Metformin but not Glyburide Prevents High Glucose–Induced Abnormalities in Relaxation and Intracellular Ca^{2+} Transients in Adult Rat Ventricular Myocytes

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We have recently demonstrated that adult rat ventricular myocytes maintained in a high glucose (HG) culture medium exhibit abnormalities in excitation-contraction coupling similar to myocytes from diabetic rats. Metformin, an insulin-sensitizing biguanide, enhances peripheral insulin action and lowers blood pressure in hyperinsulinemic animals, but its direct impact on cardiac function is not fully understood. To examine the role of metformin on HG-induced cardiac dysfunction at the cellular level, normal adult ventricular myocytes were cultured for 1 day in a serum-free insulin-containing medium with either normal glucose (5.5 mmol/l glucose) or HG (25.5 mmol/l glucose) in the presence or absence of metformin or the sulfonylurea glyburide. Mechanical properties were evaluated using a high-speed video-edge detection system, and intracellular Ca^{2+} transients were recorded in fura-2–loaded myocytes. As previously reported, culturing myocytes in HG depresses peak shortening, prolongs time to 90% relengthening, and slows Ca^{2+} transient decay. Culturing cells with metformin (50 µmol/l) prevented the HG-induced abnormalities in relaxation without ameliorating depressed peak-shortening amplitudes. Incubation of the cells with metformin also prevented slower intracellular Ca^{2+} clearing induced by HG. However, the HG-induced relaxation defects were not improved by glyburide (50–300 µmol/l). Interestingly, metformin also improved HG-induced relaxation abnormalities in the absence of insulin, whereas it failed to protect against HG in the presence of the tyrosine kinase inhibitor genistein (50 µmol/l). These data demonstrate that, unlike glyburide, metformin provides cardioprotection against HG-induced abnormalities in myocyte relaxation, perhaps through tyrosine kinase–dependent changes in intracellular Ca^{2+} handling, independent of its insulin sensitizing action. *Diabetes* **48:2059–2065, 1999**

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AP, action potential; A_R, area of relengthening; A_R/PS, area under the relengthening curve normalized to peak shortening; CL, resting cell length; E-C, excitation-contraction; HG, high glucose; IP_3, inositol 1,4,5-trisphosphate; N, normal glucose; PKA, protein kinase A; PKC, protein kinase C; PS, peak shortening amplitude; SR, sarcoplasmic reticulum; τ, time constant of the transient decay; TPS, time to peak shortening amplitude; TR, time to 90% relengthening.

Cardiovascular disease is a major complication of diabetes and is associated with high morbidity and mortality in diabetic populations (1,2). Clinical and experimental investigations have indicated that diabetes per se can lead to deterioration of cardiac function. One complication of diabetes is the development of diabetic cardiomyopathy, a condition characterized by defects in both systolic and diastolic functions independent of coronary macrovascular disease (3). Impaired diastolic function is the most prominent cardiac abnormality. It manifests before systolic dysfunction and is characterized by prolonged relaxation and decreased compliance. A number of cellular defects have been found to contribute to the abnormal excitation-contraction (E-C) coupling, including prolonged action potential durations, changes in Ca^{2+} sensitivity of myofilaments, and impaired function of Ca^{2+}–regulating proteins, such as Na/Ca exchanger and sarcolemmal and sarcoplasmic reticulum (SR) Ca^{2+}-ATPase (4–6). Although the pathogenesis is not clearly established, hyperglycemia may play a key role in the early onset of these cellular dysfunctions (7,8).

Metformin, a biguanide used to treat type 2 diabetes, is believed to exert its antihyperglycemic action by potentiating insulin action and reducing insulin resistance (9,10), but the precise mechanism(s) is not completely understood. Recent studies in type 2 diabetic patients revealed that biguanides lower blood pressure, blood glucose, plasma triglycerides, and hepatic gluconeogenesis (11–13). Metformin treatment (in vivo) also lowers blood pressure and plasma insulin in a hyperinsulinemic animal model (14) and attenuates the development of impaired cardiac performance in diabetic rats (15). Metformin (in vitro) stimulates glucose transport in skeletal muscle (16), adipocytes (17), cardiomyocytes (18), and vascular smooth muscle cells (19,20). Other effects of metformin on the vascular tissue include the attenuation of KCl- and norepinephrine-induced contraction of rat tail artery (21), decrease of agonist-induced intracellular Ca^{2+} responses, and inhibition of vascular smooth muscle cell proliferation (19,22). In contrast to other tissues, very little is known about the direct effects of metformin on the heart (18).

