British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01539.x
www.brjpharmacol.org

RESEARCH PAPER

Rosiglitazone inhibits vascular K_{ATP} channels and coronary vasodilation produced by isoprenaline

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Keywords
thiazolidinedione; type-2 diabetes mellitus; potassium channel; vascular tones; heart; cardiovascular; mouse

Received
12 January 2011
Revised
7 May 2011
Accepted
24 May 2011

BACKGROUND AND PURPOSE

Rosiglitazone is an anti-diabetic drug improving insulin sensitivity and glucose uptake in skeletal muscle and adipose tissues. However, several recent clinical trials suggest that rosiglitazone can increase the risk of cardiovascular ischaemia, although other studies failed to show such risks. Therefore, the effects of rosiglitazone on the coronary circulation and any potential vascular targets need to be elucidated. Here, we show that the vascular isoform of the ATP-sensitive K^+ (K_{ATP}) channel is inhibited by rosiglitazone, impairing physiological regulation of the coronary circulation.

EXPERIMENTAL APPROACH

The $K_{IR}6.1$/SUR2B channel was expressed in HEK293 cells and studied in whole-cell and inside-out patch configurations. The Langendorff heart preparation was used to evaluate rosiglitazone in the coronary circulation of wild-type (WT) and $K_{IR}6.1$-null (*Kcnj8*$^{-/-}$) mice.

KEY RESULTS

$K_{IR}6.1$/SUR2B channels in HEK cells were inhibited by rosiglitazone in a membrane-delimited manner. This effect was markedly enhanced by sub-micromolar concentrations of glibenclamide and the IC_{50} for rosiglitazone fell to 2μM, a therapeutically achievable concentration. In the Langendorff heart preparation rosiglitazone inhibited, concentration-dependently, the coronary vasodilation induced by isoprenaline, without affecting basal coronary tone. Effects of rosiglitazone on coronary perfusion were attenuated by more than 50% in the *Kcnj8*$^{-/-}$ mice, supporting the involvement of K_{ATP} channels in this effect of rosiglitazone on the coronary circulation.

CONCLUSIONS AND IMPLICATIONS

These results indicate that the vascular K_{ATP} channel is one of the targets of rosiglitazone action, through which this drug may compromise coronary responses to circulating vasodilators and perhaps also to metabolic stress.

Abbreviations

K_{ATP}, ATP-sensitive K^+; VSM, vascular smooth muscles; WT, wild-type

Introduction

Rosiglitazone is one of the two thiazolidinediones currently available for the treatment of type-2 diabetes mellitus. Primarily by activating the peroxisome proliferator-activated receptor-gamma (PPAR-γ) (Duan *et al.*, 2008), rosiglitazone has three major effects on the pathogenesis of type-2 diabetes mellitus and its complications: (i) it improves insulin

resistance and has been successfully used to achieve glycaemic control in a manner that is at least as effective as the sulphonylureas and metformin; (ii) rosiglitazone activates PPAR-γ and regulates adipocyte proliferation and lipid storage, improving lipid profile; and (iii) through the PPAR-γ in vascular tissues, rosiglitazone interferes with the processes of foam cell formation and inflammatory responses, reduces lipid deposition in the vessel wall, and thereby attenuates the development of atherosclerosis (Barnett, 2009). Despite these beneficial outcomes, recent clinical studies have raised the issue of the potential cardiovascular risks in rosiglitazone users (Zinn *et al.*, 2008; Kaul *et al.*, 2010). A large meta-analysis clinical trial suggested a 43% increase in risk of myocardial infarction in patients treated with rosiglitazone (Nissen and Wolski, 2007). This study was followed by a number of additional reports using an alternative analysis of the same data, new meta-analyses and observational studies on both rosiglitazone and pioglitazone, the other clinically used thiazolidinedione (Home *et al.*, 2007; Gerstein *et al.*, 2008; Mannucci *et al.*, 2008; Duckworth *et al.*, 2009; Psaty and Furberg, 2007; Vanasse *et al.*, 2009). The results, however, were rather variable and inconsistent. Thus, more direct evidence for any impairment of coronary circulation and the potential vascular targets is needed for an effective and appropriate application of the drug in the treatment of type-2 diabetes mellitus.

A potential target molecule of rosiglitazone on the vascular wall is the ATP-sensitive K^+ (K_{ATP}) channel composed of $K_{IR}6.1$/SUR2B subunits (channel nomenclature follows Alexander *et al.*, 2009) expressed in vascular smooth muscle (VSM). Numerous vasodilator and vasoconstrictor hormones act on this K^+ channel (Quayle *et al.*, 1997; Ashcroft, 2006; Nichols, 2006; Shi *et al.*, 2007a,b; Yang *et al.*, 2008). The K_{ATP} channel is also regulated by several metabolites. This metabolite sensitivity allows the channel to regulate the vascular tone and regional blood flow according to the metabolic state in local tissues under both physiological and pathophysiological conditions (Ashcroft, 2006; Yang *et al.*, 2010; 2011). Thus, dysregulation of such a critical channel in the vasculature could affect coronary responses to circulating vasodilators, which in turn may be influenced by rosiglitazone. In order to test this hypothesis we performed these studies and our results showed that the $K_{IR}6.1$/SUR2B channel was strongly inhibited by rosiglitazone and this channel inhibition compromised the vasodilatory response of the coronary circulation.

Methods

Expression of K_{ATP} channel in HEK293 cells

The HEK293 cells were cultured in DMEM/F12 medium at 37°C with 10% fetal bovine serum and penicillin/streptomycin in the presence of 5% CO_2. A eukaryotic expression vector pcDNA3.1 was used to express rat $K_{IR}6.1$ (GenBank Accession # D42145) in the cells together with SUR2B (GenBank # D86038, mRNA isoform NM_011511). Lipofectamine^{2000} (Invitrogen Inc., Carlsbad, CA) was used for transfection. Each 35 mm Petri dish containing the cells was transfected with 1 μg $K_{IR}6.1$ and 3 μg SUR2B. Green fluorescent protein cDNA (0.4 μg, pEGFP-N2, Clontech, Palo Alto, CA) was included in the cDNA mixture to facilitate the identification of positively transfected cells. Cells were split and transferred to cover slips after 12–18 h of transfection. Experiments were performed on the cells on cover slips during the following 12–48 h. The HEK cells express endogenous β-adrenoceptors whose activation enhances the K_{ATP} channel activity through the PKA signalling system (Shi *et al.*, 2007b; Yang *et al.*, 2008). Thus, the K_{ATP} channel modulation by rosiglitazone was also studied by activating the endogenous β-adrenoceptors.

