Sulfonylurea action re-revisited

Sulfonylureas (SU), commonly used in the treatment of type 2 diabetes mellitus (T2DM), stimulate insulin secretion by inhibiting adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels in pancreatic β-cells. SU are now known to also activate cyclic adenosine monophosphate (cAMP) sensor Epac2 (cAMP-GEFII) to Rap1 signaling, which promotes insulin secretion. The different effects of various SU on Epac2/Rap1 signaling, as well as K_{ATP} channels in different tissues, underlie the diverse pancreatic and extra-pancreatic actions of SU. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00014.x, 2010)

Although earlier studies have suggested various mechanisms of sulfonylurea (SU) action, the discovery of K_{ATP} channels by electrophysiology brought a breakthrough in the understanding of the mechanism of the action of SU as well as the mechanism of glucose-stimulated insulin secretion. K_{ATP} channels were first reported in cardiac cell membranes and were later described in many other tissues including pancreatic islet cells[1]. In 1985, Sturgess *et al.* found that tolbutamide inhibits K_{ATP} channels in pancreatic β-cells, suggesting that the channels are the target of SU[2]. In 1995, Aguilar-Bryan *et al.* cloned the SU receptor (now called SUR1) from the pancreatic β-cell cDNA libraries[3]. SUR1 belongs to members of the adenosine triphosphate (ATP)-binding cassette (ABC) protein superfamily. At almost the same time, we cloned Kir6.2[4], a member of the inwardly rectifying K^+ channel family, and showed for the first time that the β-cell K_{ATP} channel is composed of Kir6.2 and SUR1.[4] The K_{ATP} channel is a hetero-octameric complex comprising two subunits: a pore-forming subunit Kir6.x (Kir6.1 or Kir6.2) and a regulatory subunit SURx (SUR1, SUR2A or SUR2B)[5]. Different combinations of Kir6.1 or Kir6.2 and SUR1 or a SUR2 variant (mix and match) form K_{ATP} channels with differing nucleotides and SU sensitivities that play distinct physiological and pathophysiological roles in different tissues[5,6]. While Kir6.2 plus SUR1 constitutes pancreatic β-cell K_{ATP} channels, Kir6.2 plus SUR2A constitutes cardiac and skeletal muscle K_{ATP} channels. Kir6.2 plus SUR2B constitutes smooth muscle K_{ATP} channels and Kir6.1 plus SUR2B constitutes vascular smooth muscle K_{ATP} channels, both of which are somewhat ATP-insensitive, nucleotide diphosphate-activated and glibenclamide-sensitive K^+ channels. SU actions were revisited after the cloning of the various K_{ATP} channels[7].

Mice lacking K_{ATP} channels (Kir6.2 null mice and SUR1 null mice) were generated[6]. Neither glucose nor tolbutamide stimulation elicited any change in $[Ca^{2+}]_i$ in Kir6.2 null β-cells. Importantly, neither glucose nor tolbutamide stimulation caused a significant insulin secretion in Kir6.2 null mice. Examination of SUR1 null mice also confirmed that both glucose-stimulated and sulfonylurea-stimulated insulin secretion depend critically on the activity of β-cell K_{ATP} channels. Based on these findings, it is generally accepted that the primary target of SU is SUR1 and that action of SU is mediated by closure of the K_{ATP} channels through binding to SUR1.

Cyclic adenosine monophosphate (cAMP) is a universal intracellular second messenger involved in the regulation of various cellular functions in many cell types. cAMP has long been considered to exert its action through protein phosphorylation by protein kinase A (PKA). However, a novel cAMP-binding protein family, termed Epac (exchange protein activated by cAMP) or cAMP-GEF (cAMP-regulated guanine nucleotide exchange factor) has been identified[8]. There are two members of the Epac family, Epac1 and Epac2, both of which possess guanine nucleotide exchange factor (GEF) activity towards Rap1, the small molecular weight GTP-binding protein, in a cAMP-dependent manner. We showed that Epac2 is involved in the potentiation of cAMP-dependent, PKA-independent insulin secretion[9]. By studying Epac2 null mice, we recently found that Epac2/Rap1 signaling is especially important in early phase (first phase) potentiation by cAMP of glucose-stimulated insulin granule exocytosis[10]. We have proposed a model in which Epac2/Rap1 signaling regulates cAMP-induced insulin granule exocytosis by controlling the size of a readily releasable pool (RPP), most likely through the regulation of granule density near the plasma membrane[10].

