Troglitazone Inhibits Voltage-Dependent Calcium Currents in Guinea Pig Cardiac Myocytes

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Background—It has been suggested that intracellular Ca\(^{2+}\) overload in cardiac myocytes leads to the development of diabetic cardiomyopathy. Troglitazone, an insulin-sensitizing agent, is a promising therapeutic agent for diabetes and has been shown to prevent diabetes-induced myocardial changes. To elucidate the underlying mechanism of troglitazone action on cardiac myocytes, the effects of troglitazone on voltage-dependent Ca\(^{2+}\) currents were examined and compared with classic Ca\(^{2+}\) antagonists (verapamil and nifedipine).

Methods and Results—Whole-cell voltage-clamp techniques were applied in single guinea pig atrial myocytes. Under control conditions with CsCl internal solution, the voltage-dependent Ca\(^{2+}\) currents consisted of both T-type (\(I_{Ca,T}\)) and L-type (\(I_{Ca,L}\)) Ca\(^{2+}\) currents. Troglitazone effectively reduced the amplitude of \(I_{Ca,L}\) in a concentration-dependent manner. Troglitazone also suppressed \(I_{Ca,T}\), but the effect of troglitazone on \(I_{Ca,T}\) was less potent than that on \(I_{Ca,L}\). The current-voltage relationships for \(I_{Ca,L}\) and the reversal potential for \(I_{Ca,L}\) were not altered by troglitazone. The half-maximal inhibitory concentration of troglitazone on \(I_{Ca,L}\) measured at a holding potential of −40 mV was 6.3 μmol/L, and 30 μmol/L troglitazone almost completely inhibited \(I_{Ca,L}\). Troglitazone 10 μmol/L did not affect the time courses for inactivation of \(I_{Ca,L}\) and inhibited \(I_{Ca,L}\) mainly in a use-independent fashion, without shifting the voltage-dependency of inactivation. This effect was different from those of verapamil and nifedipine. Troglitazone also reduced isoproterenol- or cAMP-enhanced \(I_{Ca,L}\).

Conclusions—These results demonstrate that troglitazone inhibits voltage-dependent Ca\(^{2+}\) currents (T-type and L-type) and then antagonizes the effects of isoproterenol in cardiac myocytes, thus possibly playing a role in preventing diabetes-induced intracellular Ca\(^{2+}\) overload and subsequent myocardial changes. (Circulation. 1999;99:2942-2950.)

Key Words: troglitazone • myocytes • calcium • isoproterenol • diabetes • cardiomyopathy

Myocardial contractile dysfunction is a major complication of diabetes, known as diabetic cardiomyopathy.\(^1\)\(^-\)4 The subcellular mechanisms responsible for cardiomyopathy are unknown. However, several cellular defects, including depressions in sarcoplasmic reticular Ca\(^{2+}\) uptake,\(^5\) Na\(^+\)-K\(^+\) pump,\(^6\) sarcosommal Ca\(^{2+}\) pump, Na\(^+\)-Ca\(^{2+}\) exchanger activities,\(^7\) and the alteration of mitochondrial functions,\(^8\) have been suggested to be contributors to the development of this disease. The net result of these changes in Ca\(^{2+}\) homeostasis causes an intracellular Ca\(^{2+}\) overload, thereby resulting in cellular damage and, ultimately, diabetic cardiomyopathy. Moreover, diabetes prolongs the action potential duration\(^9\)\(^-\)12 and increases the number of myocardial voltage-dependent Ca\(^{2+}\) channels,\(^13,14\) something that may also play a role in causing diabetic cardiomyopathy. In fact, it has been reported that in chronically diabetic rats, elevated tissue Ca\(^{2+}\) levels are present\(^15\) and treatment with verapamil or diltiazem, a voltage-dependent L-type Ca\(^{2+}\) channel blocker, lessens cardiac dysfunction.\(^16\)\(^-\)18 Thus, it is likely that excess Ca\(^{2+}\) influx through the voltage-dependent Ca\(^{2+}\) channels contributes to induce intracellular Ca\(^{2+}\) overload and consequently diabetic cardiomyopathy.

