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Effects of troglitazone and pioglitazone on the action potentials and membrane currents of rabbit ventricular myocytes

Shota Ikeda *, Toshifumi Watanabe

Pharmaceutical Research Laboratories, Takeda Chemical Industries, 17-85, Juso-Honmachi 2 Chome, Yodogawa-ku, Osaka 532-8686, Japan

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Abstract

The effects of the antidiabetic thiazolidinediones troglitazone and pioglitazone on action potentials and membrane currents were studied in rabbit ventricular myocytes. Troglitazone (10 μM) reversibly reduced excitability of the myocytes and modified their action potential configuration. It significantly increased the stimulation threshold required to elicit action potentials and decreased action potential amplitude and the maximum upstroke velocity of the action potentials. The Inhibition of the maximum upstroke velocity by troglitazone was also significant at 1 μM. Voltage-clamp experiments revealed that troglitazone (10 μM) reversibly inhibited both the slow inward Ca^{2+} current and the steady-state K^+ current. In contrast to troglitazone, pioglitazone (1–10 μM) had no significant effect on the excitability, action potential configuration, or membrane currents of myocytes. These results suggest that troglitazone, but not pioglitazone, modulates Na^+, Ca^{2+} and K^+ currents, leading to the changes in excitability and action potential configuration of ventricular myocytes.

Keywords: Troglitazone; Pioglitazone; Action potential; Ion current; Ventricular myocyte; (Rabbit)

1. Introduction

Troglitazone and pioglitazone are orally active antidiabetic thiazolidinediones that increase tissue sensitivity to the actions of insulin (Hofmann et al., 1991; Kobayashi et al., 1992; Kellerer et al., 1994). Their antidiabetic effects have been confirmed both in NIDDM patients (Hofmann and Colca, 1992; Miura et al., 1994; Whitcomb and Saltiel, 1995) and in several diabetic animal models (Fujiwara et al., 1988; Ikeda et al., 1990). Although their actions on the peroxisomal proliferator-activated receptor have been suggested to be critical (Whitcomb and Saltiel, 1995), the precise mechanism of their antidiabetic effects has not been fully elucidated.

Recently, thiazolidinedione inhibition of certain ion currents has been reported as one possible mechanism of their antidiabetic properties. Lee et al. (1996) reported that troglitazone inhibits K_{ATP} channel activity in CRI-G1 insulin-secreting cells, suggesting a possible contribution to the stimulation of insulin secretion. Rowe et al. (1997) showed that englitazone acts as a blocker of the Ca^{2+}-activated non-selective cation channel and the K_{ATP} channels in CRI-G1 cells. Inhibition of the L-type calcium current by troglitazone (Song et al., 1997) and pioglitazone (Zhang et al., 1994) has been reported in vascular smooth muscle cells, suggesting that this action may contribute to the hypotensive effect of the thiazolidinediones observed in vivo. In addition, Lee and Boden (1997) reported that troglitazone inhibited type 2 K_{ATP} channel activity in rat neurons.

In spite of these reports, questions remain about the electrophysiological properties of thiazolidinediones. Because of the lack of comparable results in the same preparation, the differences in the electrophysiological properties among thiazolidinediones have not been described. The effects of thiazolidinediones on ion currents other than those described above are unknown. We therefore have conducted the present study to clarify (1) whether troglitazone and pioglitazone can modulate action potential configuration and ion currents in ventricular myocytes and (2) whether the electrophysiological effects of troglitazone and pioglitazone differ.

* Corresponding author. Tel.: +81-6-300-6679; Fax: +81-6-300-6306; E-mail: ikeda_shota@takeda.co.jp

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2. Materials and methods

2.1. Animals

Male New Zealand white rabbits (2–3.5 kg) were used in this study. They were housed in a room at a temperature of 25 ± 1°C under an alternating 12-h light/dark cycle (lights on at 0700). Food and water were given ad libitum. Experimental protocols were approved by Takeda Experimental Animal Care and Use Committee and performed in accordance with the Recommendations from the Declaration of Helsinki and the NIH Guide for the Care and Treatment of Laboratory Animals.

