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RESEARCH PAPER

Rosiglitazone selectively inhibits K_{ATP} channels by acting on the $K_{IR}6$ subunit

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BACKGROUND AND PURPOSE

Rosiglitazone is an anti-diabetic drug acting as an insulin sensitizer. We recently found that rosiglitazone also inhibits the vascular isoform of ATP-sensitive K^+ channels and compromises vasodilatory effects of β-adrenoceptor activation and pinacidil. As its potency for the channel inhibition is in the micromolar range, rosiglitazone may be used as an effective K_{ATP} channel inhibitor for research and therapeutic purposes. Therefore, we performed experiments to determine whether other isoforms of K_{ATP} channels are also sensitive to rosiglitazone and what their sensitivities are.

EXPERIMENTAL APPROACH

$K_{IR}6.1$/SUR2B, $K_{IR}6.2$/SUR1, $K_{IR}6.2$/SUR2A, $K_{IR}6.2$/SUR2B and $K_{IR}6.2\Delta C36$ channels were expressed in HEK293 cells and were studied using patch-clamp techniques.

KEY RESULTS

Rosiglitazone inhibited all isoforms of K_{ATP} channels in excised patches and in the whole-cell configuration. Its IC_{50} was 10 μmol·L^{-1} for the $K_{IR}6.1$/SUR2B channel and ~45 μmol·L^{-1} for $K_{IR}6.2$/SURx channels. Rosiglitazone also inhibited $K_{IR}6.2\Delta C36$ channels in the absence of the sulphonylurea receptor (SUR) subunit, with potency (IC_{50} = 45 μmol·L^{-1}) almost identical to that for $K_{IR}6.2$/SURx channels. Single-channel kinetic analysis showed that the channel inhibition was mediated by augmentation of the long-lasting closures without affecting the channel open state and unitary conductance. In contrast, rosiglitazone had no effect on $K_{IR}1.1$, $K_{IR}2.1$ and $K_{IR}4.1$ channels, suggesting that the channel inhibitory effect is selective for $K_{IR}6.x$ channels.

CONCLUSIONS AND IMPLICATIONS

These results suggest a novel K_{ATP} channel inhibitor that acts on the pore-forming $K_{IR}6.x$ subunit, affecting the channel gating.

LINKED ARTICLE

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Abbreviations

K_{IR}, inward rectifying potassium channel; SUR, sulphonylurea receptor; VSM, vascular smooth muscle

Introduction

Rosiglitazone is a potent anti-diabetic drug. By activating the nuclear transcriptional factor PPAR-γ, rosiglitazone affects the pathogenic processes of type 2 diabetes and its complications. Rosiglitazone is known to interfere with the foam cell formation and inflammatory response, reduce lipid deposition in the vessel wall, and attenuate the development of atherosclerosis (Barnett, 2009). By enhancing insulin sensitivity, it helps glycaemic control. Also, rosiglitazone regulates adipocyte proliferation and lipid storage, thus improving the lipid profile. As remarkable as these beneficial effects are, recent clinical studies suggest that rosiglitazone increases the risk of myocardial infarction (Nissen and Wolski, 2007), suggesting that the pharmacological targets of rosiglitazone may be more complicated than PPAR-γ activation.

Our recent studies (Yu *et al.*, 2011) indicate that rosiglitazone acts on the vascular wall via the inhibition of the

$K_{IR}6.1$/SUR2B isoform of ATP-sensitive K^+ (K_{ATP}) channels (nomenclature follows Alexander *et al.*, 2011). The $K_{IR}6.1$/SUR2B channel is expressed in vascular smooth muscle (VSM) and targeted by various vasodilators and vasoconstrictors (Quayle *et al.*, 1997; Ashcroft, 2006; Nichols, 2006; Shi *et al.*, 2007a,b; Yang *et al.*, 2008). The potency of rosiglitazone for vascular K_{ATP} channel inhibition is much higher than that of tolbutamide and slightly lower than glibenclamide. Therefore, rosiglitazone may be a novel K_{ATP} channel inhibitor if it also inhibits other isoforms of K_{ATP} channels. To address this issue, we performed these studies. Our results showed that rosiglitazone inhibited all the isoforms of K_{ATP} channels. Rosiglitazone appeared to act mostly on the $K_{IR}6.x$ subunit, although the K_{ATP} channel inhibition was moderately enhanced by the SURx subunit. To demonstrate the specificity of the channel inhibition, three inwards rectifier K channels ($K_{IR}1.1$, $K_{IR}2.1$ and $K_{IR}4.1$) were studied, which are expressed in the heart, kidney and nervous system. We found that rosiglitazone did not have any inhibitory effects on these K^+ channels. In several patch configurations, we showed evidence for the biophysical basis of the channel inhibition and the potential location of the targeted protein domain. These results, therefore, demonstrate a novel K_{ATP} channel inhibitor that acts on the pore-forming $K_{IR}6.x$ subunit selectively, without affecting $K_{IR}1.1$, $K_{IR}2.1$ and $K_{IR}4.1$ channels.

Methods

Expression of K_{ATP} channels and other K_{IR} channels in HEK293 cells

HEK293 cells were used to express K_{IR} channels. The cells were cultured in the DMEM/F12 medium at 37°C with 5% CO_2 with 10% fetal bovine serum and penicillin/streptomycin. The cells were transfected with cDNAs that were cloned to the eukaryotic expression vector pcDNA3.1. $K_{IR}6.1$ (GenBank accession #D42145) or $K_{IR}6.2$ (mBIR, #D50581) was transfected together with SUR2B (#D86038, mRNA isoform #NM_011511), SUR1 (#L40623) or SUR2A (#D83598). Homomeric $K_{IR}6.2\Delta C36$, a $K_{IR}6.2$ with 36 amino acids truncated at the C-terminal, was used to express functional K_{ATP} currents without the SUR subunit (Tucker *et al.*, 1997; Piao *et al.*, 2001). $K_{IR}4.1$ (#X83585), $K_{IR}1.1$ (ROMK1, #X72341) and $K_{IR}2.1$ (#X73052) were expressed in HEK293 cells individually. Green fluorescent protein (GFP) cDNA (0.4 μg, pEGFP-N2, Clontech, Palo Alto, CA) was co-transfected to facilitate the identification of positively transfected cells. Cells were split and transferred to coverslips 12–18 h after transfection. Experiments were performed on the cells in the following 12–48 h.

