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Comparative actions of insulin sensitizers on ion channels in vascular smooth muscle

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Abstract

Thiazolidinedione and isoxazolidinedione insulin sensitizers activate peroxisome proliferator-activated receptor γ (PPARγ). Some thiazolidinediones modify ion channels in smooth muscles; however, the mechanism by which their actions occur has not been clarified. We, thus, examined the effects of three thiazolidinediones (troglitazone, pioglitazone, and rosiglitazone) and isoxazolidinedione (JTT-501), as well as an intrinsic ligand for PPARγ, 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (prostaglandin J_2), on voltage-operated Ca^{2+} currents (I_{Ca}), voltage-dependent K^+ currents (I_{Kv}), and Ca^{2+}-activated K^+ currents (I_{Kca}), to clarify whether a thiazolidinedione structure or PPARγ activation is related to their actions on ion channels. The whole-cell patch clamp method was used to record currents in smooth muscle cells from guinea-pig mesenteric arteries. Thiazolidinediones inhibited I_{Ca} in a dose-dependent manner (troglitazone > pioglitazone = rosiglitazone). Troglitazone (≥ 1 μM) and rosiglitazone (100 μM), but not pioglitazone, inhibited I_{Kv}. Rosiglitazone (≥ 10 μM) enhanced, troglitazone (≥ 1 μM) inhibited, and pioglitazone did not affect I_{Kca}. A high concentration of JTT-501 (100 μM) inhibited I_{Ca}, I_{Kv}, and I_{Kca} to a similar extent. Prostaglandin J_2 enhanced I_{Kca}, but affected neither I_{Ca} nor I_{Kv}. In summary, the three thiazolidinediones and isoxazolidinedione act differently on Ca^{2+} and K^+ channels in vascular smooth muscle. The action of thiazolidinediones on I_{Ca} could be attributed to specific regions of the molecules and not to activation of PPARγ. Involvement of PPARγ activation in the stimulation of I_{Kca} is possible but should be tested further.

Keywords: Thiazolidinedione; Isoxazolidinedione; Ca^{2+} channel; K^+ channel; PPARγ (peroxisome proliferator-activated receptor γ)

1. Introduction

Thiazolidinediones, including troglitazone, pioglitazone, and rosiglitazone, have been developed as insulin sensitizers that reduce plasma glucose by increasing insulin sensitivity in target tissues (Ciaraldi et al., 1990; Hoffman et al., 1991; Oakes et al., 1994). These agents have been shown to lower blood pressure and increase blood flow in animals and humans (Yoshioka et al., 1993; Kemnitz et al., 1994; Buchanan et al., 1995; Kaufman et al., 1995; Ogihara et al., 1995; Walker et al., 1998; Fujishima et al., 1998). The mechanism by which such cardiovascular actions occur is unknown; however, an improvement of insulin resistance and a direct vasodilating action on the vasculature have been postulated. As regards vascular actions, studies using the patch–clamp method have shown that troglitazone, pioglitazone, and rosiglitazone inhibit L-type Ca^{2+} currents in vascular smooth muscle cells (Zhang et al., 1994; Nakamura et al., 1998; Knock et al., 1999). However, Knock et al. (1999) showed that rosiglitazone enhanced but troglitazone inhibited Ca^{2+} activated K^+ currents (I_{Kca}) in rat aortic cells. They also showed that both drugs inhibited delayed rectifier K^+ currents (I_{Kv}) in rat pulmonary artery muscle cells. JTT-501 (isoxazolidinedione) is a novel insulin sensitizer, which is structurally distinct from thiazolidinediones (Shibata et al., 1998). Thus, JTT-501 could be a useful tool for evaluating the mechanism of thiazolidinedione effects on ion channels; however, there are no reports regarding the action of JTT-501 on ion currents.

