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Differential sensitivities of the vascular K_{ATP} channel to various PPAR activators

Yingji Wang^{a,b}, Lei Yu^{a,b}, Ningren Cui^a, Xin Jin^a, Daling Zhu^b, and Chun Jiang^{a,b}

^a Department of Biology, Georgia State University, Atlanta, Georgia 30302-4010

^b Harbin Medical University School of Pharmacy, Harbin, Heilongjiang, China.

Abstract

Several agonists of the peroxisome proliferator-activated receptors (PPARs) are currently used for the treatment of metabolic disorders including diabetes. We have recently shown that one of them, Rosiglitazone, inhibits the vascular ATP-sensitive K^+ (K_{ATP}) channel and compromises the coronary vasodilation by the β -adrenoceptor agonist. Here, we show evidence for the channel inhibition by various PPAR agonists, information that may be useful for finding new therapeutical agents with less cardiovascular side-effects and more selective K_{ATP} channel blockers targeting at the $K_{ir}6.1$ subunit. Structural comparison of these PPAR agonists may shed insight into the critical chemical groups for the channel inhibition.

$K_{ir}6.1/SUR2B$ channel was expressed in HEK293 cells and studied in whole-cell voltage clamp. The $K_{ir}6.1/SUR2B$ channel was strongly inhibited by several $PPAR_\gamma$ agonists with potencies similar to, or higher than, that of Rosiglitazone, while other $PPAR_\gamma$ agonists barely inhibited the channel. The $K_{ir}6.1/SUR2B$ channel was also inhibited by $PPAR_\alpha$ and $PPAR_{\beta/\delta}$ agonists with intermediate potencies. The structure necessary for the channel inhibition appears to include the thiazole linked to an aromatic or furan ring. Additions of side groups such as small aliphatic chain increased the potency for channel inhibition, while additions of aromatic rings reduced it. These results indicate that the $PPAR_\gamma$ agonists with weak K_{ATP} channel inhibition may be potential candidates as therapeutical agents, and those with strong channel inhibition may be used as selective K_{ATP} channel blockers. The structural information of the PPAR agonists may be useful for the development of new therapeutical modalities with less cardiovascular side-effects.

Keywords

PPAR; thiazolidinedione; Type-2 diabetes mellitus; potassium channel; vascular tones; cardiovascular

Correspondence to: Phone: 404-413-5404 Fax: 404-413-5301 cjiang@gsu.edu.

8. Authorship Contributions

Participated in research design: Yingji Wang, Chun Jiang.

Conducted experiments: Yingji Wang, Lei Yu, Ningren Cui, Xin Jin, Daling Zhu

Performed data analysis: Yingji Wang, Chun Jiang.

Wrote or contributed to the writing of the manuscript: Yingji Wang, Chun Jiang.

3. Introduction

Glitazones, also known as thiazolidinediones (TZDs), are a group of synthetic activators of peroxisome proliferator-activated receptors (PPARs). The PPARs are nuclear receptor proteins functioning as transcriptional factors [1]. There are three subfamilies in the PPAR superfamily, i.e., PPAR γ , PPAR α and PPAR β/δ [2]. The PPARs play a role in a number of cellular function including differentiation, development and metabolism. These effects, especially the latter, have been of therapeutical interest in the development of anti-diabetic drugs.

Two anti-diabetic glitazones, Rosiglitazone and Pioglitazone, are currently available for the treatment of type-2 diabetes mellitus (DM2). Acting on the PPAR γ , they have three major beneficial effects on the pathogenic process of DM2 and its complications: 1) diminishing insulin resistance for glycemic control, 2) improving lipid profile by regulating adipocyte proliferation and lipid storage, and 3) reducing lipid deposition in the vessel wall and thus atherosclerosis by interfering with foam cell formation and inflammatory response [3]. Despite these, Rosiglitazone is known to raise potentially the risk of cardiovascular ischemia [4-6]. In contrast, Pioglitazone does not seem to have such cardiovascular side-effects [7], raising a question as to what cellular and molecular mechanisms underlie the different effects of these structurally similar glitazones on the cardiovascular system.

