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COMMENTARY

Selective block of K_{ATP} channels: why the anti-diabetic sulphonylureas and rosiglitazone have more in common than we thought

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Rosiglitazone, the thiazolidinedione class anti-diabetic withdrawn from Europe in 2010 amid reports of adverse cardiovascular effects, is revealed by Yu *et al.* in this issue of the *British Journal of Pharmacology* to be a selective blocker of ATP-sensitive potassium (K_{ATP}) channels. This seems little cause for excitement given that the closure of pancreatic K_{ATP} channels is integral to insulin secretion; and sulphonylureas, which inhibit K_{ATP} channels, are widely used to treat type II diabetes. However, rosiglitazone, whose primary targets are nuclear transcription factors that regulate genes involved in lipid metabolism, blocks K_{ATP} channels by a novel mechanism different to that of the sulphonylureas and has a worrying preference for blood flow–regulating vascular K_{ATP} channels. Identification of a new molecule that modulates K_{ATP} channel gating will not only tell us more about how these complex metabolic sensors work but also raises questions as to whether rosiglitazone suppresses the cardiovascular system's ability to cope with metabolic stress – a claim that has dogged the sulphonylureas for many years.

LINKED ARTICLE
This article is a commentary on Yu *et al.*, pp. 26–36 of this issue. To view this paper visit http://dx.doi.org/10.1111/j.1476-5381.2012.01934.x

Abbreviations
ER, endoplasmic reticulum; K_{ATP} channel, ATP-sensitive potassium channel; SUR, sulphonylurea receptor

The anti-diabetic rosiglitazone has had a chequered history. Working as an agonist at nuclear PPARγ, it was introduced in 1999 and widely prescribed for the treatment of type II diabetes mellitus based on its ability to increase insulin sensitivity in fat cells by regulating genes involved in glucose and lipid metabolism (Brown and Plutzky, 2007). Additional beneficial cardiovascular effects were soon linked to its usage, including anti-inflammatory and anti-proliferative effects that retarded the development of atherosclerosis (Zinn *et al.*, 2008). These wholly positive outcomes were tempered by reports that rosiglitazone and its fellow thiazolidinediones exacerbated fluid retention and congestive heart failure (Zinn *et al.*, 2008; Kaul *et al.*, 2010), although the drug's benefits were still considered to largely outweigh any risks. Opinion shifted in 2007 with the publication in the *New England Journal of Medicine* of a large-scale meta-analysis of 42 randomized trials involving almost 28 000 patients that indicated an alarming 43% increase in myocardial infarction in patients taking rosiglitazone (Nissen and Wolski, 2007). Publication of this report prompted the United States Food and Drug Administration (FDA) to release a safety alert flagging the possible increased risk of ischaemic cardiovascular events in patients prescribed rosiglitazone. The ensuing controversy saw a flurry of additional publications supporting or refuting the adverse cardiovascular effects of the drug (reviewed by Zinn *et al.*, 2008; Kaul *et al.*, 2010), muddying the waters to such an extent that the FDA Advisory Panel subsequently voted against removing rosiglitazone from the US market. The European Medicine Agency took a harder line and withdrew rosiglitazone from Europe in September 2010. Whether rosiglitazone produces net clinical benefit or harm is still far from clear, and in this issue of the *British Journal of Pharmacology*, Yu *et al.* add to the debate by revealing that rosiglitazone at near clinically relevant concentrations acts to inhibit ATP-sensitive potassium (K_{ATP}) channels, a family of proteins that play critical protective roles during acute metabolic stress

(Yu *et al.*, 2012). While rosiglitazone-induced block of K_{ATP} channels is not in itself novel, Yu *et al.* showed that unlike the well-characterized sulphonylureas that inhibit K_{ATP} channels by interacting with their large modulatory sulphonylurea receptor (SUR) subunit, rosiglitazone suppresses the channel's open probability by interacting with the cytosolic face of pore-forming $K_{IR}6.x$ subunit. They also demonstrated a 4-fold increase in rosiglitazone potency for K_{ATP} channels containing the $K_{IR}6.1$ pore-forming subtype. Given the primarily vascular distribution of $K_{IR}6.1$-containing channels, this raises a number of questions with regard to the potential clinical implications of rosiglitazone usage. This also echoes the ongoing debate regarding the cardiovascular safety of another mainstay of type II diabetes, the sulphonylureas (Tzoulaki *et al.*, 2009).