Thiazolidinediones and JTT-501 activate a nuclear receptor, peroxisome proliferator-activated receptor γ (PPARγ) (Lehmann et al., 1995; Shibata et al., 1999). PPARγ expression is abundant in adipose tissue. Recent studies have shown that PPARγ is also expressed in non-adipose tissues, including the heart, skeletal muscle, proximal tubules of kidney, and vascular smooth muscle (Park et al., 1997; Vidal-Puig et al., 1997; Guan et al.,

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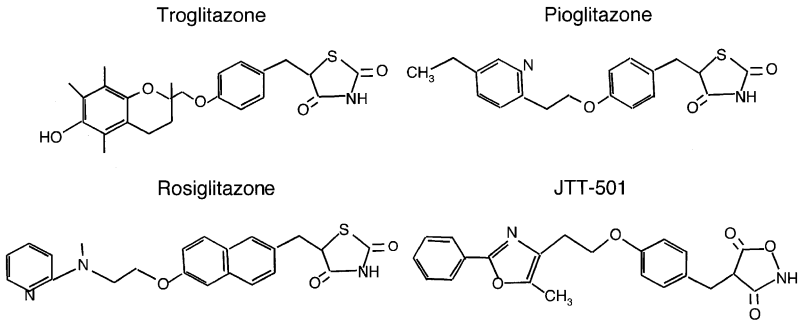

Fig. 1. Chemical structures of troglitazone, pioglitazone, rosiglitazone and JTT-501.

1997; Law et al., 2000; Iijima et al., 1999). Although thiazolidinediones affect ion channels, the association between ion channels and PPARγ has not been clarified. We thus compared the effects of three thiazolidinediones, one isoxazolidinedione, and 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2, ligands to PPARγ, for ion currents in vascular smooth muscle cells.

2. Materials and methods

2.1. Single cell preparation and voltage clamp recordings

Female guinea-pigs (body weight 200–250 g) were anesthetized with ether and then decapitated. Single smooth muscle cells were obtained by collagenase treatment (Wako, Tokyo, Japan) from the mesenteric arterial branch (diameter < 300 μm) as described previously (Ohya et al., 1993; Setoguchi et al., 1995; Nakamura et al., 1998).

Conventional whole-cell patch–clamp methods were performed with a patch pipette through a voltage clamp amplifier (Axopatch 1-D, Axon Instruments, Foster City, CA, USA). The conditions and procedures were the same as those previously described (Setoguchi et al., 1995; Nakamura et al., 1998). Current recording was carried out at room temperature (22–24 °C). Recording electrodes were made from Pyrex glass capillary tubing.

To isolate the Ca^{2+} channel current, the pipette was filled with a high Cs^+ solution (mM): Cs asparatate 130, CsCl 20, ATP-Na_2 3, $MgCl_2$ 1, EGTA 10, HEPES 10, and pH 7.3 titrated with CsOH. As a carrier of Ca^{2+} currents, Ba^{2+} ion was used (Ba^{2+} current, I_{Ba}). The Ba^{2+}-containing bath solution consisted of (mM): $BaCl_2$ 10, NaCl 150, KCl 6, glucose 5.4, HEPES 5, $MgCl_2$ 3, and pH 7.3 titrated with NaOH. To isolate K^+ currents, the pipette solution contained (mM): KCl 130, $MgCl_2$ 3, ATP-Na_2 3, EGTA 0.5 to 10, HEPES 10, and pH 7.3 titrated with KOH. The bath solution for K^+ currents consisted of (mM): NaCl 150, KCl 6, Glucose 5.4, HEPES 5, $MgCl_2$ 3 and pH 7.3 titrated with NaOH.

