ELSEVIER

European Journal of Pharmacology 368 (1999) 103–109

ejp

Differential effects of insulin-sensitizers troglitazone and rosiglitazone on ion currents in rat vascular myocytes

Gregory A. Knock, Santosh K. Mishra, Philip I. Aaronson *

Department of Pharmacology, The Guy's, King's College and St. Thomas' Hospitals Medical and Dental School, St Thomas's Hospital Campus, Lambeth Palace Road, London SE1 7EH, UK

Received 31 August 1998; revised 4 December 1998; accepted 12 January 1999

Abstract

Insulin-sensitizing thiazolidinediones such as troglitazone and pioglitazone have been shown to lower blood pressure in vivo and cause vasorelaxation in vitro. Rosiglitazone (BRL 49653) is a novel thiazolidinedione which has been reported not to cause vasoleraxation. We therefore compared the effects of troglitazone and rosiglitazone on Ca^{2+} and K^+ currents in rat aorta and pulmonary artery smooth muscle cells. Currents were recorded with the conventional whole cell patch clamp technique. Both drugs reduced the voltage-gated (L-type) Ca^{2+} current in rat aorta cells, with half-maximal current inhibition by troglitazone and rosiglitazone at 2 and 10 μM, respectively. Troglitazone, 2 μM and rosiglitazone, 20 μM caused a similar hyperpolarizing shift of 12 mV in the potential-dependence of Ca^{2+} current availability. Troglitazone (20 μM) produced a marked block of the tetraethylammonium- and paxilline-sensitive Ca^{2+} activated K^+ current, while rosiglitazone (20 μM and 60 μM) slightly enhanced this current. Rat pulmonary artery smooth muscle cells have a prominent delayed rectifier K^+ current. Troglitazone produced a potent block of this current (half-maximal inhibition at < 1 μM), while rosiglitazone caused a smaller inhibition at 10 and 60 μM. These results show that troglitazone has relatively potent blocking effects on a wide variety of ion currents in vascular smooth muscle cells. Rosiglitazone exerts less potent, but similar effects on the Ca^{2+} current and delayed rectifier K^+ current, but it enhances the Ca^{2+} activated K^+ current.

Keywords: Rosiglitazone; Troglitazone; Ca^{2+} current; K^+ current; Smooth muscle, vascular

1. Introduction

The thiazolidinediones troglitazone, rosiglitazone and pioglitazone are insulin-sensitizing agents which have been developed for the treatment of type II diabetes (Hoffman and Colca, 1992). These drugs have been shown to reduce plasma glucose, free fatty acids, and triglycerides in diabetic rodent models, but are unable to cause hypoglycemia because they do not interfere with the normal regulation of pancreatic insulin secretion by glucose. The mechanisms of action of the thiazolidinediones remain incompletely understood. It has been shown, however, that thiazolidinediones enhance the expression of glucose transporters in adipocytes, probably by increasing adipocyte differentiation (Saltiel and Olefsky, 1996). This may result from an interaction of the drugs with peroxisome proliferator activated receptors (PPARreceptorγ), a member of the nuclear receptor super family and which regulates gene transcription in adipose tissue, muscle, and liver (reviewed by Saltiel and Olefsky, 1996).

In addition to their antihyperglycaemic actions, troglitazone and pioglitazone have been reported to lower blood pressure in vivo (Pershadsingh et al., 1993; Yoshioka et al., 1993; Buchanan et al., 1995; Ogihara et al., 1995; Ghazzi et al., 1997) and to exhibit vasorelaxant activity in vitro. Troglitazone has been reported to manifest a potent inhibition of the L-type Ca^{2+} current in the A7r5 cell line and freshly isolated rat tail artery smooth muscle cells, which may explain its vasorelaxing properties (Song et al., 1997). Pioglitazone also suppresses the L-type Ca^{2+} current in rat aortic myocytes, albeit at much higher concentrations (Zhang et al., 1994). In a recent paper, this order

* Corresponding author. Tel.: +44-171-960-5654; Fax: +44-171-928-7965; E-mail: p.aaronson@umds.ac.uk

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00020-5

of potency of troglitazone and pioglitazone in blocking Ca^{2+} currents was confirmed in guinea-pig resistance artery smooth muscle cells (Nakamura et al., 1998). Possible effects of thiazolidinediones on other types of ion currents in vascular smooth muscle have not been reported, although troglitazone has been reported to potently block K_{ATP} channels in an insulinoma cell line (Lee et al., 1996).

In contrast to troglitazone, the compound rosiglitazone, which is currently in clinical trials, was reported not to cause relaxation of phenylephrine-constricted small arteries from human subcutaneous fat at concentrations up to 100 μM (Walker et al., 1998).

In view of the contrasting responses of preconstricted arteries to troglitazone and rosiglitazone, and in light of the limited information available regarding the effects of these compounds on ion channel activity, we have compared the concentration-dependency and mechanism of the effects of troglitazone and rosiglitazone on the Ca^{2+} channel current in rat aorta smooth muscle cells. In addition, we report for the first time that both compounds, and in particular troglitazone, also affect both the delayed rectifier K^+ current ($I_{K,v}$), and the Ca^{2+}-activated K^+ ($I_{K,Ca}$) currents, in vascular smooth muscle cells isolated from the rat aorta and main pulmonary artery, respectively.