Sulfonylureas such as glyburide are widely used in the treatment of type 2 diabetic patients and act primarily as insulin secretagogues (23). It is known that glyburide blocks ATP-sensitive K^+ channels (affecting membrane depolarization), thus stimulating insulin secretion by pancreatic β-cells. In addition, sulfonylureas exert extrapancreatic effects, such as inhibiting hepatic gluconeogenesis and glycolysis (24). Certain sulfonylureas have also been reported to slightly improve insulin sensitivity in peripheral tissues (24). Considerable debate has occurred as to whether insulin secretagogues have deleterious cardiovascular effects because they may exacerbate hypertension (by augmenting vasoconstriction) and increase myocardial susceptibility to ischemia and reperfusion injury (25,26).

Abnormal E-C coupling is apparent in ventricular myocytes isolated from diabetic rats even after only a few days of diabetes (27,28). We have recently shown that these abnormalities are reproduced in normal myocytes cultured in a "diabetic-like" medium containing high glucose (HG) (8). Furthermore, we found that troglitazone prevents most of the HG-induced dysfunctions in our cell system (29). Troglitazone is a member of the antihyperglycemic insulin-sensitizing thiazolidinediones and is pharmacologically distinct from the biguanides and sulfonylureas. The following study was designed to determine the efficacy of the antidiabetic agents metformin and glyburide on preventing HG-induced myocyte dysfunctions. We used our in vitro model of diabetes to evaluate whether metformin and glyburide are cardioprotective against elevated glucose at the cellular level.

RESEARCH DESIGN AND METHODS

Animals and materials. Adult male Sprague-Dawley rats (200–250 g) were purchased from Harlan Bioproducts for Sciences (Indianapolis, IN). Collagenase was obtained from Worthington Biochemical (Freehold, NJ). Metformin, glyburide, hyaluronidase, trypsin, gentamicin, genistein, and Medium 199 were purchased from Sigma Chemicals (St. Louis, MO). Laminin was obtained from Collaborative Biochemical Products (Bedford, MA). Penicillin and streptomycin were from Gibco (Grand Island, NY). Fura-2/AM was purchased from Molecular Probes (Eugene, OR).

Myocyte isolation. Ventricular myocytes were isolated by coronary perfusion and prepared for primary culture as previously described (8,28). In brief, ventricular myocytes were dissociated under sterile conditions by collagenase (176 U/ml) and hyaluronidase (0.1 mg/ml), perfused through the coronaries, and further digested by trypsin (0.02 mg/ml) during trituration (5 min) after the tissue was removed from the perfusion apparatus and minced. Isolated myocytes were plated on glass coverslips precoated with laminin (10 µg/ml) and maintained in a defined medium consisting of Medium 199 with Earle's salts containing HEPES (25 mmol/l) and $NaHCO_3$ (25 mmol/l), supplemented with albumin (2 mg/ml), L-carnitine (2 mmol/l), creatine (5 mmol/l), taurine (5 mmol/l), insulin (100 nmol/l), D-triiodothyronine (0.1 nmol/l), penicillin (100 U/ml), streptomycin (100 µg/ml), and gentamicin (5 µg/ml). This medium also contained either normal glucose (N) (5.5 mmol/l) or HG (25.5 mmol/l). The HG is comparable to serum glucose levels in diabetic rats (28). A subset of each medium was also supplemented with either metformin (50 µmol/l) or glyburide (50–300 µmol/l). The cells were maintained at 37°C in a 100% humidity and 5% CO_2 incubator for 1 day.

Cell shortening and relengthening. Mechanical properties of cultured ventricular myocytes were assessed by a video-based edge-detection system (Crescent Electronics, Sandy, UT) as described (28). In brief, coverslips with cells attached were placed in a chamber mounted on the stage of an inverted microscope (Nikon Diaphot, Melville, NY) with the temperature maintained at 37°C. The chamber was superfused (2 ml/min) with a buffer containing 131 mmol/l NaCl, 4 mmol/l KCl, 1 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$, 10 mmol/l glucose, and 10 mmol/l HEPES, at pH 7.4 and maintained at 37°C. The cells were field stimulated to contract at a frequency of 0.5 Hz. Shortening of rod-shaped myocytes was detected at both longitudinal edges at a video-sweep speed of 120 Hz, while sampling at 333-Hz. Steady-state twitches (5–10) were averaged and the following indices measured using Clampfit (Axon Instruments, Foster City, CA): peak shortening amplitude (PS), time to PS (TPS), time to 90% relengthening (TR), and area of relengthening (A_R). PS was expressed as a percentage of resting cell length, and A_R was normalized to PS (A_R/PS), as described previously (28).