Troglitazone, a novel member of the insulin-sensitizing thiazolidinediones, has been widely used to treat patients with non–insulin-dependent diabetes mellitus and other insulin-resistant diseases. Treatment with troglitazone reduced hyperglycemia, plasma triglycerides, and blood pressure.\(^19\)\(^-\)22 Recent studies show that troglitazone attenuates high-glucose–induced abnormalities in relaxation and intracellular calcium in rat ventricular myocytes\(^23\) and may improve cardiac function in diabetic patients.\(^24\) Until now, the mechanisms underlying the beneficial effects of troglitazone on hearts have

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not been clearly established, but several articles have shown that troglitazone inhibits the voltage-dependent L-type Ca\(^{2+}\) currents (\(I_{\text{Ca,L}}\)) in vascular smooth muscle cells.\(^{25,26}\)

Therefore, the purpose of the present study was to clarify the effects of troglitazone on the voltage-dependent Ca\(^{2+}\) currents (T-type \(I_{\text{Ca,T}}\) and L-type) in cardiac myocytes. We have also made comparisons with the classic Ca\(^{2+}\) antagonists verapamil and nifedipine.

**Methods**

**Cell Preparation**

Single atrial myocytes were obtained from guinea pig hearts by an enzymatic dissociation procedure described elsewhere.\(^{27,28}\) Briefly, the animals were anesthetized with sodium pentobarbital, and their hearts were rapidly removed and retrogradely Langendorff-perfused at 35°C to 37°C with an oxygenated Tyrode’s solution. The hearts were then perfused with Ca\(^{2+}\)-free Tyrode’s solution for ~10 minutes and subsequently with the same solution containing collagenase (0.04% wt/vol type 1, Sigma Chemical Co) for 17 to 20 minutes. The digested hearts were stored in a high-K+/low-Cl\(^{-}\) solution\(^{27}\) at 5°C for later experimentation. The atria were then removed, and cells were obtained by gentle mechanical agitation. This procedure consistently yielded an acceptable number of quiescent and relaxed atrial cells.

**Solution and Drugs**

The composition of the normal Tyrode’s solution was (in mmol/L) NaCl 136.5, KCl 5.4, CaCl\(_2\) 1.8, MgCl\(_2\) 0.53, glucose 5.5, and HEPES-NaOH buffer 5, pH 7.4. The Ca\(^{2+}\)-free Tyrode’s solution was identical to normal Tyrode’s solution except that CaCl\(_2\) was omitted. To record voltage-dependent Ca\(^{2+}\) currents, K\(^{+}\) currents were eliminated by the internal Cs and external Ba (5 mmol/L), and Ca\(^{2+}\)-activated currents were blocked by 10 mmol/L EGTA and 2 mmol/L BAPTA in the internal solution. The composition of the internal solution was (in mmol/L) CsCl 140, EGTA 10, BAPTA 2, Na\(_2\)-ATP 3, GTP (sodium salt, Sigma) 0.1, MgCl\(_2\) 0.53, glucose 5.5, and HEPES-CsOH buffer 5, pH 7.4. Trogolitazone was obtained from Sankyo Co Ltd. Trogolitazone was dissolved in DMSO to give a stock solution of 1 to 30 mmol/L, and the final concentration of DMSO applied to the bathing solution was 0.1%. Nifedipine and verapamil were dissolved in ethanol to give a final concentration of DMSO applied to the bathing solution was 0.1%. Nifedipine and verapamil were dissolved in ethanol to give a final concentration of DMSO applied to the bathing solution was 0.1%. 5.5, tetrodotoxin (TTX) 0.01, and HEPES-CsOH buffer 5, pH 7.3. In the experiments in which the cells were held at −80 mV, the bath was perfused with the following solution (in mmol/L) to block the voltage-dependent Na\(^{+}\) current: tetraethylammonium chloride (TEA-Cl) 140, BaCl\(_2\) 5, MgCl\(_2\) 0.53, glucose 5.5, tetrodotoxin (TTX) 0.01, and HEPES-CsOH buffer 5, pH 7.4. Trogolitazone was added to the pipette solution. (+)-Isoproterenol, cAMP, verapamil, and nifedipine were purchased from Sigma.