2.2. Isolation of rabbit ventricular myocytes

Single rabbit ventricular myocytes were isolated, in principle, by the method described previously (Watanabe et al., 1985). Male New Zealand white rabbits (2–3.5 kg) were heparinized (500 U/kg, i.v.) and anesthetized with sodium pentobarbital (40 mg/kg, i.v.). The excised heart was rapidly mounted on a Langendorff apparatus and perfused at 37°C with Ca^{2+}-free Hepes solution (in mM: NaCl, 136; KCl, 5.4; $MgCl_2$, 1.0; $NaHPO_4$, 0.33; dextrose, 10; Hepes, 10, pH adjusted to 7.3 with NaOH), followed by perfusion with enzyme Hepes solution containing 60 U/ml collagenase (Yakult, Tokyo, Japan) for 15 min. After washing out the enzyme with the Ca^{2+}-free Hepes solution, the ventricles were cut into small pieces. The myocytes were then dispersed mechanically and maintained in low Ca^{2+} Hepes solution containing 0.6 mM $CaCl_2$ at room temperature until used.

2.3. Whole-cell patch clamp

The membrane potentials and currents of rabbit ventricular myocytes were recorded by the conventional whole-cell clamp method with an amplifier (Axopatch-1B, Axon Instruments, Foster City, CA, USA) through suction pipettes (2–5 MΩ) filled with pipette solution (in mM: KCl, 140; KH_2PO_4, 0.33; EGTA, 1.0; HEPES, 10, pH adjusted to 7.3 with KOH). In a current-clamp mode, action potentials were elicited by injecting rectangular pulse currents (5 ms, ≤ 2 nA) with a stimulator (Nihon-Kohden, Tokyo, Japan) through recording pipettes. The elicited action potentials were displayed on a dual-beam storage oscilloscope (Tektronix 5113, Tektronix, Beaverton, OR, USA) and photographed. After determining the threshold stimulus current (the minimum strength of the depolarizing current required to initiate action potentials), stable action potentials were elicited by a 0.2 Hz stimulus current 5–10% above the threshold. Action potentials were electrically differentiated to obtain the maximum upstroke velocity. Action potential duration (APD_{50}) was measured at 50% repolarization. In a voltage-clamp mode, the slow inward calcium current ($I_{Ca,L}$) was measured with double-step depolarization pulses (−40 mV for 300 ms and subsequent 500 ms between −40 to +30 mV) with a holding potential of −80 mV. The magnitude of $I_{Ca,L}$ was defined as the difference between the peak inward current during the second pulse and the current at the end of the depolarization pulse. Steady-state potassium current was induced by a 20-s ramp pulse from −120 to +100 mV. This voltage command pulse generation and data acquisition were achieved using an IBM PC/AT computer with pCLAMP software (Axon Instruments). Currents were digitized at sampling intervals of 4.0 kHz and 20 Hz for the calcium and potassium current, respectively, and the digitized data were stored on the hard disk of the computer.

Myocytes were superfused (2–3 ml/min) with normal Ca^{2+} Hepes solution containing 1.2 mM $CaCl_2$ at room temperature. After the whole-cell clamp recording was established, myocytes were continuously stimulated at 0.2 Hz throughout the experiments. To minimize the 'run-down' of the $I_{Ca,L}$, experiments were started after a stabilizing period of 30 min (Wu et al., 1995). Drug- or vehicle-contained superfusate was applied for 20 min followed by a washout period of 20 min.

Based on preliminary experiments using 22 myocytes, we decided the experimental design as follows; 20 ventricular myocytes were divided into five groups (four myocytes in each group): a vehicle group, a 1 and 10 μM troglitazone group, and a 1 and 10 μM pioglitazone group. It should be noted that the preliminary results were basically the same as the present results. Because one myocyte in the troglitazone 10 μM-treated group lost viability (loss of rod shape, resting potential, and inward rectification of the background current) in the washout period, the data from that point onward in this group were obtained from only three myocytes. No myocytes in the other groups lost viability during the experiment.

2.4. Drugs

Troglitazone and pioglitazone were synthesized in our laboratories. They were dissolved in dimethlysulfoxide (DMSO) and diluted in the Hepes solution immediately before use. The final concentration of DMSO was 0.1% (v/v).

2.5. Statistical analysis

Data are expressed as means ± S.E.M. Dunnett's multiple comparison test was used to determine significant differences between the vehicle- and drug-treatment groups. When the difference in variance among groups was significant, data were logarithmically transformed. A value of $P < 0.05$ was considered significant.

