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Enhanced inhibition of L-type calcium currents by troglitazone in streptozotocin-induced diabetic rat cardiac ventricular myocytes

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1 Troglitazone, an insulin-sensitizing agent shown to improve cardiac function in both experimental animals and patients with diabetes, inhibits voltage-dependent L-type Ca^{2+} currents ($I_{Ca,L}$) in cardiac myocytes, which may underlie its cardioprotective effects. However, inhibition by troglitazone of $I_{Ca,L}$ in diabetic cardiac myocytes has not been characterized.

2 Using whole-cell voltage-clamp techniques, $I_{Ca,L}$ was measured in ventricular myocytes isolated from 4–6 weeks streptozotocin (STZ)-induced diabetic rats and age-matched control rats.

3 Under control conditions with CsCl internal solution, diabetic myocytes did not differ from control myocytes in membrane capacitance, current density or voltage-dependent properties of $I_{Ca,L}$.

4 Troglitazone decreased amplitude of $I_{Ca,L}$ in both control and diabetic myocytes in a concentration-dependent manner. This inhibition was more potent in diabetic than in control myocytes; half-maximum inhibitory concentrations of troglitazone measured at a holding potential of -50 mV were 4.3 and 9.5 μmol l^{-1}, respectively.

5 Troglitazone at 5 μmol l^{-1} did not significantly influence the voltage dependency of steady-state inactivation or the inactivation time course of $I_{Ca,L}$ in either control or diabetic myocytes.

6 Since troglitazone inhibits $I_{Ca,L}$ more effectively in STZ-induced diabetic ventricular myocytes, this agent may prevent cardiac dysfunction in diabetes.

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Abbreviations: $I_{Ca,L}$, voltage-dependent L-type Ca^{2+} current; STZ, streptozotocin; TEA-Cl, tetraethylammonium chloride

Introduction

An explosive increase has occurred in our understanding of how diabetes mellitus increases the risk of cardiac dysfunction. This effect can occur independently of coronary atherosclerosis, hypertension, and valvular disease (Hamby *et al*., 1974; Kannel *et al*., 1974; Regan *et al*., 1977). A variety of abnormalities in contraction and relaxation have been identified in the streptozotocin (STZ)-induced diabetic heart (Fein *et al*., 1980; Penpargkul *et al*., 1980). In this regard, intracellular Ca^{2+} overload may underlie subcellular mechanisms responsible for these abnormalities (Teshima *et al*., 2000; Kjeldsen *et al*., 1987; Makino *et al*., 1987; Pierce & Dhalla, 1985; Penpargkul *et al*., 1981).

Troglitazone, a thiazolidinedione derivative, is an oral hypoglycaemic agent that reduces insulin resistance (Fujiwara *et al*., 1988; Suter *et al*., 1992). Troglitazone treatment reportedly improves cardiac function in STZ-induced diabetic rats and also in diabetic patients (Shimabukuro *et al*., 1996; Chazzi *et al*., 1997). Although the precise mechanisms responsible for cardioprotective effects of this agent remain unclear, inhibition of voltage-dependent L-type Ca^{2+} currents ($I_{Ca,L}$) may be involved. In vascular smooth muscle cells, thiazolidinediones including troglitazone reduce the amplitude of $I_{Ca,L}$ (Zhang *et al*., 1994; Song *et al*., 1997; Nakamura *et al*., 1998), an effect also demonstrated in cardiac myocytes (Nakajima *et al*., 1999; Katoh *et al*., 2000; Ikeda & Watanabe, 1998). Because treatment with verapamil or diltiazem lessens cardiac dysfunction in diabetes (Afzal *et al*., 1988; 1989; Fein *et al*., 1991), cardioprotective effects of troglitazone could be explained in terms of antagonism of $I_{Ca,L}$. However, no reports have described the inhibitory action of troglitazone on $I_{Ca,L}$ in diabetic myocytes.

In the present study, the whole-cell voltage-clamp technique was used to compare effects of troglitazone on $I_{Ca,L}$ in isolated rat ventricular myocytes in STZ-induced diabetes with effects in control rat myocytes.

Methods

Induction of diabetes

Male Wistar King A rats (8 weeks old, 250–300 g) were anaesthetized with diethyl ether and then received a single injection of STZ (60 mg kg^{-1}; Sigma Chemical, St. Louis, MO, U.S.A.) *via* the tail vein. STZ was dissolved in sterile sodium citrate buffer solution (0.1 mol l^{-1} citric acid and 0.2 mol l^{-1} sodium phosphate, pH 4.5). Age-matched control rats received an equivalent volume of citrate buffer solution alone.

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All experimental procedures were performed in accordance with the guidelines of the Physiological Society of Oita Medical University, Japan, for the care and use of laboratory animals.