**Recording Technique and Data Analysis**

Membrane currents were recorded with patch electrodes in a whole-cell clamp configuration\(^{27,28}\) and a patch-clamp amplifier (EPC-7, List Electronics). The heat-polished patch electrodes had a tip resistance of 3 to 6 MΩ. The membrane currents were monitored with a high-gain storage oscilloscope (COS 5020-ST, Kikkasu Electronics). At the start of each experiment, the series resistance was compensated. The data were stored on video cassettes with a PCM converter system (RP-880, NF electronic circuit design). Later, the data were reproduced, low-pass–filtered at 2 kHz (−3 dB) with a Bessel filter (FV-665, NF, 48-DB/octave slope attenuation), sampled at 5 kHz, and analyzed off-line on a computer with p-Clamp software (Axon Instruments). In general, we used a holding potential of −40 mV at a frequency of 0.2 Hz to inactivate the voltage-dependent Na\(^{+}\) current. In experiments to evaluate the contribution of \(I_{\text{Ca,L}}\) or voltage-dependence of the drug, a holding potential of −80 mV was used in combination with the high-TEA solution containing Ba\(^{2+}\) 5 mmol/L in place of Ca\(^{2+}\) (see Methods). Statistical results are expressed as mean±SD. Student’s \(t\) tests were performed, with a value of \(P<0.05\) considered significant.

The first data were usually taken after the current amplitude of Ca\(^{2+}\) currents had been stabilized (2 to 3 minutes after the rupture of the membrane). After that, we could investigate the effects of drugs on the voltage-dependent Ca\(^{2+}\) currents for ~15 to 20 minutes. In experiments with cAMP, data were taken immediately after the rupture of the membrane. To measure the amplitude of the voltage-dependent Ca\(^{2+}\) currents, we subtracted from the peak amplitude of Ca\(^{2+}\) currents the amplitude of the Ca\(^{2+}\) currents with conditioning pulse and that without conditioning pulse was plotted as lines in A. Amplitude of \(I_{\text{Ca,L}}\) was measured from zero current level. Current traces obtained at times indicated by a through f in B are shown in A (a through f).

**Results**

Effects of Troglitazone on Voltage-Dependent \(I_{\text{Ca,L}}\)

The effects of troglitazone on the voltage-dependent \(I_{\text{Ca,L}}\) were examined in single atrial myocytes (Figure 1). The membrane potential was held at −40 mV, and command voltage pulses (320 ms in duration) to +10 mV were applied at 0.2 Hz. In control cells, a transient inward current was elicited during each voltage pulse. The inward current was

![Figure 1](image-url)
Effects of troglitazone (Tro) on voltage-dependent $I_{\text{Ca,L}}$. Cell was held at $-40\,\text{mV}$, and command voltage pulses (320 ms in duration) were applied at 0.2 Hz to various membrane potentials. In A, original current traces are shown in control (a) and in presence of troglitazone 5 μmol/L (b) and 30 μmol/L (c). Current-voltage relationships of $I_{\text{Ca,L}}$ peak in absence and presence of troglitazone 5 and 30 μmol/L obtained by subtraction from $I_{\text{Ca,L}}$ peak amplitude in original trace (A) to current level in presence of Cd²⁺ (1 mmol/L) are shown in B.

Figure 2. Effects of troglitazone (Tro) on voltage-dependent $I_{\text{Ca,L}}$. Cell was held at $-40\,\text{mV}$, and command voltage pulses (320 ms in duration) were applied at 0.2 Hz to various membrane potentials. In A, original current traces are shown in control (a) and in presence of troglitazone 5 μmol/L (b) and 30 μmol/L (c).