3. Results

In the vehicle-treated group, stable action potentials were obtained throughout the experiments (Fig. 1A, right). The pretreatment values of threshold stimulus current, peak amplitude, and APD_{50} were 1.00 ± 0.05 nA, 114 ± 5 mV, and 374 ± 71 ms, respectively, in the vehicle-treated group (mean $\pm$ S.E.M., $n = 4$). In the myocytes treated with troglitazone at 10 μM, however, both the excitability and action potential configuration of the myocytes were

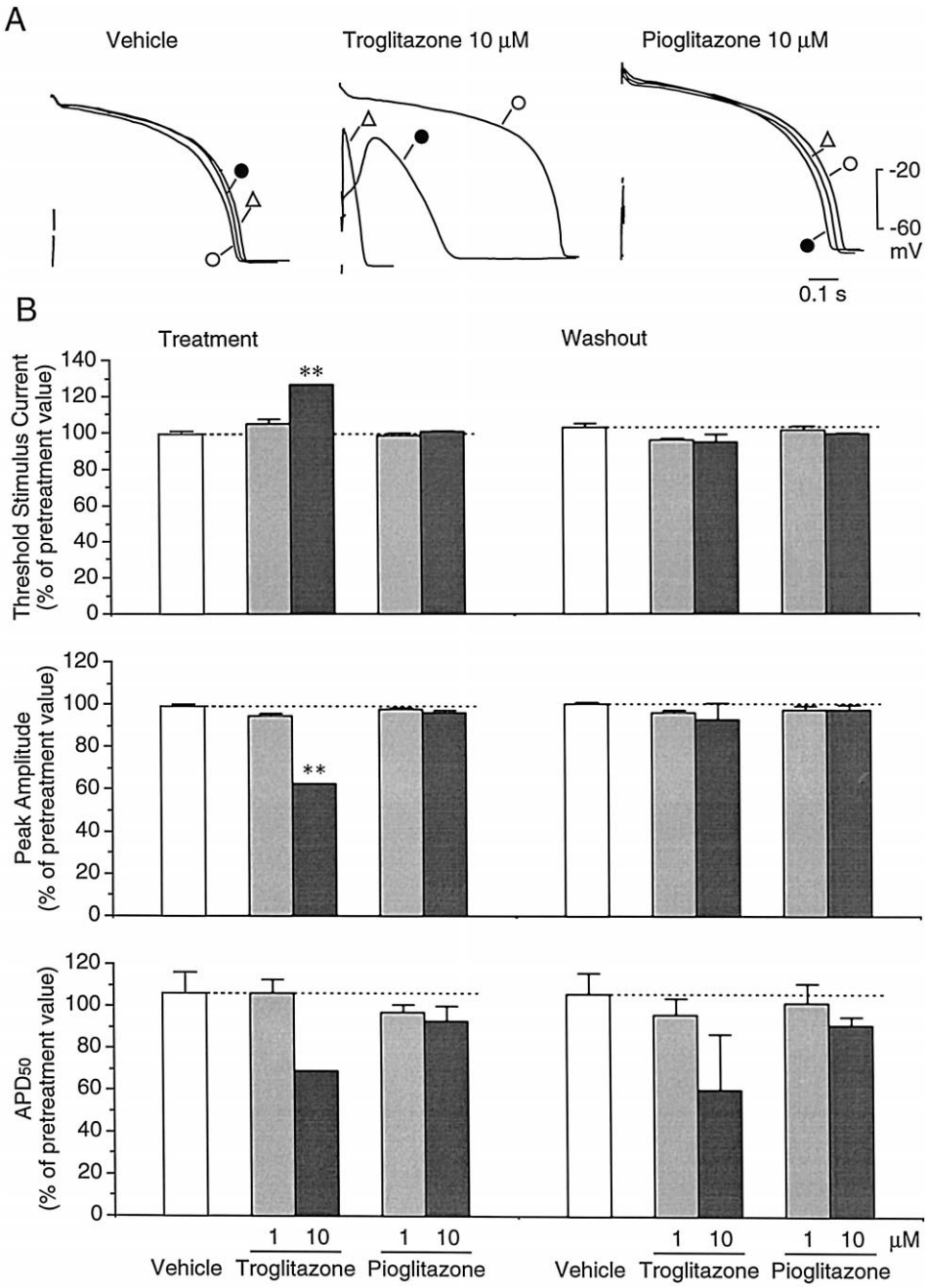

Fig. 1. Effects of troglitazone and pioglitazone on action potentials in rabbit ventricular myocytes. (A) Representative records of action potentials in myocytes treated with vehicle (left), troglitazone 10 μM (middle), and pioglitazone 10 μM (right). Action potentials were elicited by injecting rectangular pulses (5 ms) at 0.2 Hz. The superimposed records were obtained before (○), 20 min after (●) treatment, and 20 min after washout (△). (B) Effects of troglitazone and pioglitazone on threshold stimulus current (top), peak amplitude (middle), and duration (APD_{50}, bottom) of action potentials in rabbit ventricular myocytes. Parameters obtained 20 min after treatment (left) and 20 min after washout (right) were calculated as percentages of pretreatment values. The pretreatment values for threshold stimulus current, peak amplitude, and APD_{50} were 1.12 ± 0.05 nA, 117 ± 2 mV and 349 ± 39 ms, respectively (means $\pm$ S.E.M., $n = 20$). Data are expressed as means $\pm$ S.E.M. ($n = 3$–4), except for the data during treatment with troglitazone at 10 μM (mean without S.E.M., $n = 2$); in this group, only two of four myocytes were able to generate action potentials at maximum stimulation (2 nA). $** P < 0.01$ vs. vehicle.