Electrophysiology

Patch clamp experiments were carried out at room temperature as described previously (Wang *et al.*, 2003; Shi *et al.*, 2007a,b; 2008a,b; 2010; Yang *et al.*, 2008; 2010). The bath solution contained (in mM): KCL 10, potassium gluconate 135, EGTA 5, glucose 5 and HEPES 10 (pH 7.4). The pipette was filled with a solution containing (in mM): KCl 10, potassium gluconate 133, EGTA 5, glucose 5, K_2ATP 1, NaADP 0.5, $MgCl_2$ 1 and HEPES 10 (pH 7.4). Whole-cell currents were recorded in single-cell voltage clamp with holding potential 0 mV and step to –80 mV for 1 s. To avoid nucleotide degradation, all intracellular solutions were freshly made and used within 4 h.

Recordings were made with the Axopatch 200B amplifier (Axon Instruments Inc., Foster City, CA). The data were low-pass filtered (2 kHz, Bessel 4-pole filter, –3 dB), and digitized (10 kHz, 16-bit resolution) with Clampex 9 (Axon Instruments Inc.). Single-channel currents were recorded from inside-out patches with a constant single voltage of –60 mV. Higher sampling rate (20 kHz) was used to digitize the currents recorded from inside-out patch. Data were analysed using Clampfit 9 (Axon Instruments Inc.).

Langendorff-perfused hearts

All animal care and experimental procedures were in compliance with an approved protocol by the Institutional Animal Care and Use Committees (IACUC) at Georgia State University. Male wild-type (WT) C57BL/6 mice weighing 20 to 30 g (8 to 14 weeks of age) were deeply anaesthetized followed by removal of the heart. The heart was transferred to ice-cold (4°C) Krebs-Henseleit (KH) solution (composition in mM): 119.0 NaCl, 4.7 KCl, 2.5 $CaCl_2$, 2.5 $MgSO_4$, 25.0 $NaHCO_3$, 1.2 KH_2PO_4, 0.5 disodium EDTA and 10 glucose (pH 7.4). The aortic root was quickly cannulated and flushed gently with KH solution to remove blood in the coronary arteries. The heart was then placed in an organ bath and perfused with KH solution at a constant pressure. The reservoir was maintained at a fixed height above the heart to keep the perfusion pressure at approximately 80 cm H_2O. The KH solution was bubbled with a mixture of 95% O_2–5% CO_2 and the perfusate temperature was maintained at 35°C using a warming coil. The isolated heart was constantly bathed in a small chamber (~2 mL) with the perfusate constantly flowing through the coronary arteries. The flow rate from the heart was measured by collecting the overflow fluid from the chamber at five minute intervals.

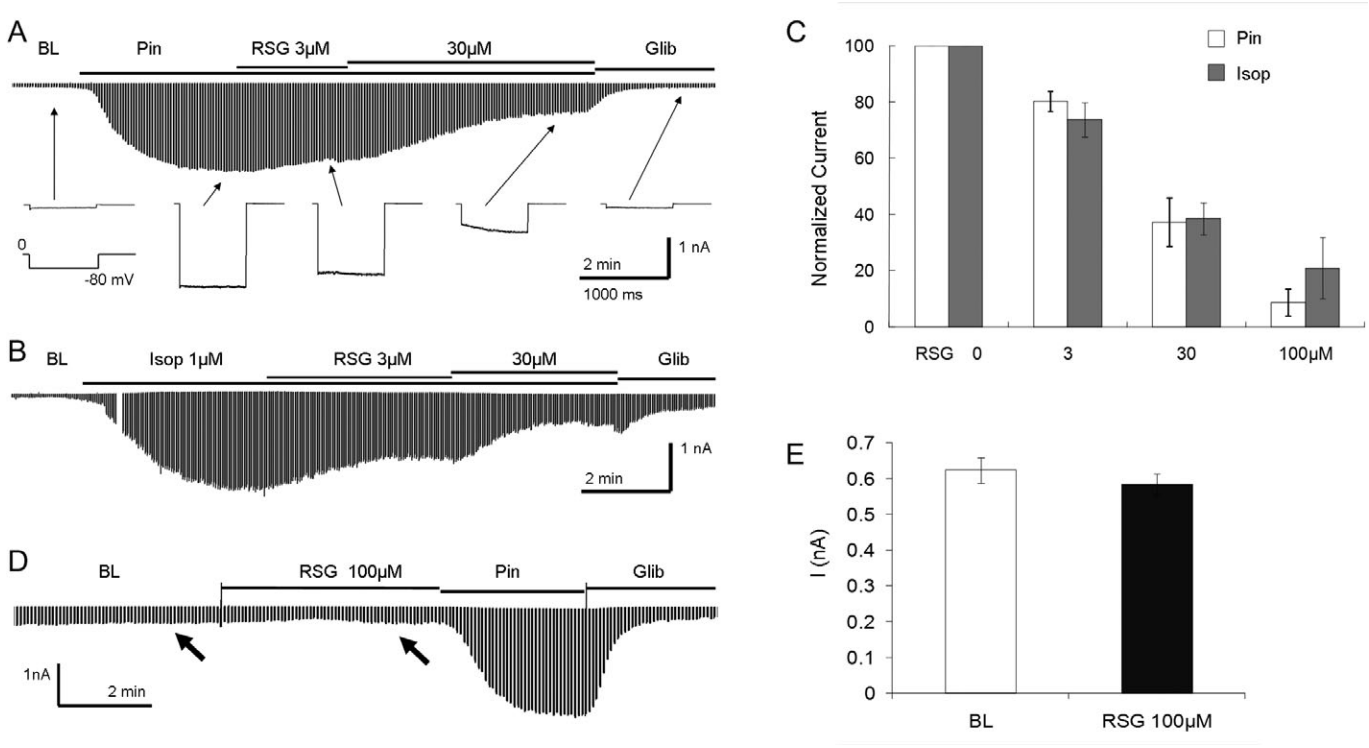

Figure 1

Inhibition of the $K_{IR}6.1$/SUR2B channel by rosiglitazone. (A) The HEK cell was co-transfected with $K_{IR}6.1$ and SUR2B. Inward currents were studied 2 days after transfection using symmetric concentrations of K^+ (145 mM) applied to the bath and pipette solutions. Membrane potentials were held at 0 mV and stepped to –80 mV in every 3 s. The cell showed small currents at baseline (BL). The currents were strongly activated by 10 μM pinacidil (Pin). At the maximum channel activation the application of rosiglitazone (RSG) led to concentration-dependent inhibitions of the $K_{IR}6.1$/SUR2B currents. The currents were further inhibited by 10 μM glibenclamide (Glib). (B) In the same condition, the $K_{IR}6.1$/SUR2B currents were activated by isoprenaline (Isop) via the HEK cell-endogenous β-adrenoceptor as shown previously (Shi *et al.*, 2007b). The currents had almost the same sensitivity to rosiglitazone. Note that there is a 1 min gap during isoprenaline exposure. (C) When both currents are plotted against rosiglitazone concentrations, the pinacidil-activation currents overlie the isoprenaline-activated currents. Rosiglitazone produced concentration-dependent inhibition of the $K_{IR}6.1$/SUR2B currents activated by both pinacidil and isoprenaline. (D,E) Rosiglitazone did not have any evident effect on the basal $K_{IR}6.1$/SUR2B currents before the currents were activated by pinacidil. The current amplitude with rosiglitazone treatment remained the same as the baseline (arrows). Similar results were found in another cell (n = 4 to 7 patches from different cells).