Electrophysiology

Patch clamp was performed using a bath solution containing the following (in mmol·L^{-1}): 10 KCl, 105 potassium gluconate ($KC_6H_{11}O_7$), 5 KF, 5 potassium pyrophosphate ($K_4P_2O_7$), 0.1 sodium vanadate ($NaVO_3$), 5 EGTA, 5 glucose and 10 HEPES at pH 7.4. The pipette was filled with the same solution when $K_{IR}6.2$/SURx channels were recorded (Wang *et al.*, 2005), while K_2ATP (1 mmol·L^{-1}) and KADP (0.5 mmol·L^{-1}) were added into the pipette solution when $K_{IR}6.1$/SUR2B channels were studied (Beech *et al.*, 1993). Spermine (10 μmol·L^{-1}) was added to the pipette solution for the $K_{IR}1.1$, $K_{IR}2.1$ and $K_{IR}4.1$ studies (Oliver *et al.*, 1998; Xu *et al.*, 2000). The same internal and external solutions were used for inside-out patches and outside-out patches. Pyrophosphate and vanadate are widely used to alleviate channel rundown. In millimolar concentrations, they do not have evident effects on other channel activities (Nakashima *et al.*, 1993; Wang *et al.*, 2005). With the solution, we found that there was only modest or no channel rundown in 10 min when recordings were done in most patches. In a few patches in which the channel rundown was shown at the end of the experiment, the data were rejected for further analysis. To avoid nucleotide degradation, all internal solutions were freshly made and used within 4 h. Rosiglitazone (Cayman Chemical Company, Ann Arbor, MI, USA), pinacidil and glibenclamide (Sigma Chemicals, St. Louis, MO, USA) were dissolved in DMSO, which had no effect on channel activity in the final concentration (~0.5%).

Recordings were made with the Axopatch 200B amplifier (Molecular Devices, Union City, CA). The data were low-pass filtered (1 kHz, Bessel four-pole filter, –3 dB) and digitized (10 kHz, 16-bit resolution) with Clampex 9 (Molecular Devices). Single-channel currents were recorded from inside-out or outside-out patches with a membrane potential of –60 mV. Higher sampling rate (20 kHz) was chosen for single-channel studies when fast openings and closures were studied. In some patches, command ramp potentials from –100 to 100 mV were applied at a holding potential of 0 mV. Whole-cell currents were recorded in voltage clamp at a holding potential 0 mV and step to –80 mV for 1 s, and the protocol was repeated in every 3 s.

Single-channel conductance was measured with slope command potentials from 100 to –100 mV. The open-state probability (P_{open}) was calculated by first measuring the time, t_j, spent at current levels corresponding to $j = 0, 1, 2, \ldots N$ channels open, based on all evident openings during the entire period of record (Zhu *et al.*, 1999; Yang *et al.*, 2000). The P_{open} was then obtained as $P_{open} = (\sum_{j=1}^{N} t_j j)/TN$ (Equation 1), where N was the number of channels active in the patch, and T was the duration of recordings. P_{open} values were calculated from a single stretch of recording using the Fetchex 6.0 software (Molecular Devices) in a duration of 40–70 s. Open and closed times were measured from records in which only a single active channel was observed. The open-time and closed-time distributions were fitted using the Marquardt-LSQ method in the Pstat6 software (Molecular Devices). Open/closed events smaller than 0.2 ms were ignored, as a result of the use of 1000 Hz offline filter (Yang *et al.*, 2000). The open dwell-time histograms were fit with one exponential. In some patches, the current amplitude was described using Gaussian distributions, and the difference between two adjacent peaks was taken as the unitary current amplitude.

Data analysis

Data are presented as the means ± SE. Differences in means were tested with the Student's *t*-test for two groups and ANOVA for three groups or more. The differences were accepted as significant if $P \leq 0.05$. The relationship of rosiglitazone dose with channel activity was expressed using the Hill equation: $y = 1 / [1 + (x / IC_{50})^h]$ (Equation 2), where y is normalized

channel activity, x is ligand concentration, h is the Hill coefficient and IC_{50} is half maximal inhibitory concentration.

Results

Rosiglitazone inhibited all isoforms of K_{ATP} channels

$K_{IR}6.x$/SURx channels were expressed in HEK293 cells. Channel activity was studied in inside-out patches with symmetric concentrations of K^+ (145 mM) applied to both sides of patch membranes. Under these conditions, inward currents were analysed with the membrane potential held at –60 mV. The $K_{IR}6.1$/SUR2B channel showed small basal currents. The channel was strongly activated by the K_{ATP} channel activator pinacidil (10 μmol·L^{-1}). Following the channel activation by pinacidil, the $K_{IR}6.1$/SUR2B channel was inhibited dose-dependently by rosiglitazone (Figure 1A) as shown previously (Yu *et al.*, 2011). The channel was inhibited by 11% with rosiglitazone (1 μmol·L^{-1}) applied to the internal solution. Rosiglitazone (100 μmol·L^{-1}) inhibited the channel activity by 98%. The channel showed very weak inward rectification at physiological pH, without polyamine. Measured with slope command potential (–100 to 100 mV), the unitary conductance was 35 ± 0 pS (n = 7) in the control condition (Figure 1B) and remained the same in the presence of 10 μmol·L^{-1} rosiglitazone (Figure 1C). These results indicated that the effect of rosiglitazone was mediated by suppression of the P_{open} without affecting the unitary conductance.

Also, rosiglitazone strongly inhibited $K_{IR}6.2$/SUR1, $K_{IR}6.2$/SUR2A and $K_{IR}6.2$/SUR2B channels known to be expressed in the pancreatic beta cells, the striated muscle and the myocardium, respectively (Figure 2). Since the $K_{IR}6.2$-containing channels are mostly open without ATP, K_{ATP} channel openers were not used to activate these channels. With ramp commands from 100 to –100 mV at a holding potential of 0 mV, the $K_{IR}6.2$-containing channels showed weak inward rectification. When the patches were exposed to rosiglitazone, concentration-dependent inhibition of these $K_{IR}6.2$-containing channels was clearly seen (Figure 3). The relationship of channel activity versus rosiglitazone concentrations was described using Equation 2. The IC_{50} was 10 μmol·L^{-1} (h 1.3) for the $K_{IR}6.1$/SUR2B channel, 45 μmol·L^{-1} for the $K_{IR}6.2$/SUR1 (h 1.2), 37 μmol·L^{-1} (h 1.1) for the $K_{IR}6.2$/SUR2A and 50 μmol·L^{-1} (h 1.2) for the $K_{IR}6.2$/SUR2B.