2. Methods

2.1. Cell isolation and electrophysiology

Adult male Wistar rats were killed by cervical dislocation. The thoracic aorta and pulmonary artery were isolated, transferred to cold physiological saline solution (PSS) and cleaned of fat and connective tissue under a dissecting microscope. The vessels were then cut into small pieces and incubated at 37°C in PSS containing 15 μM Ca^{2+} for 15 min. Aortic myocytes were prepared by transferring tissue pieces to the same solution containing 0.23 mg of elastase Type I (Sigma) and incubated for 20 min at 37°C. At the end of the elastase treatment, the tissue was transferred to an enzyme mixture that contained collagenase Type I (1 mg/ml), collagenase Type XI (1 mg/ml), papain (0.5 mg/ml) and dithiothreitol in low Ca^{2+} PSS and incubated further for 20 min. Pulmonary artery myocytes were prepared by a similar collagenase digestion but without the prior digestion with elastase and with 0.25 mg/ml of papain. After the enzymatic digestion, tissue pieces were washed in enzyme-free low Ca^{2+} PSS and triturated by sucking in and out in a wide bore glass pipette for single cell dispersion. The cells were stored in low Ca^{2+} PSS at 4°C and used within 5-6 h of their isolation. The whole-cell membrane currents were recorded at room temperature using the standard patch clamp technique (Smirnov and Aaronson, 1996), with PCLAMP-6 software and axopatch 200B amplifier (Axon Instruments, CA). Data analysis, including fitting of curves to data points was performed with SigmaPlot 4.01 (Jandel Scientific, CA) and Microsoft Excel software on an Elonex MTX-6233/II computer.

2.2. Solutions

PSS contained (mM) NaCl, 130; KCl, 5.0; $MgCl_2$, 1.2; $CaCl_2$, 1.5; HEPES, 10; glucose, 10. The pH was adjusted to 7.4 with NaOH. Low-Ca^{2+} PSS contained 15 μM Ca^{2+}. Ca^{2+}-free high-K^+ solution was made by replacing NaCl with equimolar concentration of KCl and removing Ca^{2+}. Ba^{2+} PSS, which was used to record the inward current through Ca^{2+} channels, contained (in mM): NaCl 110, TEA-Cl 4, CsCl 1, $MgCl_2$ 1.2, $BaCl_2$ 20, HEPES 10, and glucose 10. The pipette solution used for recording the inward Ca^{2+} current contained: CsCl 135, $MgCl_2$ 2.5, MgATP 5, HEPES 10, and EGTA 10 and the pH was adjusted to 7.2 using NaOH. The pipette solution for measuring the K^+ current contained KCl, 110; $MgCl_2$, 2.5; MgATP, 1.0; HEPES, 10; EGTA, 10 and the pH was adjusted with KOH.

2.3. Drugs and chemicals

Rosiglitazone and troglitazone were the kind gifts of Dr Robin Buckingham, SmithKline Beecham Pharmaceuti-

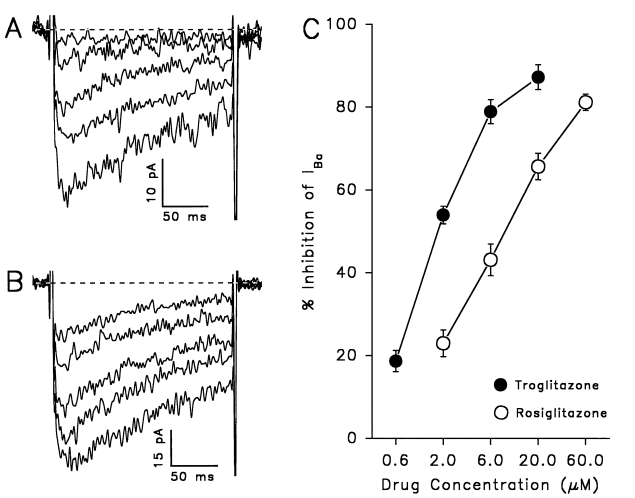

Fig. 1. Inhibition of I_{Ba} by troglitazone and rosiglitazone in rat aortic myocytes. (A) Representative traces showing I_{Ba} under control conditions and in the presence of 0.6, 2, 6, and 20 μM troglitazone. (B) Representative traces showing I_{Ba} under control conditions and the presence of 2, 6, 20, and 60 μM rosiglitazone. Dashed lines represent zero current. (C) Percent inhibition of I_{Ba} by troglitazone (filled circles) and rosiglitazone (empty circles), using measurements of the integral of the inward current over 200 ms for each concentration. Values shown are the mean and S.E.M. calculated from 6–8 cells. Inhibition of I_{Ba} was significant for all points illustrated ($P < 0.05$).

cals, Harlow, UK. Stock solutions of rosiglitazone (20 mM) and troglitazone (2 mM) were prepared in dimethylsulphoxide (DMSO). All other chemicals were obtained from Sigma. Tetraethylammonium (1 M) was dissolved in distilled water. 4-Aminopyridine (100 mM) was dissolved in distilled water and the pH was adjusted to 7.4 using HCl.

2.4. Statistics

The statistical significance of differences between sets of values was assessed using students paired or unpaired two-tailed *T*-test, as appropriate, with $P < 0.05$ taken to reject the null hypothesis.