We have recently shown that Rosiglitazone acts on the vascular wall by selective inhibition of the ATP-sensitive K⁺ (K_{ATP}) channel that is composed of K_{ir}6.1/SUR2B and expressed in vascular smooth muscle (VSM)[8] This isoform of K_{ATP} channels is targeted by various endogenous vasodilators and vasoconstrictors that work on the K_{ATP} channel via protein phosphorylations by PKA and PKC, respectively [9-13]. The channel inhibition by therapeutical concentrations of Rosiglitazone may compromise the activity-dependent vasodilation, consistent with the ischemic coronary side-effect of Rosiglitazone found in its users[14]. Therefore, the discovery of glitazones that have less inhibitory effect on the vascular K_{ATP} channel may have impact on the development of new anti-diabetic agents.

The fact that the vascular K_{ATP} channel plays a role in both vasodilation and vasoconstriction suggests that selective and potent K_{ATP} channel inhibitors may be used in the control of vascular tones. Unlike sulfonylureas, Rosiglitazone inhibits the vascular K_{ATP} channel by acting on the pore-forming K_{ir}6 subunit and has no inhibitory effect on K_{ir}1.1, K_{ir}2.1 and K_{ir}4.1 channels [8,14]. Thus, the demonstration of more potent glitazones may have impact on the therapeutical design for several cardiovascular diseases such as hypertension and shock as well as the development of pharmacological tools targeting at the K_{ir}6.1 subunit of the K_{ATP} channels for research purposes.

For these reasons, we performed the studies in which a variety of glitazones were investigated. Special attention was paid to those that either strongly inhibited the K_{ir}6.1/SUR2B channel or those that had less inhibitory effect. Meanwhile, we also compared the structures of the highly potent glitazones with those that weakly inhibited the K_{ir}6.1/SUR2B channel, as the structural similarity of Rosiglitazone with Pioglitazone suggests that glitazones with different side groups may have different effects on the vascular K_{ATP}

channel. In addition to PPAR γ activators, we examined several activators of PPAR α and PPAR β/δ . Our results showed that the K $_{ir}6.1$ /SUR2B channel was inhibited by all PPAR agonists studied, although their potencies varied widely. The differential potencies suggest that some of the glitazones may have potential risks for diminishing the K $_{ATP}$ channel dependent vasodilation, while others have less such risks. Our parallel comparisons of various glitazones also provided several suggestions about the critical structures of the glitazones for their channel inhibition.

4. Materials and Methods

4.1. Chemicals and Reagents

The following chemicals were purchased from Sigma(St. Louis, MO, USA) (5Z)-5-[[5-(4-fluoro-2-hydroxyphenyl)furan-2-yl]methylidene]-1,3-thiazolidine-2,4-dione (AS252424); (5Z)-5-(quinoxalin-6-ylmethylidene)-1,3-thiazolidine-2,4-dione (AS605240); 3-(2-aminoethyl)-5-[(4-ethoxyphenyl)methylidene]-1,3-thiazolidine-2,4-dione hydrochloride (A6355); propan-2-yl 2-[4-(4-chlorobenzoyl)phenoxy]-2-methylpropanoate (Fenofibrate); 5-[[4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Pioglitazone); 5-(2,5-dimethylphenoxy)-2,2-dimethylpentanoic acid (Gemfibrozil); 2,4-thiazolidinedione and 5-(4-hydroxybenzyl)-2,4-thiazolidinedione (CDS005865); 2,4-thiazolidinedione(TZD) ; *N*-cyano-*N'*-pyridin-4-yl-*N''*-(1,2,2-trimethylpropyl)guanidine(Pinacidil); 5-chloro-*N*-(4-[*N*-(cyclohexylcarbonyl)sulfamoyl]phenethyl)-2-methoxybenzamide(Glibenclamide).5-[[4-[2-[methyl(pyridin-2-yl)amino]ethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione(Rosiglitazone); 5-[[4-[(1-methylcyclohexyl)methoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Ciglitazone);5-[[4-[(6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydrochromen-2-yl)methoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Troglitazone); 5-[[4-[2-(5-ethylpyridin-2-yl)-2-oxoethoxy]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Cay10415); 2-[4-[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-1,3-thiazol-5-yl]methylsulfanyl]-2-methylphenoxy]acetic acid(GW0742) were purchased from Cayman Chemical (Ann Arbor, MI,USA). 5-[(2-benzyl-3,4-dihydro-2H-chromen-6-yl)methyl]-1,3-thiazolidine-2,4-dione (Englitazone); 5-[[4-[3-(5-methyl-2-phenyl-1,3-oxazol-4-yl)propanoyl]phenyl]methyl]-1,3-thiazolidine-2,4-dione (Darglitazone) were purchased from Dalton Chemical (Wildcat Rd., Toronto, CA). 2-[2-methyl-4-[[4-methyl-2-[4-(trifluoromethyl)phenyl]-1,3-thiazol-5-yl]methylsulfanyl]phenoxy]acetic acid (GW501516) was purchased from RCS Chemicals(Radiator Rd., NC, USA). Agents were prepared as concentrated stocks in double-distilled water or dimethyl sulfoxide (DMSO). The final DMSO concentration in all experimental solutions was less than 0.1%, which did not cause any detectable effect on the channel activity.