The $K_{IR}6.x$ subunit was likely to be the target of rosiglitazone

Because these K_{ATP} channels consist of $K_{IR}6.x$ and SURx subunits, we were interested in knowing whether rosiglitazone acted on the $K_{IR}6.x$ or the SURx subunit. Therefore, we studied the $K_{IR}6.2\Delta C36$ channel, known to express functional K_{ATP} currents without the SUR subunit (Tucker *et al.*, 1997; Piao *et al.*, 2001). Under the same experimental condition as for other $K_{IR}6.2$-containing channels, the $K_{IR}6.2\Delta C36$ channel was inhibited by rosiglitazone (Figure 4A). The IC_{50} of rosiglitazone was 45 μmol·L^{-1} (h 1.3, Figure 3). The similar sensitivity to rosiglitazone found for the $K_{IR}6.2\Delta C36$ and other $K_{IR}6.2$/SURx combinations strongly suggest that the $K_{IR}6.x$ subunit is likely to be the target of the rosiglitazone, although the SUR subunit enhances rosiglitazone sensitivity modestly. The currents with inward rectification had unitary conductance of 74 ± 0 pS (n =

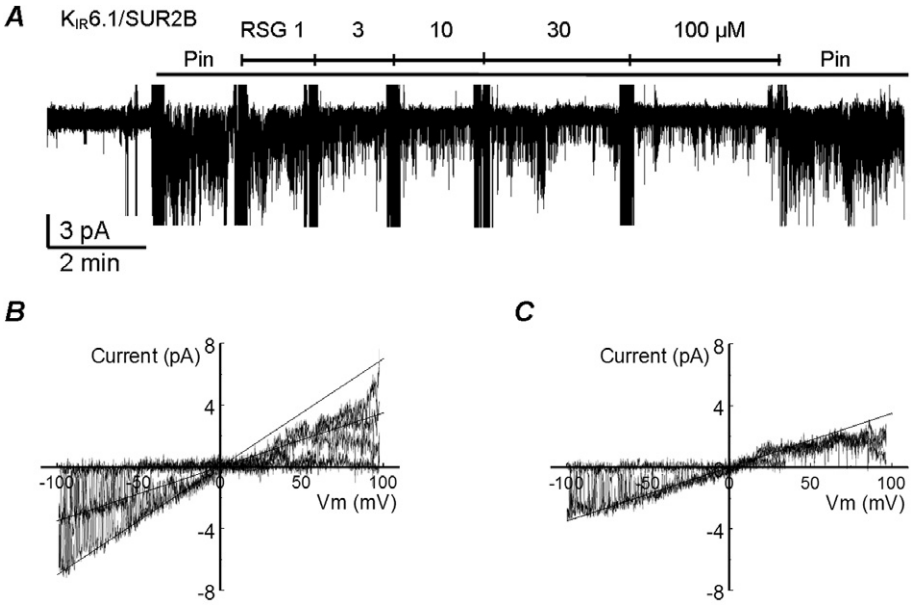

Figure 1

Inhibition of $K_{IR}6.1$/SUR2B channel by rosiglitazone (RSG) in an inside-out patch. (A) An HEK cell was co-transfected with $K_{IR}6.1$ and SUR2B. The holding potential for the patch was –60 mV. The channels were activated by 10 μM pinacidil (Pin) and then dose-dependently inhibited by rosiglitazone. Washout led to complete recovery. (B) The conductance of $K_{IR}6.1$/SUR2B channel (35 pS) was not changed after a treatment with 10 μM rosiglitazone (C, 35 pS).

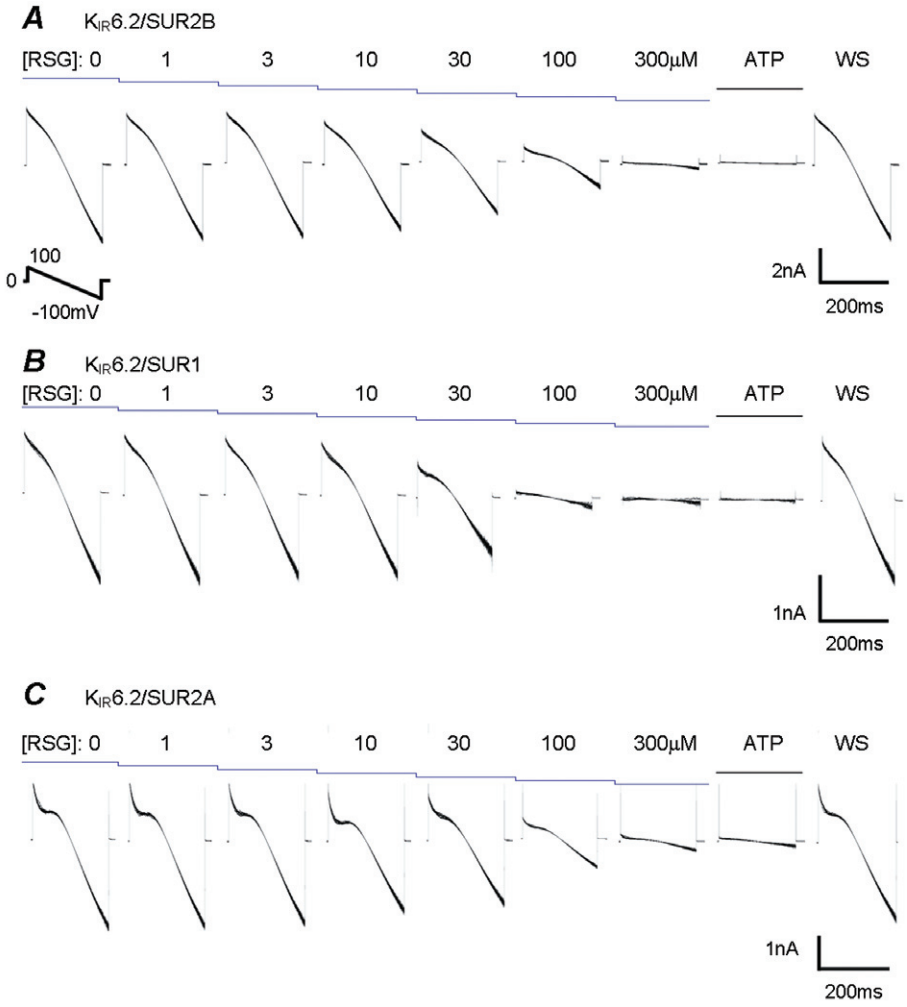

Figure 2

Concentration-dependent inhibition of three K_{IR}6.2 channel isoforms by rosiglitazone (RSG). At baseline, all K_{IR}6.2-containing channels were active without ATP and K_{ATP} channel opener. Exposure to rosiglitazone produced dose-dependent inhibition of the K_{IR}6.2/SUR2B (A), K_{IR}6.2/SUR1 (B) and K_{IR}6.2/SUR2A (C). Complete channel inhibition was seen with 1 mM ATP. The channel inhibition was reversible, and the current amplitudes almost returned to baseline levels after washout (WS). Note that 8 superimposed traces are shown in each panel.