3. Results

3.1. Effects of troglitazone and rosiglitazone on the voltage-gated Ca^{2+} current

It has previously been shown that the Ca^{2+} current in rat aortic myocytes is of the L-type; no T-component was observed, even when the holding potential was −100 mV (Quignard et al., 1996). We also observed that the inward Ba^{2+} current (I_{Ba}) in aortic myocytes demonstrated kinetic and electrophysiological properties characteristic of an L-type current. These were: firstly, the slow decay of I_{Ba} over 200 ms at +10 mV (Fig. 1); secondly, a threshold of activation at around −40 mV with a holding potential of −60 mV and a potential of maximum activation between 0 and +10 mV (Fig. 2); and thirdly, inactivation over a positive range of potentials (Fig. 3).

The Ca^{2+} current was elicited by stepping cells for 200 ms at 0.1 Hz to a test potential of +10 mV, from a resting potential of −60 mV. Fig. 1 shows typical examples of the effect of various concentrations of troglitazone (A) and rosiglitazone (B) on I_{Ba}. Troglitazone caused a rapidly developing and concentration-dependent inhibition of I_{Ba}. Rosiglitazone exerted a similar effect, although over a higher concentration range. Fig. 1C summarizes the mean concentration-dependency of the inhibition of the Ca^{2+} channel current by each drug obtained from 6–8 similar experiments. Both drugs inhibited the current, with IC_{50}'s of approximately 2 and 10 μM for troglitazone and rosiglitazone, respectively.

Fig. 2 illustrates that the inhibition of I_{Ba} by 20 μM rosiglitazone occurred over a wide range of test potentials, and that the threshold of activation and potential of maximum activation were not shifted. A similar observation has previously been made for 2 μM troglitazone (Song et al., 1997; Nakamura et al., 1998).

Many Ca^{2+} channel antagonists, including those of the dihydropyridine and benzothiazepine classes, have a higher

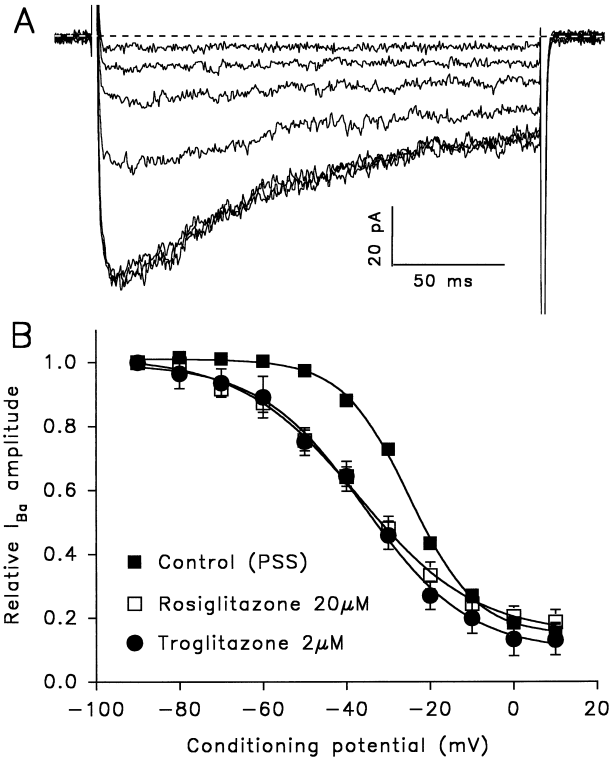

Fig. 3. The effect of troglitazone and rosiglitazone on voltage-dependence of inactivation of I_{Ba}. 5 s conditioning potentials to between −90 and +10 mV were applied followed by 200 ms test pulses to +10 mV. (A) An example of currents at the +10 mV test potentials following conditioning pulses to −90, −70, −50, −30, −20, −10 and +10 mV under control conditions. The dashed line represents zero current. (B) The mean ± S.E.M. of current measurements at the +10 mV test pulses, firstly under control conditions (filled squares, $n = 16$) and then in the presence of either 2 μM troglitazone (filled circles, $n = 6$) or 20 μM rosiglitazone (empty squares, $n = 9$). Points are normalised to the current at the test pulse following the −90 mV conditioning pulse and plotted against membrane potential. Smooth curves were fitted to data points with the Boltzmann function (see text).

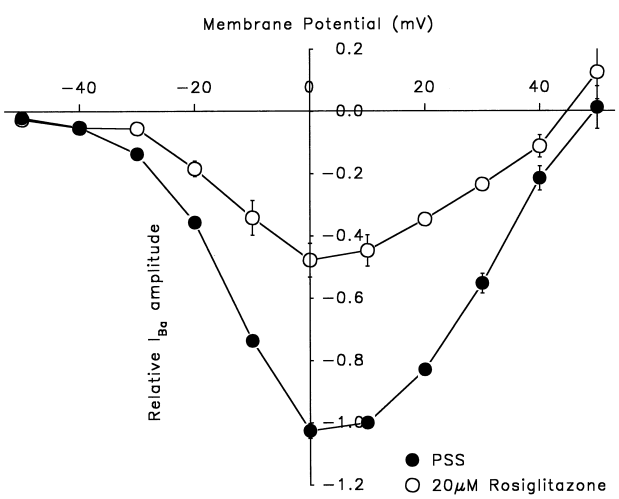

Fig. 2. Current–voltage relationship for I_{Ba} in the absence (solid circles) and presence (open circles) of 20 μM rosiglitazone. Points are the mean and S.E.M. of integrated current, normalised to the current present at +10 mV in the absence of rosiglitazone ($n = 3$).