In the course of the studies of Epac2-mediated mechanisms of insulin secretion, we developed a fluorescence resonance energy transfer (FRET)-based Epac2 sensor (termed C-Epac2-Y) in which the full-length Epac2 is fused amino-terminally to enhanced cyan fluorescent protein (ECFP) and carboxyl-terminally to enhanced yellow fluorescent protein (EYFP)[11]. Epac2 is a closed form in the inactive state[8], so that ECFP and EYFP are located very closely to each other (within 10 nm), which causes FRET. On cAMP binding to Epac2, Epac2 changes its conformation to an open form. As a result, ECFP and EYFP separate away, so that FRET does not occur (active state)[8]. Utilizing this principle, we are able to monitor the activation status of Epac2. In the search for agents that activate Epac2 using this FRET

*Corresponding author. Susumu Seino
Tel.: +81-78-382-5860 Fax: +81-78-382-6762
E-mail address: seino@med.kobe-u.ac.jp
Received 19 January 2010; accepted 22 January 2010

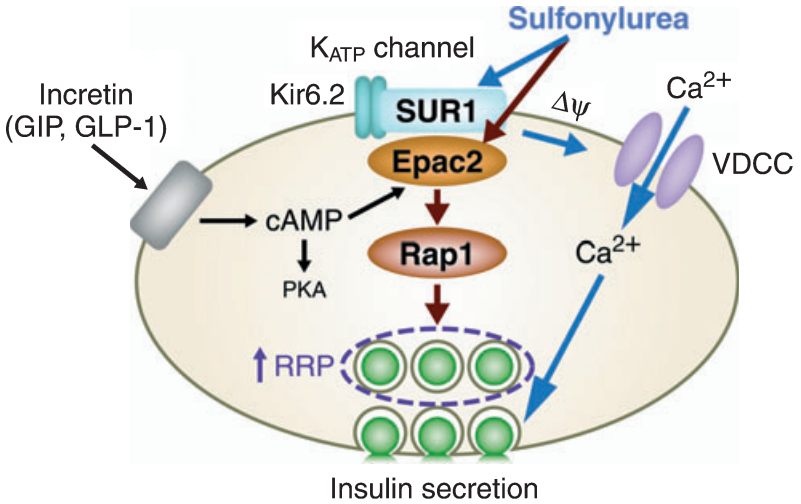

Figure 1 | Model of sulfonylurea (SU) action in insulin secretion. Closure of K_{ATP} channels is essential for SU to stimulate insulin secretion. Activation of Epac2/Rap1 signaling is required for SU to exert their full effect on insulin secretion. cAMP, cyclic adenosine monophosphate; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide 1; PKA, protein kinase A; RRP, readily releasable pool; SUR, SU receptor; VDDC, voltage-dependent Ca^{2+} channels.

sensor, we found that tolbutamide, glibenclamide, chlorpropamide, acetohexamide and glipizide significantly decreased the FRET response in COS-1 cells transfected with the Epac2 FRET sensor in different degrees and varying kinetics, suggesting strongly that these SU activate Epac2. However, gliclazide, another SU, did not decrease the FRET response. Direct binding of SU to Epac2 was confirmed by specific binding of radiolabeled glibenclamide to Epac2 expressed in COS-1 cells. We also found that tolbutamide and glibenclamide activate Rap1 in clonal pancreatic β-cells (MIN6 cells), but gliclazide does not. In addition, tolbutamide-stimulated insulin secretion and glibenclamide-stimulated insulin secretion from isolated pancreatic islets of Epac2 null mice were significantly reduced, compared with those of wild-type mice. However, there was no significant difference in insulin secretion in response to gliclazide. Furthermore, the insulin response to the oral administration of tolbutamide alone or concomitant administration of glucose and tolbutamide in Epac2 null mice was significantly reduced, compared with that in wild-type mice, and the glucose lowering effect of tolbutamide in Epac2 null mice was significantly less than that in wild-type mice.

As described above, it is well established that SU stimulate insulin secretion by eliciting a series of ionic events including closure of K_{ATP} channels, opening of voltage-dependent Ca^{2+} channels (VDCC), and Ca^{2+} influx into the β-cells. Although closure of the K_{ATP} channels is a prerequisite for SU to stimulate insulin secretion, the activation of Epac2/Rap1 signaling is required for SU to exert their full effects in insulin secretion (except in the case of gliclazide). Considering the role of Epac2/Rap1 signaling in insulin granule exocytosis[10], SU might increase the size of a readily releasable pool of insulin granules near the plasma membrane (Figure 1).