The cells were held at -80 or -70 mV, and command potentials were applied every 10 or 20 s. Data were

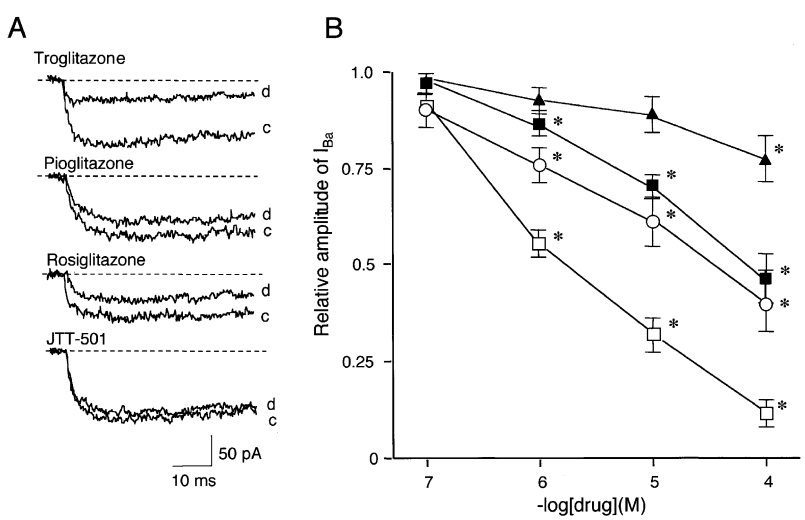

Fig. 2. Effects of troglitazone, pioglitazone, rosiglitazone and JTT-501 on voltage-operated Ca^{2+} channel currents: Ba^{2+} was used for the current carrier (I_{Ba}). (A) Current traces recorded at a command potential of 20 mV from a holding potential of -70 mV, before (c) and after (d) application of 10 μM drugs. Dotted line indicates zero current level. (B) Dose–response relationships of drugs on the current amplitude. Relative amplitudes of the currents (mean ± S.E.) with various drug-concentrations are plotted; troglitazone (open square, $n = 6$), pioglitazone (filled square, $n = 6$), rosiglitazone (open circle, $n = 5$) and JTT-501 (filled triangle, $n = 11$). The control amplitude (before application of drugs) was normalized to 1.0. $^*P < 0.05$ compared with 1.0.

obtained after the current amplitude had stabilized (usually 3–5 min after the whole-cell configuration was obtained). The current did not run-down over the next 20 min under these conditions (Ohya and Sperelakis, 1989; Setoguchi et al., 1995). Membrane currents were low-pass-filtered at 2 kHz, digitized with a sampling frequency of 5–10 kHz, and stored in a personal computer system for subsequent analysis. The liquid junctions among solutions were corrected. For I_{Ba}, the P/4 protocol was used to subtract the leak and capacitive currents. For I_{Kv} and I_{Kca}, no correction of the leak and capacitive currents was made. The traces are shown after being low-pass-filtered at 1 kHz.

2.2. Chemicals

Three thiazolidinediones, troglitazone ((±)-5-[4-(6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy) benzyl]-2,4-thiazolidinedione), pioglitazone ((±)-5-[*p*-(5-ethyl-2-pyridyl-2-ethoxy) benzyl]-2,4 thiazolidinedione) and rosiglitazone (5-[4-([*N*-methyl-*N*-(2-pyridyl) amino] ethoxy) benzyl] thiazolidine-2, 4-dione), and a non-thiazolidinedione, JTT-501 (4-[4-(2-[5-methyl-2-phenyl-4-oxazolyl] ethoxy) benzyl]-3,5-isoxazolidinedione) were gifts from Sankyo, Tokyo, Japan (Fig. 1). 15-deoxy-$\Delta^{12,14}$-prostaglandin (prostaglandine J_2) was from Cayman Chemical (Ann Arbor, MI, USA). Charybdotoxin and 4-aminopyridine were from Sigma (St. Louis, MO, USA).

Troglitazone, pioglitazone, rosiglitazone, and JTT-501 were dissolved in 100% dimethylsulfoxide (DMSO) as a 10–100 mM stock solution. PGJ_2 was dissolved in distilled water before each experiment. Drug solution was diluted at least 1000 times. DMSO at a concentration of 0.1% did not apparently affect the currents.