Fluorescence measurement. A separate cohort of myocytes were loaded with fura-2/AM (0.5 µmol/l) for 15 min at room temperature, and fluorescence measurements were recorded with a dual-excitation fluorescence photomultiplier system (Ionoptix, Milton, MA) as described (7,28). Myocytes were placed on an inverted microscope equipped with a heated (37°C) and light-tight chamber, imaged through a 40 × oil objective, and field stimulated to contract at a frequency of 0.5 Hz. Cells were exposed to light emitted by a 75-W lamp and passed through either a 360- or a 380-nm filter (bandwidths were 15 nm). Fluorescence emissions were detected between 480–520 nm by a photomultiplier tube after first illuminating cells at 360 nm for 0.5 s, then at 380 nm for the duration of the recording protocol (333-Hz sampling rate). The 360-nm excitation scan was repeated at the end of the protocol, and an interpolated signal was calculated and used to calculate the ratio with the 380-nm emission. Steady-state intracellular Ca^{2+} transients (5–10 traces) were averaged, and the following indices were measured using Clampfit software: resting (or baseline) 360/380 ratios, peak 360/380 ratios, and the time constant of the transient decay (τ) estimated using a single exponential equation.

Statistics. Data are presented as means ± SE. Statistical significance was ascertained by analysis of variance. Appropriate follow-up tests for multiple comparisons were chosen depending on whether significance ($P < 0.05$) was identified in main effects or interaction terms. The metformin and glyburide data were analyzed separately. Recordings from control groups (i.e., N and HG cells) were always made on the same day as those for experimental groups (i.e., with and without drugs) to control for any potential interculture variability.

TABLE 1
Mechanical indices of myocytes maintained for 1 day in medium containing N or HG or in these media supplemented with 50 µmol/l metformin or 100 µmol/l glyburide.

	CL (µm)	PS (% CL)	TPS (msec)	*n*
Metformin				
N	129 ± 3	9.4 ± 0.4	52 ± 2	53
HG	137 ± 2	6.3 ± 0.3*	53 ± 2	58
N + MET	127 ± 3	8.8 ± 0.4	47 ± 2	53
HG + MET	132 ± 3	7.6 ± 0.3*†	52 ± 2	53
Glyburide				
N	114 ± 2	8.2 ± 0.6	48 ± 2	43
HG	114 ± 3	8.6 ± 0.9	53 ± 4	32
N + GLB	108 ± 3	8.6 ± 0.5	46 ± 2	37
HG + GLB	114 ± 2	9.8 ± 0.9*	47 ± 2	36

Data are means ± SE. *Statistically significant ($P < 0.05$) when compared with N; †statistically significant ($P < 0.05$) when compared with HG. GLB, glyburide; MET, metformin; PS (% CL), peak shortening amplitude expressed as a percentage of resting cell length (CL). *n* = number of cells. TPS, time to 90% PS from 10% above baseline.