Data analysis

Data were evaluated using Student's *t*-tests and ANOVA, and statistical significance was deemed acceptable when $P < 0.05$.

Materials

Rosiglitazone was purchased from Cayman Chemical Compamy (Ann Arbor, MI, USA). Pioglitazone, pinacidil, glybenclamide and isoprenaline were purchased from Sigma Chemicals (St. Louis, MO, USA).

Results

Whole-cell K^+ currents were recorded from HEK cells transfected with $K_{IR}6.1$/SUR2B in single-cell voltage clamp. Symmetric concentrations of K^+ (145 mM) were applied to the bath and pipette solutions. The membrane potential was held at 0 mV and stepped to –80 mV for 1 s. This protocol was repeated every 3 s. Under this condition, the cells showed a very small basal current (Yang *et al.*, 2010; 2011). Administration of 10 μM pinacidil, a selective K_{ATP} channel activator, strongly activated the inward K^+ currents that were subsequently inhibited by 10 μM glibenclamide, a K_{ATP} channel blocker. These pinacidil/glibenclamide-sensitive currents were exogenous as pinacidil/glibenclamide had very little effect on the HEK cells transfected with the expression vector alone (Figure S1A). Therefore, these K_{ATP} channel activator and inhibitor were used to determine the expression of the channel in HEK cells.

Exposure to rosiglitazone produced an inhibition of the pinacidil-activated current (Figure 1A). The channel inhibition occurred within 1 min, and showed a clear concentration dependence (Figure 1C). A complete reversal was seen after removal of rosiglitazone (Figure S1B). When the $K_{IR}6.1$/SUR2B current was activated by the β-adrenoceptor agonist isoprenaline (1 μM), treatment of the cell with rosiglitazone led to a similar channel inhibition (Figure 1B). Indeed, the current activated by isoprenaline had almost the same sensitivity to rosiglitazone as the current activated by pinacidil (Figure 1C). Rosiglitazone had no evident effect on the basal current before the $K_{IR}6.1$ /SUR2B channel was activated by pinacidil (Figure 1D,E). In the presence of rosiglitazone, pinacidil (10 μM or 100 μM) did not activate the $K_{IR}6.1$ /SUR2B

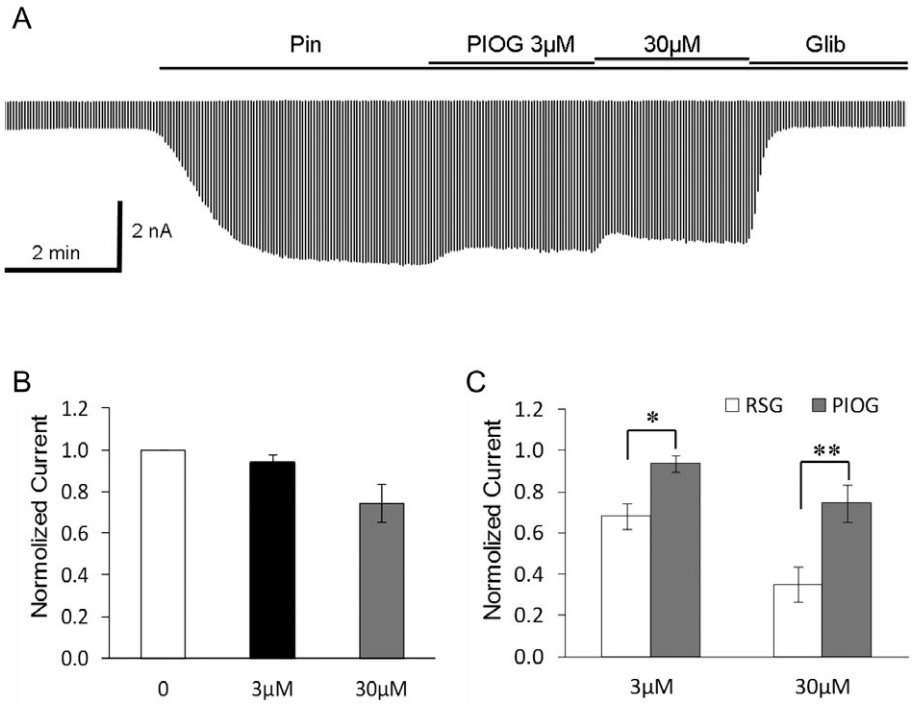

Figure 2

(A) Pioglitazone (PIOG) inhibited the $K_{IR}6.1$/SUR2B currents only modestly. Pin, pinacidil; Glib, glibenclamide. (B) $K_{IR}6.1$/SUR2B currents were insensitive to 3 μM pioglitazone and showed rather small response to 30 μM pioglitazone. (C) In comparison to rosiglitazone (RSG), the effect of pioglitazone was significantly smaller. *$P < 0.05$, **$P < 0.01$, compared with each other ($n = 9$ to 11).

current, although the channel activity normalised after the washout of rosiglitazone (Figure S1B,C). In contrast, another thiazolidinedione, pioglitazone, caused only modest inhibition of the $K_{IR}6.1$ /SUR2B current, at 3 and 30 μM (Figure 2A,B). Thus, compared with rosiglitazone, the effect of pioglitazone was much weaker (Figure 2C).

In order to understand the biophysical mechanisms of the channel inhibition, we studied the $K_{IR}6.1$/SUR2B currents in inside-out patches. The effect of rosiglitazone was mediated by suppression of the channel open state probability without affecting the unitary conductance (Figure 3A,B). The channel inhibition was reversible and showed concentration dependence. The current–concentration relationship is described using the Hill equation with IC_{50} of 10 μM and h of 1.2 (Figure 3C).

As a number of type-2 diabetes mellitus patients are prescribed rosiglitazone together with sulphonylureas which inhibit the K_{ATP} channel, we studied the combined effect of rosiglitazone and glibenclamide. In the presence of 0.25 μM glibenclamide, the effect of rosiglitazone was markedly potentiated with the IC_{50} now falling to 2 μM (h 2.0) (Figure 3C). Both these rosiglitazone and glibenclamide concentrations are achieved therapeutically. Serum concentration of rosiglitazone may be raised above these levels as a result of impaired drug metabolism and genetic variations of individual patients and hence, an inhibition of the K_{ATP} channel may well take place in these patients, compromising their cardiovascular function.