2.3. Analyses and statistics

The concentration for half inhibition (IC_{50}) was obtained by fitting the data to the Michaelis–Menten equation by the nonlinear least-squares method. Data are expressed as means ± S.E. Statistical significance was determined by one-way analysis of variance (ANOVA) with post-hoc test. A level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects on Ba^{2+} currents (I_{Ba})

Fig. 2 shows the effects of troglitazone, pioglitazone, rosiglitazone, and JTT-501 on I_{Ba}. Fig. 2A shows the changes in current traces in the presence of 10 μM drugs. All thiazolidinediones inhibited the I_{Ba}; however, their potencies were different. Ten μM JTT-501 had little effect. The dose–response relationships of these agents on the current amplitudes are shown in Fig. 2B (vs. 1.0 by

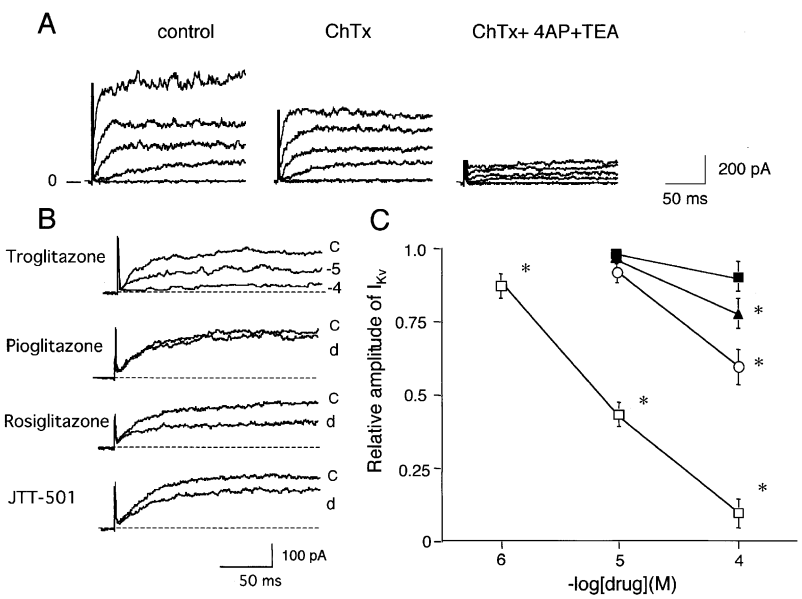

Fig. 3. Effects of troglitazone, pioglitazone, rosiglitazone, and JTT-501 on the voltage-dependent K^+ current (I_{Kv}). (A) Charybdotoxin (ChTx, 0.1 μM) inhibited I_{KCa} and 4-aminopiridine (4AP, 3 mM) and tetraethyleammonium (TEA, 10 mM) inhibited I_{Kv}. Currents were evoked by command potentials to between −20 and 60 mV in 20-mV increments. The pipette solution contained 1 mM EGTA. (B) Current traces before (c) and after application of 0 μM (−5) and 100 μM (−4) troglitazone, and 100 μM pioglitazone (d), rosiglitazone (d), and JTT-501 (d). Currents were recorded at a command potential of 40 mV from a holding potential of −70 mV in the presence of 0.1 μM ChTx. Dotted line indicates zero current level. The pipette solution contained 10 mM EGTA. (C) Dose–response relationship of the effects of drugs on the current amplitudes. Relative amplitudes of the currents (measured at command potential 40 mV; mean ± S.E.) with various drug-concentrations are plotted; troglitazone (open square, $n = 6$), pioglitazone (filled square, $n = 5$), rosiglitazone (open circle, $n = 5$) and JTT-501 (filled triangle, $n = 5$). The control amplitude (before application of drugs) was normalized to 1.0. * $P < 0.05$ compared with 1.0.