Cell isolation

Four to 6 weeks after STZ or vehicle injection, single ventricular myocytes from the diabetic rats and age-matched control rats were isolated using an enzymatic dissociation procedure as previously described (Taniguchi *et al*., 1981). Briefly, the animals were heparinized (500 IU kg^{-1}, i.p.) and anaesthetized with sodium pentobarbital (100 mg kg^{-1}, i.p.). Hearts were removed quickly, and the aorta was cannulated and perfused by a Langendorff perfusion apparatus with normal Tyrode's solution (see below). The heart was then perfused with nominally Ca^{2+}-free Tyrode's solution for 5 min at a rate of 12 ml min^{-1} followed by perfusion with the same solution containing collagenase (0.13%, w v^{-1}; Wako Pure Chemical, Osaka, Japan) and type XIV protease (0.005%, w v^{-1}; Sigma Chemical, St. Louis, MO, U.S.A.) at 37°C. Twenty to 25 min later the heart was perfused with recovery solution. The right ventricle was dissected, and cells were mechanically dispersed and stored in recovery solution at 4°C. This procedure consistently yielded an acceptable number of quiescent, relaxed ventricular cells. The cell yield and viability in the Tyrode's solution were not much different among both groups.

Solutions and chemicals

The Tyrode's solution contained the following (as mmol l^{-1}): NaCl 140, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, HEPES 5, and glucose 5.5 (pH adjusted to 7.4 with NaOH). Nominally Ca^{2+}-free Tyrode's solution was prepared by simply omitting $CaCl_2$. The recovery solution contained the following (as mmol l^{-1}): KCl 30, L-glutamic acid 70, KH_2PO_4 10, taurine 20, HEPES 10, EGTA 0.3, and glucose 10 (pH adjusted to 7.4 with KOH). The composition of the external solution was (as mmol l^{-1}): NaCl 140, CsCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, HEPES 5, and glucose 5.5 (pH adjusted to 7.4 with NaOH). The internal solution contained the following (as mmol l^{-1}): CsCl 140, tetraethylammonium chloride (TEA-Cl) 10, EGTA 10, BAPTA 2, Na_2ATP 3, Na_3GTP 0.1, $MgCl_2$ 1, and HEPES 5 (pH adjusted to 7.3 with CsOH). Troglitazone, obtained from Sankyo Co Ltd, Japan, was dissolved in DMSO to give a stock solution of 30 μmol l^{-1} to 300 mmol l^{-1}. The final concentration of DMSO in the bathing solution was 0.1%. Before experiments, this concentration of DMSO was confirmed not to significantly influence $I_{Ca,L}$.

Electrophysiological recordings

Membrane currents were measured at room temperature (22 ± 1°C) using the whole-cell configuration of the patch-clamp technique (Hamil *et al*., 1981). Myocytes were allowed to settle in the bath for 5 min before being superfused with external solution at a rate of 1 ml min^{-1}. Heat-polished patch electrodes with a tip resistance of 2 to 6 MΩ were used. After obtaining a gigaseal, a suction pulse was applied to establish the whole-cell mode. Command pulses were delivered and data were acquired with an EPC-8 patch-clamp amplifier controlled by the PULSE software (HEKA, Lambrecht, Germany) connected to a Power Macintosh G4 computer (Apple Computer, Cupertino, CA, U.S.A.). High-resolution currents were low-pass filtered at 3 kHz, acquired at a sampling rate of 10 kHz, and stored on a hard disk for off-line analysis.

Data analysis and statistics

The amplitude of $I_{Ca,L}$ was defined as the difference between the peak inward current and the current at the end of the test pulse. Initial data were obtained after the amplitude of $I_{Ca,L}$ had stabilized within 10 min after the rupture of the membrane. The amplitude of $I_{Ca,L}$ diminishes as a function of time during patch-clamp recording, a phenomenon termed 'run down' (Belles *et al*., 1988). In the experiments presented here, the rate of $I_{Ca,L}$ run down was similar between control and diabetic myocytes and resulted in $<10\%$ reduction in peak current by the 10 min recording period ($5.7 \pm 1.2\%$, $n=30$ vs $5.9 \pm 1.2\%$, $n=30$, $P=$ns). Thereafter, the effects of troglitazone on $I_{Ca,L}$ could be investigated for 15–20 min. At the start of each experiment, the series resistance was compensated. For off-line data analysis, IGOR PRO software (WaveMetrics, Lake Oswego, OR, U.S.A.) was used. Data are presented as the mean ± s.e.m. Statistical significance was determined with the unpaired Student's *t*-test in conjunction with the Newman-Keuls test where applicable. Differences were considered significant at the level of $P<0.05$.

Results

Body weight and fasting plasma glucose concentrations of experimental animals

The basic characteristics of STZ-induced diabetic rats and age-matched control rats used in the present study are summarized in Table 1. Body weight was lower ($P<0.01$) and plasma glucose concentrations were higher ($P<0.01$) in the diabetic rats than in control rats.