A two-site (A-site and B-site) model for the interaction of SU and glinides with SUR has been proposed[6]. The A-site is located on the eighth (between transmembrane segment (TM) 15 and 16) cytosolic loop, which is specific for SUR1, and the B-site involves the third (between TM 5 and 6) cytosolic loop, which is very similar in all SUR. Based on this model, SU and glinides can be divided into three groups. The first group (which includes tolbutamide, gliclazide and nateglinide) binds specifically to the A-site of SUR1; the second group (which includes glibenclamide and glimepiride) binds to the B-sites of both SUR1 and SUR2A as well as the A-site of SUR1; and the third group (which includes meglinitide and repaglinide) binds to the B-site of SUR1 and SUR2A. In addition, SU, with the exception of gliclazide, activate Epac2/Rap1 signaling, whereas glinides do not. Thus, different SU and glinides have different mechanisms of action in insulin secretion in terms of specificities for SUR1 and Epac2.

Mutations of Kir6.2 have recently been shown to cause neonatal diabetes mellitus (ND) with varying degrees of severity[12]. In most ND patients, insulin injection can be replaced by high-dose SU orally. Studies by Zhang *et al.* suggest that the effectiveness of SU in the treatment of ND patients might vary, depending on the properties of the SU[11].

Incretin-related drugs such as analogs of glucagon-like peptide 1 (GLP-1) and dipeptidyl peptidase IV (DPP-IV) inhibitors, which potentiate insulin secretion through cAMP signaling in pancreatic β-cells, are currently being used as new hypoglycemic agents to treat T2DM. Because Epac2 is also required for potentiation of insulin secretion by cAMP, it is a target of both SU and incretin-related drugs. There are many basic and clinical questions yet to be answered. Where is the SU binding site in Epac2? Is there any additive or synergistic effect of cAMP and SU on activation of Epac2/Rap1 signaling? Is there any accessory protein that might facilitate direct interaction of SU and Epac2? Is Epac2/Rap1 signaling involved in the extrapancreatic effects of SU and incretin-related drugs and which SU has the least adverse effect? Is Epac2/Rap1 signaling involved in the secondary failure of SU and incretin-related drugs, and which SU shows least secondary failure? What is the best combination of SU and incretin-related drugs for the most beneficial effect for treatment of T2DM in terms of insulin secretion, glycemic control and adverse effects? Answers to these questions are required

to provide a basis of beneficial treatment of T2DM. Thus, the actions of the SU must be re-revisited.

Susumu Seino[1,2,3]*, Chang-Liang Zhang[1,4], Tadao Shibasaki[1]
[1]*Division of Cellular and Molecular Medicine, Department of Physiology and Cell Biology and* [2]*Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe,* [3]*Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Saitama and* [4]*Department of Neurology, Kyoto University Graduate School of Medicine, Kyoto, Japan*

REFERENCES

1. Ashcroft FM. Adenosine 5′-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* 1988; 11: 97–118.
2. Sturgess NC, Cook DL, Ashford ML, *et al*. The sulfonylurea receptor may be an ATP-sensitive potassium channel. *Lancet* 1985; 2: 474–475.
3. Aguilar-Bryan L, Nichols CG, Wechsler SW, *et al*. Cloning of the β cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 1995; 268: 423–426.
4. Inagaki N, Gonoi T, Clement JP, *et al*. Reconstitution of IK_{ATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 1995; 270: 1166–1170.
5. Seino S. ATP-sensitive potassium channels: a model of heteromultimeric potassium channel/receptor assemblies. *Annu Rev Physiol* 1999; 61: 337–362.
6. Seino S, Miki T. Physiological and pathophysiological roles of ATP-sensitive K^+ channels. *Prog Biophys Mol Biol* 2003; 81: 133–176.
7. Gribble FM, Reimann F. Sulphonylurea action revisited: the post-cloning era. *Diabetologia* 2003; 46: 875–891.
8. Bos JL. Epac proteins: multi-purpose cAMP targets. *Trends Biochem Sci* 2006; 31: 680–686.
9. Seino S, Shibasaki T. PKA-dependent and PKA-independent pathways for cAMP-regulated exocytosis. *Physiol Rev* 2005; 85: 1303–1342.
10. Shibasaki T, Takahashi H, Miki T, *et al*. Essential role of Epac2/Rap1 signaling in regulation of insulin granule dynamics by cAMP. *Proc Natl Acad Sci USA* 2007; 104: 19333–19338.
11. Zhang CL, Katoh M, Shibasaki T, *et al*. The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. *Science* 2009; 325: 607–610.
12. Pearson ER, Flechtner I, Njolstad PR, *et al*. Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *New Engl J Med* 2006; 355: 4367–4477.