Current-voltage relationships of $I_{\text{Ca,L}}$ peak in absence and presence of troglitazone 5 and 30 μmol/L obtained by subtraction from $I_{\text{Ca,L}}$ peak amplitude in original trace (A) to current level in presence of Cd²⁺ (1 mmol/L) are shown in B.

Troglitazone 30 μmol/L almost completely blocked $I_{\text{Ca,L}}$. These results suggest that troglitazone inhibited $I_{\text{Ca,L}}$ in cardiac myocytes. The effects of various concentrations of troglitazone on the amplitude of $I_{\text{Ca,L}}$ are shown in Figure 3. The cells were held at $-40\,\text{mV}$, and the command pulses to $+0\,\text{mV}$ were applied at 0.2 Hz. Troglitazone at concentrations $>1$ μmol/L decreased $I_{\text{Ca,L}}$, and the half-maximal inhibitory concentration (IC₅₀) of troglitazone on $I_{\text{Ca,L}}$ was 6.3 μmol/L.

Figures 4 and 5 illustrate the effects of troglitazone on isoproterenol- and cAMP-enhanced $I_{\text{Ca,L}}$. Isoproterenol 1 μmol/L increased the amplitude of $I_{\text{Ca,L}}$ (Figure 4, b). Immediately after application of isoproterenol, there was a rapid small increase in $I_{\text{Ca,L}}$. Probably reflected by direct activation of the GTP-binding proteins (Gₛ), then a large increase in $I_{\text{Ca,L}}$ was observed. The additional application of troglitazone 30 μmol/L completely abolished $I_{\text{Ca,L}}$ (Figure 4, c).

Moreover, when cAMP was applied through the patch pipette, $I_{\text{Ca,L}}$ increased from $-370$ to $-1080\,\text{pA}$ in this cell.
Effects of Troglitazone on the Voltage-Dependent \( I_{\text{Ca,L}} \) and \( I_{\text{Ca,T}} \)

