Rapid Publications

Troglitazone Attenuates High-Glucose–Induced Abnormalities in Relaxation and Intracellular Calcium in Rat Ventricular Myocytes

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Diabetes is associated with impaired cardiac diastolic dysfunction. Isolated ventricular myocytes from diabetic animals demonstrate impaired relaxation concomitant with prolonged intracellular Ca^{2+} transients. We have recently shown that maintaining normal adult rat ventricular myocytes in a "diabetic-like" culture medium (low insulin and high glucose) produces abnormalities in excitation-contraction coupling similar to in vivo diabetes. Troglitazone (TRO), a novel insulin-sensitizing agent, significantly lowers blood pressure and modestly increases cardiac output in vivo, but its direct impact on cardiac function is unknown. To determine whether TRO could prevent high-glucose–induced dysfunction, normal myocytes were maintained in culture for 1–2 days in either normal medium containing 5 mmol/l glucose or high-glucose medium containing 25 mmol/l glucose. TRO (5 μmol/l) was added to both normal and high-glucose media. Mechanical properties were evaluated using a high-resolution video-edge detection system, and Ca^{2+} transients were recorded in fura-2–loaded myocytes. Relaxation from peak contraction was significantly longer in myocytes cultured in high glucose. Treating cells with TRO either attenuated or prevented the high-glucose effects, without changing the mechanical properties of myocytes cultured in normal medium. TRO also prevented the abnormally slow rates of Ca^{2+} transient decay induced by high glucose. Collectively, these data demonstrate that TRO can protect against the high-glucose–induced relaxation defects, perhaps through changes in intracellular Ca^{2+} handling. If TRO has both vasodilatory actions and beneficial cardiac properties (e.g., improvement of diastolic function) in the presence of hyperglycemia, this antidiabetic agent may prove to have significant salutary cardiovascular effects in type II diabetes. *Diabetes* 45:1822–1825, 1996

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A_R, area under the relengthening portion of the twitch; TR_{90}, time to 90% relengthening; TRO, troglitazone.

Diabetic cardiomyopathy, a major complication of diabetes, is characterized by both systolic and diastolic dysfunctions (1). Impaired diastolic function is the most prominent cardiac abnormality and is characterized by prolonged relaxation and decreased compliance. Several cellular defects have been found to contribute to the abnormal relaxation, including prolonged action potential duration, depressed myosin ATPases and sensitivity of myofilaments, and impaired function of Ca^{2+}-regulating proteins such as sarcolemmal and sarco(endo)plasmic reticulum Ca^{2+} ATPase (2,3). Although the pathogenesis of these cellular defects is not clearly established, hyperglycemia may play a key role (4,5).

Troglitazone (TRO), a novel member of the insulin-sensitizing thiazolidinediones, has been shown to lower blood pressure, blood glucose, and plasma triglycerides and enhance insulin action in diabetic and insulin-resistant humans and rodents (6–8). TRO enhances insulin sensitivity by stimulating glucose uptake, glucose oxidation, and lipogenesis in both adipose tissue and muscle, as well as stimulating lipogenesis and inhibiting gluconeogenesis in liver (9). TRO also prevents high-glucose–induced insulin resistance in cultured rat fibroblasts (10). Recent studies from our group have demonstrated that TRO, as well as another thiazolidinedione, pioglitazone, blocks voltage-dependent Ca^{2+} channels in vascular smooth muscle cells (11) and induces vasodilation (12). However, the mechanisms of effects of TRO on myocardial function have not been previously studied.

Impaired relaxation is apparent in ventricular myocytes isolated from diabetic rats, even after only a few days of diabetes, and this abnormality is reproduced in normal myocytes cultured in a "diabetic-like" medium containing high glucose (4,13). We used this in vitro model of diabetes to investigate whether TRO prevents high-glucose–mediated changes in excitation-contraction coupling. Our data indicate that by culturing myocytes with TRO, impaired relaxation and Ca^{2+} handling are attenuated in high-glucose–treated myocytes. These data support the view that TRO may provide myocardial protection against the detrimental effects of high glucose.