ANOVA; troglitazone, $P < 0.001$, $n = 6$; pioglitazone, $P < 0.001$, $n = 6$; rosiglitazone, $P < 0.001$, $n = 5$; JTT-501, $P < 0.00$, $n = 11$). Troglitazone inhibited I_{Ba} to more extent than other drugs (by ANOVA; $P < 0.001$). Similarly, three thiazolidinediones inhibited I_{Ba} to more extent than JTT-501 ($P < 0.001$). The IC_{50} for troglitazone, pioglitazone, and rosiglitazone were 2, 99 and 34 μM, respectively. The IC_{50} of JTT-501 was not calculated, because 100 μM JTT-501 inhibited I_{Ba} by only about 20%.

3.2. Effects on voltage-dependent K^+ current ($I_{K\upsilon}$)

The effects of the three thiazolidinediones and JTT-501 on I_{Kv} were examined. The I_{Kv} was recorded with 0.1 μM charybdotoxin, a K_{Ca} channel blocker (Fig. 3A). Fig. 3B shows the changes in the current traces in the presence of 10 and 100 μM troglitazone, and 100 μM pioglitazone, rosiglitazone, and JTT-501. Troglitazone dose-dependently ($IC_{50} = 18$ μM) inhibited I_{Kv}, whereas only a high concentration of rosiglitazone and JTT-501 inhibited I_{Kv}