4.2. Expression K $_{ATP}$ channel in HEK293 cells

K $_{ATP}$ channels were expressed in HEK 293 cells. The HEK293 cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies) containing 4.5 g L $^{-1}$ glucose with 10% fetal bovine serum (Helena Biosciences) and 1% penicillin/streptomycin (Life Technologies). Cells were incubated at 37°C in a humidified incubator containing 5% CO $_2$. The cells were plated to 35 mm culture dishes (Falcon 3001) to reach 90% confluent prior to

transfection. A eukaryotic expression vector pcDNA3.1 was used to express rat $K_{ir}6.1$ (GenBank accession no. **D42145**) in the cells together with SUR2B (GenBank accession no. **D86038**, mRNA isoform GenBank accession no. **NM-011511**), were transfected to the HEK293 cells. Green fluorescent protein (GFP) cDNA (0.4 μ g, 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 in cover slips during the following 12-48 h.

4.3. Electrophysiology

Patch clamp experiments were carried out at room temperature as we described previously [12, 13, 15-20]. In brief, fire-polished patch pipettes with 2-5 M resistance were made from 1.2 mm borosilicate glass capillaries. The patch clamp was done using a bath solution containing 10 mM KCl, 135 mM potassium gluconate, 5mM EGTA, 5 mM glucose, and 10 mM HEPES, and adjust pH7.4 with KOH when $K_{ir}6.1$ /SUR2B was recorded. The pipette was filled with a solution containing: 10 mM KCl, 135 mM potassium gluconate, 5mM EGTA, 5 mM glucose, 1mM K_2 ATP, 0.5 mM NaADP, 1 mM $MgCl_2$, and 10mM HEPES (pH=7.4) [21]. To avoid nucleotide degradation, all intracellular solutions were freshly made and used within 4 h. Whole-cell currents were recorded in single-cell voltage clamp with holding potential of 0 mV and a hyperpolarizing step to -80 mV for 600 ms.

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 8 (Axon Instruments). Data were analyzed using Clampfit 10.3(Axon Instruments). The current amplitude was measured at the plateau level when the cell was exposed to agents. The relationship of the current amplitude and agent concentration was described with the Hill equation after the current amplitude was normalized to the window of currents with K_{ATP} channel activator and inhibitor. The contraction dependent current inhibition was described using the Hill equation: $y = 1 / [1 + (x / IC_{50})^h]$, where x is ligand concentration, y is normalized channel activity, h is the Hill coefficient.

4.4. Data analysis

Data are presented as the means $\pm$ S.E. Differences were evaluated using Student *t*-tests for a pair data and ANOVA for three groups or more. Statistical significance was accepted if $P < 0.05$.