15) and 74 ± 0 pS (n = 14) in the absence and presence of 100 μmol·L^{-1} rosiglitazone respectively (Figure 4B).

Rosiglitazone did not have any effect on K_{IR}1.1, K_{IR}2.1 and K_{IR}4.1 channels

Several other inward rectifying K^+ channels (i.e. K_{IR}1.1, K_{IR}2.1 and K_{IR}4.1) were studied. All these channels have high basal activity (Tucker *et al.*, 1997; Zhu *et al.*, 2000; Rojas *et al.*, 2007). Exposures to different concentrations of rosiglitazone (30, 100, 300 μmol·L^{-1}) did not produce any detectable channel inhibition (Figure 5), suggesting that the effect of rosiglitazone is rather specific for K_{ATP} channels.

The rosiglitazone interaction site appeared to be located on the cytosolic side

In outside-out patches, the inhibitory effect of rosiglitazone was much less potent in comparison with that seen in inside-out patches (Figure 6A). In K_{IR}6.2/SUR2B channels, the IC_{50} of rosiglitazone was increased to 350 from 50 μmol·L^{-1} in inside-out patches (Figure 6B). A similar effect was found in K_{IR}6.1/SUR2B channels, with IC_{50} of 150 μmol·L^{-1} with outside exposure, compared with 10 μmol·L^{-1} with inside exposure (Figure 6C). The final concentration (~0.5%) of the solvent DMSO had no effect on channel activity from either inside or outside the membrane.

Consistent with these data, whole-cell currents of K_{IR}6.1/SUR2B (Yu *et al.*, 2011), K_{IR}6.2/SUR2B (Figure 7A) and K_{IR}6.2ΔC36 channels were suppressed by rosiglitazone. As rosiglitazone acted on K_{IR}6.1 and K_{IR}6.2 channels with different potencies, 30 and 100 μmol·L^{-1} rosiglitazone was tested on K_{IR}6.1- and K_{IR}6.2-containing channels respectively. The inhibitory effect of rosiglitazone was less in the whole-cell configuration than in inside-out patches in all three combinations of K_{IR}6.x channels (Figure 7B–D). These data suggest

that rosiglitazone is likely to act on the cytosolic side of the channel protein, although they do not rule out the possibility of the existence of an additional low-affinity extracellular interaction site.

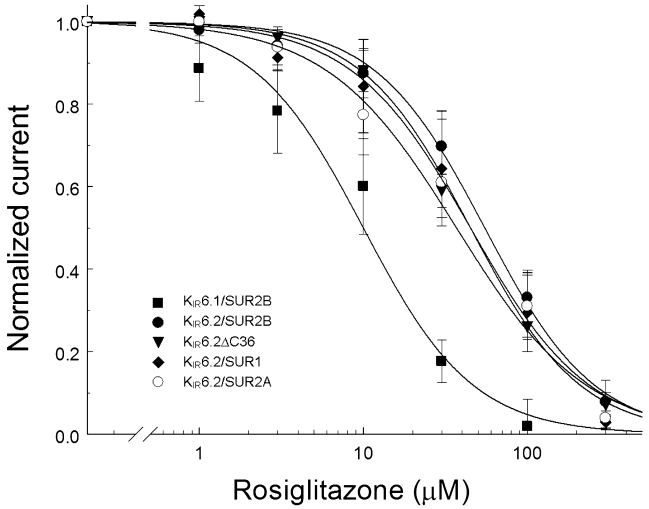

Figure 3

The relationship of channel activity with rosiglitazone (RSG) concentration was described using Equation 2. All combinations of $K_{IR}6.x$ and SURx subunits showed clear concentration dependence. The IC_{50} was 10 μM for $K_{IR}6.1$/SUR2B (h 1.3, $n = 10$ patches), 45 μM for $K_{IR}6.2$/SUR1 (h 1.2, $n = 5$), 37 μM for $K_{IR}6.2$/SUR2A (h 1.1, $n = 5$), 50 μM for $K_{IR}6.2$/SUR2B (h 1.2, $n = 6$–7) and 45 μM for $K_{IR}6.2\Delta C36$ (h 1.3, $n = 5$–8). See the text for the h values.

Specific single-channel properties were targeted by rosiglitazone

Single-channel activity was analysed on the $K_{IR}6.2\Delta C36$ channel in inside-out patches (Figure 8A,B). In the control condition, the mean open time (T_O) of the currents averaged about 2 ms and the mean closed time (T_C) was about 17 ms (Table 1, $n = 5$ patches). In the presence of 100 μmol·L^{-1} rosiglitazone, the T_O did not change, while the T_C was doubled (Table 1).

In these patches ($n = 5$), the dwell-time histograms of channel openings of the $K_{IR}6.2\Delta C36$ channel were described with a single exponential equation with the time constant $\tau_O = 1.9 \pm 0.1$ ms (Figure 8C, Table 2). We tried to model the dwell-time histograms for closure with two and three exponentials. Our results showed that the data were well described with three, but not two, exponentials (τ_{C1}, τ_{C2}, τ_{C3})

Table 1

Effects of rosiglitazone on mean open and closed times of K_{ATP} channels

		Control	Rosiglitazone
$K_{IR}6.1$/SUR2B	T_O	4.98 ± 1.41 ms	2.97 ± 0.48 ms
	T_C	8.08 ± 1.69 ms	34.89 ± 8.09 ms**
$K_{IR}6.2\Delta C36$	T_O	2.33 ± 0.17 ms	2.17 ± 0.26 ms
	T_C	16.92 ± 4.19 ms	34.18 ± 7.46 ms**