In the Langendorff heart preparation, the perfusate through the coronary circulation was collected and measured at 5 min intervals under constant pressure (80 cm H_2O). The viability of the preparation was confirmed by the following criteria (i) was the heart beating spontaneously or responsive to isoprenaline in the perfusate, and (ii) was the perfusion volume increased in the presence of isoprenaline. Our results showed that rosiglitazone (30 μM or 100 μM) had no significant effect on the basal perfusion volume of the heart from the WT mice (Figure S2). The perfusion volume rose rapidly when the perfusate contained isoprenaline (a 1.30 mL increase or 86.8%). During the period of isoprenaline-induced coronary vasodilation, exposure to rosiglitazone resulted in a concentration-dependent decrease in the perfusion volume (Figure 4A), that is, 30 or 100 μM rosiglitazone reduced the perfusion volume by 0.41 mL or 0.85 mL respectively and this effect of rosiglitazone was reversible (Figure 4A,B). Similar results were obtained in studies using pinacidil-induced coronary vasodilation (Figure 4C, D). Moreover, another known $K_{IR}6.1$ inhibitor glibenclamide showed similar inhibitory effects on perfusion volume in the presence of isoprenaline or pinacidil (Figure S3A,B).

In the Langendorff hearts of *Kcnj8*$^{-/-}$ mice (lacking the $K_{IR}6.1$ channel) prepared identically to the WT mice, an exposure to 100 nM isoprenaline led to a rather small and inconsistent vasodilation, with no discernable effects of rosiglitazone. Therefore 300 nM isoprenaline was used instead of 100 nM in the hearts of the *Kcnj8*$^{-/-}$ mice and this concentration of isoprenaline produced an increase in the coronary perfusion volume (Figure 5A). The isoprenaline-induced coronary vasodilation, however, was not affected by 30 μM rosiglitazone in the $K_{IR}6.1$-null hearts (Figure 5B).

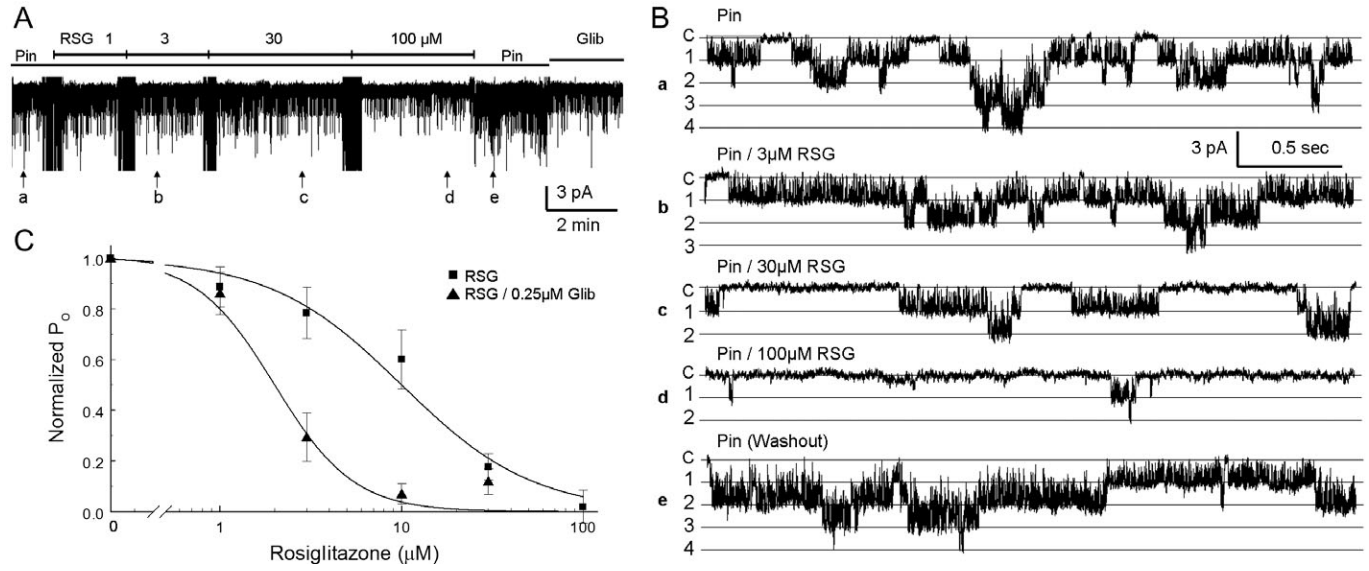

Figure 3

Membrane-delimited inhibition of the $K_{IR}6.1$/SUR2B channel. (A) Single-channel $K_{IR}6.1$/SUR2B currents were recorded in an inside-out patch from an HEK cell with symmetric K^+ (145 mM) and a –60 mV membrane potential. (B) Single-channel activity shown in expanded scales (traces from top to bottom are obtained from A at times marked a – e respectively). The currents were activated by pinacidil (Pin) and inhibited dose-dependently by rosiglitazone (RSG). Washout of rosiglitazone with pinacidil-containing perfusate led to a complete recovery. Labels on the left: C, closure; 1, 2, . . . , *n*, the first, second . . . *n*th openings. (C) The channel activity is a function of rosiglitazone concentration. Their relationship is described using the Hill equation with IC_{50} 10 μM and h 1.2 (n = 4 patches from different cells) for rosiglitazone alone and 2 μM (h 2.0) in the presence of 0.25 μM glibenclamide (Glib; n = 4).

However, a concentration of 100 μM rosiglitazone reduced the isoprenaline-induced coronary vasodilation by 18.4 ± 9.5% (n = 6, Figure 5C in which the isoprenaline-augmented flow was reduced from 1.23 mL to 1.07 mL). This weak effect may be mediated by mechanisms other than interactions with the K_{ATP} channel. In the WT heart, the same concentration of rosiglitazone reduced coronary vasodilation by 67.2 ± 5.9% (n = 5, a 0.85 mL decrease from 1.30 mL; $P < 0.001$).

Pinacidil had no vasodilatory effects in the *Kcnj8*$^{-/-}$ hearts (Figure S3A). In comparison with the 84.6% inhibition seen in the WT hearts, glibenclamide did not reduce the isoprenaline-induced coronary dilation in these mutant hearts (Figure S3A,B). These results suggest strongly that the $K_{IR}6.1$/SUR2B channel is a major vascular target of rosiglitazone in the coronary circulation's vasodilator response to isoprenaline.

Discussion and conclusions

These studies are the first demonstration that rosiglitazone, at therapeutically-achieved concentrations, prevented coronary vasodilatory responses to the β-adrenoceptor agonist isoprenaline. The effect is likely to involve the $K_{IR}6.1$/SUR2B channel that is expressed primarily in the VSM. Channel activity was strongly inhibited by rosiglitazone when the channel was activated by pinacidil or isoprenaline using the whole-cell configuration. Cytosolic soluble factors did not seem necessary for the channel inhibition as the rosiglitazone effect was seen in excised inside-out patches. Indeed the IC_{50} of rosiglitazone was lower in inside-out patches than in the whole-cell configuration.