5. Results

5.1. Baseline properties of $K_{ir}6.1$ /SUR2B currents

The $K_{ir}6.1$ /SUR2B channel was expressed in HEK293 cells. K^+ currents were recorded 2-3 days after transfection in whole-cell voltage clamp. Symmetric concentrations of K^+ (145 mmol L^{-1}) were applied to the bath and pipette solutions. The membrane potential was held at 0 mV and stepped to -80mV for 600ms. The protocol was repeated every 3 s. Under this condition, the HEK293 cells showed small basal currents. An exposure to 10 μ mol L^{-1} pinacidil (Pin), a K_{ATP} channel activator, strongly activated the inward currents that were

subsequently suppressed by $10\mu\text{mol L}^{-1}$ glibenclamide (Glib), a K_{ATP} channel inhibitor (Fig. 1A). These Pin/Glib-sensitive currents were exogenous as Pin/Glib had very little effect on the HEK293 cells transfected with the expression vector alone (Fig. 1B). Therefore, these K_{ATP} channel activator and inhibitor were used for determination of the expression of the channel and normalization of agent effects on the K_{ATP} currents.

5.2. PPAR γ agonists as potent $K_{\text{ir}}6.1/\text{SUR2B}$ channel inhibitors

Whole-cell $K_{\text{ir}}6.1/\text{SUR2B}$ currents were strongly inhibited by the administration of AS-252424, Darglitazone, Cay10415 and A6355 (Fig. 2). The channel inhibition took place in 1-2 min and reached the plateau level in 3-4 min. The channel was further inhibited in some cases when the cell was exposed to Glib. When the currents were plotted against drug concentrations, clear concentration dependence was seen (Fig. 2). The current-concentration relationship was described with the Hill equation in which the concentration for 50% channel inhibition (IC_{50}) was calculated. Our data showed that the IC_{50} was $4\mu\text{mol L}^{-1}$ for AS-252424, $7\mu\text{mol L}^{-1}$ for Englitazone, $8\mu\text{mol L}^{-1}$ for A6355, $12\mu\text{mol L}^{-1}$ for Rosiglitazone, $15\mu\text{mol L}^{-1}$ for Cay10415, and $25\mu\text{mol L}^{-1}$ for Darglitazone, respectively (Fig. 3, Table 1). These results suggest that these glitazone PPAR γ activators appear to be strong inhibitors of the $K_{\text{ir}}6.1/\text{SUR2B}$ channel. At $30\mu\text{M}$, they inhibited the $K_{\text{ir}}6.1/\text{SUR2B}$ currents by $92.7 \pm 4.2\%$ ($n = 5$), $89.1 \pm 6.5\%$ ($n = 4$), $84.2 \pm 9.2\%$ ($n = 5$), $74.8 \pm 8.7\%$ ($n = 4$), $70.1 \pm 10.7\%$ ($n = 7$), and $56.2 \pm 9.4\%$ ($n = 5$), respectively.

5.3. PPAR γ agonists with weak inhibitory effects on $K_{\text{ir}}6.1/\text{SUR2B}$ currents

Several glitazone-PPAR γ activators had rather weak inhibitory effect on the $K_{\text{ir}}6.1/\text{SUR2B}$ channel. We have previous shown that Pioglitazone inhibits the $K_{\text{ir}}6.1/\text{SUR2B}$ channel only modestly [8]. Similarly, the channel was inhibited weakly by Ciglitazone, Troglitazone and AS605240 (Fig. 4A-C). They had barely any inhibitory effect on the channel at $3\text{-}10\mu\text{mol L}^{-1}$, and inhibited the channel by 20-40% at $100\mu\text{mol L}^{-1}$. The IC_{50} was $60\mu\text{mol L}^{-1}$ for AS605240, $95\mu\text{mol L}^{-1}$ for Pioglitazone, $100\mu\text{mol L}^{-1}$ for Troglitazone, and $120\mu\text{mol L}^{-1}$ for Ciglitazone (Fig. 4D). At $30\mu\text{M}$, they inhibited the $K_{\text{ir}}6.1/\text{SUR2B}$ currents by $35.8 \pm 12.5\%$ ($n = 4$), $26.0 \pm 9.0\%$ ($n = 9$), $35.3 \pm 10.1\%$ ($n = 4$), and $32.9 \pm 5.3\%$ ($n = 6$), respectively.