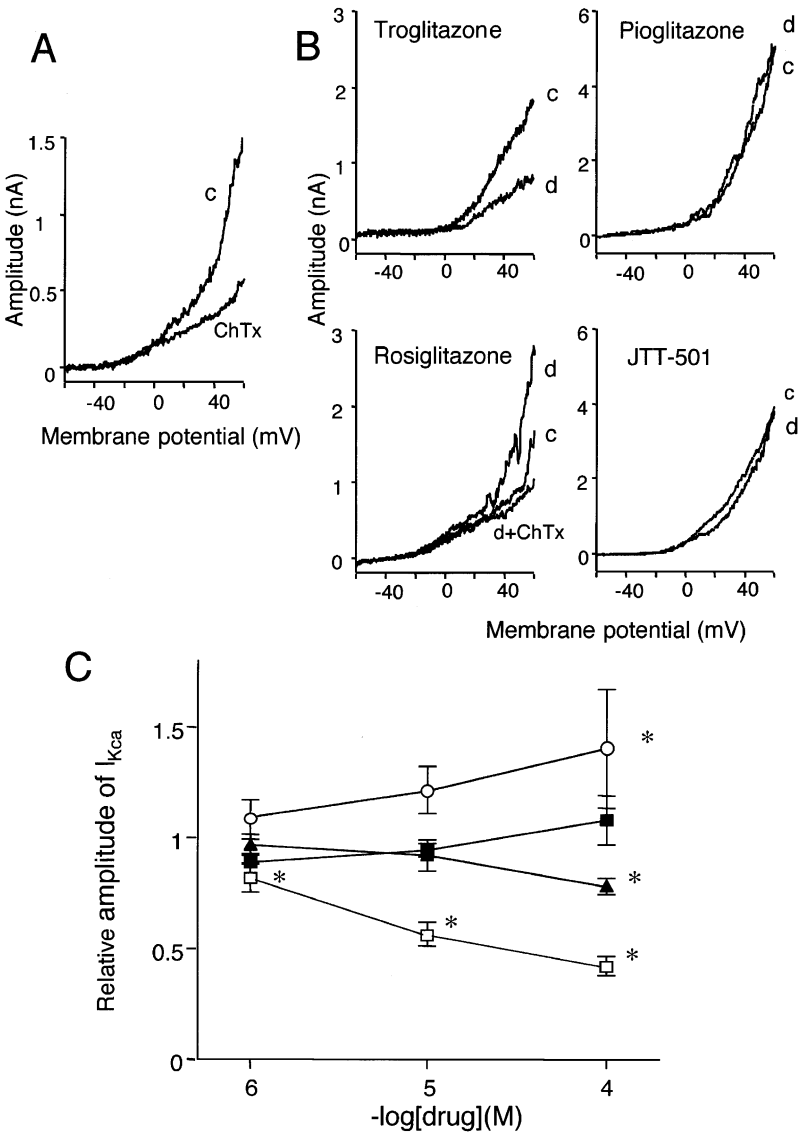

Fig. 4. Effects of troglitazone, pioglitazone, rosiglitazone and JTT-501 on the Ca^{2+}-activated K^+ current (I_{Kca}). Currents were recorded during a voltage-ramp from −60 to 60 mV in Ca^{2+}-free bath solutions containing 1–3 mM 4AP. (A) The rectifying component of the outward current was inhibited by 0.1 μM ChTx. The pipette contained 0.3 mM EGTA in order to increase I_{Kca} in this particular experiment. (B) Current traces before (c) and after (d) application of 100 μM of the drugs. ChTx (0.1 μM) inhibited the rosiglitazone-enhanced component. The pipette solution contained 1 mM EGTA. (C) Relative amplitudes of the currents (mean ± S.E.) with various drug-concentrations are plotted: troglitazone (open squares, $n = 6$), pioglitazone (filled squares, $n = 5$), rosiglitazone (open circles, $n = 8$ or 21), JTT-501 (filled triangles, $n = 6$). The control amplitude (before drug application) was normalized to 1.0. The amplitude of the current was determined as the area of the outward rectifying component at membrane potentials from −10 to +60 mV. $^*P < 0.05$ vs. 1.0.

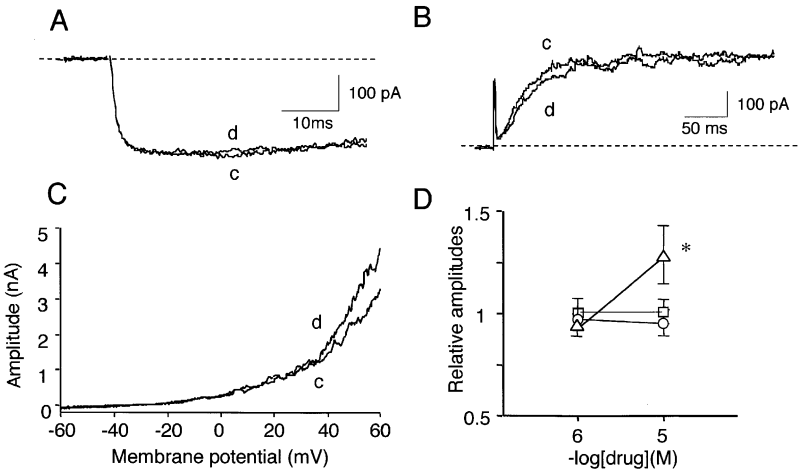

Fig. 5. Effects of 15-deoxy-$\Delta^{12,14}$ prostaglandin J_2 (PGJ_2) on I_{Ba}, I_{Kv} and I_{Kca}. Current traces of I_{Ba} (A), I_{Kv} (B), and I_{Kca} (C) before (c) and after (d) application of 10 μM of PGJ_2. (D) Relative amplitudes of I_{Ba} (open square), I_{Kv} (open circle), and I_{Kca} (open triangle) (mean ± S.E.) with various drug-concentrations are shown ($n = 5$ or 7). The control amplitude (before application of drugs) was normalized to 1.0. I_{Ba}, I_{Kv} and I_{Kca} were recorded with the same conditions as in Figs. 2, Fig. 3 and Fig. 4, respectively. $^{*}P < 0.05$ vs. 1.0.

(vs.1.0 by ANOVA; troglitazone, $P < 0.001$, $n = 6$; pioglitazone, $P = 0.22$, $n = 5$; rosiglitazone, $P < 0.001$, $n = 5$; JTT-501, $P < 0.005$, $n = 5$; Fig. 3C).