Previous clinical studies have suggested that type-2 diabetes mellitus patients treated with rosiglitazone are at increased risk of cardiovascular ischaemic events, (Nissen and Wolski, 2007; Zinn *et al.*, 2008; Kaul *et al.*, 2010), although such observations are not consistent (Home *et al.*, 2007; Psaty and Furberg, 2007; Gerstein *et al.*, 2008; Mannucci *et al.*, 2008; Duckworth *et al.*, 2009; Vanasse *et al.*, 2009). The discrepancies may be related to the many factors involved in clinical studies, such as placebo- versus active-controlled trials, patient demographics and treatment variations.

However, data from animal experiments suggest that rosiglitazone has more beneficial than harmful cardiovascular effects. The beneficial effects are likely to be due to the improvement of metabolic profile and VSM remodelling, whilst the deleterious effects might involve oxidative stress and endothelial damage. (Wang *et al.*, 2006; How *et al.*, 2007; Lu *et al.*, 2008b; Kanda *et al.*, 2009; Savoia *et al.*, 2010; Torres Tda *et al.*, 2010; Yu *et al.*, 2010). Some cardiovascular effects of rosiglitazone have been studied using various animal models of ischaemia and cardiac protection (Knock *et al.*, 1999; Khandoudi *et al.*, 2002; Abe *et al.*, 2008; Kilter *et al.*, 2009; Potenza *et al.*, 2009; Wang *et al.*, 2009; 2010). But direct effects of rosiglitazone on the vessel tone and cardiac regional blood flow has not been demonstrated.

As ion channel activity determines membrane potential, excitability and contractility in VSM cells, the potential involvement of these channels in the effects of rosiglitazone

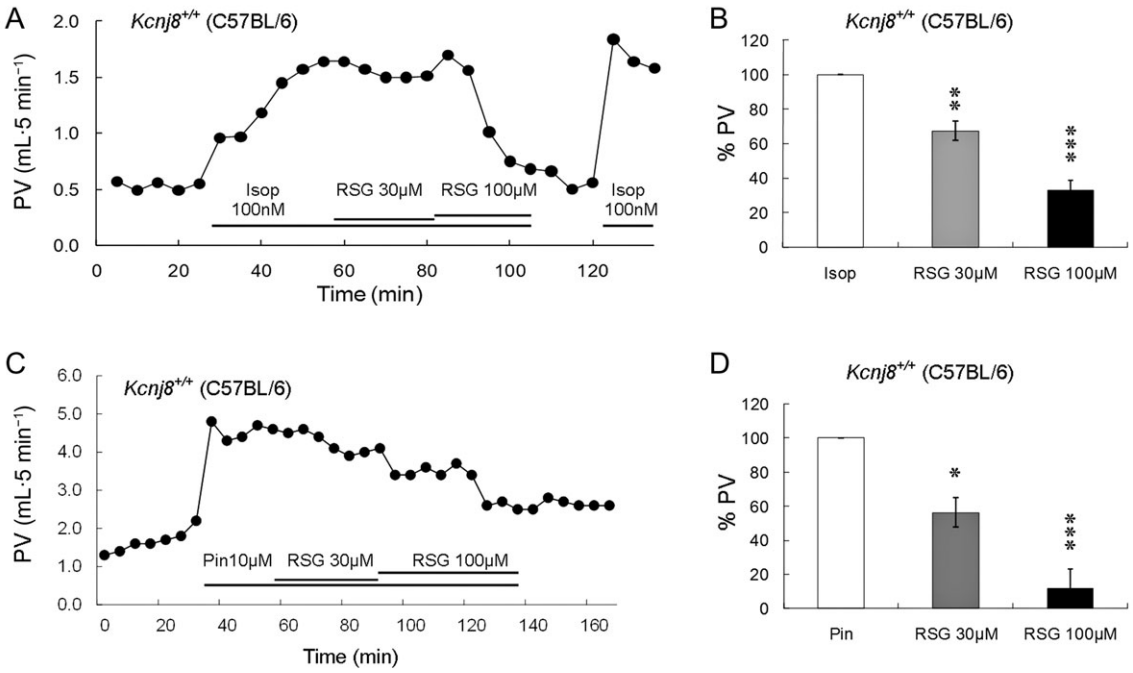

Figure 4

Effects of rosiglitazone on the coronary circulation of WT (*Kcnj*$^{+/+}$) mice. (A) The perfusion volume (PV) increased markedly when there was isoprenaline (Isop; 100 nM) in the perfusate. In the presence of isoprenaline, rosiglitazone (RSG) reduced the PV in a dose-dependent manner. (B) Dose-dependent inhibition of isoprenaline-induced coronary vasorelaxation in two strains of mice in which the $K_{IR}6.1$ channel is present. The PV was normalized to the maximum PV during isoprenaline exposure as 100%. The isoprenaline-induced coronary vasorelaxation was suppressed by 32.6% and 67.2% in response to 30 μM and 100 μM rosiglitazone respectively. (C,D) Dose-dependent inhibition of pinacidil (Pin)-induced coronary vasodilation. The pinacidil-induced coronary vasodilation was suppressed by 43.7% and 83.1% in response to 30 μM and 100 μM rosiglitazone respectively. Data are presented as mean ± SEM (n = 3 to 5 hearts). *P < 0.05; **P < 0.01; ***P < 0.001 compared with control.

has been studied and reported previously (Knock *et al.*, 1999; Mishra and Aaronson, 1999; Eto *et al.*, 2001; Lu *et al.*, 2008a; Chang *et al.*, 2009). Rosiglitazone inhibits Ca^{2+} currents and voltage-activated K^+ currents that play a role in cAMP-mediated vasodilation (Eto *et al.*, 2001; Li *et al.*, 2003). Moreover, rosiglitazone activates Ca^{2+}-activated K^+ currents in acutely dissociated mesenteric VSM cells (Eto *et al.*, 2001; Lu *et al.*, 2008a). However, rosiglitazone does not induce vasorelaxation of human subcutaneous small arterial rings preconstricted with noradrenaline (Walker *et al.*, 1998). Also, rosiglitazone inhibits glibenclamide-sensitive K^+ currents in freshly isolated aortic myocytes (Chang *et al.*, 2009), but does not affect the vasorelaxation of endothelium-denuded human internal mammary artery rings preconstricted with noradrenaline and KCl (Irat *et al.*, 2006). These inconsistent findings may be due to differences in the experimental preparations employed and/or the presence of multiple ion channel species in the preparations. Focusing on a specific subunit, rosiglitazone has been shown to stimulate insulin secretion in pancreatic beta cells via phosphorylation of the $K_{IR}6.2$ channel by AMP-dependent protein kinase (Chang *et al.*, 2009). In other studies, rosiglitazone has been shown to block cardiac K_{ATP} channels and promote the onset of ventricular fibrillation during severe ischaemia (Mishra and Aaronson, 1999).