5.4. Effects of PPAR α and PPAR β/δ activators

Pioglitazone is a dual activator of PPAR γ and PPAR α . To test the possibility that the $K_{\text{ir}}6.1/\text{SUR2B}$ channel may be affected by other PPAR agonists, we studied several PPAR α and PPAR β/δ agonists. Fenofibrate and Gemfibrozil are two selective PPAR α agonists that are available commercially. They belong to a group of drugs known as the fibrate class of amphipathic carboxylic acids that is used for many forms of metabolic disorders including hypercholesterolemia [22]. Structurally, they are different from glitazones (Fig. 5). Both Fenofibrate and Gemfibrozil caused a moderate inhibition of the $K_{\text{ir}}6.1/\text{SUR2B}$ currents. At $30\mu\text{M}$, they inhibited the $K_{\text{ir}}6.1/\text{SUR2B}$ currents by $18.2 \pm 4.3\%$ ($n = 4$) and $14.8 \pm 4.9\%$ ($n = 4$). At $100\mu\text{M}$, they inhibited the $K_{\text{ir}}6.1/\text{SUR2B}$ currents by $47.9 \pm 12.7\%$ ($n = 4$) and $15.8 \pm 4.6\%$ ($n = 4$), respectively (Fig. 5A,B). The IC_{50} was $80\mu\text{mol L}^{-1}$ for Fenofibrate and $290\mu\text{mol L}^{-1}$ for Gemfibrozil (Fig. 5C, Table 1).

We also tested two commercially available PPAR $_{\beta/\delta}$ agonists, GW0742 and GW501516. GW0742 is a highly selective PPAR $_{\beta/\delta}$ agonist that exhibits 1,000-fold selectivity over the human PPAR $_{\alpha}$ and PPAR $_{\gamma}$, and is being investigated as a potential therapeutic agent [23]. GW0742 produced weak inhibition of the K $_{ir}6.1$ /SUR2B currents by $22.1 \pm 9.8\%$ ($n = 4$) at 30 μM and $52.5 \pm 13.0\%$ ($n = 4$) at 100 μM (Fig. 6A). The effect of GW501516, another PPAR $_{\beta/\delta}$ agonist, was also weak (Fig. 6B). The IC $_{50}$ was 80 $\mu\text{mol L}^{-1}$ for GW0742 and 100 $\mu\text{mol L}^{-1}$ for GW501516, respectively (Fig. 6C).

5.5. The core structures of glitazones for the K $_{ir}6.1$ /SUR2B currents inhibition

The core of all glitazones is the thiazole ring linked to an aromatic or furan ring (Table 2). With the thiazole alone, thiazolidinedione (TZD) did not produce any K $_{ir}6.1$ /SUR2B channel inhibition. Instead, the channel was slightly activated (Fig. 7A), suggesting that the thiazole ring alone is inadequate for K $_{ir}6.1$ /SUR2B channel inhibition. With both the thiazole and aromatic rings, CDS005865 has all core structures of glitazones, and was capable of inhibiting K $_{ir}6.1$ /SUR2B channel (Fig. 7B). Its channel inhibitory effect, however, was rather moderate in comparison to several potent PPAR agonists shown in Figures 2 and 3, suggesting that the glitazone core may be necessary but not sufficient for the K $_{ir}6.1$ /SUR2B channel inhibition. We found that several side groups appeared to play a role in the channel inhibition potency (see below).

6. Discussion and Conclusions

This work represents the first attempt to analyze systematically the differential sensitivities of the vascular K $_{ATP}$ channel to most glitazones that are available commercially at present. Accumulating experimental evidence indicates that the vascular K $_{ATP}$ channel in a heterologous expression system responds to its regulators similarly as its VSM endogenous counterpart. The channels in both conditions are activated by several vasodilating hormones and neurotransmitters in a PKA dependent manner [13, 17]. Both the K $_{ir}6.1$ /SUR2B and VSM-endogenous K $_{ATP}$ channels are strongly inhibited by vasoconstricting hormones and neurotransmitters via the activation of PKC [24, 25]. They are both inhibited by Rosiglitazone leading to a disruption of the coronary vasodilatory response to the β -adrenergic receptor agonist isoproterenol [14]. Hence, it is reasonable to believe that the glitazone sensitivities of the K $_{ir}6.1$ /SUR2B channel shown in the present study resemble those of the VSM-endogenous K $_{ATP}$ channel.