dramatically changed (Fig. 1A, middle). In two of the four myocytes in this group, troglitazone at 10 μM completely suppressed the excitability of the myocytes, so that the maximum stimulation current (2 nA) failed to elicit any action potentials. Washout of the drug for 20 min completely reinstated the excitability of these myocytes. In the other two myocytes with troglitazone at 10 μM, the threshold stimulus currents increased significantly and reversibly during drug treatment (Fig. 1B). The peak action potential amplitude was significantly reduced and the APD_{50} was shortened by the treatment with troglitazone at 10 μM (Fig. 1A and B). The change in APD_{50} was hardly recovered at all after the washout period. In contrast to troglitazone, pioglitazone had no effect on excitability and action potential configuration (Fig. 1A, right). All myocytes treated with pioglitazone (1–10 μM) generated stable action potentials throughout the experiments and showed no changes in threshold stimulus currents, peak amplitude, or APD_{50} (Fig. 1B). In addition, the resting membrane potentials of the myocytes (pretreatment value: -77 ± 1 mV, $n = 20$, mean ± S.E.M.) were unchanged by the vehicle or drug treatment.

Fig. 2 shows effects of troglitazone and pioglitazone on the maximum upstroke velocity of the action potential. Troglitazone (1–10 μM) significantly decreased the maximum upstroke velocity of the action potential in a concentration-dependent manner (Fig. 2B). At the low concentration of 1 μM troglitazone significantly reduced the maximum upstroke velocity to less than 50% (47 ± 5%, % of pretreatment value, $P < 0.01$ vs. vehicle, Fig. 2B). The inhibitory effects of troglitazone were partially reversed by washout of the drug. In contrast to troglitazone, pioglitazone (1–10 μM) had little effect on the maximum upstroke velocity of the action potential (Fig. 2B). Although