**$P < 0.01$ (paired Student's t-test; n = 5–6 patches).

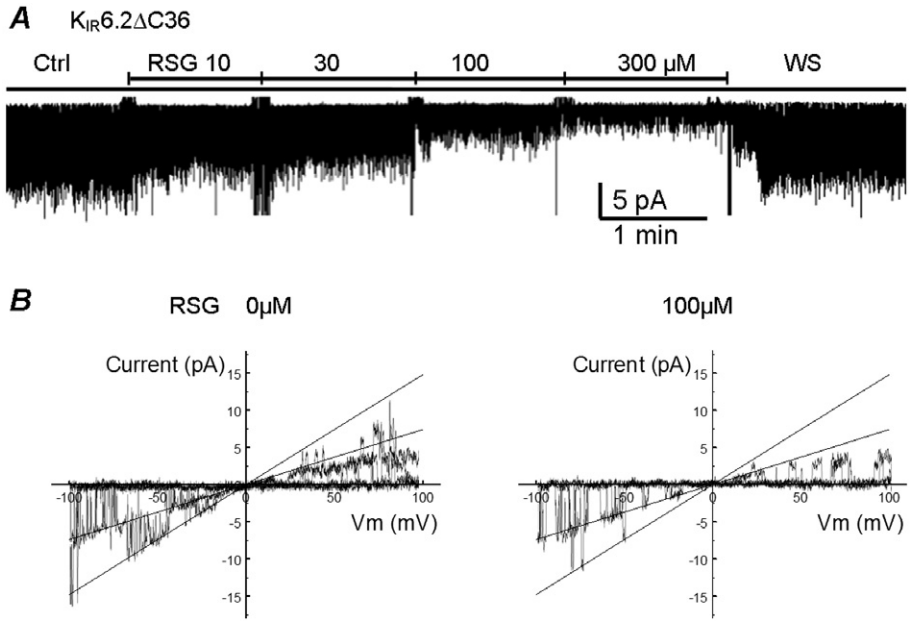

Figure 4

$K_{IR}6.2\Delta C36$ also showed dose-dependent inhibition by rosiglitazone (RSG) (A) The channel inhibition was reversible with washout (WS). Also, the unitary conductance was not changed with 100 μM rosiglitazone treatment, which was 74 pS with or without RSG (B).

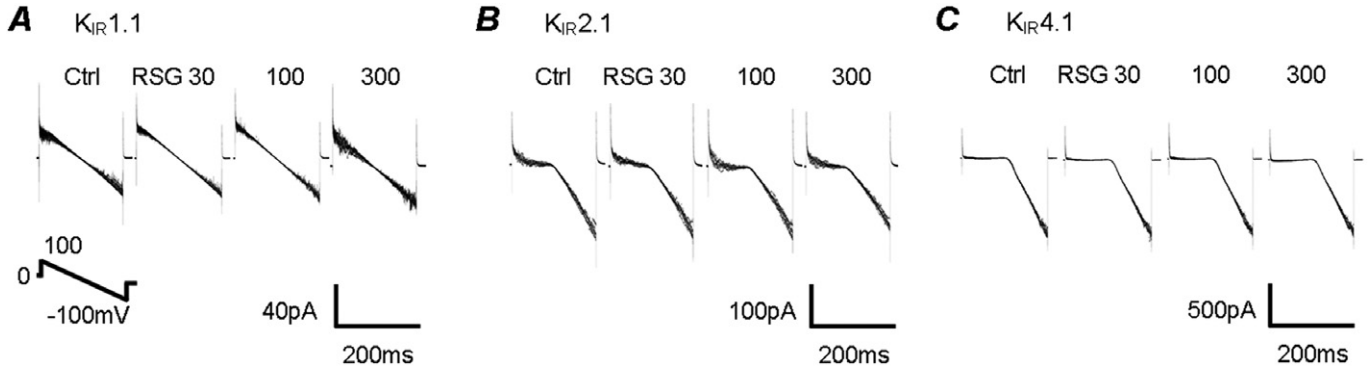

Figure 5

Rosiglitazone (RSG) had no inhibitory effect on $K_{IR}1.1$, $K_{IR}2.1$ and $K_{IR}4.1$ channels. These K_{IR} channels were expressed in HEK cells and studied in the inside-out patches under the same condition in Figure 2. None of these channels were inhibited by rosiglitazone at 30, 100 and 300 μM.

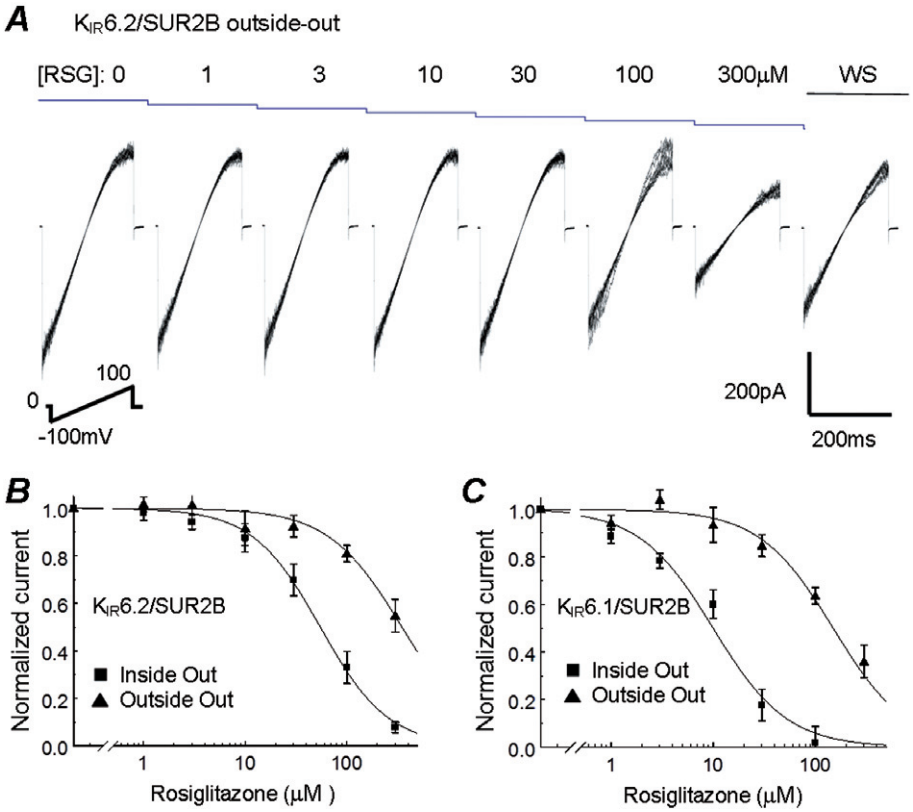

Figure 6

Comparison of channel inhibition between outside-out and inside-out patches. (A) In an outside-out patch, the $K_{IR}6.2$/SUR2B channel was partially inhibited with external exposure to rosiglitazone (RSG) up to 300 μM. (B) With the external exposure of rosiglitazone, the relationship of $K_{IR}6.2$/SUR2B channel activity with rosiglitazone concentration was shifted by ~7-fold toward the higher concentration level, where the IC_{50} was 350 μM ($n = 4$), and 50 μM with internal exposure. (C) Similar effects were seen in the $K_{IR}6.1$/SUR2B channel inhibition where the IC_{50} was 150 μM with external exposure ($n = 15$) and 10 μM with internal exposure.