3.3. Effects on Ca^{2+}-activated K^+ currents (I_{Kca})

The effects of drugs on Ca^{2+}-activated K^+ currents (I_{Kca}) were then evaluated. The I_{Kca} was recorded with the same protocol as that reported by Knock et al. (1999): ramps were applied from −60 to +60 mV in the presence of 1–3 mM 4-aminopyridine, a K_v channel blocker. Ca^{2+} was not included in the bath solution in order to avoid a secondary effect on I_{Kca} due to inhibition of I_{Ca}. Fig. 4A illustrates I_{Kca} and its inhibition by 0.1 μM charybdotoxin. Charybdotoxin inhibited most of the outward rectifying component. Thus, the effects of drugs were estimated by observing the changes in the calculated area of the outward rectifying component at potentials from −10 to +60 mV. Fig. 4B illustrates the changes in current in the presence of 100 μM troglitazone, pioglitazone, rosiglitazone, and JTT-501. Fig. 4C summarizes the action of drugs on I_{Kca}. Troglitazone inhibited I_{Kca} (vs. 1.0 by ANOVA, $P < 0.001$, $n = 6$), whereas pioglitazone ($P = 0.16$, $n = 5$) did not affect the outward current (vs. 1.0 by ANOVA). A high concentration of JTT-501 inhibited I_{Kca} ($P = 0.01$, $n = 6$). Rosiglitazone enhanced I_{Kca} in most cells, but it also inhibited I_{Kca} in some cells: the current was 79–176% at 10 μM and 75–220% at 100 μM ($P = 0.04$, $n = 8$–21). This enhanced component was inhibited by charybdotoxin, suggesting that I_{Kca} was stimulated by rosiglitazone.

3.4. Effects of PGJ_2 on these currents

To investigate whether the effects of these drugs on I_{Ba}, I_{Kv}, and I_{Kca} are related to activation of PPARγ, we examined the effects of PGJ_2 on these currents. Fig. 5A, B and C illustrate the changes in I_{Ba}, I_{Kv} and I_{Kca}, respectively, in the presence of 10 μM PGJ_2. Ten micromolar is reportedly a high enough concentration to activate PPARγ in various cells (Satoh et al., 1999; Law et al., 2000). PGJ_2 enhanced I_{Kca} (vs. 1.0 by ANOVA; $P = 0.007$, $n = 6$), but affected neither I_{Ba} nor I_{Kv} (I_{Ba}, $P = 0.998$, $n = 5$; I_{Kv}, $P = 0.862$, $n = 7$; Fig. 5D).

4. Discussion

The present study is the first to directly compare the actions of three thiazolidinediones and one isoxazolidinedione on ion channels. The main findings were as follows: (1) the thiazolidinediones, troglitazone, pioglitazone, and rosiglitazone inhibited I_{Ba} (troglitazone > pioglitazone = rosiglitazone); (2) troglitazone and rosiglitazone but not pioglitazone inhibited I_{Kv}; (3) rosiglitazone enhanced I_{Kca}; (4) a high concentration of JTT-501 (100 μM) inhibited I_{Ba}, I_{Kv}, and I_{Kca} to a similar extent; and (5) PGJ_2 enhanced I_{Kca} but affected neither I_{Ba} nor I_{Kv}.

The three thiazolidinediones inhibited I_{Ba}. Troglitazone had a greater potency to inhibit I_{Ba} than did pioglitazone and rosiglitazone. Their potencies to inhibit I_{Ba} were comparable to those in the previous reports (Nakamura et al., 1998; Asano et al., 1999; Knock et al., 1999). Since the thiazolidinediones inhibited I_{Ba}, whereas JTT-501 (≤ 10 μM) and PGJ_2 did not inhibit I_{Ba}, the differential effect of thiazolidinediones on Ca^{2+} channels can be attributed to specific regions of the molecules and not to activation of PPARγ. The right half of the structure, which includes benzyl-thiazolidinedione, appears to be essential for the inhibition of Ca^{2+} channels (Fig. 1). The other half of the structure may affect the ability to inhibit Ca^{2+} channels. Since only a high concentration of JTT-501 inhibited I_{Ba}, I_{Kv} and I_{Kca} to a similar extent, JTT-501 stabilizes mem-

brane currents through the non-specific blockade of all the ion channels studied.