The existence of 2 distinct Ca\(^{2+}\) currents has been shown for cardiac myocytes in several kinds of mammalian hearts.\(^{30-33}\) \( I_{\text{Ca,T}} \) activates at low voltages and inactivates quickly; \( I_{\text{Ca,L}} \) activates at high voltages and inactivates slowly. In addition, the T-type Ca\(^{2+}\) channel is about equally permeable to Ca\(^{2+}\) and Ba\(^{2+}\) ions and has the same inactivation kinetics in Ba\(^{2+}\) as in Ca\(^{2+}\); the L-type Ca\(^{2+}\) channel is more permeable to Ba\(^{2+}\) and has a dramatically slower inactivation time in Ba\(^{2+}\) than Ca\(^{2+}\). To clarify whether both types of Ca\(^{2+}\) currents can be identified in guinea pig atrial myocytes, we carried out tests under the conditions in which extracellular Na\(^{+}\) ions were replaced by impermeable TEA\(^{+}\) ions, and 5 mmol/L BaCl\(_2\) was added in place of Ca\(^{2+}\). Sodium removal induced cell contracture, but under our conditions with EGTA 10 mmol/L and BAPTA 2 mmol/L in the patch pipette, the cell attached to the patch electrode survives, probably owing to the diffusion of EGTA and BAPTA into the cytosol. The cells were held at \(-40\) or \(-80\) mV (Figure 6A), and command voltage steps (320 ms in duration) were applied to various membrane potentials. The current-voltage relationships of the peak inward current are shown in Figure 6C. At a holding potential of \(-40\) mV, the inward current was elicited at positive potentials to \(-30\) mV (Figure 6A, right). A small fraction of current was inactivated at the command pulses to \(-20\) and \(-10\) mV. Conversely, when the cell was held at \(-80\) mV, the transient inward current was recorded at a command potential of \(-30\) mV and was overlapped on the noninactivated component at a command potential of \(-20\) mV (Figure 6A, left). The current traces subtracted from the current of a holding potential of \(-80\) mV to that of a holding potential of \(-40\) mV at command potentials of \(-30, -20,\) and \(+0\) mV are shown in Figure 6B. The transient inward current rapidly inactivated within 50 ms and could be discriminated from the sustained component. Cd\(^{2+}\) 1 mmol/L abolished both types of inward current, but nifedipine 1 \(\mu\)mol/L (data not shown) failed to inhibit the transient component. These findings suggest that both types of Ca\(^{2+}\) currents in guinea pig atrial myocytes, A, Cell was held at \(-40\) mV (V\(_h\) = \(-40\) mV) and \(-80\) mV (V\(_h\) = \(-80\) mV), respectively, and command voltage pulses (V\(_c\), 320 ms in duration) were applied at 0.2 Hz to various membrane potentials. Bath was perfused with high-TEA solution with TTX 10 \(\mu\)mol/L in place of Na\(^+\). Extracellular Ca\(^{2+}\) was totally replaced by 5 mmol/L Ba\(^{2+}\), and patch pipette contained CsCl internal solution with 10 mmol/L EGTA and 2 mmol/L BAPTA. Original current traces obtained at a holding potential of \(-40\) and \(-80\) mV are shown at various command voltage steps. B, Current traces subtracted from current of a holding potential of \(-80\) to that of \(-40\) mV at a command potential of \(-30, -20,\) and \(+0\) mV. C, Current-voltage relationship of peak of voltage-dependent Ca\(^{2+}\) current are shown for a holding potential of \(-40\) mV (C) and \(-80\) mV (C). Note that transient component (arrows) was observed at command steps \((-40\) and \(-20\) mV) from a holding potential of \(-80\) mV.
currents exist in guinea pig atrial myocytes. The fast inward current consisted of \( I_{\text{Ca,T}} \) and the slow component consisted primarily of \( I_{\text{Ca,L}} \). Figure 7 shows the effects of troglitazone on both types of \( \text{Ca}^{2+} \) currents. \( I_{\text{Ca,T}} \) and \( I_{\text{Ca,L}} \) were elicited at a command voltage to \(-30\) and \(+10\) mV from a holding potential of \(-80\) mV, respectively. Troglitazone \( 10 \mu\text{mol/L} \) inhibited both types of \( \text{Ca}^{2+} \) currents (Figure 7A and 7B) but inhibited \( I_{\text{Ca,L}} \) more effectively than \( I_{\text{Ca,T}} \) (Figure 7B).

**Effects of Troglitazone on the Kinetic Parameters of the Voltage-Dependent \( I_{\text{Ca,L}} \).** Figure 8 shows the effects of troglitazone on the inactivation time courses of \( I_{\text{Ca,L}} \). Under conditions in which the cell was perfused with normal Tyrode’s solution, the inactivation time courses of \( I_{\text{Ca,L}} \) were well fitted by the sum of 2 exponentials (Figure 8A and Table) as previously described. Troglitazone \( 10 \mu\text{mol/L} \) did not affect the time courses of inactivation of \( I_{\text{Ca,L}} \) significantly (Figure 8 and Table). The differences between the values of \( \tau_1 \) and \( \tau_2 \) in the control and those in the presence of troglitazone were not statistically significant.

The use-dependent block of troglitazone was also examined and compared with the classic \( \text{Ca}^{2+} \) antagonists verapamil and nifedipine as shown in Figure 9. The changes in the amplitude of \( I_{\text{Ca,L}} \) elicited by successively applied command pulses were measured in the absence or presence of each drug with a test depolarizing pulse to \(+0\) mV from a holding potential of \(-40\) mV at 0.2 Hz. The amplitude of \( I_{\text{Ca,L}} \) recorded by the last pulse of a train stimulation before application of the agents (Figure 9A through D) was normalized to 1.0. In control conditions (Figure 9A), the amplitude of \( I_{\text{Ca,L}} \) elicited by the first command pulse (b) was not inhibited and remained nearly constant during the successive repetitive pulses (c). The small decrease of the current (8 ± 3% of the first pulse, \( n=5 \)) during 1-minute application of repetitive stimulation was thought to be induced simply by \( \text{Ca}^{2+} \) channel rundown. Verapamil (1 \( \mu\text{mol/L} \), Figure 9B) produced very little inhibition of \( \text{Ca}^{2+} \) current in the absence of test pulses (b), but blockade increased with repeated depolarizations (c). Conversely, in studies with the same pulse protocol, nifedipine blockade was different (Figure 9C). The first current after the quiescent period gave a good estimate of the final level of blockade (b and c). Figure 9D shows the use-dependent effects of troglitazone \( 10 \mu\text{mol/L} \). As in the case of nifedipine, the inward current elicited by the