(Figure 8D; Table 2; Supplementary Figure S1). In the presence of 100 μmol·L^{-1} rosiglitazone, τ_{C3} was increased (Figure 8E,F), while none of the other time constants showed significant changes. Thus, the results are consistent with the rosiglitazone effect on the T_C shown above and indicate that rosiglitazone selectively enhances long-lasting closure.

Similar results were obtained for the $K_{IR}6.1$/SUR2B channel, where in the presence of 10 μmol·L^{-1} rosiglitazone, the T_C was increased and T_O was not changed (Table 1, $n = 6$ patches). The dwell-time histograms of $K_{IR}6.1$/SUR2B channel openings were described with a two exponential equation (Table 2), and the dwell-time histograms for closures also

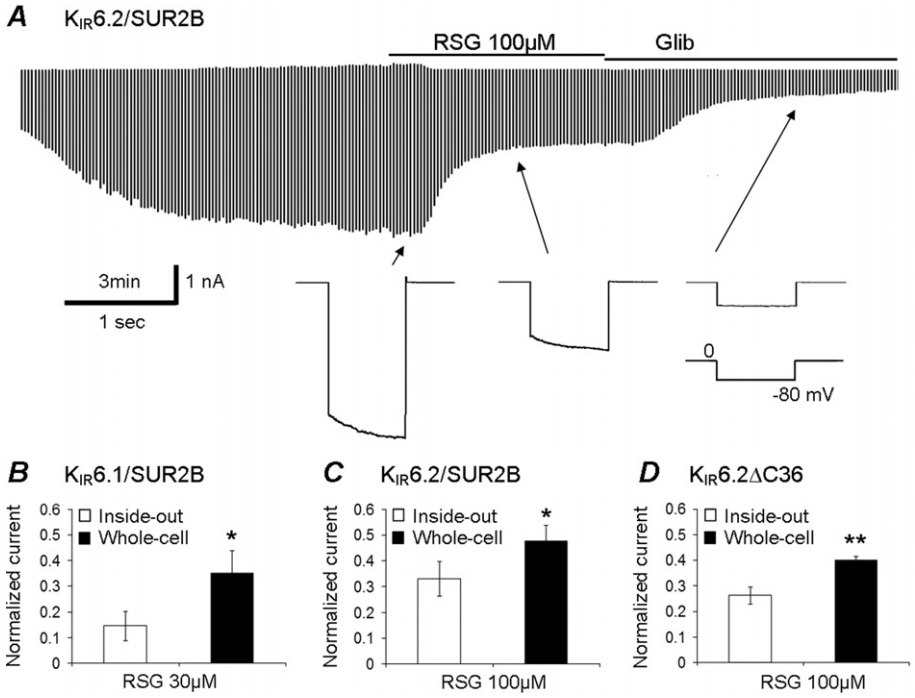

Figure 7

The currents were recorded in the whole-cell configuration with a high concentration (145 mM) of K^+ applied to either side of the plasma membrane. The membrane potential was held at 0 mV and stepped to −80 mV every 3 s as shown in the lower panel of A. The $K_{IR}6.2$/SUR2B channel spontaneously opened without activator application and without ATP in the pipette solution; the channel activation was inhibited by 100 μM rosiglitazone (RSG) and further suppressed by 10 μM glibenclamide (Glib). Whole-cell currents of $K_{IR}6.1$/SUR2B (B, $n = 8$), $K_{IR}6.2$/SUR2B (C, $n = 4$) and $K_{IR}6.2\Delta C36$ channels (D, $n = 5$) were inhibited by 30, 100 and 100 μM rosiglitazone respectively. The current inhibition was significantly smaller than that seen in inside-out patches.

contained only two exponential components. After 10 μmol·L^{-1} rosiglitazone treatment, only τ_{C2} showed significant change. Therefore, the single-channel analysis suggested that rosiglitazone augmented the long-lasting closures of the K_{ATP} channels without affecting the open state and shorter closed states.

Discussion and conclusions

Our results suggest that rosiglitazone is a novel K_{ATP} channel inhibitor, which was surprising as rosiglitazone is better known as a PPAR-γ activator and has been widely used for the treatment of type 2 diabetes. We have found that rosiglitazone at micromolar concentrations inhibits all isoforms of K_{ATP} channels in cell-free isolated membrane patches. Interestingly, rosiglitazone seemed to inhibit the K_{ATP} channels by acting on the pore-forming $K_{IR}6.x$ subunit, as it had similar potencies for $K_{IR}6.2$-containing channels with or without the SUR subunit. Its potency is much higher for the $K_{IR}6.1$/SUR2B channel than $K_{IR}6.2$-containing channels. The K_{ATP} channel inhibition is specific as rosiglitazone has no effect on $K_{IR}1.1$, $K_{IR}2.1$ and $K_{IR}4.1$ channels.

In the treatment of type 2 diabetes, rosiglitazone has several beneficial cardiovascular effects, which are likely to derive from the improvement of the metabolic profile and VSM remodelling (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). Despite these beneficial outcomes, recent clinical studies have raised the issue of the potential cardiovascular risks in users of rosiglitazone (Zinn *et al.*, 2008; Kaul *et al.*, 2010). The ischemic cardiovascular effects of rosiglitazone have also been studied in animal models, and results of the studies are inconsistent with the reports from patients with type 2 diabetes (Knock *et al.*, 1999; Khandoudi *et al.*, 2002; Abe *et al.*, 2008; Kilter *et al.*, 2009; Potenza *et al.*, 2009; CX Wang *et al.*, 2009; Y Wang *et al.*, 2010).