Our current studies indicate that the $K_{IR}6.1$/SUR2B channel is indeed a target of rosiglitazone as the Kir6.1/SUR2B channel is inhibited by rosiglitazone. In WT and $K_{IR}6.1$-null mice, inhibition of the $K_{IR}6.1$/SUR2B channel leads to an impairment of coronary vasodilatory responses. This $K_{IR}6.1$/SUR2B channel is a common target of both vasodilator and vasoconstrictor hormones and transmitters that activate and inhibit the channel via distinct protein phosphorylation (Ashcroft, 2006; Shi *et al.*, 2007a,b; Yang *et al.*, 2008; Orie *et al.*, 2009). Experimental genetic disruption of either $K_{IR}6.1$ or SUR2B subunits leads to a defective coronary circulation and sudden death (Chutkow *et al.*, 2002; Miki *et al.*, 2002). We have shown that the inhibition of this K_{ATP} channel by rosiglitazone disrupted coronary vasodilatory responses to a β-adrenoceptor agonist. As the basal level of channel activity is low under physiological conditions (Quayle *et al.*, 1997; Nichols, 2006), the inhibition of the K_{ATP} channel in VSM cells may not produce depolarization sufficient to cause muscle contraction, which could explain the lack of effect of rosiglitazone on basal vascular tone, as shown in previous studies (Walker *et al.*, 1998; Irat *et al.*, 2006). Our results have also shown that rosiglitazone did not affect the basal $K_{IR}6.1$/SUR2B currents. However, during stimulation of the $K_{IR}6.1$/SUR2B channel by activation with vasoactive agents, rosiglitazone did impair coronary vasodilation. Under patho-physiological conditions where critical and continuous regulation of the coronary circulation is controlled by the sympathetic/adrenal gland systems, it is very likely that K_{ATP} channel inhibition induced by rosiglitazone therapy may

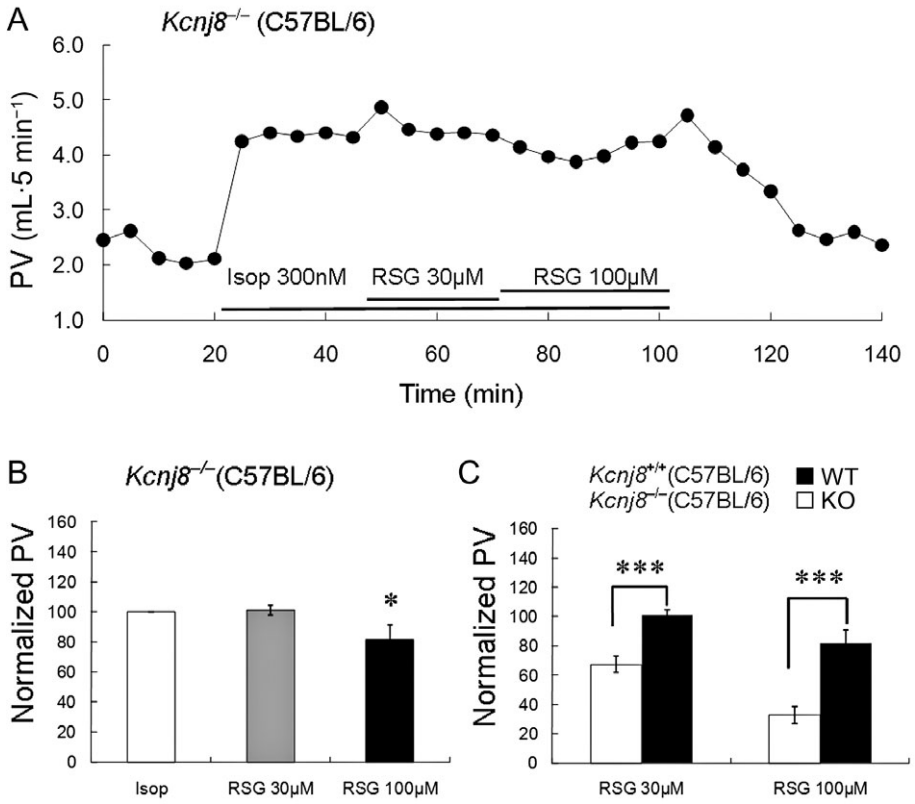

Figure 5

The effect of rosiglitazone on coronary PV in *Kcnj8*$^{-/-}$ mice. (A) In a Langendorf heart from a *Kcnj8*$^{-/-}$ mouse, isoprenaline (Isop; 300 nM) produced coronary vasodilation. (B) The isoprenaline-induced coronary vasorelaxation was suppressed by 18.4% in response to 100 μM rosiglitazone (RSG), while rosiglitazone at 30 μM had no evident effect on the PV. (C) In comparison with the WT hearts, the rosiglitazone effect on coronary circulation was significantly lower in 30 μM and 100 μM (*P < 0.05; ***P < 0.001; n = 6).

compromise coronary circulation under circumstances when vasodilation is essential, such as during elevated metabolic activity, exercise and stress.

Using the *Kcnj8*$^{-/-}$ mice allowed us to estimate the contributions of both the K_{ATP} channel-dependent and the K_{ATP} channel-independent effects of rosiglitazone on the coronary circulation. Rosiglitazone (100 μM) inhibited coronary perfusion by ~19% in *Kcnj8*$^{-/-}$ mice and ~68% in the WT mice. This difference indicates that >50% of the isoprenaline-induced coronary vasodilation was inhibited by rosiglitazone via the K_{ATP} channel, which is consistent with the idea that the vascular K_{ATP} channel is the major target of rosiglitazone.

Our results indicated that the Kir6.1/SUR2B channel was inhibited by rosiglitazone alone with an IC_{50} of 10 μM but the IC_{50} was lowered to 2 μM, in the presence of 0.25 μM glibenclamide. Both of these concentrations are within the range of therapeutic concentrations in the treatment of type-2 diabetes mellitus (Coppack *et al.*, 1990; Cox *et al.*, 2000). Many diabetics are prescribed combinations of rosiglitazone and glibenclamide, hence it is likely that the vascular K_{ATP} channel is inhibited, at least partially, in these patients.

Interestingly, we found that pioglitazone at 30 μM produced only a modest inhibition of the vascular K_{ATP} channel. We were unable to use pioglitazone in higher concentrations as the compound from Sigma and LKT Lab were insoluble even with DMSO as a solvent. As 30 μM pioglitazone is a higher concentration than the blood level in patients given this drug, our results suggest that the vascular K_{ATP} channel would not be significantly suppressed by therapeutic concentrations of pioglitazone, consistent with data from several clinical trials (Wong *et al.*, 2004).