**Effects of Troglitazone on the Time Courses of Inactivation of the Voltage-Dependent \( I_{\text{Ca,L}} \) in Atrial Myocytes**

<table>
<thead>
<tr>
<th>Condition</th>
<th>( A_1 ), pA</th>
<th>( \tau_1 ), ms</th>
<th>( A_2 ), pA</th>
<th>( \tau_2 ), ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>(-705 \pm 376)</td>
<td>(4.8 \pm 0.8)</td>
<td>(-419 \pm 68)</td>
<td>(31.2 \pm 2.4)</td>
</tr>
<tr>
<td>Troglitazone</td>
<td>(-336 \pm 237^*)</td>
<td>(5.4 \pm 0.9)</td>
<td>(-246 \pm 126^*)</td>
<td>(33.3 \pm 6.0)</td>
</tr>
</tbody>
</table>

\( A_1, \tau_1 \) indicate amplitude and time constant of the fast component of \( I_{\text{Ca,L}} \); \( A_2, \tau_2 \) amplitude and time constant of the slow component of \( I_{\text{Ca,L}} \). Data were obtained from 6 different atrial cells (mean ± SD).

\(^*P<0.05\) vs control.
first command pulse after a 2-minute quiescent period was consistently inhibited by 63±10% (n=5, P<0.01), which was different from that recorded with verapamil. With the repetitive stimulations, the inward current decreased slightly, by 13±7% (n=5) from the first pulse during repetitive stimulation (Figure 9D, b), but could not discriminate the simple rundown of the Ca²⁺ channel. These results suggest that troglitazone inhibited \textit{I}\textsubscript{Ca,L} mainly in a use-independent manner.

The influence of the holding potential on the inhibitory effects of nifedipine and troglitazone was compared as shown in Figure 10. In these experiments, the command voltage steps (320 ms in duration) to +10 mV from a holding potential of –80 mV (V\textsubscript{h} = –80 mV) and –40 mV (V\textsubscript{h} = –40 mV), respectively, and command voltage pulses (V\textsubscript{c}, 320 ms in duration) were applied at 0.2 Hz to +10 mV. Bath was perfused with TEA solution with TTX 10 μmol/L, and 5 mmol/L Ba²⁺ was added to bath solution in place of Ca²⁺.

In B, percentage inhibition of \textit{I}\textsubscript{Ca,L} by nifedipine and troglitazone is shown at each holding potential. Amplitude of \textit{I}\textsubscript{Ca,L} peak after application of these agents was compared with control level. Mean±SD is indicated (n=5 in each case). *P<0.05.
maximal Ca\(^{2+}\) channel availability (0.69±0.05 of the control, n=5), with a significant shift of the curve toward the negative (−18±4 mV, n=5). Conversely, in the absence of troglitazone 10 μmol/L, f\(_{\text{max}}\)=1, a=−21.3 mV, and b=5.33 mV. In the presence of troglitazone, f\(_{\text{max}}\)=0.39, a=−22.3 mV, and b=6.0 mV (Figure 11B). Thus, troglitazone reduced the maximal Ca\(^{2+}\) channel availability (0.56±0.1 of control, n=5) but did not show any significant shift of the voltage-dependent inactivation curve (−23.4±3.4 mV in the control versus −25.9±5.0 mV in the presence of troglitazone, n=5, P=NS).