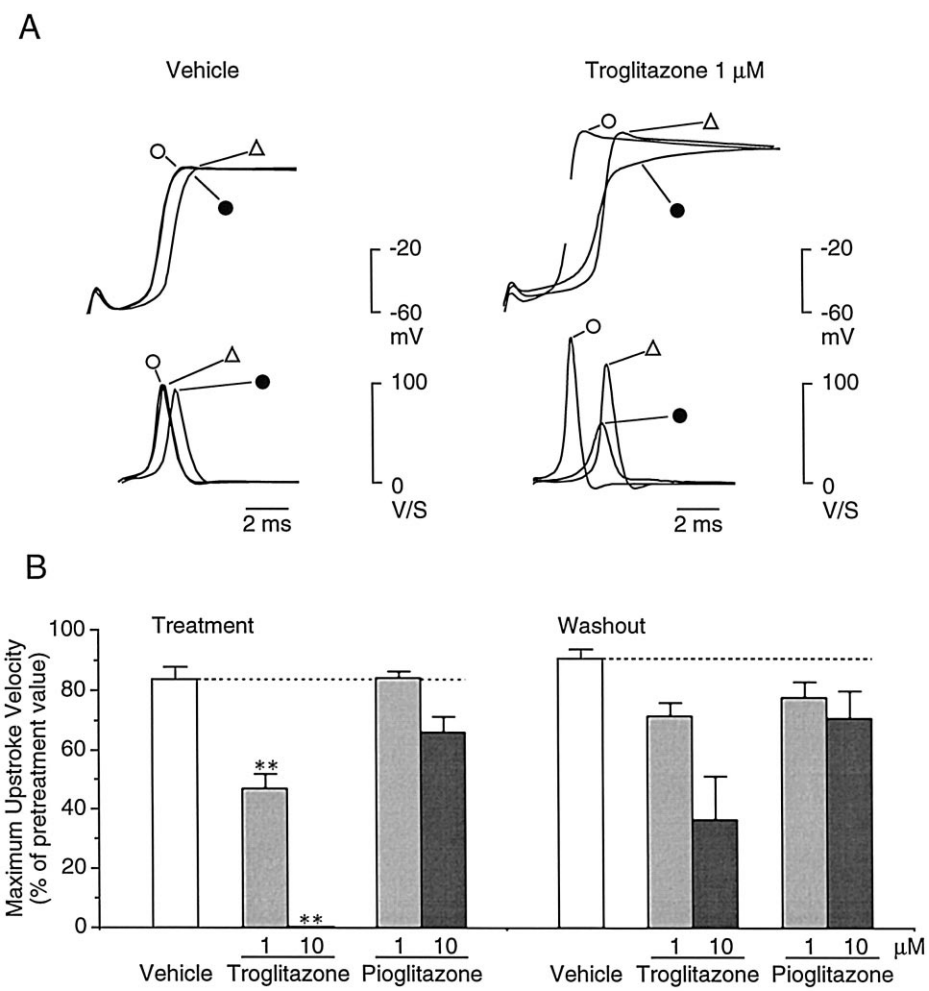

Fig. 2. Effects of troglitazone and pioglitazone on the upstroke velocity of action potentials in rabbit ventricular myocytes. (A) Representative records of early depolarization phases (top) and upstroke velocity (bottom) of action potentials in the myocytes treated with vehicle (left) and troglitazone at 1 μM (right). The superimposed records were obtained before (○), 20 min after (●) treatment, and 20 min after washout (△). (B) The maximum upstroke velocity of the action potentials 20 min after treatment (left) and 20 min after washout (right). Data were calculated as percentages of the pretreatment values (100 ± 6 V/s, mean ± S.E.M., $n = 20$). Means ± S.E.M. ($n = 3$–4) are shown except for the data during treatment with troglitazone at 10 μM (mean without S.E.M., $n = 2$, see the legend of Fig. 1). $**$ $P < 0.01$ vs. vehicle.

10 μM pioglitazone reduced the maximum upstroke velocity slightly, the degree of the effect was not statistically significant.

Effects of the drugs on the slow inward calcium currents ($I_{Ca,L}$) were investigated (Fig. 3). In the voltage-clamp mode at a holding potential of −80 mV, $I_{Ca,L}$ was elicited by double-step depolarization. The maximum inward currents were elicited at 0 mV (Fig. 3A). Although the amplitude of $I_{Ca,L}$ gradually decreased with time ('rundown' phenomenon), stable $I_{Ca,L}$ values were obtained in vehicle-treated myocytes throughout the experiments. The amplitude of maximum $I_{Ca,L}$ at the end of the experiment was 89 ± 8% of the pretreatment value ($n = 4$, mean ± S.E.M.). As shown in Fig. 3A (middle) and B, troglitazone at 10 μM significantly decreased the amplitude of the maximum $I_{Ca,L}$ (48 ± 15%, % of pretreatment