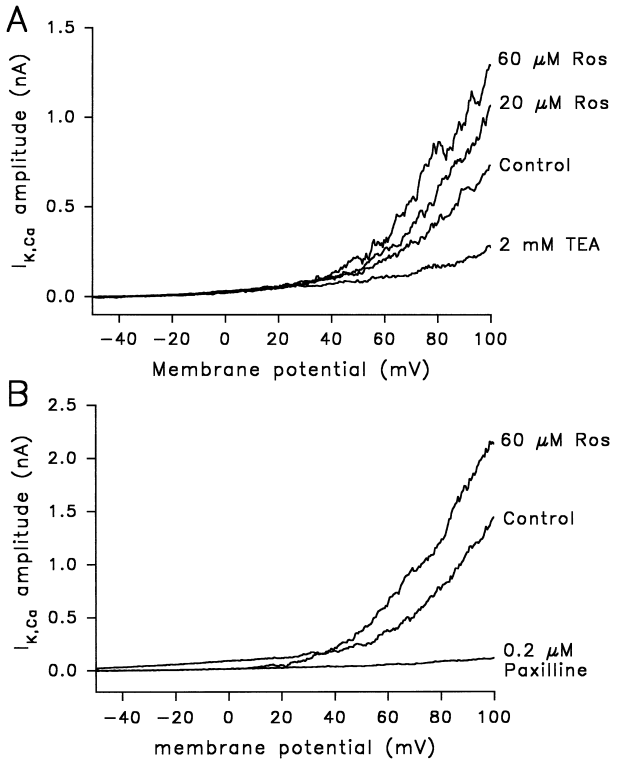

Fig. 4. Examples of the effect of rosiglitazone (Ros) on $I_{K,Ca}$ in typical rat aortic myocytes recorded during voltage ramps from −50 to +100 mV. Currents were recorded in Ca^{2+} free PSS containing 1 mM 4-aminopyridine. The current is enhanced by 20 and 60 μM rosiglitazone, and is blocked by either 2 mM tetraethylammonium (TEA) added after the removal of rosiglitazone (A), or 0.2 μM paxilline in the continued presence of rosiglitazone (B), confirming the predominance of $I_{K,Ca}$ in these cells.

affinity for the inactivated state of the Ca^{2+} channel, compared to the resting state (Sanguinetti and Kass, 1984; Uehara and Hume, 1985). Drugs with this property produce a characteristic hyperpolarizing shift of I_{Ba} potential–inactivation curve, which describes the tendency for the channels to become inactivated (refractory) as the resting membrane potential becomes more positive. In order to define this function, cells were held at −60 mV, and then stepped for 5 s to one of a series of conditioning potentials of between −90 and 0 mV. The cells were then stepped to a test potential of +10 mV and the amplitude of I_{Ba} was measured. Fig. 3 shows the resulting current amplitudes, normalised to that recorded at −90 mV, plotted against the conditioning potential. Data from each individual experiment was fitted to the Boltzmann function:

$$I = \left(\left[1 - C\right]/1 + \exp\left[\left(V - V_H\right)/k\right]\right) + C \quad (1)$$

where I is the normalised current at any potential, C is the fraction of the current which was not inactivated by depolarisation, V_H is the conditioning potential at which the inactivating current was half-inactivated, and k is the slope factor. Under control conditions, V_H was -24.3 ± 0.65 mV ($n = 16$). Troglitazone (2 μM) shifted V_H to -35.9 ± 1.3 mV ($n = 6$, $P < 0.001$), while with rosiglitazone (20 μM) V_H was -36.0 ± 1.1 mV ($n = 9$, $P < 0.001$).

3.2. Effects of rosiglitazone and troglitazone on K^+ channel currents

The Ca^{2+}-activated K^+ ($I_{K,Ca}$) current was the predominant current observed in the rat aortic cells. This current was defined pharmacologically, using a low concentration (2 mM) of tetraethylammonium (Nelson and Quayle, 1995) or the highly selective blocker paxilline (0.2 μM) (Knaus et al., 1994). We recorded this current using membrane ramps from −50 to +100 mV (ie, the membrane potential was increased linearly from −50 to +100 mV over a period of 750 ms). These experiments were also performed in the absence of external Ca^{2+} (Ca^{2+}-free PSS) in order to eliminate an indirect effect on the current caused by block of the Ca^{2+} current.