Cell capacitance, current densities and current-voltage relationships of $I_{Ca,L}$

In each voltage-clamp experiment, membrance capacitance was measured immediately after disruption of the membrane patch. Membrane capacitance of control myocytes did not differ significantly from that of diabetic myocytes (196.1 ± 15.1 pF, $n=17$ vs 175.7 ± 9.7 pF, $n=28$, $P=$ns). Membrane currents were elicited by stepwise voltage changes of 300 ms duration from an initial −50 mV to a range between −60 and +60 mV. The changes were applied in increments of 10 mV at 0.2 Hz frequency (Figure 1A). Transient inward currents were elicited in control and diabetic myocytes, peaking within 10 ms after onset of depolarization and gradually declining, maximal peak current occurred at a potential of +0 mV. The inward current was completely blocked by Cd^{2+} at 0.1 mmol l^{-1} (data not shown), indicating that it consisted of $I_{Ca,L}$. Maximum densities of $I_{Ca,L}$ were not significantly different between control and diabetic myocytes (7.5 ± 0.4 pA pF^{-1}, $n=17$ vs

8.3 ± 0.4 pA pF^{-1}, $n = 28$, $P =$ ns). Current-voltage relationships of $I_{Ca,L}$ in control and diabetic myocytes nearly overlapped (Figure 1B).

Steady-state inactivation and activation of $I_{Ca,L}$

Steady-state inactivation and activation of $I_{Ca,L}$ was determined in five control myocytes and five diabetic myocytes. For estimating steady-state inactivation, a double-pulse voltage-clamp protocol was used. After establishing conditioning holding potentials at different voltage levels between -60 mV and $+60$ mV for 1 s, a test pulse to $+0$ mV with a duration of 300 ms was applied to elicit $I_{Ca,L}$. Normalized current amplitudes (I/I_{max}) were plotted against conditioning holding potentials. A steady-state inactivation curve was drawn by fitting the data to the Boltzmann distribution, $I/I_{max} = 1/\{1 + \exp[(V\text{-}V_h)/k]\}$, with I/I_{max} representing the relative current amplitude compared with the maximum current amplitude; V, the conditioning holding potential; V_h, the potential required for half-inactivation of the current; and k, the Boltzmann coefficient. The steady-state inactivation curves of $I_{Ca,L}$ were similar between control and diabetic myocytes (Figure 1C and Table 2).

Conductance (G) was calculated using the equation, $G = I_{peak}/(V\text{-}V_{rev})$, with I_{peak} as the peak amplitude of the current; V, the test potential; and V_{rev}, the reversal potential. The potential at zero current level, tentatively used as the reversal potential, was estimated from the current-voltage curve or determined in some cases by extrapolating the current-voltage relationship until the inward current became outwardly directed. For estimating steady-state activation, $I_{Ca,L}$ conductances were plotted against test potentials. The derived activation curve was fitted to data with a Boltzmann equation, $G/G_{max} = 1/\{1 + \exp[(V_{1/2}\text{-}V)/k]\}$, with G/G_{max} as relative conductance normalized to the maximal conductance; $V_{1/2}$, the potential required for half-activation of the current; and k, the Boltzmann coefficient. Steady-state activation curves of $I_{Ca,L}$ nearly overlapped between control myocytes ($V_{1/2} = -10.7 \pm 1.4$ mV, $k = 6.2 \pm 0.6$ mV, $n = 5$) and diabetic myocytes ($V_{1/2} = -11.5 \pm 1.5$ mV, $k = 6.0 \pm 0.3$ mV, $n = 5$, Figure 1D).

Table 1 Basic characteristics of control and streptozotocin-induced diabetic rats

	Control (n = 10)	*Diabetes* (n = 10)
Body weight (g)	420.0 ± 10.6	239.0 ± 7.7*
Plasma glucose concentration (mg dl^{-1})	163.2 ± 3.9	511.3 ± 23.7*

*$P < 0.01$ vs control.