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 and deoxyribonuclease were obtained from Worthington Biochemical (Freehold, NJ). Hyaluronidase, trypsin, and medium 199 with Earle's salts were purchased from Sigma (St. Louis, MO). Laminin was obtained from Collaborative Biochemical Products (Bedford, MA). Fura-2/AM was purchased from Molecular Probes (Eugene, OR). TRO was provided by Parke-Davis (Ann Arbor, MI).

Cell isolation and culture. Ventricular myocytes were isolated by coronary perfusion and prepared for primary culture as previously described (4). 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 they were further digested by trypsin (0.02 mg/ml) and deoxyribonuclease (0.02 mg/ml) after the tissue has been 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 25 mmol/l HEPES and $NaHCO_3$ supplemented with albumin (2 mg/ml), L-carnitine (2 mmol/l), creatine (5 mmol/l), taurine (5 mmol/l), insulin (0.1 μmol/l), D-triiodothyronine (0.1 nmol/l), penicillin (100 U/ml), streptomycin (100 μg/ml), and gentamicin (100 μg/ml). This medium also contained either normal (5 mmol/l) or high (25 mmol/l) glucose concentrations. The high glucose concentration is comparable to serum glucose levels in diabetic rats (13). A subset of each medium was also supplemented with TRO (5 μmol/l, dissolved in 100% ethanol, final concentration of ethanol <0.02%). Media without TRO were supplemented with equal concentrations of ethanol. The cells were maintained at 37°C in a 100% humidity and 5% CO_2 incubator for 1–2 days.

Cell shortening/relengthening. Mechanical properties of cultured ventricular myocytes were assessed by a video-based edge detection system (Crescent Electronics, Sandy, UT) as described previously (13). In brief, coverslips with cells attached were placed in a plastic chamber mounted on the stage of an inverted microscope (Nikon Diaphot) with the temperature maintained at 37°C. The chamber was superfused (~2 ml/min) with a buffer containing (in millimoles per liter): 131 NaCl, 4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 10 glucose, 10 HEPES, at pH 7.4. 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. Cell shortening and relengthening properties recorded with this optical-video system have been shown to be linearly related to contractility (14) and will thus be referred to as contraction and relaxation, respectively. Cell length, peak twitch amplitude, and time and area of both contraction and relaxation were measured as described previously (13).

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 previously described (4,13). Myocytes were placed on an inverted microscope equipped with a heated (37°C) and light-tight chamber and 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 and 520 nm by a photomultiplier tube after illuminating cells first at 360 nm for 0.5 s then at 380 nm for the duration of the recording protocol (333-Hz sampling rate). The 360 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.

Statistics. Data are presented as means ± SE. Statistical significance was estimated 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 and/or interaction terms.

RESULTS

TRO protects against high-glucose–induced mechanical dysfunction. Culturing myocytes for 1–2 days in either high-glucose or TRO-containing media had no effect on overt myocyte phenotype compared to control cells. For example, cell shape, cell length, and presence of distinct striations were similar among all cells. Properties associated with myocyte shortening were similar in all groups of cells, independent of culture medium constituents. These indexes of myocyte contraction (shortening) included peak twitch amplitude (PT), time to peak contraction, and area under contraction phase (Table 1) (13). The indexes used to describe relaxation were time to 90% relengthening (TR_{90}) and area under the relengthening portion of the twitch normalized to PT (A_R). Both TR_{90} and A_R were increased in myocytes cultured in high glucose when compared with myocytes cultured in normal glucose medium. Abnormal (prolonged) relaxation in high-glucose myocytes was partially prevented when TRO was added to the culture medium, yet TRO had no effect on either TR_{90} or A_R in cells cultured in normal glucose (Fig. 1).

TABLE 1
Indexes of shortening in myocytes maintained for 1–2 days in culture in normal-glucose (N), high-glucose (HG), normal-glucose with TRO (N+T), or high-glucose with TRO (HG+T) medium

	N	HG	N+T	HG+T
CL (μm)	119 ± 3	122 ± 3	115 ± 3	121 ± 3
PT (% of CL)	11 ± 1	11 ± 1	13 ± 1	10 ± 1
TPT (ms)	47 ± 2	51 ± 2	50 ± 2	50 ± 1
A_C (μm · ms · μm^{-1})	48 ± 2	55 ± 2	51 ± 2	55 ± 2

Data are means ± SE. Sample sizes are 65–93 cells/group. CL, cell length; PT, peak twitch amplitude; TPT, time to PT; A_C, area under the contractile portion of the twitch normalized to PT.