**Discussion**

We have demonstrated here that troglitazone had inhibitory effects on voltage-dependent Ca\(^{2+}\) currents in single atrial myocytes from the guinea pig. The inward Ba\(^{2+}\) current in place of Ca\(^{2+}\) could easily be divided into 2 components with distinct physiological properties, as described in mammalian cardiac myocytes.\(^{30–33}\) One component had characteristics identical to the I\(_{\text{Ca,L}}\), including slow inactivation (Figure 6) and sensitivity to dihydropyridine (Figures 9 and 10) and isoproterenol (Figure 4). The second component, I\(_{\text{Ca,T}}\), had a fast inactivation (Figures 6 and 7) and small amplitude (≈−100 pA) even in the presence of Ba\(^{2+}\) and insensitivity to dihydropyridine (nifedipine 1 μmol/L) and isoproterenol (data not shown). We found that troglitazone inhibited both types of voltage-dependent Ca\(^{2+}\) currents in atrial myocytes, although it inhibited I\(_{\text{Ca,T}}\) more effectively than I\(_{\text{Ca,L}}\). Thus, the effects of troglitazone on Ca\(^{2+}\) currents might not be restricted to L-type Ca\(^{2+}\) channels, in comparison with the classic Ca\(^{2+}\) channel antagonists nifedipine and verapamil, because nifedipine and verapamil 1 μmol/L did not inhibit I\(_{\text{Ca,T}}\) significantly (data not shown). Troglitazone 1 μmol/L reduced I\(_{\text{Ca,L}}\) by 10% to 20% within 2 minutes of application, and 10 μmol/L troglitazone reduced it by 60% to 80%. Troglitazone 30 μmol/L almost completely abolished I\(_{\text{Ca,L}}\), and the IC\(_{50}\) value was estimated at 6.3 μmol/L. The inhibitory potency of troglitazone on I\(_{\text{Ca,L}}\) was less than that of nifedipine and verapamil. However, because the therapeutic plasma concentration of troglitazone was estimated to be 0.6 to 2.7 μmol/L,\(^{35}\) these concentrations are nearly the same as those required for the inhibition of I\(_{\text{Ca,L}}\) in this study. Thus, troglitazone may affect cardiac function by inhibiting the channel. The direct evidence showing that troglitazone inhibits I\(_{\text{Ca,L}}\) has been shown in vascular smooth muscle cells.\(^{25,26}\) The IC\(_{50}\) of troglitazone on I\(_{\text{Ca,L}}\) of vascular smooth muscle cells was ≈3 μmol/L,\(^{26}\) which was relatively lower than that in the present study. However, we conclude that troglitazone inhibited the L-type Ca\(^{2+}\) channels in cardiac myocytes as well as vascular smooth muscle cells in therapeutic concentrations.