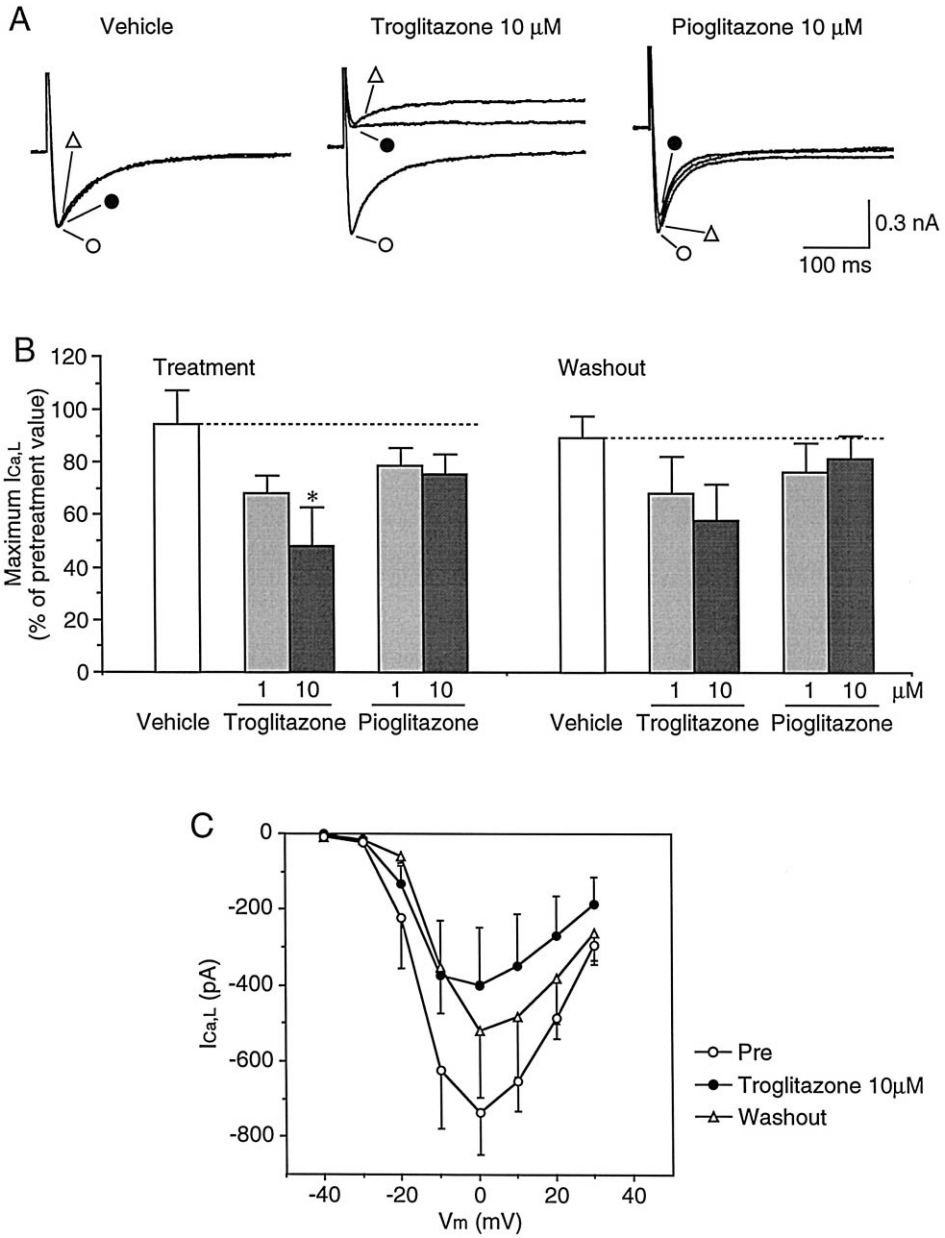

Fig. 3. Effects of troglitazone and pioglitazone on the slow inward calcium current ($I_{Ca,L}$) in rabbit ventricular myocytes. (A) Representative records of $I_{Ca,L}$ in myocytes treated with vehicle (left), troglitazone 10 μM (middle), and pioglitazone 10 μM (right). $I_{Ca,L}$ was elicited by double-step depolarization from −80 to −40 mV for 300 ms and subsequent 500 ms step pulses of 0 mV. The superimposed records were obtained before (○), 20 min after (●) treatment, and 20 min after washout (△). (B) The maximum $I_{Ca,L}$ 20 min after treatment (left) and 20 min after washout (right). Data (mean ± S.E.M., $n = 3$–4) are expressed as percentages of pretreatment values (598 ± 41 pA, mean ± S.E.M., $n = 20$). * $P < 0.05$ vs. vehicle. (C) Current–voltage (V_m) relations for $I_{Ca,L}$ in the troglitazone (10 μM)-treated myocytes. $I_{Ca,L}$ was elicited by double-step depolarization from −80 to −40 mV for 300-ms and subsequent 500-ms step pulses between −40 to +30 mV before (○), 20 min after (●) treatment, and 20 min after washout (△). Data are expressed as means ± S.E.M. ($n = 3$–4).