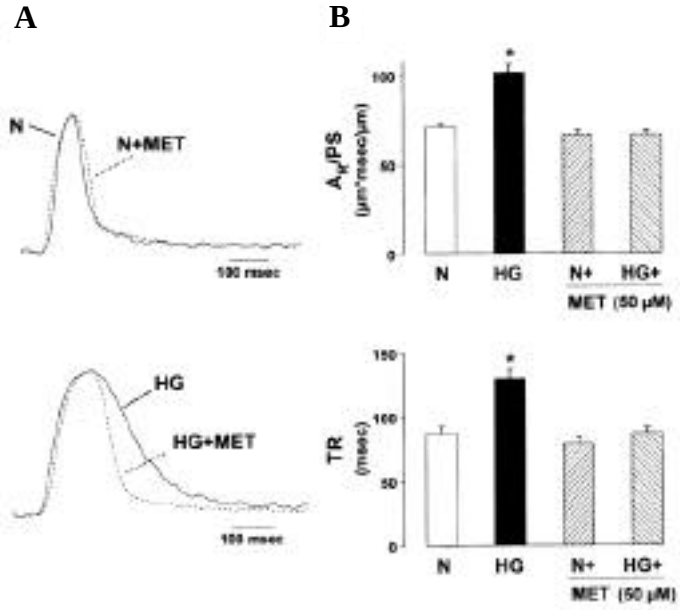

FIG. 1. *A*: Representative twitches (nonaveraged/nonfiltered) of ventricular myocytes cultured for 1 day in medium containing N glucose (5.5 mmol/l), HG (25.5 mmol/l), N with 50 µmol/l metformin (N+MET), and HG with 50 µmol/l metformin (HG+MET). The PS of the HG, N+MET, and HG+MET cells was normalized to that of the N cell to better illustrate differences in relengthening. *B*: Graphs illustrate relaxation properties of myocytes cultured for 1 day in each medium. Relaxation indices are TR and A_R/PS. Data represent means ± SE. Sample sizes are 53–58 cells/group. *Statistically significant (P < 0.05) when compared with all other groups.

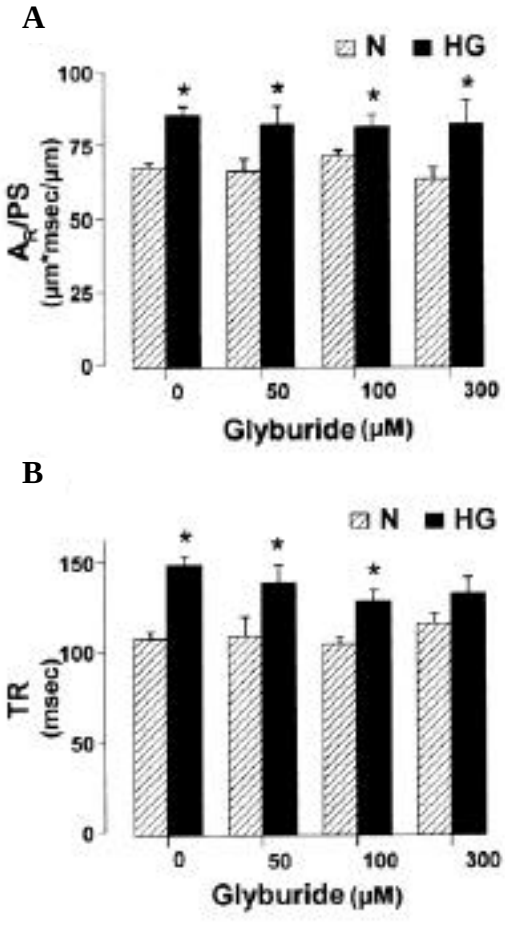

FIG. 2. Graphs illustrate relaxation properties of myocytes cultured for 1 day in medium containing N, HG, or N and HG media supplemented with glyburide (50–300 µmol/l). The relaxation indices are A_R/PS (*A*) and TR (*B*). Data represent means ± SE. Sample sizes are 26–64 cells/group. *Statistically significant (P < 0.05) when compared with N.

RESULTS

Metformin but not glyburide protects against HG-induced prolonged relengthening. Culturing myocytes for 1 day with either HG or the antidiabetic agents (metformin or glyburide) had no overt effect on cell phenotype. For example, cell shape, resting cell length (CL), and presence of distinct striations were similar to normal cells in each experimental group. As we have previously reported (8,29), ~50% of our cultures' myocytes maintained in HG medium exhibited a reduced PS, without changes in TPS, compared with myocytes maintained in N medium (Table 1). Representative traces of cell shortening and relengthening are shown in Fig. 1*A*. Indices for relaxation (TR and the area under the relengthening curve normalized to peak shortening [A_R/PS]) were significantly increased in myocytes cultured in HG when compared with those of N cells (Fig. 1*B*). Prolonged relengthening in HG myocytes was prevented when the medium was supplemented with 50 µmol/l metformin (Fig. 1*B*). Higher concentrations of metformin (e.g., 100 µmol/l) exerted toxic effects on myocytes (e.g., reduced cell viability in both N and HG cells) and were not used further in our study (data not shown). In addition to its effects on TR and A_R/PS, metformin also partially prevented the HG-induced decrease in PS (Table 1). HG-induced relaxation defects were not affected by glyburide supplementation (50–300 µmol/l), with the exception of the TR index at 300 µmol/l (Fig. 2). Neither drug adversely affected PS or TPS in any of the groups studied (Table 1), nor did either drug have an effect on TR or A_R/PS in cells cultured in N medium (Figs. 1 and 2). PS was greater in cells cultured with HG and 100 µmol/l glyburide when compared with that of N cells, but not different from other groups (Table 1). This increase in PS was also seen in cells cultured in 300 µmol/l glyburide, although not at 50 µmol/l (data not shown).