Potential involvement of ion channels in the effects of rosiglitazone has been examined previously by several research groups (Knock *et al.*, 1999; Mishra and Aaronson, 1999; Eto *et al.*, 2001; Lu *et al.*, 2008a; Chang *et al.*, 2009). Rosiglitazone inhibited Ca^{2+} currents and voltage-activated K^+ currents that play a role in cAMP-mediated vasodilation (Eto *et al.*, 2001; Li *et al.*, 2003). Rosiglitazone also activated Ca^{2+}-activated K^+ currents in acutely dissociated mesenteric VSM cells (Eto *et al.*, 2001; Lu *et al.*, 2008a), although it did not produce vasorelaxation in human subcutaneous small arterial rings (Walker *et al.*, 1998). The glibenclamide-sensitive K^+ currents of freshly isolated aortic myocytes were inhibited by rosiglitazone (Chang *et al.*, 2009). Moreover, 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). Rosiglitazone

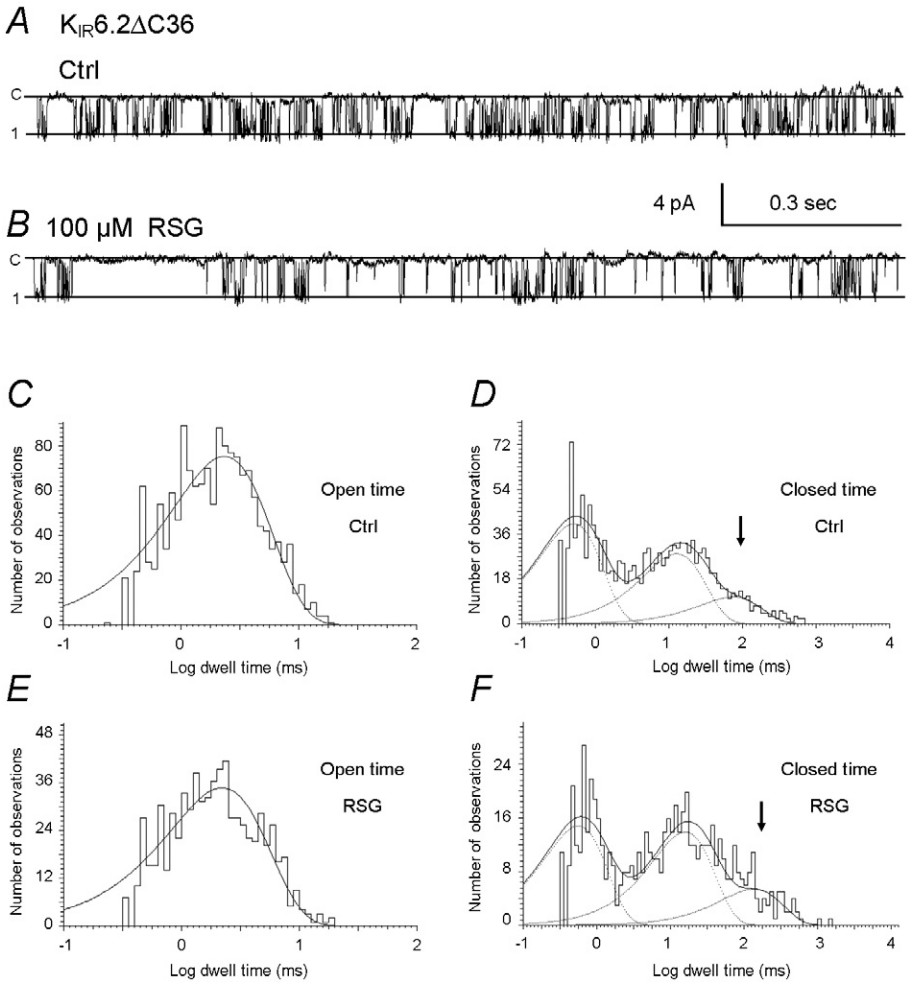

Figure 8

Single-channel kinetic analysis. Single-channel activity of the $K_{IR}6.2\Delta C36$ channel was studied in inside-out patches. (A) $K_{IR}6.2\Delta C36$ single-channel current at baseline. (B) The $K_{IR}6.2\Delta C36$ channel was inhibited by 100 μM rosiglitazone (RSG). (C) The dwell-time histogram of channel openings was described by a single-exponential with the constant τ_O = 2.3 ms. (D) The dwell-time histogram for channel closures contained three components of time constants: τ_{C1} = 0.5 ms, τ_{C2} = 12.7 ms, τ_{C3} = 74.6 ms. (E,F) With 100 μM rosiglitazone treatment, the dwell-time histograms of the channel openings and closures did not show marked changes (τ_O = 2.2 ms, τ_{C1} = 0.6 ms, and τ_{C2} = 15.9 ms) except τ_{C3} = 136.4 ms.

Table 2

Effects of rosiglitazone on open and closed time constants of K_{ATP} channels

		τ_{O1}	τ_{O2}	τ_{C1}	τ_{C2}	τ_{C3}
$K_{IR}6.1$/SUR2B	CTL	1.12 ± 0.02 ms	5.71 ± 1.96 ms	1.46 ± 0.82 ms	11.96 ± 4.44 ms	
		(0.38 ± 0.07)	(0.62 ± 0.07)	(0.72 ± 0.14)	(0.29 ± 0.14)	
	RSG	1.41 ± 0.27 ms	4.86 ± 0.77 ms	0.77 ± 0.12 ms	84.08 ± 14.83 ms**	
		(0.60 ± 0.16)	(0.40 ± 0.16)	(0.72 ± 0.15)	(0.28 ± 0.15)	
$K_{IR}6.2\Delta C36$	CTL	1.90 ± 0.11 ms		0.60 ± 0.15 ms	9.81 ± 2.05 ms	41.73 ± 1.15 ms
		(1.00 ± 0.00)		(0.56 ± 0.09)	(0.32 ± 0.08)	(0.12 ± 0.02)
	RSG	1.91 ± 0.14 ms		0.47 ± 0.08 ms	10.72 ± 1.56 ms	84.20 ± 20.97 ms**
		(1.00 ± 0.00)		(0.53 ± 0.09)	(0.31 ± 0.07)	(0.16 ± 0.04)

CTL, control; RSG, rosiglitazone. Numbers in parentheses are proportions of the time constant above.
**$P < 0.01$ (paired Student's t-test, n = 5–6 patches).

blocked cardiac K_{ATP} channels and promoted the onset of ventricular fibrillation during severe ischaemia (Mishra and Aaronson, 1999).