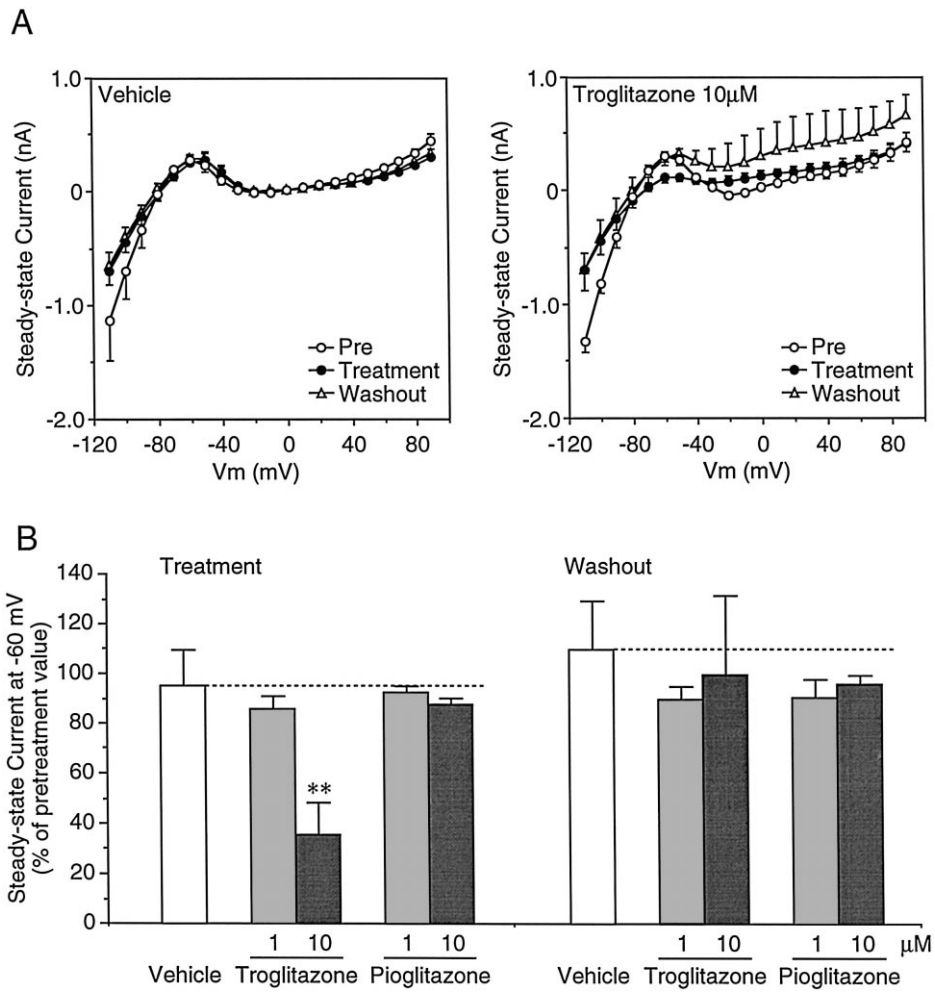

Fig. 4. Effects of troglitazone and pioglitazone on the steady-state potassium currents in rabbit ventricular myocytes. (A) Current–voltage (V_m) relations for the steady-state potassium current in the vehicle (left)- and troglitazone (10 μM, right)-treated myocytes. The currents were induced by a 20-s ramp pulse from −120 to +100 mV before (○), 20 min after (●) treatment, and 20 min after washout (△). (B) The steady-state potassium current at −60 mV 20 min after treatment (left) and 20 min after washout (right). Data (mean ± S.E.M., $n = 3–4$) are expressed as percentages of pretreatment values (319 ± 18 pA, mean ± S.E.M., $n = 20$). ** $P < 0.01$ vs. vehicle.

value, $P < 0.05$ vs. vehicle, mean ± S.E.M., $n = 4$). The inhibitory effects of 10 μM troglitazone on $I_{Ca,L}$ were partially reversed after the washout period (Fig. 3A, middle) and did not show strong voltage-dependency (Fig. 3C). Pioglitazone at 1–10 μM, however, had no significant effects on $I_{Ca,L}$ (Fig. 3A, right and Fig. 3B).

Fig. 4 shows the effects of the drugs on the steady-state potassium current. In pretreatment myocytes, a ramp-pulse protocol of 20 s from −120 to +100 mV revealed 'N-shaped' current–voltage relationship of the current, i.e., strong inward rectification and an outward 'hump' between −70 mV to −30 mV (Fig. 4A). The reversal potential of the current was −79 ± 1 mV ($n = 20$, mean ± S.E.M.) in the pretreatment myocytes near to the equilibrium potential of potassium ions under the experimental conditions. In vehicle-treated myocytes, the currents were stably elicited throughout the experiments (Fig. 4A, left). Troglitazone at 1 μM and pioglitazone at 1–10 μM did not modify this current, while troglitazone at 10 μM changed the current–voltage relationship of the current (Fig. 4A, right). The magnitude of the outward current at −60 mV was reversibly and significantly decreased by troglitazone at 10 μM (Fig. 4B). The outward current at membrane potentials positive to −40 mV, however, increased slightly 20 min after treatment, and increased even more 20 min after the washout (Fig. 4A).

4. Discussion

The principal findings of this study are: (1) troglitazone changes both the excitability and action potential configuration of rabbit ventricular myocytes; and in particular, greatly reduces the maximum upstroke velocity of the action potential; (2) troglitazone inhibits L-type calcium

and steady-state potassium currents in the myocytes; (3) in contrast to troglitazone, pioglitazone has little effect on myocyte excitability, action potential configuration, and membrane currents.