Metformin protects against HG-induced abnormal Ca^{2+} handling. We have recently shown that prolonged relaxation in HG-treated cells is accompanied by slower intracellular Ca^{2+} clearing (7,8). Since metformin prevented HG-induced relaxation dysfunctions, we wanted to determine whether this effect coincided with changes in intracellular Ca^{2+}. Resting and systolic (peak) Ca^{2+} ratios and the time course of intracellular Ca^{2+} transients were evaluated in fura-2–loaded myocytes. The time course of the fluorescence signal decay was described by a single exponential equation, and the time constant τ was used as a measure of the rate of cytosolic Ca^{2+} extrusion. Consistent with our previous reports (7,8), culturing myocytes in HG slowed the rate of intracellular Ca^{2+} decay (indicated by a longer τ), which likely contributes to prolonged relengthening. Culturing myocytes in HG with metformin (50 µmol/l) prevented this slower intracellular Ca^{2+} clearing (Fig. 3). Metformin had no effect on τ when added to N medium. HG had no effect on either resting or peak Ca^{2+} ratios (Table 2). Metformin elevated resting intracellular Ca^{2+} ratios (which affected peak Ca^{2+}) in myocytes cultured in HG medium, but did not affect those indices of myocytes maintained in N medium. Metformin apparently increased peak Ca^{2+} ratio in HG cells; however, the

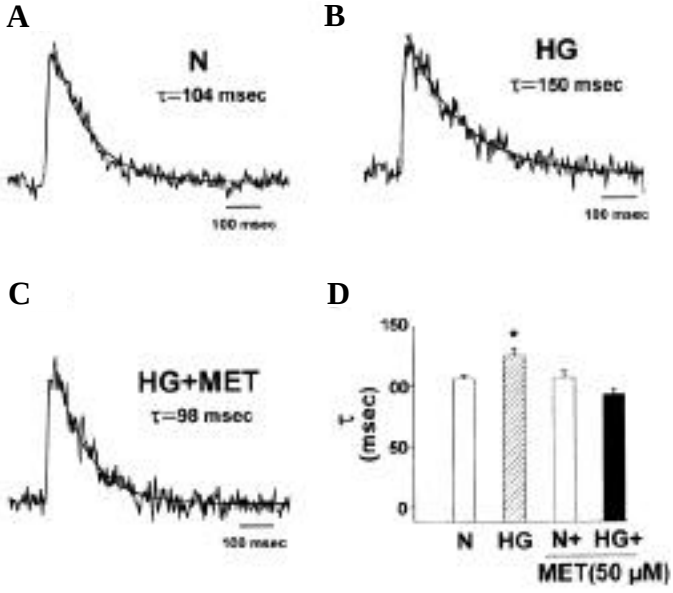

FIG. 3. *A–C*: Intracellular Ca^{2+} transients (nonaveraged/nonfiltered) from fura-2–loaded ventricular myocytes cultured for 1 day in medium containing N, HG, or HG with 50 μmol/l metformin (HG+MET). The time course of the Ca^{2+} transient decay (τ) was evaluated by curve fitting with a single exponential equation. Curve fits are shown in solid lines. *D*: Average τs recorded from myocytes cultured in each medium. Data are expressed as means ± SE. Sample sizes are 11 cells/group. *Statistically significant ($P < 0.05$) when compared with all other groups.

amplitude of the Ca^{2+} transients (difference between peak and resting) was similar among all groups (Table 2).

Metformin protects against HG-induced prolonged relengthening in the absence of insulin. To explore whether the cardioprotective effect of metformin was related to its insulin-sensitizing action, the influence of metformin on HG-induced prolonged relengthening was re-examined in culture medium devoid of insulin. Interestingly, metformin (50 μmol/l) prevented the HG-induced abnormal relengthening even in the absence of insulin, as indicated by both TR and A_R/PS (Fig. 4). Similar to data presented in Table 1 (in which all media contained 100 nmol/l insulin), metformin failed to prevent the HG-induced depression of PS (data not shown).