TRO protects against high-glucose–induced abnormal Ca^{2+} handling. The time course of Ca^{2+} transients was evaluated in fura-2–loaded myocytes cultured for 1–2 days in either normal or high-glucose medium, with or without TRO. 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 duration of free intracellular Ca^{2+}. Culturing myocytes in high glucose slowed the rate of intracellular Ca^{2+} decay (longer τ), which likely contributes to prolonged relaxation (Fig. 2). Culturing myocytes in high glucose and TRO partially prevented this slower cytosolic Ca^{2+} clearing. TRO had no effect on τ when added to normal medium (data not shown).

DISCUSSION

The principal finding of this investigation is that impaired relaxation induced by culturing myocytes in high glucose can be attenuated by TRO. Prolonged relaxation is associated with slower intracellular Ca^{2+} clearing in high-glucose cells, and TRO partially prevents this abnormal Ca^{2+} handling (Figs. 1 and 2). Hyperglycemia has often been considered to be the most important predisposing factor in diabetes-related vascular disorders (15,16) and may also contribute to the development of cardiac dysfunction. We have previously shown that freshly isolated ventricular myocytes from diabetic rats, and normal myocytes cultured in a diabetic-like medium (low insulin and high glucose), exhibit abnormal relaxation (4,5,13). High glucose alone produces the same dysfunctions as the diabetic-like medium, inducing prolonged intracellular Ca^{2+} transients (longer τs) and prolonged relaxation (increased TR_{90} and A_R). There is evidence that elevated

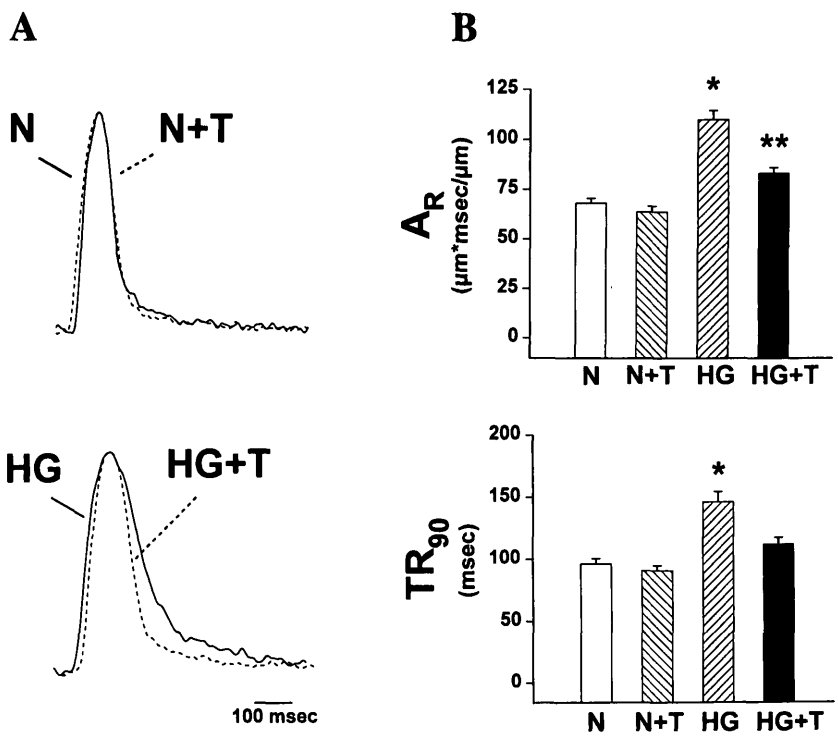

FIG. 1. *A*: representative twitches of ventricular myocytes cultured for 1 day in normal (N), high-glucose (HG), normal with troglitazone (5 μmol/l; N+T), or high-glucose with troglitazone (HG+T) medium. *B*: graphs illustrate relaxation properties of myocytes cultured for 1–2 days in either of the above-mentioned media. The relaxation indexes are TR_{90} and A_R. Data represent means ± SE. Sample sizes are 65–93 cells/group. *Indicates statistical significance ($P < 0.05$) when compared to all other groups. **Indicates significance when compared to normal medium.

extracellular glucose leads to increased intracellular Ca^{2+} in both vascular smooth muscle (17,18) and cardiac myocytes (19), which may contribute to cardiovascular complications associated with hyperglycemia. These data are consistent with the proposal that myocardial intracellular Ca^{2+} overload is either pathogenic or a pathophysiologic consequence of diabetes (20,21).