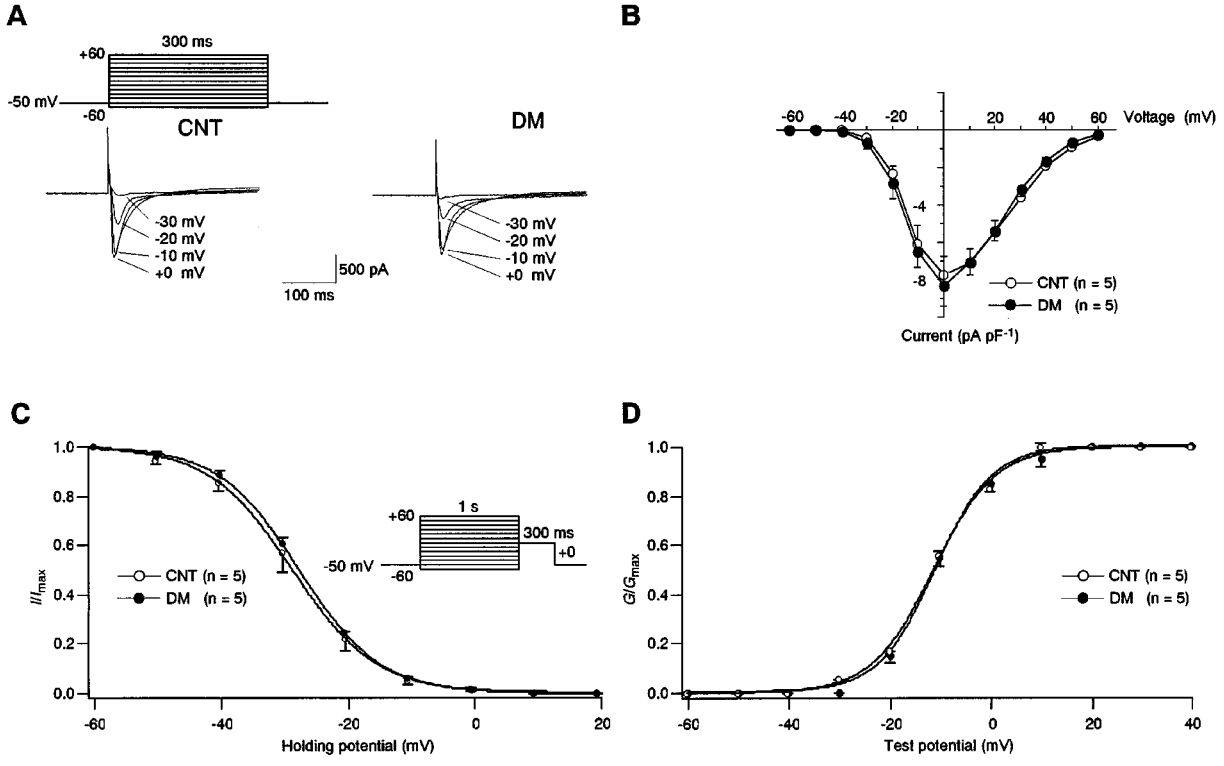

Figure 1 L-type Ca^{2+} currents ($I_{Ca,L}$) in control (CNT) and diabetic (DM) myocytes. (A), representative tracings of $I_{Ca,L}$. $I_{Ca,L}$ were elicited in 300 ms steps from a holding potential of -50 mV to a range between -60 and $+60$ mV in increments of 10 mV applied at a frequency of 0.2 Hz. (B), voltage-dependency of $I_{Ca,L}$. (C), steady-state inactivation of $I_{Ca,L}$ as demonstrated with a double-pulse voltage-clamp protocol. After applying conditioning holding potentials at different voltage levels between -60 mV and $+60$ mV for 1 s, a test pulse to $+0$ mV with a duration of 300 ms was used to elicit $I_{Ca,L}$. Normalized current amplitudes (I/I_{max}) were plotted against conditioning holding potentials. The steady-state inactivation curve was drawn by fitting data to the Boltzmann distribution. (D), steady-state activation of $I_{Ca,L}$ determined as ratio of conductances (G) to maximum conductance (G_{max}). The derived activation curve was fitted to data with a Boltzmann equation. Data are expressed as the mean ± s.e.m.

Inactivation time course of $I_{Ca,L}$

The time course of inactivation of $I_{Ca,L}$ was determined by analysis of the decay phase of current traces. Myocytes were held at −50 mV, and command pulses to +0 mV were applied at 0.2 Hz. Best fit was obtained with an equation including a sum of two exponentials plus a constant expressed as $A_{fast}\exp(-t/\tau_{fast})+A_{slow}\exp(-t/\tau_{slow})+A_0$, with τ and A as the time constant and the initial amplitude of the two components subscripted fast and slow, respectively; and A_0, the amplitude of the time-independent component. Pooled mean values for the fast (τ_{fast}) and slow (τ_{slow}) time constants of inactivation were similar in control and diabetic myocytes (Table 3).