Metformin-induced protection against HG-induced prolonged relengthening is abolished by genistein. To further explore the signaling pathway(s) involved in the cardioprotective effect of metformin, the influence of metformin on HG-induced prolonged relengthening was re-examined

TABLE 2
Fluorescence properties of Ca^{2+} transients from myocytes maintained for 1 day in medium containing N or HG or in these media supplemented with 50 μmol/l metformin.

	Resting 360/380	Peak 360/380	Amplitude
N	1.06 ± 0.02	1.24 ± 0.03	0.18 ± 0.01
HG	1.01 ± 0.02	1.18 ± 0.03	0.17 ± 0.02
N+MET	1.09 ± 0.01	1.24 ± 0.02	0.15 ± 0.01
HG+MET	1.18 ± 0.02*	1.35 ± 0.02*	0.17 ± 0.01

Data are means ± SE; sample sizes are 11 cells/group. *Statistically significant ($P < 0.05$) when compared with all other groups. MET, metformin.

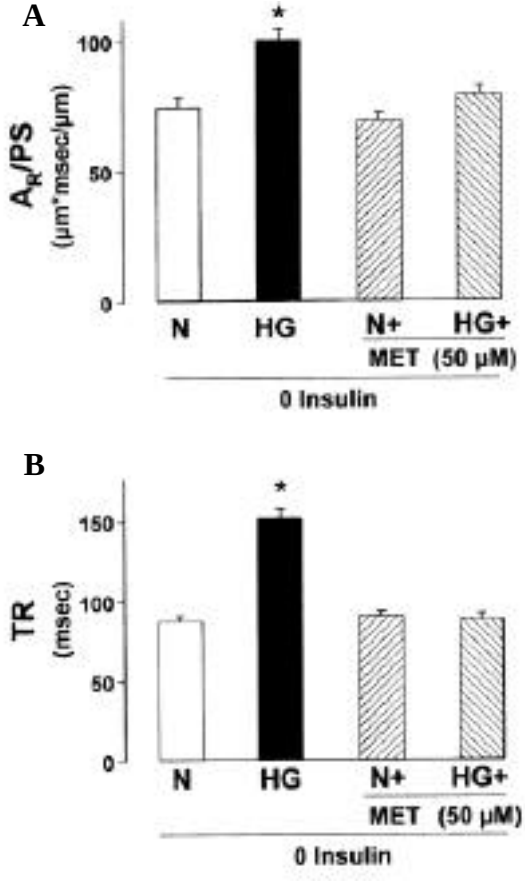

FIG. 4. Graphs depict relaxation properties of myocytes cultured for 1 day in insulin-free medium containing N, HG, or N and HG media supplemented with 50 μmol/l metformin (MET). The relaxation indices are A_R/PS (*A*) and TR (*B*). Data represent means ± SE. Sample sizes are 36–44 cells/group. *Statistically significant ($P < 0.05$) when compared with all other groups.

in the presence of genistein, a nonselective inhibitor of tyrosine protein kinases. Co-culture with genistein (50 μmol/l) prevented metformin-induced protection against HG-induced abnormal relengthening, as indicated by both TR and A_R/PS (Fig. 5). Genistein alone had no effect on myocyte shortening (i.e., PS and TPS) in N or HG media (data not shown).

DISCUSSION

The results described in this investigation indicate that the antihyperglycemic agent metformin protected against the HG-induced cardiac relaxation dysfunctions in an insulin-independent manner. However, the hypoglycemic drug glyburide did not possess this cardiac-protective property. Prolonged relaxation associated with slowed intracellular Ca^{2+} extrusion is the most consistent feature in diabetic cardiomyopathy and in our cell culture model (5,7,28). Metformin but not glyburide improved HG-induced relaxation dysfunction without affecting mechanical indices in normal cells. This action is associated with the improvement of cytosolic Ca^{2+} clearing, which may underlie metformin-induced actions.