Knock et al. (1999) showed that troglitazone and rosiglitazone inhibited I_{Kv} in rat pulmonary arteries. The present study further extended the observation that both drugs inhibited this current, but pioglitazone, JTT-501 and PGJ_2 did not affect I_{Kv} in guinea-pig mesenteric arteries. Thus, it is possible that the inhibitory action on I_{Kv} is not associated with the thiazolidinedione residue or activation of PPARγ.

Knock et al. (1999) reported that rosiglitazone enhanced I_{Kca} in rat aortic cells. In the present study, we confirmed that rosiglitazone enhanced I_{Kca}. We also demonstrated that troglitazone, pioglitazone, and JTT-501 did not enhance I_{Kca}. Thus, the stimulatory effect on I_{Kca} is a unique characteristic of rosiglitazone. In the present study, rosiglitazone also slightly inhibited I_{Kca} in some cells. The reason for the discrepant responses of rosiglitazone among cells is not known. It is possible that rosiglitazone may have both stimulating and inhibitory actions on K_{Ca} channels.

In the present study, PGJ_2, an intrinsic ligand for PPARγ, enhanced I_{Kca}. Since rosiglitazone has the highest affinity for PPARγ among the various insulin sensitizers (Lehmann et al., 1995; Murakami et al., 1998), this raises the possibility that its stimulatory effect on I_{Kca} may be associated with PPARγ activation. However, it has been reported that prostaglandin I_2 and prostaglandin E_2 activate Ca^{2+}-dependent K^+ channels and cause relaxation in pig pial arteries (Armstead, 1999), and that prostaglandin E_1 activates Ca^{2+}-dependent K^+ channels in human corporal smooth muscle (Lee et al., 1999). It is thus also possible that the stimulating action of PGJ_2 on I_{Kca} did not depend on PPARγ activation. Further studies are required to clarify the intracellular mechanism for PGJ_2-induced I_{Kca} enhancement.

Only a few studies have directly compared the potencies of thiazolidinediones to cause vasodilation and to exert antihypertensive actions. Troglitazone has a greater potency to dilate arteries than does pioglitazone (Nakamura et al., 1998). This is in accordance with the observation that troglitazone inhibits Ca^{2+} currents with a greater potency than pioglitazone. The present study also showed that the concentration of rosiglitazone required was 15 times higher than that of troglitazone. However, rosiglitazone enhanced I_{Kca}. Since the opening of K_{Ca} channels would hyperpolarize the membrane potential of smooth muscle and decrease vascular tone (Nelson and Quayle, 1995), the vasodilating action of rosiglitazone could result from stimulation of K_{Ca} channels together with inhibition of Ca^{2+} channels. Rosiglitazone administration prevented a rise in blood pressure in Zucker fatty rats (Buckingham et al., 1998); however, the vasodilating effect of rosiglitazone has not been demonstrated in human arteries in vivo (Walker et al., 1998). The effects of JTT-501 on the vasculature and on blood pressure have not been evaluated so far. Since the effects of JT-501 on various currents were weak, this drug may not have a direct action on vascular tissues by modifying ion channels.

In conclusion, three thiazolidinediones and an isoxazolidinedione act differently on ion channels in vascular smooth muscle cells. These differences may characterize their actions in the vasculature. The effects of thiazolidinediones on Ca^{2+} channels may be attributed to specific regions of their molecules rather than to PPARγ activation. The possible involvement of PPARγ activation in the enhancement of I_{Kca} should be tested further.

Acknowledgements

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