Effects of troglitazone on $I_{Ca,L}$

The effects of troglitazone on $I_{Ca,L}$ were compared between control and diabetic myocytes. Figure 2 represents typical examples. Cells were held at −50 mV, and command pulses (300 ms in duration) to +0 mV were applied at 0.2 Hz. Application of troglitazone at 3 μmol l^{-1} began to reduce the amplitude of $I_{Ca,L}$ within 30 s in both control and diabetic myocytes, but the decline of the current amplitude was greater in latters. In both control and diabetic myocytes, washout of troglitazone caused partial recovery of $I_{Ca,L}$. During 120 s application of troglitazone at 3 μmol l^{-1}, the per cent inhibition of $I_{Ca,L}$ was greater in diabetic myocytes than in control myocytes (34.5±4.5%, $n=11$ vs 22.2±2.4%, $n=14$; $P<0.05$). Concentration-dependent inhibition of $I_{Ca,L}$ by troglitazone is shown in Figure 3. The concentration-dependent curve of $I_{Ca,L}$ to troglitazone was significantly shifted to the left in diabetic myocytes compared with control myocytes ($P<0.01$). The concentration of drug giving half-maximum inhibition (IC_{50}) measured at a holding potential of −50 mV was 9.5 μmol l^{-1} in control myocytes, but only 4.3 μmol l^{-1} in diabetic myocytes. Figure 4 shows the effects of troglitazone on the current-voltage relationships of $I_{Ca,L}$ in four myocytes from each group. Troglitazone at 5 μmol l^{-1} reduced the amplitude of $I_{Ca,L}$ at any command voltage without affecting the voltage dependence of $I_{Ca,L}$ in either control or diabetic myocytes. The reversal potential for $I_{Ca,L}$ was not altered significantly by troglitazone in control or diabetic myocytes (data not shown).

Table 2 Effects of troglitazone (5 μmol l^{-1}) on the steady-state inactivation of $I_{Ca,L}$

	Control (n=4)	*Diabetes* (n=4)
Absence of troglitazone		
V_h (mV)	−28.2±1.9	−27.2±0.4
k (mV)	6.2±0.2	6.4±0.4
Presence of troglitazone		
V_h (mV)	−29.7±2.3	−29.7±1.0
k (mV)	6.9±0.3	6.7±0.3

V_h represents potential required for half-inactivation of the current; k represents the Boltzmann coefficient.

Table 3 Fast and slow components of inactivation time constant of $I_{Ca,L}$ in the absence and presence of troglitazone (5 μmol l^{-1})

	Control (n=4)	*Diabetes* (n=4)
Absence of troglitazone		
τ_{fast} (ms)	14.2±0.7	13.6±1.7
τ_{slow} (ms)	72.4±4.4	71.3±3.2
Presence of troglitazone		
τ_{fast} (ms)	16.7±1.3	16.0±2.2
τ_{slow} (ms)	73.0±6.4	77.0±6.0

τ_{fast}, τ_{slow} indicate the fast and slow time constant of inactivation.

Effects of troglitazone on steady-state inactivation and inactivation time course of $I_{Ca,L}$

Figure 5 shows the voltage dependencies of steady-state inactivation of $I_{Ca,L}$ recorded in control and diabetic myocytes in the absence and presence of 5 μmol l^{-1} of troglitazone. Troglitazone only slightly shifted the steady-state inactivation curve to the left in both groups of myocytes (Table 2). Values of τ_{fast} and τ_{slow} in the presence of 5 μmol l^{-1} of troglitazone were not significantly different between control and diabetic myocytes (Table 3).

Discussion

Main findings

The main findings of the present study are that while troglitazone rapidly inhibited $I_{Ca,L}$ in a concentration-dependent manner in both control and STZ-induced diabetic ventricular myocytes, the inhibitory effect was stronger in the latter myocytes. Troglitazone did not influence the voltage-dependent properties of $I_{Ca,L}$ in control and diabetic myocytes.

Effects of diabetes on $I_{Ca,L}$

In the absence of troglitazone, control and diabetic myocytes showed no significant differences with respect to membrane capacitance, current densities, or any voltage-dependent properties of $I_{Ca,L}$. The effects of diabetes on current density of $I_{Ca,L}$ remain to be controversial. Our findings are in agreement with those reported by Jourdon & Feuvray (1993). However, Wang *et al.* (1995) and Chattou *et al.* (1999) reported that the density of $I_{Ca,L}$ was reduced in ventricular myocytes isolated from diabetic rats. The effect of diabetes on $I_{Ca,L}$ may depend on the duration of diabetes. It might be inferred that the longer duration of the diabetic state, the more severe is the depression of the $I_{Ca,L}$ likely to develop. Alternatively, the differences in the basal phosphorylation state of the myocardial $I_{Ca,L}$ between diabetic and control myocytes may be involved. It is also possible that each study was performed under different experimental conditions, leading to the different results.