In the presence of 1 mM 4-aminopyridine to block $I_{K,v}$ (see below), 20 μM or 60 μM rosiglitazone caused enhancement of $I_{K,Ca}$ at positive potentials (20 μM, 34 ± 8%,

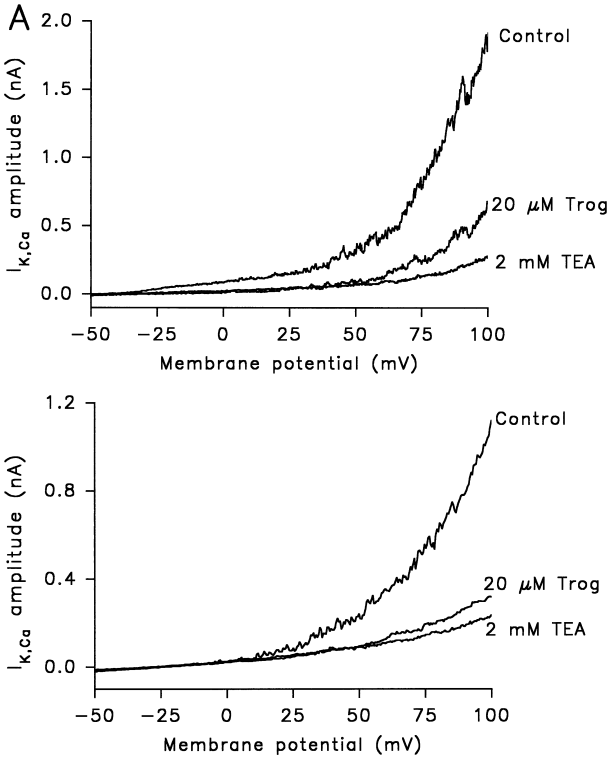

Fig. 5. Examples of the effect of troglitazone (Trog) on $I_{K,Ca}$ in typical rat aortic myocytes recorded during voltage ramps from −50 to +100 mV in Ca^{2+}-free PSS. (A) The current was markedly blocked by 20 μM troglitazone and by 2 mM tetraethylammonium (TEA), confirming it was predominantly mediated by K,Ca channels. (B) In another cell in Ca^{2+}-free solution containing 1 mM 4-aminopyridine, to rule out any influence of block of voltage-gated currents, the current was also almost completely abolished by 2 mM tetraethylammonium and 20 μM troglitazone.

$n = 5$; 60 μM, 52 ± 8%, $n = 10$ at +80 mV). The predominance of $I_{K,Ca}$ in these experiments was confirmed by near complete block with either 2 mM tetraethylammonium (81 ± 2%, $n = 5$) or 0.2 μM paxilline (88 ± 2%, $n = 5$). Examples of the activation of $I_{K,Ca}$ by 20 and 60 μM rosiglitazone (Ros) and the block by either tetraethylammonium or paxilline are shown in Fig. 4A,B, respectively.

The effect of troglitazone (Trog) on $I_{K,Ca}$ is illustrated in Fig. 5. The predominance of $I_{K,Ca}$ was first confirmed with 2 mM tetraethylammonium and then after washout, 20 μM troglitazone was applied. In contrast to the effect of rosiglitazone, $I_{K,Ca}$ was inhibited by troglitazone (Fig. 5A). A similar effect was obtained when troglitazone was applied with the addition of 1 mM 4-aminopyridine to block the small component of delayed rectifier K^+ current ($I_{K,v}$) which is sometimes present in these cells (Fig. 5B). The influence of several concentrations of rosiglitazone ($n = 10$) and troglitazone ($n = 4$–5) on the amplitude of $I_{K,Ca}$ in Ca^{2+}-free solution in the presence of 1 mM 4-aminopyridine are summarised in Fig. 6.

As shown above, $I_{K,V}$ in rat aortic cells is small or sometimes negligible; it is however quite prominent in rat pulmonary arterial smooth muscle cells (Smirnov et al., 1994). We therefore used pulmonary artery myocytes to evaluate the effects of troglitazone and rosiglitazone on this current.

$I_{K,V}$ in pulmonary artery myocytes could be recorded in isolation by stepping cells from −60 to +80 mV in the presence of 10 mM tetraethylammonium, which completely blocks $I_{K,Ca}$, but has no effect on $I_{K,V}$, in these cells (Smirnov et al., 1994). Fig. 7A shows that $I_{K,V}$ was slightly but significantly inhibited by rosiglitazone at 10 and 60 μM. Conversely, troglitazone caused a potent

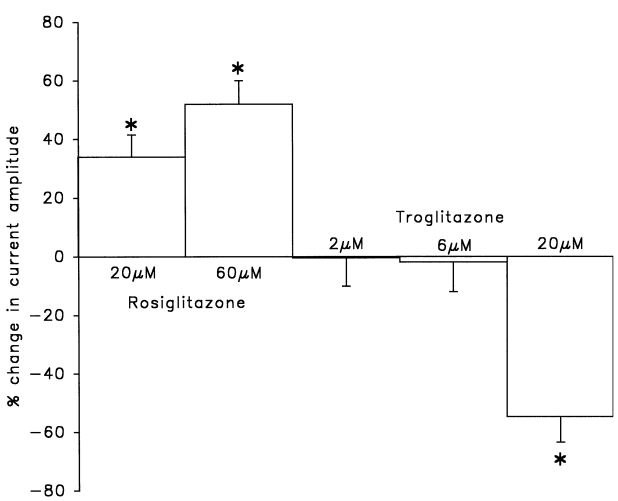

Fig. 6. Effect of several concentrations of troglitazone ($n = 5$) and rosiglitazone ($n = 5$–10) on the outward current measured at +80 mV in the absence of Ca^{2+} and in the presence of 1 mM 4-aminopyridine. Asterisks indicate a significant change in the current amplitude compared to the control ($P < 0.05$).

