyte function.

O-GlcNAcylation is increasingly recognized as an important and widespread posttranslational modification. The number of identified proteins capable of posttranslational O-glycosylation is quickly growing, including a wide range of proteins, such as NF-κB, annexin, endothelial nitric oxide synthase, αB-crystallin, OGT, α-tubulin, c-myc, and heat shock protein 70 (50, 51). Increased levels of O-glycosylation have been implicated in a range of cellular processes, including the development of insulin resistance in muscle (2), hyperglycemia-induced apoptosis (28), and impaired excitation-contraction coupling (9). Recently, it also was shown that increased protein O-GlcNAcylation occurs in a range of different cells in response to stress, suggesting that activation of this pathway may be a component of an endogenous cell survival pathway (55).
viability (7). Thus, because the experiments described herein were much shorter than previous studies, it is unlikely that the effects of alloxan are due to nonspecific toxicity. Furthermore, we also found that alloxan alone did not prevent the ANG II- or thapsigargin-induced increase in basal [Ca^{2+}]_i (Figs. 1 and 2). One limitation is that we did not determine whether alloxan had any impact on the effects of PUGNAc. However, alloxan does not block the PUGNAc-induced increase in O-GlcNAc levels (data not shown); consequently, we think it is unlikely that alloxan would inhibit the effects of PUGNAc shown in the present study. Nevertheless, we cannot entirely rule out the possibility that glucosamine and PUGNAc may act via different mechanisms to block the increase in basal [Ca^{2+}]_i.

The effects of ANG II on cardiomyocytes are mediated via the formation of DAG and IP_3 (4, 17, 39); however, the mechanisms leading to the positive inotropic effect of ANG II are still unclear. IP_3 acts on specific receptors on the SR, and inhibition of these receptors has been shown to block the increase in basal [Ca^{2+}]_i induced by IP_3-generating agonists (57) but not their positive inotropic effects (39). It has been suggested that both the Na^+/H^+ and Na^+/Ca^{2+} exchangers may play a role in the positive inotropic effects of ANG II (11, 39). We found that glucosamine and PUGNAc abolished the increase in basal [Ca^{2+}]_i elicited by both ANG II and thapsigargin; however, neither intervention significantly affected peak Ca^{2+} levels or the ANG II-induced increase in spontane-
uous beating frequency. We previously showed that thapsigargin increased basal \([Ca^{2+}]_i\) levels in NRVMs via a CCE-mediated pathway that was independent of both L-type Ca\(^{2+}\) channels and the Na\(^+\)/Ca\(^{2+}\) exchanger (15, 16) and that ANG II also increased basal \([Ca^{2+}]_i\) levels via a similar mechanism (15, 16). Therefore, these new data, combined with our findings published in earlier reports (15, 16), suggest that the effect of \(O\)-GlcNAcylation on Ca\(^{2+}\) homeostasis is most likely due to the inhibition of Ca\(^{2+}\) entry via CCE rather than to decreased flux through L-type Ca\(^{2+}\) channels or the Na\(^+\)/H\(^+\)/Ca\(^{2+}\) exchanger. However, we cannot rule out the possibility that increased levels of \(O\)-GlcNAc might also affect Ca\(^{2+}\) homeostasis mediated via the SR IP\(_3\) receptor or the Na\(^+\)/H\(^+\) exchanger.

It should be noted that we used a fairly high (1 \(\mu\)M) concentration of ANG II, which is outside the normal physiological range and raises the possibility that the effects of ANG II that we observed might be more pharmacological than physiological. The 1 \(\mu\)M concentration of ANG II was used in the present study because in a previous study we found that stimulation of cardiomyocyte hypertrophy with 1 \(\mu\)M ANG II was inhibited by glucosamine (15). Furthermore, the use of ANG II in the micromolar range in cardiomyocyte studies is fairly common (39, 43, 46). Although we have not examined the effects of lower ANG II concentrations in our studies, previously published reports have demonstrated similar effects of ANG II in the 10–100 nM range on cardiomyocyte Ca\(^{2+}\) homeostasis (20, 46). Consequently, we think it likely that alterations in \(O\)-GlcNAc levels also affect the response of cardiomyocytes to changes in ANG II concentration in the physiological range; however, this hypothesis remains to be proved.

As shown in Fig. 5, glucosamine and PUGNAC lead to increased \(O\)-GlcNAc levels in several different protein bands. The specific proteins affected by \(O\)-GlcNAcylation that might mediate the effects of ANG II have yet to be identified. However, the transient receptor potential channel (TRPC) protein family is the prime candidate for the long-sought CCE channel proteins and thus may be involved in the \(O\)-GlcNAc-mediated effects on Ca\(^{2+}\) homeostasis. Analysis of the protein sequence for TRPC1 suggests a high-affinity site for \(O\)-GlcNAc close to the NH\(_2\)-terminal region (for further information, see the Center for Biological Sequence Analysis web site, available at: http://www.cbs.dtu.dk/services/YinOYang/).

In conclusion, we have shown that increasing levels of \(O\)-GlcNAc by either increasing flux through the HBP with glucosamine or inhibiting \(O\)-GlcNAcase with PUGNAC inhibits the increase in basal \([Ca^{2+}]_i\) in NRVMs induced by either ANG II or thapsigargin. Alloxan, an inhibitor of OGT, prevented the increase in \(O\)-GlcNAc induced by glucosamine but not the increase in UDP-HexNAc. Alloxan also blocked the effect of glucosamine on ANG II- and thapsigargin-mediated changes in \([Ca^{2+}]_i\). These data, in combination with data published in our earlier reports (15, 16), provide strong evi-
dence for the regulation of [Ca$^{2+}$]$_i$ in cardiomyocytes by O-GlcNAcylation. This finding has important implications for the regulation of cardiomyocyte function because changes in Ca$^{2+}$ homeostasis play a central role in the cardiomyocyte stress response, initiating numerous signaling cascades that can lead to apoptosis, hypertrophy, arrhythmia, or cell death (3, 27, 30, 33, 38, 41, 56). Increased flux through the HBP also has led to apoptosis, hypertrophy, arrhythmia, or cell death (3, 27, 30). 

Increased flux through the HBP also has lead to apoptosis, hypertrophy, arrhythmia, or cell death (3, 27, 30). Stress response, initiating numerous signaling cascades that can alter Ca$^{2+}$-GlcNAc levels may contribute to the deleterious effects of prolonged hyperglycemia (9). It is also possible that regulation of cardiomyocyte [Ca$^{2+}$]$_i$ by protein O-GlcNAcylation may contribute to the cytoprotection associated with increased levels of protein O-GlcNAc (55).

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DISCLOSURES

R. B. Marchase and J. C. Chatham have a patent pending that relates to the work presented in this article.

REFERENCES