In comparison to Rosiglitazone, Pioglitazone has rather weak inhibitory effect on the K $_{ir}6.1$ /SUR2B channel [14]. In this study, we have found another three glitazones, AS605240, Ciglitazone and Troglitazone, inhibit the K $_{ir}6.1$ /SUR2B channel similarly as Pioglitazone. Troglitazone was a therapeutic drug, and later was withdrawn from the market because of liver toxicity [26]. With a channel inhibition potency similar to Pioglitazone, AS605240 and Ciglitazone do not have obvious effect on the K $_{ir}6.1$ /SUR2B channel at 3-10 $\mu\text{mol L}^{-1}$, a concentration that resembles a high plasma level of Rosiglitazone and Pioglitazone for the treatment of DM2 [14]. Since Pioglitazone does not seem to have the myocardial ischemic side-effect at its therapeutical concentrations, AS605240 and Ciglitazone may not affect the K $_{ATP}$ channel dependent coronary vasodilation at low concentrations as well. Clearly,

further studies of these glitazones are needed to show whether some of them may be suitable as new therapeutical agents.

Our results suggest that several glitazones are potent inhibitors of the $K_{ir}6.1/SUR2B$ channel, including AS-252424, Englitazone, A6335, Rosiglitazone, Cay10415 and Darglitazone. Their potencies for the channel inhibition are higher than, or comparable to, that of Rosiglitazone. Unlike sulfonylureas, these highly potent K_{ATP} channel inhibitors target at the $K_{ir}6.1$ subunit. They may be useful when the pore-forming subunit needs to be selectively inhibited in certain pathophysiological conditions such as septic shock [27], although they may not be the optimal choice for the treatment of DM2 as therapeutical agents without significant structural modifications. Since some of these glitazones are known to be tested as therapeutic agents, the information of these highly potent K_{ATP} channel inhibitors may be useful for the further development of the drugs.

The $PPAR_{\alpha}$ and $PPAR_{\beta/\delta}$ are ubiquitously expressed in most tissues. The $PPAR_{\alpha}$ is found in the liver, muscle, kidney, and heart [28], while the $PPAR_{\beta/\delta}$ is expressed considerably in the brain, skin and adipose tissue [29]. The fact that Pioglitazone is dual $PPAR_{\gamma}$ and $PPAR_{\alpha}$ agonist motivated us to investigate whether the $K_{ir}6.1/SUR2B$ channel was affected by other PPAR agonists. Our results have shown that the channel is inhibited by the $PPAR_{\alpha}$ agonists Fenofibrate and Gemfibrozil. The former is much more potent than the latter with IC_{50} 3-4 times greater. The only available $PPAR_{\beta/\delta}$ activators are GW0742 and GW501516, both of which are nearly identical in their structures. We have found that they both inhibit the $K_{ir}6.1/SUR2B$ channel with a similar potency. This finding is novel and striking, as these PPAR agonists have different structures from the $PPAR_{\gamma}$ activators, and as they do not seem to have cardiovascular side-effects. Apparently, they are capable of inhibiting the $K_{ir}6.1/SUR2B$ channel and compromising the K_{ATP} channel dependent coronary vasodilation at high concentrations, especially when they are jointly used with sulfonylureas or other glitazones.

Our studies of various PPAR agonists allow us to appreciate some structural insights into their $K_{ir}6.1/SUR2B$ channel inhibition. Most of the PPAR agonists share a few common elements: There is a thiazole head connected to an aromatic ring through a short linker. A second linker is found connecting the aromatic ring to a lipophilic tail represented by either an aromatic ring or an aliphatic chain. The linkers can also contain additional substituents. The critical structure of all glitazones is the thiazole ring. With the thiazole alone, however, TZD does not produce any $K_{ir}6.1/SUR2B$ channel inhibition, suggesting that other essential structures are required for the $K_{ir}6.1/SUR2B$ channel inhibition. With the addition of an aromatic ring, CDS005865 produces the channel inhibition, indicating the glitazone core is critical. With further modifications on the thiazole and aromatic rings, A6355 inhibits the channel strongly. In comparison to CDS005865, A6355 has a methyl ether tail attached to the aromatic ring, and an aminoethyl chain to the thiazole. The aliphatic chain on the thiazole ring does not seem critical, as the potent channel inhibitors Englitazone and AS252424 do not have it. Interestingly, with additional aromatic rings in the tail, potencies for the $K_{ir}6.1/SUR2B$ channel inhibition decrease as Pioglitazone, Ciglitazone, Troglitazone and AS605240 all are weak channel inhibitors. Although the aromatic ring is seen in the core of most glitazones, a furan ring in AS252424 can have an effect similar to the strong