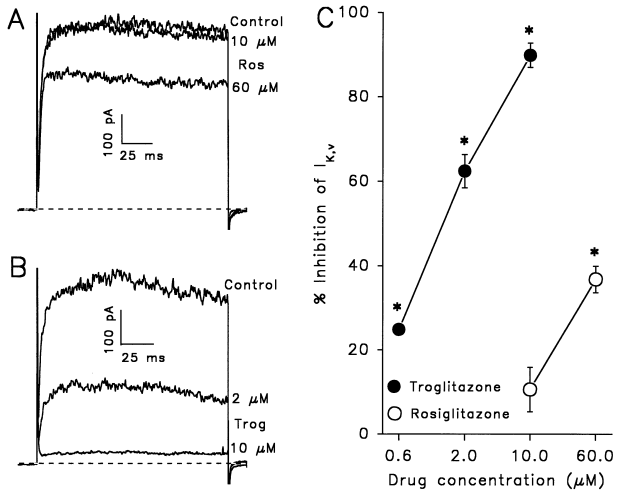

Fig. 7. Inhibition of $I_{K,V}$ by rosiglitazone and troglitazone in rat pulmonary arterial myocytes. (A, B) Examples of the effect of 10 and 60 μM rosiglitazone (Ros) and 2 and 10 μM troglitazone (Trog), respectively, on $I_{K,V}$ in two typical cells recorded during 200 ms step to +80 mV from a holding potential of −60 mV. Currents were recorded in the presence of 10 mM tretraethylammonium to eliminate the $I_{K,Ca}$ current. Dashed lines represent zero current. (C) concentration-dependency of the inhibition of $I_{K,V}$ by troglitazone ($n = 4$–5) and rosiglitazone ($n = 4$–5). Asterisks indicate significant inhibition of the current ($P < 0.05$).

inhibition of $I_{K,V}$, significantly reducing the amplitude of the current at concentrations of 0.6 μM and higher (Fig. 7B). Block of this current by both drugs was prompt and reversible. Fig. 7C illustrates the concentration–response curve for the inhibition of $I_{K,V}$ by both drugs ($n = 4$–5).

4. Discussion

We find that troglitazone and rosiglitazone exert rather different effects on 3 important types of ion channel current found in rat vascular smooth muscle cells. Troglitazone inhibits each of these currents, with a potency order $I_{K,V} \geq I_{Ca} > I_{K,Ca}$. Although rosiglitazone also inhibited I_{Ca}, this effect occurred over a higher concentration range than did the equivalent blocking effect of troglitazone. At much higher concentrations, rosiglitazone inhibited $I_{K,V}$ but stimulated the $I_{K,Ca}$ current.

Troglitazone has been shown to cause vasodilatation in both animals (Fujiwara et al., 1995) and man (Ghazzi et al., 1997). Troglitazone also causes vasorelaxation in vitro (Song et al., 1997; Walker et al., 1998), as does pioglitazone (Buchanan et al., 1995). Several mechanisms may be involved. In addition to blocking the voltage-gated Ca^{2+} channel current (Song et al., 1997; Nakamura et al., 1998), troglitazone increased prostacyclin production by aortic rings (Fujiwara et al., 1995). Consistent with this observation, the troglitazone-mediated relaxation of the norepinephrine contracture in small arteries from human subcutaneous fat was suppressed by indomethacin (Walker et al., 1998). Kotchen et al. (1996) provided evidence that

another thiazolidinedione, pioglitazone, may attenuate the norepinephrine contraction in rat aorta by unmasking a latent endothelium-dependent vasodilating action of insulin. It has also been recently reported that troglitazone abolished the activation by high glucose of membrane bound protein kinase C in cultured rabbit coronary myocytes (Yasunari et al., 1997). Ciglitazone, another thiazolidinedione which reduced blood pressure in obese Zucker rats, reduced the sustained rise in intracellular [Ca^{2+}] evoked by platelet derived growth factor in the A10 rat vascular smooth muscle cell line (Pershadsingh et al., 1993).

Much less is currently known about the effects of rosiglitazone on the vasculature. A single recent report by Walker et al. (1998) has demonstrated that rosiglitazone at concentrations up to 100 μM did not relax human subcutaneous small arteries preconstricted with norepinephrine.

The results presented in the present study bear on a number of issues. One relates to the potencies of the effect of both drugs on the voltage-gated Ca^{2+} current. We observed that troglitazone inhibited the Ca^{2+} current by 50% at a concentration of 2 μM. Similar values have previously been reported by Nakamura et al. (1998) (IC_{50} = 3 μM in myocytes from guinea-pig small mesenteric arteries) and Song et al. (1997) (IC_{50} between 6 and 10 μM in myocytes from rat tail arteries). We have now found, however, that troglitazone (and also rosiglitazone) caused a hyperpolarizing shift of the steady-state availability of I_{Ca}. According to the approach of Bean (1984) the affinity of a drug for the inactivated state of the Ca^{2+} channel can be calculated on the basis of this shift (ΔV_h):