Hyperglycemia is one of the most important predisposing factors in diabetes-related cardiovascular disorders (2,30) and most likely contributes to the development of cardiac dysfunction. We have previously reported that ventricular

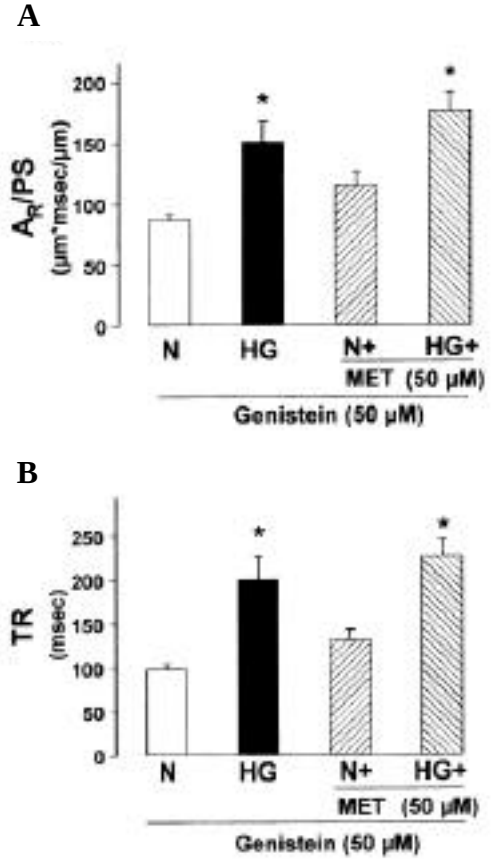

FIG. 5. Graphs illustrate relaxation properties of myocytes cultured for 1 day in genistein (50 μmol/l) containing medium with N, HG, or N and HG media supplemented with metformin (50 μmol/l). The relaxation indices are A_R/PS (*A*) and TR (*B*). Data represent means ± SE. Sample size is 26 cells/group. *Statistically significant ($P < 0.05$) when compared with N.

myocytes freshly isolated from diabetic rats, and normal myocytes cultured in HG-containing medium, exhibit prolonged relaxation and slowed cytosolic Ca^{2+} removal (7,8,28). Elevated extracellular glucose leads to increased intracellular Ca^{2+} in both vascular smooth muscle (29) and cardiac myocytes (32,33), which may be responsible for altered E-C coupling, cellular enzyme activity, or gene expression. These alterations may cause changes in intracellular Ca^{2+} homeostasis and, in turn, the cardiac defects (34). However, the precise signaling pathway(s) by which glucose affects E-C coupling or the cellular targets (e.g., ion channels and pumps) has not been determined. Preliminary evidence has revealed prolonged action potential (AP) duration in HG-cultured myocytes (8), which is likely due to depressed transient outward K^+ current (I_{To}) rather than enhanced L-type Ca^{2+} current (I_{Ca}) (27,35). Prolonged AP would be sufficient to impair relaxation in our HG cells, but other cellular changes, including depressed SR Ca^{2+}-ATPase and Na/Ca exchange, have been shown in myocytes from diabetic animals. Schaffer and colleagues (36) recently observed decreased Na/Ca exchange activity in myocytes exposed to HG, although the effect of HG on SR Ca^{2+}-ATPase still remains to be elucidated. Results from our recent study (8) may shed some light on the signaling pathway involved in glucose-induced cardiac E-C coupling abnormalities. We have recently shown that in adult ventricular myocytes exposed to HG, ~24 h is required to manifest its effect on E-C coupling. Additionally, neither nonenzymatic glycation nor osmotic stress can account for changes in myocyte E-C coupling in our model. Our data also suggested that glucose must enter the cell and be metabolized, perhaps through the hexosamine pathway. In other cell types, it has been shown that the increases in cellular glucose can be shunted preferentially through the hexosamine biosynthesis pathway (37).

Our preliminary evidence suggests that the tyrosine kinase–signaling pathway may be involved in metformin-induced cardiac protection against the adverse effects of HG. It is well documented that metformin modulates tyrosine kinase activity through inositol 1,4,5-trisphosphate (IP_3) production (19,38). However, the following are other aspects that should also be considered.

Inhibition of rise of intracellular Ca^{2+}. Abnormal intracellular Ca^{2+} handling (either elevated diastolic levels or slowed removal) may play a key role in diabetic cardiomyopathy. Several studies have shown that HG increases intracellular Ca^{2+} acutely (in minutes) (32,33,39). Symonian et al. (39) suggest that glucose induces Ca^{2+} influx in a dose- and time-dependent manner. They were able to prevent the rise of intracellular Ca^{2+} by a number of interventions, including treating myocytes with inhibitors of cAMP–protein kinase A (PKA) and protein kinase C (PKC). These acute effects on intracellular Ca^{2+} may be linked to the longer-term effects of HG on E-C coupling that we have observed. Metformin has been shown to inhibit Ca^{2+} influx (19,21,22) and phenylephrine-induced intracellular Ca^{2+} oscillations through IP_3-sensitive stores in several cell types (40). Therefore, metformin may exert its cardiac protection against HG through attenuating increases in intracellular Ca^{2+}. Contrary to this speculation is the observation that resting cytosolic Ca^{2+} appeared higher in metformin-treated HG myocytes than in any other group (Table 2). It is uncertain whether these data are meaningful, since the amplitude of the transients (Table 2) was similar among all groups of cells.