An anti-diabetic drug that selectively inhibits K_{ATP} channels seems on the face of it to be little cause for concern. Inhibition of these channels by high ATP (glucose) levels in pancreatic beta cells induces membrane depolarization, Ca^{2+} influx via voltage-gated Ca^{2+} channels and Ca^{2+}-dependent secretion of insulin (Ashcroft and Gribble, 1999). Indeed, sulphonylureas such as tolbutamide, glibenclamide (glyburide) and glimepiride have been used clinically to treat type II diabetes mellitus for many years, often in combination with the insulin-sensitizing thiazolidinediones. Pancreatic beta-cell K_{ATP} channels most likely form as octomers of four pore-forming $K_{IR}6.2$ and 4 modulatory SUR1 subunits, but other distinct K_{ATP} channel isoforms also exist in cardiac and smooth muscle. Activation of vascular K_{ATP} channels ($K_{IR}6.1$/SUR2B) by vasodilating transmitters causes membrane hyperpolarization, decreased Ca^{2+} entry and vasorelaxation (Flagg *et al.*, 2010), and drugs that open vascular K_{ATP} channels to increase arterial diameter and blood flow are used to treat angina pectoris (nicorandil) and intractable hypertension (minoxidil and diazoxide). The cardiac isoform of K_{ATP} channel ($K_{IR}6.2$/SUR2A) opens during ischaemia, promoting membrane repolarization and a shortening of the action potential, which reduces Ca^{2+} entry in an attempt to conserve ATP and thus minimize cell damage (Flagg *et al.*, 2010). Blockade of vascular K_{ATP} channels by rosiglitazone would therefore be expected to have adverse effects at times when the coronary circulation needs to dilate, for example during exercise or stress, and inhibition of cardiac K_{ATP} channels has been shown to severely compromise the heart's ability to cope with ischaemic assault. What the study by Yu *et al.* highlights is that micromolar concentrations of rosiglitazone inhibit the activity of all isoforms of K_{ATP} channel, but with a marked preference for K_{ATP} channels containing the $K_{IR}6.1$ isoform. The IC_{50} of rosiglitazone is calculated to be 45 μM for $K_{IR}6.2$/SURx (pancreatic and heart) channels and 10 μM for the vascular $K_{IR}6.1$/SUR2B, which is reduced to 2 μM in the presence of therapeutic concentrations of sulphonylureas (Yu *et al.*, 2011). Plasma concentrations of rosiglitazone in the treatment of type II diabetes are generally in the region of 3 μM (Cox *et al.*, 2000), which places the vascular channel in particular in the firing line. A caveat to this is that the IC_{50} values stated above were obtained by directly applying rosiglitazone to the cytoplasmic face of the channel in excised inside-out membrane patches. The effects of rosiglitazone were considerably weaker when applied extracellularly. This difference in potency was taken by the authors to indicate that rosiglitazone acts primarily on the cytosolic face of the channel and that the weakened effects associated with extracellular application were due to the drug having to cross the membrane to reach its active site. It should be remembered that rosiglitazone's primary targets are nuclear transcription factors and the drug has the ability to enter cells quite readily. Indeed, the authors have previously shown that 30 μM rosiglitazone reduces by a third the ability of the coronary arteries to dilate in response to β-adrenoceptor stimulation in a Langendorff-perfused heart preparation (Yu *et al.*, 2011). Thus, while it is unclear if clinical doses of rosiglitazone would reach levels sufficient to significantly inhibit vascular K_{ATP} channels, the potential for rosiglitazone to uncouple the coronary circulation from autonomic control is serious enough to warrant further investigation. Similar unresolved fears have of course existed since the early 1970s regarding the sulphonylureas and their ability to block both cardiac and vascular K_{ATP} channels.

Yu *et al.* use $K_{IR}6.2\Delta C36$ channels to show that rosiglitazone acts predominantly on the pore-forming $K_{IR}6.x$ subunit. These truncated subunits lack a C-terminal ER retention signal and form functional channels at the cell surface without the SUR subunit (Zerangue *et al.*, 1999). $K_{IR}6.2\Delta C36$ channels are as sensitive to rosiglitazone as the complete $K_{IR}6.2$/SURx complex, which distinguishes rosiglitazone's action from that of the sulphonylureas and places it in the same class as the pore-acting K_{ATP} channel blocker PNU-37883A. As discussed above, differences in the potency of rosiglitazone when applied to the intracellular or extracellular side of the membrane also led the authors to conclude that the main site of action is on the pore-forming subunit's intracellular domain. These intracellular regions contain the sites where ATP binds to inhibit channel activity and an amphipathic 'slide' helix that lies parallel to the cytosolic face of the membrane (Nichols, 2006). This helix is tethered to the cytoplasmic end of the channel pore where the channel 'gate' is believed to reside and thus may link ATP binding to opening/closure of the potassium conduction pathway. Analysis of the behaviour of single K_{ATP} channels in the presence and absence of rosiglitazone shows that while the drug has no effect on the size of single channel openings, it suppresses channel activity by extending long-lasting channel closures, almost certainly by modulating the channel's complex gating mechanism. Channel openings or closures result from the balance between the inhibitory drive of ATP binding to the pore-forming subunit and the activating drive of MgADP binding the modulatory SUR subunit. Additionally, membrane lipids such as phosphatidylinositol 4,5-bisphosphate interact with sites on $K_{IR}6.x$ that overlap with the ATP binding site and promote channel activity by antagonizing ATP inhibition (Flagg *et al.*, 2010). Clues as to rosiglitazone's action may come from the fact that $K_{IR}6.1$ and 6.2 share around 70% amino acid sequence identity, with much of the divergency occurring in the C-terminal intracellular region. Identification of rosiglitazone's exact binding site and mechanism of action may provide additional insight into the channel's gating mechanism and the reason why rosiglitazone has a fourfold preference for $K_{IR}6.1$. This in turn may highlight key differences between the pore-forming isoforms that aid the design of isoform-specific modulators.

Rosiglitazone is unlikely to be re-introduced into the European market anytime in the near future but is still available under restriction in the States. Despite many positive reports on its clinical cardiovascular effects (reviewed by Zinn *et al.*, 2008), there remains the lingering suspicion that in certain patients under certain conditions, rosiglitazone promotes unfavourable outcomes. The finding that it acts as a selective inhibitor of K_{ATP} channel and may thus inadvertently inhibit a population of ion channels involved in ischaemic protection and the regulation of blood flow may help focus future clinical studies.

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