$$-\Delta V_h = k\ln\left[(1 + D/K_i)/(1 + D/K_r)\right] \quad (2)$$

where k = the slope factor of the Boltzmann function describing steady-state inactivation, D is the drug concentration, K_r is the affinity constant for the binding of the channel to the resting state of the channel (taken as 2 μM for troglitazone and 10 μM for rosiglitazone see above), and K_i is the affinity constant of the drug for the inactivated channel. Taking ΔV_h as −12 mV for both 2 μM troglitazone and 20 μM rosiglitazone, K_i is 0.25 and 1.5 μM for troglitazone and rosiglitazone, respectively. Vascular smooth muscle cells in vivo are likely to be partially depolarized due to tonic effects of sympathetic tone and autocoid release, and may as a result have an increased fraction of inactivated Ca^{2+} channels. Therefore, these drugs, particularly troglitazone, may have a somewhat more potent effect in vivo than would expected on the basis of the concentration-response data previously reported (Song et al., 1997; Nakamura et al., 1998). It should be mentioned, however, that our results differ from those of Nakamura et al. (1998), who reported that troglitazone did not shift the steady state inactivation curve of the Ca^{2+} channel current in smooth muscle cells from guinea-pig mesenteric small arteries. The reason for this disparity are not apparent, but might reflect species or vessel heterogeneity.

Troglitazone also has a quite potent effect on the delayed rectifier K^+ current, blocking this by approximately 50% at 1 μM. Rosiglitazone had a similar effect, but at much higher concentrations. Inhibition of $I_{K,V}$ by troglitazone might be expected to ameliorate the effects of its Ca^{2+} channel antagonism, and could also have an impact on the effectiveness of vasodilators such as β-adrenoceptor agonists, which enhance this K^+ current (Aiello et al., 1995). The delayed rectifier is thought to be involved in suppressing the excitability of vascular smooth muscle (Nelson and Quayle, 1995), and is also important in setting the resting membrane potential in some arteries, particularly those of the pulmonary vasculature (Smirnov and Aaronson, 1996). Both acute and chronic hypoxic pulmonary vasoconstriction are though to result, at least in part, from the inhibition of the delayed rectifier K^+ current described above (Yuan et al., 1993; Smirnov and Aaronson, 1996).

We have previously shown that arachidonic acid inhibits $I_{K,V}$ (Smirnov and Aaronson, 1996), and this fatty acid has been found also to enhance the $I_{K,Ca}$ current (Kirber et al., 1992). In rat aorta, rosiglitazone similarly had opposite effects on both currents at high concentrations. It has been suggested that thiazolidinediones may cause prostaglandin production by displacing fatty acids from a binding protein (Walker et al., 1998), and it is possible that rosiglitazone was exerting its effects on these channels by releasing arachidonic acid or a similar fatty acid. This would not, however, explain the responses of these currents to troglitazone, since this drug was uniformly inhibitory. The inhibition of $I_{K,Ca}$ by troglitazone was not an indirect result of its Ca^{2+} channel antagonism, since this response persisted in Ca^{2+} free solution.

The results presented above indicate that the thiazolidinediones may have diverse effects on the vasculature which, unlike their insulin-sensitizing activity, do not require gene transcription. However, it seems likely that each drug will differ in its ability to influence ion channel function. In particular, in isolated smooth muscle cells troglitazone blocks the Ca^{2+} and delayed rectifier K^+ currents in a concentration range which is similar to that over which it binds to PPA receptor γ, the putative therapeutic target for its antidiabetic action (Cobb et al., 1997). Rosiglitazone was a less potent blocker of the Ca^{2+} channel, which may explain why it has been found to lack vasorelaxing properties in vitro.

Acknowledgements

This study was supported by a grant from SmithKline Beecham Pharaceuticals, Harlow, Essex, United Kingdom. Dr. Knock's salary was from the Tommy's Campaign, UK.

References

Aiello, E.A., Walsh, M.P., Cole, W.C., 1995. Phosphorylation by protein kinase A enhanced delayed rectifier K^+ current in rabbit vascular smooth muscle cells. Am. J. Physiol. 268, H926–H934.

Bean, B.P., 1984. Nitrendipine block of cardiac calcium channels: high-affinity binding to the inactivated state. Proc. Natl. Acad. Sci. U.S.A. 81, 6388–6392.

Buchanan, T.A., Meehan, W.P., Jeng, Y.Y., Yang, D., Chan, T.M., Nadler, J.L., Scott, S., Rude, R.K., Hseuh, W.A., 1995. Blood pressure lowering by pioglitazone: evidence for a direct vascular effect. J. Clin. Invest. 96, 354–360.

Cobb, J., Kliewer, S., Lehmann, J., Blanchard, S., Parks, D., Moore, L., Lenhard, J., Beck, K., Thomson, S., 1997. Troglitazone is a peroxisome proliferator activated receptor γ agonist: a novel mechanism for an antidiabetic agent. Diabetologia 40, A314, Suppl. 1.

Fujiwara, T., Ohsawa, T., Miyamoto, M., Ushiyama, S., Matsuda, K., Horikoshi, H., 1995. Troglitazone (CS-045) acutely increases skin blood flow in dexamethasone-induced diabetic obese Zucher rats and normal rats. Diabetes 44, 72A, Suppl. 1.

The Troglitazone Study Group, Ghazzi, M.N., Perez, J.E., Antonucci, T.K., Driscoll, J.H., Huang, S.M., Faja, B.W., Whitcomb, R.W., 1997. Cardiac and glycaemic benefits of troglitazone treatment in NIDDM. Diabetes 46, 433–439.