In conclusion, the VSM isoform of the K_{ATP} channel was inhibited by rosiglitazone. The channel inhibition is membrane-delimited and seems to occur by direct interaction with the channel protein. In the isolated perfused heart, rosiglitazone did not affect the basal coronary circulation but inhibited the β-adrenoceptor agonist-mediated and K_{IR}6.1/SUR2B channel activator-induced coronary vasodilation. The effect is likely to be mediated via the K_{IR}6.1 channel as it was markedly attenuated in hearts from *Kcnj8*$^{-/-}$ mice. Therefore, K_{IR}6.1/SUR2B channel inhibition may be an important contributory factor in myocardial ischaemia in diabetics who have an underlying cardiovascular condition.

Acknowledgements

This work was supported by the NIH (HD060959), the American Heart Association (09GRNT2010037). L.Y. was partially supported by a scholarship of Harbin Medical University. Y.Y.

is a Brains & Behavior fellow of Georgia State University. Thanks for Timothy C. Trower's technique support.

Conflict of interest

There is no conflict of interest for any author.

References

Abe M, Takiguchi Y, Ichimaru S, Kaji S, Tsuchiya K, Wada K (2008). Different effect of acute treatment with rosiglitazone on rat myocardial ischemia/reperfusion injury by administration method. Eur J Pharmacol 589: 215–219.

Alexander SPH, Mathie A, Peters JA (2009). Guide to Receptors and Channels (GRAC), 4th edn. Br J Pharmacol 158 (Suppl. 1): S1–S254.

Ashcroft FM (2006). From molecule to malady. Nature 440: 440–447.

Barnett AH (2009). Redefining the role of thiazolidinediones in the management of type 2 diabetes. Vasc Health Risk Manag 5: 141–151.

Chang TJ, Chen WP, Yang C, Lu PH, Liang YC, Su MJ *et al.* (2009). Serine-385 phosphorylation of inwardly rectifying K+ channel subunit (Kir6.2) by AMP-dependent protein kinase plays a key role in rosiglitazone-induced closure of the K(ATP) channel and insulin secretion in rats. Diabetologia 52: 1112–1121.

Chutkow WA, Pu J, Wheeler MT, Wada T, Makielski JC, Burant CF *et al.* (2002). Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K(ATP) channels. J Clin Invest 110: 203–208.

Coppack SW, Lant AF, McIntosh CS, Rodgers AV (1990). Pharmacokinetic and pharmacodynamic studies of glibenclamide in non-insulin dependent diabetes mellitus. Br J Clin Pharmacol 29: 673–684.

Cox PJ, Ryan DA, Hollis FJ, Harris AM, Miller AK, Vousden M *et al.* (2000). Absorption, disposition, and metabolism of rosiglitazone, a potent thiazolidinedione insulin sensitizer, in humans. Drug Metab Dispos 28: 772–780.

Duan SZ, Usher MG, Mortensen RM (2008). Peroxisome proliferator-activated receptor-gamma-mediated effects in the vasculature. Circ Res 102: 283–294.

Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD *et al.* (2009). Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 360: 129–139.

Eto K, Ohya Y, Nakamura Y, Abe I, Fujishima M (2001). Comparative actions of insulin sensitizers on ion channels in vascular smooth muscle. Eur J Pharmacol 423: 1–7.

Gerstein HC, Miller ME, Byington RP, Goff DC Jr, Bigger JT, Buse JB *et al.* (2008). Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 358: 2545–2559.

Home PD, Pocock SJ, Beck-Nielsen H, Gomis R, Hanefeld M, Jones NP *et al.* (2007). Rosiglitazone evaluated for cardiovascular outcomes–an interim analysis. N Engl J Med 357: 28–38.

How OJ, Larsen TS, Hafstad AD, Khalid A, Myhre ES, Murray AJ *et al.* (2007). Rosiglitazone treatment improves cardiac efficiency in hearts from diabetic mice. Arch Physiol Biochem 113: 211–220.

Irat AM, Aslamaci S, Karasu C, Ari N (2006). Alteration of vascular reactivity in diabetic human mammary artery and the effects of thiazolidinediones. J Pharm Pharmacol 58: 1647–1653.

Kanda T, Brown JD, Orasanu G, Vogel S, Gonzalez FJ, Sartoretto J *et al.* (2009). PPARgamma in the endothelium regulates metabolic responses to high-fat diet in mice. J Clin Invest 119: 110–124.

Kaul S, Bolger AF, Herrington D, Giugliano RP, Eckel RH (2010). Thiazolidinedione drugs and cardiovascular risks: a science advisory from the American Heart Association and American College Of Cardiology Foundation. J Am Coll Cardiol 55: 1885–1894.

Khandoudi N, Delerive P, Berrebi-Bertrand I, Buckingham RE, Staels B, Bril A (2002). Rosiglitazone, a peroxisome proliferator-activated receptor-gamma, inhibits the Jun NH(2)-terminal kinase/activating protein 1 pathway and protects the heart from ischemia/reperfusion injury. Diabetes 51: 1507–1514.

Kilter H, Werner M, Roggia C, Reil JC, Schafers HJ, Kintscher U *et al.* (2009). The PPAR-gamma agonist rosiglitazone facilitates Akt rephosphorylation and inhibits apoptosis in cardiomyocytes during hypoxia/reoxygenation. Diabetes Obes Metab 11: 1060–1067.

Knock GA, Mishra SK, Aaronson PI (1999). Differential effects of insulin-sensitizers troglitazone and rosiglitazone on ion currents in rat vascular myocytes. Eur J Pharmacol 368: 103–109.

Li H, Chai Q, Gutterman DD, Liu Y (2003). Elevated glucose impairs cAMP-mediated dilation by reducing Kv channel activity in rat small coronary smooth muscle cells. Am J Physiol Heart Circ Physiol 285: H1213–H1219.

Lu L, Reiter MJ, Xu Y, Chicco A, Greyson CR, Schwartz GG (2008a). Thiazolidinedione drugs block cardiac KATP channels and may increase propensity for ischaemic ventricular fibrillation in pigs. Diabetologia 51: 675–685.

Lu YL, Jimbu YM, Chen Y, Zhao JB, Ye TT, Yang H (2008b). The effects of rosiglitazione on renal artery endothelium in diabetic rats. Exp Clin Endocrinol Diabetes 116: 537–540.

Mannucci E, Monami M, Lamanna C, Gensini GF, Marchionni N (2008). Pioglitazone and cardiovascular risk. A comprehensive meta-analysis of randomized clinical trials. Diabetes Obes Metab 10: 1221–1238.

Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K *et al.* (2002). Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. Nat Med 8: 466–472.

Mishra SK, Aaronson PI (1999). Differential block by troglitazone and rosiglitazone of glibenclamide-sensitive K(+) current in rat aorta myocytes. Eur J Pharmacol 386: 121–125.

Nichols CG (2006). KATP channels as molecular sensors of cellular metabolism. Nature 440: 470–476.

Nissen SE, Wolski K (2007). Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 356: 2457–2471.