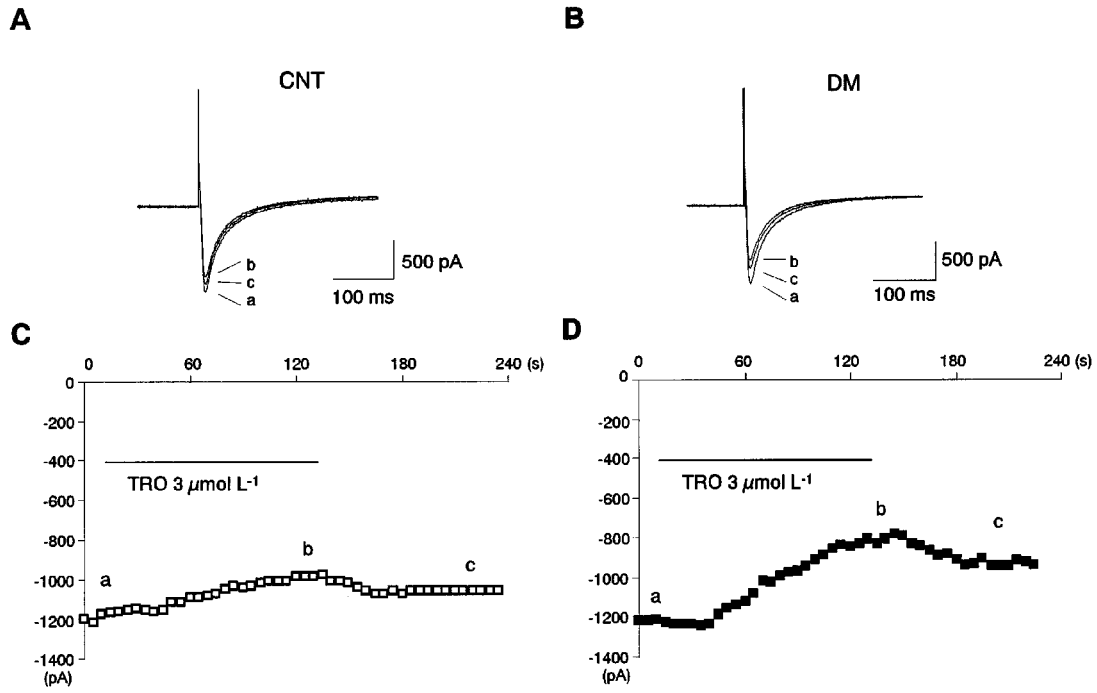

Figure 2 Effects of troglitazone (TRO) on L-type Ca^{2+} currents ($I_{Ca,L}$) in control (CNT) and diabetic (DM) myocytes. Cells were held at -50 mV, and command pulses 300 ms in duration causing voltage elevation to $+0$ mV were applied at 0.2 Hz. A and B, representative tracings of $I_{Ca,L}$ obtained at times indicated by a through c in C and D. C and D, time courses of alterations of $I_{Ca,L}$ amplitude. Drug administration sequences also are shown.

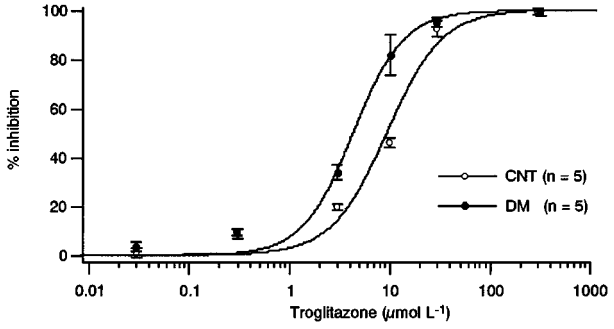

Figure 3 Concentration-dependent inhibition of L-type Ca^{2+} currents ($I_{Ca,L}$) by troglitazone in control (CNT) and diabetic (DM) myocytes. Amplitude of $I_{Ca,L}$ after application of troglitazone was compared with the control value. Percentage inhibition of troglitazone on $I_{Ca,L}$ is plotted against concentration of troglitazone. Data are expressed as the mean $\pm$ s.e.m. and fit to a Hill equation.

Effects of troglitazone on $I_{Ca,L}$ *in control and diabetic myocytes*

In the present study, troglitazone inhibited $I_{Ca,L}$ immediately after addition to control and diabetic myocytes without any modification of the kinetics or voltage dependence of the current. Nakajima *et al.* (1999) also demonstrated that the inhibitory effect of troglitazone on $I_{Ca,L}$ in guinea-pig atrial myocytes was neither voltage-dependent nor use-dependent. Together with our observations, it is suggested that the inhibitory action of troglitazone on $I_{Ca,L}$ could be a direct effect. As presented in Figure 2C,D, the troglitazone slowly inhibited $I_{Ca,L}$. Because washout of troglitazone caused partial recovery of $I_{Ca,L}$, we believe that the gradual decrease in $I_{Ca,L}$ was not due to the rundown. In the previous studies (Katoh *et al.*, 2000; Nakajima *et al.*, 1999; Nakamura *et al.*, 1998), the inhibition of $I_{Ca,L}$ by troglitazone was also reported to be slow. The precise binding mechanisms of troglitazone remain to be elucidated.