channel inhibitors Englitazone and A6355. These structural analyses suggest that new therapeutical agents may be developed by the modification of the tail of glitazones to diminish their side-effects on the vascular K_{ATP} channels.

The finding that all PPAR agonists have more or less inhibitory effects on the $K_{ir6.1}/SUR2B$ channel is not only a surprise but also has potential impacts on the therapeutical modalities and clinical practice. Several PPAR activators are currently available for the treatment of DM2 and other metabolic disorders, while some may have potential side-effects on cardiac ischemia [30-35]. The information of the channel inhibition by various PPAR activators may help to avoid the channel inhibition in people with coronary conditions. The relationship of glitazone structures with the channel inhibition may help the future development of the PPAR $_{\gamma}$ agonists with less cardiovascular side-effects. The demonstration of the differential sensitivities of the vascular K_{ATP} channel to various glitazones thus provides useful information for the application of the PPAR agonists for therapeutical purposes and for the new drug design.

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Abbreviations

DM2	Type-2 diabetes mellitus
K_{ATP}	ATP-sensitive K^{+}
K_{ir}	inwardly rectifying potassium channel
PPAR-γ	peroxisome proliferator-activated receptor
SUR	sulfonylurea receptor
TZDs	thiazolidinediones
VSM	vascular smooth muscles

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Fig. 1.

Expression of $K_{ir}6.1/SUR2B$ channel in the HEK293 cell line. **A.** In an HEK cell that was co-transfected with $K_{ir}6.1$ and $SUR2B$, inward currents were studied 2 days after transfection using symmetric concentrations of K^+ (145 mmol L^{-1}) applied to the bath and pipette solutions. Membrane potentials were held at 0 mV and stepped to -80 mV in every 3 sec. The cell showed small currents at baseline (BL). The currents were strongly activated by $10 \text{ } \mu\text{mol L}^{-1}$ pinacidil (Pin). At the maximum channel activation the application of $10 \text{ } \mu\text{mol L}^{-1}$ glibenclamide (Glib) led to current inhibition. **B.** The same experiment was performed in another HEK that was transfected with the expression vector only (pcDNA3.1). No evident current activation by Pin was found, neither the channel inhibition by Glib.

Fig. 2.

Inhibition of the $K_{ir}6.1/SUR2B$ currents by glitazones. Whole-cell currents were studied in an HEK cell in the same condition as shown in Figure 1. AS-252424, a $PPAR_{\gamma}$ activator, inhibited the $K_{ir}6.1/SUR2B$ currents almost completely at 30 μ mol L^{-1} (A). Similar $K_{ir}6.1/SUR2B$ channel inhibitions were seen with Darglitazone (Darg, B), Cay10415 (C) and A6355 (D).

Fig. 3. Concentration-dependent inhibition of the $K_{ir}6.1/SUR2B$ channel by glitazones. The current amplitude was measured at the plateau level when the cell was exposed to agents. The relationship of the current amplitude and agent concentration was described with the Hill equation (Methods) after the current amplitude was normalized to the baseline level before drug treatment. IC_{50} and h are presented in Table 1. Abbreviations: Eng, Englitazone; Darg, Darglitazone; RSG, Rosiglitazone.

Fig. 4.