Orie NN, Thomas AM, Perrino BA, Tinker A, Clapp LH (2009). Ca2+/calcineurin regulation of cloned vascular K ATP channels: crosstalk with the protein kinase A pathway. Br J Pharmacol 157: 554–564.

Potenza MA, Gagliardi S, De Benedictis L, Zigrino A, Tiravanti E, Colantuono G *et al.* (2009). Treatment of spontaneously hypertensive rats with rosiglitazone ameliorates cardiovascular pathophysiology via antioxidant mechanisms in the vasculature. Am J Physiol Endocrinol Metab 297: E685–E694.

Psaty BM, Furberg CD (2007). The record on rosiglitazone and the risk of myocardial infarction. N Engl J Med 357: 67–69.

Quayle JM, Nelson MT, Standen NB (1997). ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. Physiol Rev 77: 1165–1232.

Savoia C, Ebrahimian T, Lemarie CA, Paradis P, Iglarz M, Amiri F *et al.* (2010). Countervailing vascular effects of rosiglitazone in high cardiovascular risk mice: role of oxidative stress and PRMT-1. Clin Sci (Lond) 118: 583–592.

Shi W, Cui N, Shi Y, Zhang X, Yang Y, Jiang C (2007a). Arginine vasopressin inhibits Kir6.1/SUR2B channel and constricts the mesenteric artery via V1a receptor and protein kinase C. Am J Physiol Regul Integr Comp Physiol 293: R191–R199.

Shi Y, Wu Z, Cui N, Shi W, Yang Y, Zhang X *et al.* (2007b). PKA phosphorylation of SUR2B subunit underscores vascular KATP channel activation by beta-adrenergic receptors. Am J Physiol Regul Integr Comp Physiol 293: R1205–R1214.

Shi Y, Chen X, Wu Z, Shi W, Yang Y, Cui N *et al.* (2008a). cAMP-dependent protein kinase phosphorylation produces interdomain movement in SUR2B leading to activation of the vascular KATP channel. J Biol Chem 283: 7523–7530.

Shi Y, Cui N, Shi W, Jiang C (2008b). A short motif in Kir6.1 consisting of four phosphorylation repeats underlies the vascular KATP channel inhibition by protein kinase C. J Biol Chem 283: 2488–2494.

Shi W, Cui N, Wu Z, Yang Y, Zhang S, Gai H *et al.* (2010). Lipopolysaccharides up-regulate Kir6.1/SUR2B channel expression and enhance vascular KATP channel activity via NF-kappaB-dependent signaling. J Biol Chem 285: 3021–3029.

Torres Tda S, Aguila MB, Mandarim-de-Lacerda CA (2010). Rosiglitazone reverses cardiac adverse remodeling (fibrosis and vascularization) in perinatal low protein rat offspring. Pathol Res Pract 206: 642–646.

Vanasse A, Carpentier AC, Courteau J, Asghari S (2009). Stroke and cardiovascular morbidity and mortality associated with rosiglitazone use in elderly diabetic patients. Diab Vasc Dis Res 6: 87–93.

Walker AB, Naderali EK, Chattington PD, Buckingham RE, Williams G (1998). Differential vasoactive effects of the insulin sensitizers rosiglitazone (BRL 49653) and troglitazone on human small arteries in vitro. Diabetes 47: 810–814.

Wang X, Wu J, Li L, Chen F, Wang R, Jiang C (2003). Hypercapnic acidosis activates KATP channels in vascular smooth muscles. Circ Res 92: 1225–1232.

Wang K, Zhou Z, Zhang M, Fan L, Forudi F, Zhou X *et al.* (2006). Peroxisome proliferator-activated receptor gamma down-regulates receptor for advanced glycation end products and inhibits smooth muscle cell proliferation in a diabetic and nondiabetic rat carotid artery injury model. J Pharmacol Exp Ther 317: 37–43.

Wang CX, Ding X, Noor R, Pegg C, He C, Shuaib A (2009). Rosiglitazone alone or in combination with tissue plasminogen activator improves ischemic brain injury in an embolic model in rats. J Cereb Blood Flow Metab 29: 1683–1694.

Wang Y, Lau WB, Gao E, Tao L, Yuan Y, Li R *et al.* (2010). Cardiomyocyte-derived adiponectin is biologically active in protecting against myocardial ischemia-reperfusion injury. Am J Physiol Endocrinol Metab 298: E663–E670.

Wong H, Ozalp Y, Lainesse A, Alpan RS (2004). In vivo bioequivalence of oral antidiabetic agents: pioglitazone tablets. Arzneimittelforschung 54: 618–624.

Yang Y, Shi Y, Guo S, Zhang S, Cui N, Shi W *et al.* (2008). PKA-dependent activation of the vascular smooth muscle isoform of KATP channels by vasoactive intestinal polypeptide and its effect on relaxation of the mesenteric resistance artery. Biochim Biophys Acta 1778: 88–96.

Yang Y, Shi W, Cui N, Wu Z, Jiang C (2010). Oxidative stress inhibits vascular K(ATP) channels by S-glutathionylation. J Biol Chem 285: 38641–38648.

Yang Y, Shi W, Chen X, Cui N, Konduru AS, Shi Y *et al.* (2011). Molecular basis and structural insight of vascular KATP channel gating by S-glutathionylation. J Biol Chem 286: 9298–9307.

Yu J, Zhang Z, Li Z, Feng X, He L, Liu S *et al.* (2010). Peroxisome proliferator-activated receptor-gamma(PPARgamma) agonist improves coronary artery endothelial function in diabetic patients with coronary artery disease. J Int Med Res 38: 86–94.

Zinn A, Felson S, Fisher E, Schwartzbard A (2008). Reassessing the cardiovascular risks and benefits of thiazolidinediones. Clin Cardiol 31: 397–403.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 (A) Pinacidil did not activate currents in non-transfected HEK cells. (B) Reversible inhibition of Kir6.1/SUR2B currents. Whole-cell recording was performed in an HEK cell under the conditions shown in Figure 1. The currents activated by pinacidil were inhibited by 30 μM rosiglitazone by ~60%. The exposure to 10 μM glibenclamide produced further current inhibition. The currents returned to almost the pre-exposure level after washout with pinacidil-containing solution, indicating that inhibition does not result from channel rundown. (C) pinacidil (10 μM and 100 μM did not activate the Kir6.1/SUR2B currents in presence of rosiglitazone, and the channel activation occurred immediately after washout of rosiglitazone.
Figure S2 (A,B) Rosiglitazone in either 30 μM or 100 μM had very little effect on basal perfusion volume.
Figure S3 (A,B) Glibenclamide produced about 85% inhibition of the isoprenaline-induced coronary dilation in WT hearts. (C,D) In the *Kcnj8*$^{-/-}$ mouse heart, pinacidil did not produce coronary dilation although isoprenaline was capable of increasing the coronary perfusion to a less degree. Glibenclamide failed to reduce the perfusion volume. ***$P < 0.001$; $n = 3$.

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