The mechanism by which TRO attenuates the detrimental effects of high glucose is not known. However, thiozolidinediones (including TRO) have been shown to inhibit L-type Ca^{2+} channels in vascular smooth muscle cells (11,22). Elevating extracellular glucose from 5 to 25 mmol/l leads to a twofold increase in ventricular myocyte intracellular Ca^{2+} (19), which may influence excitation-contraction coupling directly (23) or may alter enzyme activity (e.g., protein kinase C, Ca^{2+}-calmodulin–dependent protein kinase) or gene expression. The high-glucose effects may be due, in part, to changes in Ca^{2+} homeostasis. TRO may attenuate high-glucose–induced Ca^{2+} overload by blocking Ca^{2+} influx through L-type channels and thus protect against the cascade of events triggered by elevated intracellular Ca^{2+}. Alternatively, TRO may compensate for the adverse effects of high glucose by enhancing the cellular mechanisms that promote cytosolic Ca^{2+} removal (e.g., increasing sarcoplasmic reticulum Ca^{2+} uptake and/or Ca^{2+} efflux through Na/Ca exchange). Further studies will be needed to determine whether the TRO effects are genomic or direct on these proteins.

We are currently investigating the underlying cellular mechanisms associated with prolonged relaxation and the signaling mechanisms that contribute to these changes, as well as the cellular effects of TRO on cardiac myocytes. To date, few studies have used 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 high glucose, indepen-

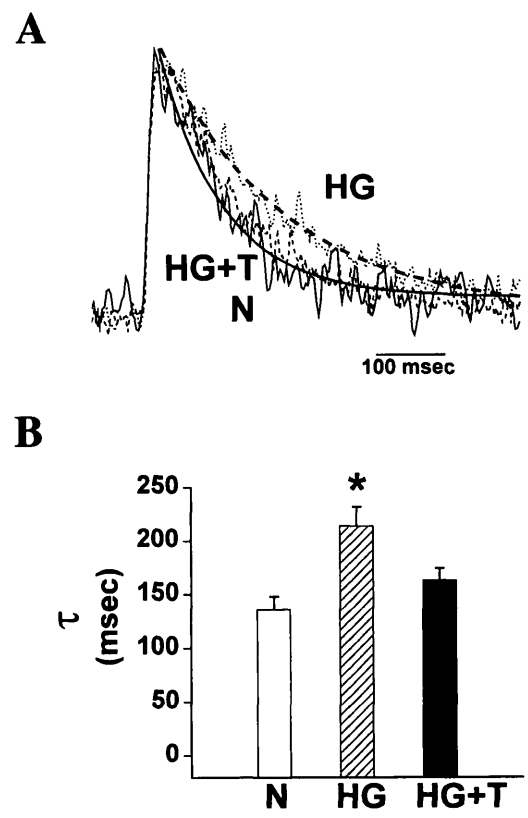

FIG. 2. *A*: Ca^{2+} transients from fura-2–loaded ventricular myocytes cultured in normal (N), high-glucose (HG), or high-glucose with troglitazone (HG+T) medium. Time course of the Ca^{2+} transient decay (τ) was evaluated by curve fitting with a single exponential equation (N, 102 ms [—]; HG, 222 ms [···]; HG+T, 144 ms [- - -]). Curve fits are shown for N and HG transients. *B*: average τs recorded from myocytes cultured in each medium. Data are expressed as means ± SE. Sample sizes are 18–33 cells/group. *Indicates statistical significance ($P < 0.05$) when compared with all other groups.

dent 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 TRO attenuates the high-glucose–induced myocyte dysfunctions. In conjunction with its vascular effects, this suggests that TRO may be beneficial to both cardiac and vascular disease associated with hyperglycemia.

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