Glitazones as weak Kir6.1/SUR2B channel inhibitors. Whole-cell currents were studied in an HEK cell in the same condition as shown in Figure 1. Similar Kir6.1/SUR2B channel inhibitions were seen with Cig (A), Trog (B) and AS605240 (C). Concentration-dependent inhibition of the Kir6.1/SUR2B channel by these weak channel inhibitory glitazones (D). Abbreviations: Cig, Ciglitazone; Trog, Troglitazone; Piog, Pioglitazone; RSG, Rosiglitazone. See Table 1 for their IC₅₀ and h values.

Fig. 5. Effects of PPAR α agonists on the $K_{ir6.1}$ /SUR2B currents. **A.** Fenofibrate (Fen) has a clear difference in its structure from glitazones. Despite this, it inhibited the $K_{ir6.1}$ /SUR2B currents at 100 nmol L $^{-1}$. **B.** Gemfibrozil, however, inhibited the $K_{ir6.1}$ /SUR2B currents only modestly. **C.** The IC $_{50}$ was 80 μ mol L $^{-1}$ for Fen and 290 μ mol L $^{-1}$ for Gem.

Fig. 6. Inhibition of the $K_{ir6.1}/SUR2B$ currents by PPAR $_{\beta/\delta}$ agonists. GW0742 (**A**) and GW501516 (**B**) are almost identical in their structures. They both inhibited the $K_{ir6.1}/SUR2B$ currents moderately. The IC_{50} was $80 \mu\text{mol L}^{-1}$ for GW0742 and $100 \mu\text{mol L}^{-1}$ for GW501516, respectively (**C**).

Fig. 7.

Effects of the core structures of glitazones. Whole-cell currents were studied in the same condition as shown in Figure 1.A. With the thiazole ring only, 2,4-Thiazolidinedione (TZD) did not produce any $K_{ir}6.1$ /SUR2B channel inhibition. Instead, the channel was slightly activated. **B.** With both the thiazole and the aromatic rings, CDS005865 had the basic core structures of glitazones, and was capable of inhibiting $K_{ir}6.1$ /SUR2B channel, although its

channel inhibitory effect was rather weak. **C.** Summary of the effects of 100 $\mu\text{mol L}^{-1}$ TZD and 100 $\mu\text{mol L}^{-1}$ CDS005865 on $\text{K}_{\text{ir}}6.1/\text{SUR2B}$ currents. *, $P < 0.05$.

Table 1

The IC₅₀ and h values of PPAR agonists and other TZDs.

Name		IC ₅₀ (μM)	h
PPAR _γ agonists	AS-252424	4	2
	Englitazone	7	1.6
	A6335	8	1.2
	Rosiglitazone	12	1.2
	Cay10415	15	1.4
	Darglitazone	25	1.4
	AS605240	60	0.8
	Pioglitazone	95	0.8
	Troglitazone	100	0.8
	Ciglitazone	120	0.7
PPAR _α agonists	Fenofibrate	80	1.5
	Gemfibrozil	290	0.9
PPAR _{β/δ} agonists	GW0742	80	1
	GW501516	120	0.8
Other TZDs	TZD	+	
	CDS005865	>>100	

Where h is the Hill coefficient.

Table 2

TZDs analogs	Link to the Thiazole Ring	Lipophilic Tail	IC ₅₀ (μM)	Solubility (mg mL ⁻¹)
Ciglitazone	Single bond		120	DMSO ~16
Troglitazone	Single bond		100	DMSO ~20 Water ~ 1.2e ⁻³
Pioglitazone	Single bond		95	DMSO >10
AS605240	Double bond		60	DMSO > 5
Darglitazone	Single bond		25	DMSO > 5
Cay10415	Single bond		15	DMSO >30
Rosiglitazone	Single bond		12	DMSO >10 Water ~3.80e ⁻²
A6355	Double bond		8	DMSO > 4
Englitazone	Single bond		7	DMSO > 5
AS252424	Double bond		4	DMSO >20

TZDs analogs	Link to the Thiazole Ring	Lipophilic Tail	IC ₅₀ (μM)	Solubility (mg mL ⁻¹)
TZD	N/A		+	DMSO >30
CDS005865	Single bond		>> 100	DMSO >30

The glitazone core is shown on top of the Table. Structures of all compounds except last six are drawn with an omission of the glitazone core. The link to the core is presented with a dash line.