Our recent study indicates that the $K_{IR}6.1$/SUR2B channel is a target of rosiglitazone (Yu *et al.*, 2011). $K_{IR}6.1$/SUR2B channel inhibition leads to an impairment of the coronary vasodilator response as it is a common target of both vasodilating and vasoconstricting hormones (and neurotransmitters) that activate and inhibit the channel by distinct protein phosphorylation respectively (Ashcroft, 2006; Shi *et al.*, 2007a,b; Yang *et al.*, 2008; Orie *et al.*, 2009). The basal level of K_{ATP} channel activity is low under physiological conditions (Quayle *et al.*, 1997; Nichols, 2006). When the channels are mostly closed, they cannot significantly contribute to the membrane potential, and further inhibition of these channels may not allow sufficient depolarization to cause muscle contraction. Therefore, rosiglitazone does not have significant effects on basal vascular tones as suggested in several previous studies (Walker *et al.*, 1998; Irat *et al.*, 2006).

Our current studies suggest that rosiglitazone is a selective and potent K_{ATP} channel inhibitor. It inhibits all $K_{IR}6.x$-containing channels without affecting $K_{IR}1.1$, $K_{IR}2.1$ and $K_{IR}4.1$. The IC_{50} of rosiglitazone is 10 $\mu mol \cdot L^{-1}$ for the $K_{IR}6.1$/SUR2B channels and ~45 $\mu mol \cdot L^{-1}$ for the $K_{IR}6.2$/SURx channels. Although the IC_{50} values are slightly higher than the therapeutic concentrations for the treatment of type 2 diabetes, where the plasma concentration was around 3 μM (Cox *et al.*, 2000), the IC_{50} is greatly reduced in the presence of a therapeutic concentration of glibenclamide, as shown previously (Coppack *et al.*, 1990; Cox *et al.*, 2000; Yu *et al.*, 2011). Therefore, it is possible that K_{ATP} channels in various tissues may be partially inhibited when rosiglitazone is used for therapeutic purposes, especially in combination with a sulphonylurea.

A remarkable finding of the study is that rosiglitazone inhibited the K_{ATP} channels independently of the SUR subunit. The potency of rosiglitazone (IC_{50} ~45 $\mu mol \cdot L^{-1}$) for the $K_{IR}6.2\Delta C36$ channel is the same as for $K_{IR}6.2$/SURx channels, suggesting that the SUR subunit plays a rather small role in the channel inhibition. Currently, there are two inhibitors for the pore-forming $K_{IR}6.x$ subunit (i.e. Ba^{2+} and PNU-37883A, as well as its derivatives) (Hill, 1992; Takano and Ashcroft, 1996). Ba^{2+} blocks K_{IR} currents more effectively from the extracellular side, suppressing inward rectification of the channels. Since Ba^{2+} is a non-selective K_{IR} channel inhibitor, it cannot be used to inhibit the K_{ATP} channels *in vivo* where many other K_{IR} channels may also be inhibited. PNU-37883A was originally synthesized and tested as a diuretic agent (Perricone *et al.*, 1994; Humphrey *et al.*, 1995). It acts on the pore-forming subunit of K_{ATP} channels with similar potencies to rosiglitazone. Also, the vascular K_{ATP} channel preference between rosiglitazone and PNU-37883A is similar (Cui *et al.*, 2003; Teramoto, 2006). Both inhibit the $K_{IR}6.1$/SUR2B channel more potently than $K_{IR}6.2$/SURx channels. However, unlike PNU-37883A, rosiglitazone is a practical therapeutic agent and has been used clinically for over 10 years. During that period, rosiglitazone has been extensively tested clinically, and its beneficial and adverse effects have been well documented. Thus, knowledge of the rosiglitazone effect on K_{ATP} channels, as shown in the present studyn may help drug design by avoiding or deliberately acting on these novel targets of rosiglitazone. A previous autoradiographic study showed that the sulphonylurea glibenclamide may interact with the $K_{IR}6.2$ subunit in the COS cell line, although whether such interaction has a functional consequence is still unknown (Gros *et al.*, 1999),

Rosiglitazone appeared to act on the intracellular domains of $K_{IR}6.x$ subunits. The potency of rosiglitazone was over 10 times lower when the drug was used extracellularly. Such a weak extracellular effect may result from the relatively high hydrophobicity of the drug, allowing it to pass through the membrane and act on the intracellular domain of the channel protein after being diluted by the cytoplasm or intracellular solution. Consistent with this idea, similar extracellular exposure to rosiglitazone produced less K_{ATP} channel inhibition in the whole-cell configuration where rosiglitazone was diluted by the cytoplasm. Despite this, our data cannot rule out the possibility that there is an extracellular site in the channel protein interacting with less affinity to rosiglitazone. Clearly, further studies are needed to understand the structure-function relationship for the rosiglitazone-channel interaction.

Our analysis of single-channel properties indicated that rosiglitazone suppressed the P_{open} rather than the unitary conductance. This would explain the selective augmentation of the long-lasting closures by rosiglitazone, without affecting the open state time constant and the mean open time. These results suggest that rosiglitazone acts on the gating mechanisms of the $K_{IR}6.x$ subunit, which is located intracellularly, consistent with our observation of the potential interaction site at an intracellular location.

In conclusion, rosiglitazone was a potent inhibitor of all isoforms of K_{ATP} channels. It inhibited these K^+ channels in a membrane-delimited manner by a direct interaction with the $K_{IR}6.x$ subunit. The interaction site appeared to be on the intracellular domain of the $K_{IR}6.x$ involved in channel gating. The channel inhibition was specific for $K_{IR}6.x$ channels. The biophysical basis of the channel inhibition was the selective augmentation of long-lasting closure, without affecting the open state. Therefore, our results have demonstrated a novel K_{ATP} channel inhibitor that could be used for experimental intervention. As the drug is already used clinically, our reesluts also suggest new therapeutic uses to manipulate membrane excitability and metabolic state by targeting K_{ATP} channel function in various tissues.

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Conflict of interest

None of the authors has conflict of interest.

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Supporting information

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

Figure S1 In both control (A) and 100 μM rosiglitazone (RSG) treatment (B) conditions, the $K_{IR}6.2\Delta C36$ channel dwell-time histograms of channel openings were not fit with two exponentials.

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