Protein kinases. Another potential mechanism of action for metformin may be related to the inhibition of PKC or the activation of tyrosine kinase. Preliminary evidence showed that HG-induced cardiac E-C coupling abnormalities are prevented by nonspecific PKC inhibition (41). This would be consistent with evidence that certain PKC isoforms are elevated in animal models of diabetes (42). The link between metformin's cardioprotection and PKC has not been established. However, metformin's actions are completely prevented in the presence of a tyrosine inhibitor, genistein. Further studies are needed to determine whether metformin affects other kinase activity in the hearts and whether these actions, if any, are genomic or post-translational (i.e., protein kinases).

Glucose metabolism. Metformin increases both basal and insulin-stimulated glucose transport in various types of tissue (10,19,20). However, whether this is part of the basis of metformin's cardioprotective action is unlikely. Fischer et al. (18) show that metformin's beneficial effects (in vivo and in vitro) do not involve changes in glucose transport in heart cells from normal or diabetic rats.

An interesting feature of this investigation is that metformin exerted its cardiac protection against HG-induced relaxation defects in the absence of insulin. Despite its recognition as an insulin-sensitizing agent, it was reported that

metformin stimulates glucose transport in the absence of insulin in cultured skeletal muscle cells (16). Several other reports have also confirmed that metabolic changes engendered by metformin are independent of insulin (17,18). In a recent study, we found that metformin in-creases basal tyrosine kinase activity, increases glucose uptake, and attenuates agonist-induced intracellular Ca^{2+} responses in the absence of insulin in vascular smooth muscle cells (19), as reported elsewhere in other cell types (17,42). These actions of metformin suggest that the biguanide is apparently not only "insulin-sensitizing" but also "insulin-independent," perhaps through modifications of intracellular Ca^{2+} homeostasis.

Troglitazone may also have cellular effects that are independent of insulin. Troglitazone has been shown to decrease L-type Ca^{2+} channel currents in vascular smooth muscle cells in the absence of insulin (43). We recently demonstrated that troglitazone also protects cardiomyocytes from the HG-induced relaxation abnormalities and slower intracellular Ca^{2+} clearing (29). Cardioprotective effects of this thiazolidinedione have also been demonstrated in some type 2 diabetic patients (44). These data suggest that both metformin and troglitazone, which appertain to different drug classes (i.e., biguanides and thiazolidinediones, respectively), may have similar vascular and cardiac effects through modifications on cytosolic Ca^{2+} handling.

The heart and vascular tissues may be targets of sulfonylurea action, since both have sulfonylurea receptors and ATP-dependent K^+ channels (45,46). These channels play an important role in the protection of the myocardium against ischemia-reperfusion damage, and their closure by sulfonylurea could lead to amplified ischemic damage (25,26), reduce recovery of contractile function, increase the myocardial infarct size, and ultimately may modify the clinical outcome of cardiovascular function in type 2 diabetic patients (47). We find that high concentrations of glyburide (300 µmol/l) tend to prolong (albeit not significantly) myocyte relengthening in normal myocytes (Fig. 2*B*), which may account for its apparent efficacy in preventing HG-induced prolonged TR. The observation that glyburide lacks cardioprotective properties further reinforces the notion that cardioprotective function of antihyperglycemic drugs does not extend to the sulfonylurea derivatives.

We are investigating the underlying cellular mechanisms associated with HG-induced prolonged relaxation and the signaling mechanisms contributing to these changes, as well as the cardioprotective effects of metformin on cardiac myocytes. To date, few studies have utilized a cell culture system to address the pathogenesis of diabetes-related cardiac dysfunctions. Our in vitro diabetes model allows us to explore the direct actions of HG, independent of other complications associated with diabetes, such as hyperlipidemia, hyper- or hypoinsulinemia, and hypothyroidism, that may also contribute to depressed myocardial function. This work provides evidence that metformin prevents the HG-induced myocyte dysfunctions (probably through activation of tyrosine kinases), similar to another antihyperglycemic agent troglitazone and in contrast to the lack of efficacy of glyburide.

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