In the present study, inhibition by troglitazone of $I_{Ca,L}$ was enhanced in STZ-induced diabetic myocytes. In related observations, increased sensitivity of the diabetic heart to Ca^{2+} channel antagonists has been demonstrated (Lee *et al.*, 1992; Heijnis *et al.*, 1991). In isolated perfused hearts in STZ-induced diabetic hearts, several L-type Ca^{2+} channel antagonists were reported to cause more depression of contractile force than they caused in control hearts (Lee *et al.*, 1992; Heijnis *et al.*, 1991. Lee *et al.* (1992) found the number of L-type Ca^{2+} channels, as measured by ^{3}H-nitrendipine binding, in the myocardial membrane to be decreased in STZ diabetic rats, while the channels showed increased affinity for antagonists. Because no difference was observed in $I_{Ca,L}$ current density in the present study, enhanced inhibition of $I_{Ca,L}$ in STZ-induced diabetic myocytes may be explained by increased affinity of troglitazone for L-type Ca^{2+} channels. This hypothesis may be supported by a recent report demonstrating that relative responsiveness of $I_{Ca,L}$ to dihydropyridines was much higher in vascular smooth muscle cells isolated from STZ-induced diabetic rats compared to control rats (Wang *et al.*, 2000).

In our unpublished data using a nystatin-perforated patch method, the inhibition of $I_{Ca,L}$ was also greater in diabetic myocytes than in control myocytes when 3 or 10 μmol l^{-1} troglitazone was applied. However, the inhibitory effect was

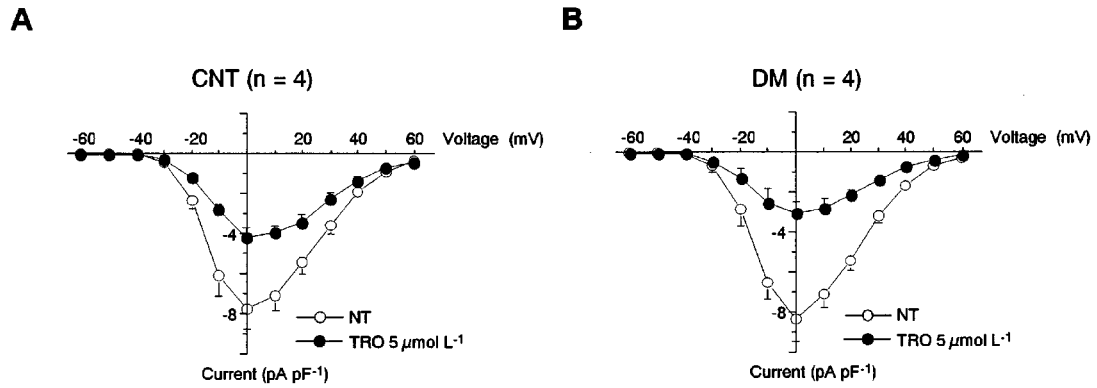

Figure 4 Effects of troglitazone (TRO) on the current-voltage relationships of L-type Ca^{2+} currents ($I_{Ca,L}$) in control (CNT) and diabetic (DM) myocytes. (A), current-voltage relationships of $I_{Ca,L}$ in the absence and presence of troglitazone 5 μmol l^{-1} in control myocytes. (B), current-voltage relationships of $I_{Ca,L}$ in the absence and presence of troglitazone 5 μmol l^{-1} in diabetic myocytes. Data are the mean$\pm$s.e.m. NT, normal Tyrode's solution.