One of the intriguing findings of this study was troglitazone inhibition of the upstroke velocity of the action potential. At the low concentration of 1 μM, troglitazone significantly reduced the maximum upstroke velocity to less than 50% (Fig. 2B) without showing significant effects on any other parameters of the action potential, $I_{Ca,L}$, or the steady-state potassium current. Moreover, the maximum upstroke velocity was greatly suppressed by troglitazone at 10 μM. This finding strongly suggests that troglitazone inhibits the fast sodium inward current (I_{Na}) in rabbit ventricular myocytes, although the relation between the maximum upstroke velocity and sodium conductance is known to be nonlinear (Sheets et al., 1988). The suppression of myocyte excitability by troglitazone (Fig. 1) may be due to inhibition of I_{Na}, but not to an increase in the background outward current, since the steady-state outward potassium current at membrane potentials negative to −40 mV (around the threshold potential) was not increased by troglitazone (Fig. 4A, left). It should be noted that inhibition of sodium currents by troglitazone has not been described previously.

In contrast to troglitazone, pioglitazone did not have any significant effect on excitability, action potential configuration, or the membrane currents of rabbit ventricular myocytes. It is unlikely that the lack of electrophysiological effects of pioglitazone is due to the potency of pioglitazone being much less than that of troglitazone. The following observations indicate that pioglitazone is a more potent (or, at least, equipotent) antidiabetic insulin-sensitizing drug than troglitazone. Pioglitazone at 10 nM significantly increased insulin-stimulated autophosphorylation and kinase activity in IM-9 cells (Kobayashi et al., 1992), while troglitazone antagonized the glucose-induced inhibition of the insulin receptor kinase with half-maximum inhibition at 0.05 μg/ml (approximately 100 nM) in rat-1 fibroblasts (Kellerer et al., 1994). In Zucker fatty rats, the effective dose at which the two thiazolidinediones exhibited antidiabetic effects were reported to be 0.3–10 mg/kg for pioglitazone (Ikeda et al., 1990) and 100–150 mg/kg for troglitazone (Fujiwara et al., 1988). Thus, the differences between the electrophysiological effects of troglitazone and pioglitazone observed in the present study may be attributable to a difference in selectivity between the two drugs, but not to a difference in their potency: troglitazone can exert multiple effects other than insulin-sensitizing at high concentrations, while pioglitazone may exert antidiabetic insulin-sensitizing effects more selectively.

Some of the results of this study are inconsistent with findings reported previously. First, we failed to observe significant effects of pioglitazone on $I_{Ca,L}$, while Zhang et al. (1994) reported that pioglitazone at 5–10 μM inhibited $I_{Ca,L}$ in a7r5 vascular smooth muscle cells. This discrepancy may be due to the difference in kind of cells used (we used freshly dissociated ventricular myocytes in this study), since they failed to detect significant inhibition of $I_{Ca,L}$ by 10 μM pioglitazone in freshly dissociated tail artery vascular smooth muscle cells. Second, Lee et al. (1996) and Lee and Boden (1997) reported inhibition of K_{ATP} channel activity by troglitazone, whereas we can only report inhibition of the steady-state potassium current by troglitazone. Our experimental conditions, however, did not allow us to detect the inhibitory effects of drugs on K_{ATP} channel activity, since cardiac K_{ATP} channels are known to be inactivated under normal (not ischemic) conditions (Noma, 1983).

Although most of the effects of troglitazone observed in this study were reversible, the troglitazone-induced increase in steady-state outward potassium current was irreversible (Fig. 4A). The fact that damaged ventricular myocytes loose the inward rectification of the background current seems to suggest that troglitazone at high concentrations damages myocytes irreversibly to some extent. The observation that one myocyte treated with 10 μM troglitazone lost its viability in the washout period seems consistent with this possibility.

Does the electrophysiological effects of troglitazone on ventricular myocytes observed in the present study cause significant effects in vivo? In patients treated with therapeutic doses of troglitazone, maximum plasma concentrations (C_{max}) of the drug were reported to be 0.4–2.4 mg/l (corresponds to 0.9–5.5 μM, Spencer and Markham, 1997). It should be noted that the C_{max} are close to the effective concentration of troglitazone in the present study (1–10 μM). One may suggest that its free plasma concentration should be lower than the C_{max} because troglitazone bound to serum albumin with a high ratio (Spencer and Markham, 1997). Kawai et al. (1997), however, showed that the binding of troglitazone to serum albumin was reversible and that area under the concentration–time curve $(AUC)_{0-24\ h}$ of ^{14}C-labeled troglitazone in muscle was comparable to that in plasma, indicating that a significant amount of troglitazone can be incorporated to muscle. Taken together, It is possible that troglitazone at therapeutic doses can affect certain ion currents of ventricular myocytes in vivo. Interestingly, we found that troglitazone (10 μM), but not pioglitazone prolonged atrioventricular conduction time and reduced cardiac contractility in isolated perfused rabbit hearts (unpublished observations). In future studies, it will be interesting to examine the direct effects of troglitazone on cardiac functions in vivo.

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