It has been reported that voltage-dependent L-type Ca\(^{2+}\) channel blockers such as verapamil and diltiazem prevent the development of diabetic cardiomyopathy.\(^{16–18}\) These cardioprotective effects of Ca\(^{2+}\)-blocking drugs have also been reported in Syrian cardiomyopathic hamsters\(^{36}\) and in patients with hypertrophic cardiomyopathy.\(^{37}\) Therefore, the mode of action of troglitazone on I\(_{\text{Ca,L}}\) was compared with that of the classic Ca\(^{2+}\) antagonists verapamil and nifedipine. As shown in Figures 10 and 11, troglitazone reduced I\(_{\text{Ca,L}}\) but did not cause a significant shift in the steady-state inactivation curve. Conversely, nifedipine, a dihydropyridine Ca\(^{2+}\) antagonist, which has a high affinity for the inactivated state of the channel but much less affinity for other states (eg, closed, open), showed strong voltage-dependent effects and caused a distinct negative shift of the steady-state inactivation curve. Thus, it is unlikely that troglitazone inhibits I\(_{\text{Ca,L}}\) by preferentially binding the inactivated states of the channels. Also, troglitazone did not exhibit significant use-dependent characteristics, which was different from verapamil (Figure 9), as previously described.\(^{38}\) Potencies of the use-dependent inhibition might be closely related to the ionization constants of the drug as shown by Sanguinetti and Kass.\(^{39}\) According to this model, charged forms of the drug can reach their receptors inside the channel by a hydrophilic pathway available only when the channel gates are open and hence are characterized by a significant use-dependent block. In con-
trast, an uncharged form of the drug easily reaches its receptors via a hydrophobic region of the membrane without channel opening and thereby does not show significant use-dependent effects. Verapamil (pKₐ=8.7) is almost entirely in the charged form at pH 7.4, whereas troglitazone (pKₐ=6.1)⁴⁰ exists almost entirely in the neutral form at the same pH. Thus, opening of the channels may not be necessary for troglitazone to affect IC₅₀, as shown in Figure 9. Furthermore, the time courses of Ca²⁺ current decay were little affected by troglitazone. From these observations, troglitazone did not appear to inhibit the Ca²⁺ channels by binding to activated Ca²⁺ channels. Thus, the mechanisms by which troglitazone affects the voltage-dependent Ca²⁺ channels are unknown at present, but troglitazone may interact with L-type Ca²⁺ channels in a manner distinct from the classic Ca²⁺ antagonists.⁴¹

The present study indicates that troglitazone inhibited the voltage-dependent Ca²⁺ currents (I_Ca,L and I_Ca,T) in cardiac myocytes in therapeutic concentrations. Under normal circumstances, the current through the T-type Ca²⁺ channel is unlikely to be very important in atrial and ventricular myocytes, because in a well-polarized cell, such as atrial and ventricular cells, Na⁺ current is much larger and activates in a similar voltage range. Also, because L-type Ca²⁺ channels inactivate more slowly, they are likely to be more important than T-type channels. However, T-type Ca²⁺ channels may contribute to the generation of pacemaker activities in pacemaker cells⁴² and may make hyperpolarized ventricular myocytes more prone to spontaneous action potentials and increase the likelihood of arrhythmia in partially depolarized hypertrophied myocardium.⁴³ Troglitazone may affect the electrical activities under these conditions by inhibiting I_Ca,T. Conversely, troglitazone inhibited I_Ca,L more effectively than I_Ca,T. The inhibitory effects of troglitazone on I_Ca,L did not show significant voltage- and use-dependent properties as observed in classic Ca²⁺ antagonists.⁴⁶ From these unique actions, troglitazone may inhibit cardiac Ca²⁺ channels in a similar way in well-polarized cells as well as in partially depolarized cells. Also, it may antagonize the effects of isoproterenol on I_Ca,L. Several studies have shown that in diabetic animals, the duration of action potential in cardiac myocytes is markedly longer, whereas the resting membrane potential is not altered.⁹–¹² In addition, an augmented number of Ca²⁺ antagonist receptor binding sites and an increase of voltage-dependent L-type Ca²⁺ channels have been reported in diabetic hearts.¹³,¹⁴ The increased influx of Ca²⁺ through the voltage-dependent Ca²⁺ channels may cause Ca²⁺ overload, which appears to be linked to the cardiac pathology in diabetic cardiomyopathy.¹⁶–¹⁸ The present study shows that troglitazone inhibits voltage-dependent Ca²⁺ currents (I_Ca,T and I_Ca,L) and then antagonizes the effects of isoproterenol in diabetic cardiomyocytes, which may play a role in preventing diabetes-induced intracellular Ca²⁺ overload and then myocardial changes. In fact, recent studies have shown that troglitazone attenuates high-glucose–induced abnormalities in relaxation and intracellular calcium in rat ventricular myocytes⁴³ and improves cardiac function in diabetes mellitus.⁴⁴ From these observations, troglitazone may be a unique agent for diabetic cardiomyopathy, but further studies are needed to clarify this possibility in diabetic patients.

References


Troglitazone Inhibits Voltage-Dependent Calcium Currents in Guinea Pig Cardiac Myocytes
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