Hoffman, C.A., Colca, J.R., 1992. New oral thiazolidinedione antidiabetic agents act is insulin sensitizers. Diabetes Care 15 (8), 1075–1077.

Kirber, M.T., Ordway, R.W., Clapp, L.H., Walsh, J.V., Singer, J.J., 1992. Both membrane stretch and fatty acids directly activate large conductance Ca^{2+} activated K^+ channels in vascular smooth muscle cells. F.E.B.S. Lett. 297, 24–28.

Knaus, H.-G., McManus, O.B., Lee, S.H., Schmalhofer, W.A., Garcia-Calvo, M., Helms, L.M.H., Sanchez, M., Giangiacomo, K., Reuben, J.P., Smith, A.B. III, Kaczorowiski, G.J., Garcia, M.L., 1994. Tremorgenic indole alkaloids potently inhibit smooth muscle high-conductance calcium-activated potassium channels. Biochemistry 33, 5819–5828.

Kotchen, T.A., Zhang, H.Y., Reddy, S., Hoffmann, R.G., 1996. Effect of pioglitazone on vascular reactivity in vivo and in vitro. Am. J. Physiol. 270, R660–R666.

Lee, K., Ibbotson, T., Richardson, P.J., Boden, P.R., 1996. Inhibition of K_{ATP} channel activity by troglitazone in CRI-G1 insulin-secreting cells. Eur. J. Pharmacol. 313, 163–167.

Nakamura, Y., Ohya, Y., Onaka, H., Fujii, K., Abe, I., 1998. Inhibitory action of insulin-sensitizing agents on calcium channels in smooth muscle cells from resistance arteries of guinea-pig. Br. J. Pharmacol. 123, 675–682.

Nelson, M.T., Quayle, J.M., 1995. Physiological roles and properties of potassium channels in arterial smooth muscle. Am. J. Physiol. 268, C799–C822.

Ogihara, T., Rakugi, H., Ikegami, H., Mikami, H., Masuo, K., 1995. Enhancement of insulin sensitivity by troglitazone lowers blood pressure in diabetic hypertensives. Am. J. Hypertens. 8, 316–320.

Pershadsingh, H.A., Szollosi, J., Benson, S., Hyun, W.C., Feuerstein, B.G., Kurtz, T.W., 1993. Effects of ciglitazone on blood pressure and intracellular calcium metabolism. Hypertension 21, 1020–1023.

Quignard, J.-F., Grazzini, E., Guillon, G., Harricane, M.-C., Nargeot, J., Richard, S., 1996. Absence of calcium channels in neonatal rat aortic myocytes. Pflug. Arch. Eur. J. Physiol. 431, 791–793.

Saltiel, A.R., Olefsky, J.M., 1996. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 45, 1661–1669.

Sanguinetti, M.C., Kass, R.S., 1984. Voltage-dependent block of calcium channel current in the calf cardiac purkinje fiber by dihydropyridine calcium channel antagonists. Circ. Res. 55, 336–348.

Smirnov, S.V., Aaronson, P.I., 1996. Modulatory effects of arachidonic acid on the delayed rectifier K^+ current in rat pulmonary arterial myocytes: structural aspects and involvement of protein kinase C. Circ. Res. 79, 20–31.

Smirnov, S.V., Robertson, T.P., Ward, J.P.T., Aaronson, P.I., 1994. Chronic hypoxia is associated with reduced delayed rectifier K^+ current in rat pulmonary artery muscle cells. Am. J. Physiol. 266, H265–H370.

Song, J., Walsh, M.F., Igwe, R., Ram, J.L., Barazi, M., Dominguez, L.J., Sowers, J.R., 1997. Troglitazone reduces contraction by inhibition of vascular smooth muscle cell Ca^{2+} currents and not endothelial nitric oxide production. Diabetes 46, 659–664.

Uehara, A., Hume, J.R., 1985. Interaction of organic calcium channel antagonists with calcium channels in single frog atrial cells. J. Gen. Physiol. 85, 621–648.

Walker, A.B., Naderali, E.K., Chattington, P.D., Buckingham, R.E., Williams, G., 1998. Differential vasoactive effects of the insulin-sensitizers rosiglitazone (BRL 49653) and troglitazone on human small arteries in vitro. Diabetes 47 (5), 810–820.

Yasunari, K., Kohno, M., Kano, H., Yokokawa, K., Minami, M., Yoshikawa, J., 1997. Mechanisms of action of troglitazone in the prevention of high glucose-induced migration and proliferation of cultured coronary smooth muscle cells. Circ. Res. 81, 953–962.

Yoshioka, S., Nishino, H., Shiraki, T., Ikeda, K., Kioke, H., Okuno, A., Wada, M., Fujiwara, T., Horikoshi, H., 1993. Antihypertensive effects of CS-045 treatment in obese Zucher rats. Metabolism 42, 75–80.

Yuan, X.-J., Goldman, W., Tod, M.L., Rubin, L.J., Blaustein, M.P., 1993. Hypoxia reduces potassium current in cultured rat pulmonary but not mesenteric arterial myocytes. Am. J. Physiol. 264, L116–L123.

Zhang, F., Sowers, J.R., Ram, J.L., Standley, P.R., Peuler, J.D., 1994. Effects of proglitazone on calcium channels in vascular smooth muscle. Hypertension 24, 170–175.