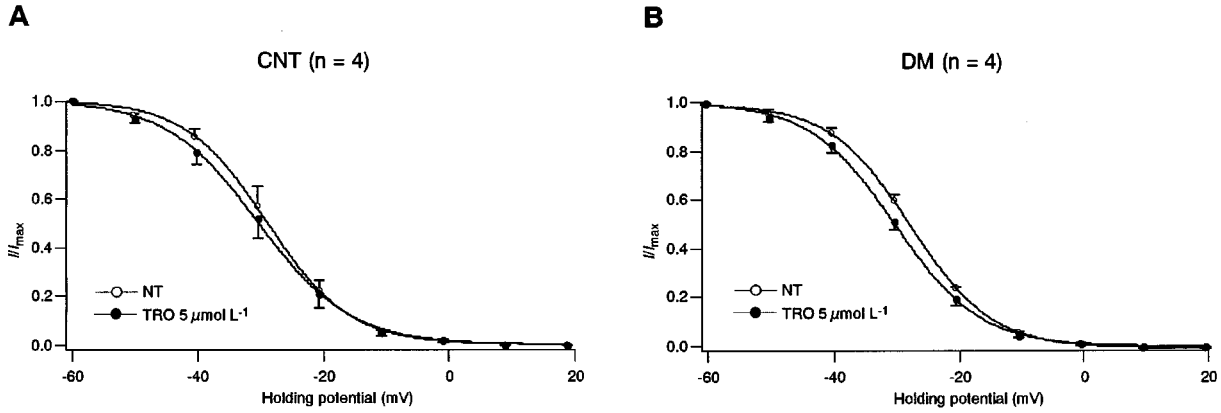

Figure 5 Effects of troglitazone (TRO) on steady-state inactivation of L-type Ca^{2+} currents ($I_{Ca,L}$) in control (CNT) and diabetic (DM) myocytes. (A), steady-state inactivation of $I_{Ca,L}$ in the absence and presence of troglitazone 5 μmol l^{-1} in control myocytes. (B), steady-state inactivation of $I_{Ca,L}$ in the absence and presence of troglitazone 5 μmol l^{-1} in diabetic myocytes. Data are the mean$\pm$s.e.m. NT, normal Tyrode's solution.

less compared to the condition where intracellular Ca^{2+} was highly buffered. Thus, under the condition of a more physiological intracellular Ca^{2+}, troglitazone also inhibited $I_{Ca,L}$ more effectively in STZ-diabetic myocytes than in control myocytes. The effects of troglitazone under the condition with Ca^{2+} overloading should be assessed in future studies.

Cardioprotective effects of troglitazone in diabetes

In diabetic myocytes, intracellular Ca^{2+} homeostasis is impaired by several mechanisms likely to contribute to intracellular Ca^{2+} overload (Teshima *et al*., 2000; Kjeldsen *et al*., 1987; Makino *et al*., 1987; Pierce & Dhalla, 1985; Penpargkul *et al*., 1981). Hyperglycaemia may play a key role in these disturbances; for instance, function of cardiac sarcoplasmic reticulum Ca^{2+} ATPase is depressed in STZ-induced diabetic heart, leading to prolonged relaxation (Penpargkul *et al*., 1981). We recently demonstrated that expression of sarcoplasmic reticulum Ca^{2+} ATPase mRNA was diminished in STZ-induced diabetic rats and that expression was restored by normalization of plasma glucose level with insulin treatment (Teshima *et al*., 2000). Ren *et al*. (1996) demonstrated that rat ventricular myocytes cultured with medium containing high glucose for 1–2 days exhibited prolonged relaxation and that troglitazone attenuated the abnormality. The authors speculated that by blocking Ca^{2+} influx through L-type Ca^{2+} channels, troglitazone may attenuate Ca^{2+} overload induced by high glucose. In this manner, troglitazone could protect myocytes against the cascade of events triggered by elevated intracellular Ca^{2+}. Thus, cardioprotective effects of troglitazone may represent compensation by enhanced cellular mechanisms reducing intracellular Ca^{2+} overload, counteracting the detrimental effects of hyperglycaemia.

Limitations

There are several limitations in the present study. First, we evaluated the effects of troglitazone on $I_{Ca,L}$ using 4–6 weeks STZ-induced diabetic rats. Because Wang *et al*. (1995) reported that $I_{Ca,L}$ was significantly smaller in 24–30 weeks

STZ diabetic rats compared to age-matched controls, further studies are necessary to assess the effects of troglitazone using diabetic rats with longer duration of diabetes. Second, our hypothesis that troglitazone may reduce intracellular Ca^{2+} overload is purely speculative. To prove this, measurements of cytosolic Ca^{2+} must be made. Lastly, experiments using Ba^{2+} instead of Ca^{2+} are required to support our hypothesis that inhibitory action of troglitazone on $I_{Ca,L}$ is a direct effect.

Relevance

Troglitazone is an insulin-sensitizing agent, used to treat patients with type 2 diabetes mellitus who show hyperinsulinemia (Suter *et al*., 1992). The present study used STZ-induced diabetic rat, which is an insulin-deficient diabetic model. The choice of model, then, may be a limitation of the present study. However, some beneficial effects of troglitazone may represent compensation for adverse effects of hyperglycaemia (Ren *et al*., 1996). Therefore, our observations suggest that troglitazone might benefit for a wide variety of hyperglycaemic patients. In the present study, the IC_{50} of troglitazone for $I_{Ca,L}$ in diabetic myocytes was 4.3 μmol l^{-1}, which is near the established therapeutic plasma concentration (Shibata *et al*., 1993). Thus, the inhibitory action upon $I_{Ca,L}$ by troglitazone may be clinically important.

Conclusions

In the present study, troglitazone inhibited $I_{Ca,L}$ more effectively in STZ-induced diabetic ventricular myocytes than in age-matched control myocytes, possibly because of increased sensitivity to troglitazone of L-type Ca^{2+} channels in diabetic myocardium. These results suggest that troglitazone may be useful in preventing diabetic cardiac dysfunction because of actions that reduce intracellular Ca^{2+} overload.

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