

Epac2: A Molecular Target for Sulfonylurea-Induced Insulin Release

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Abstract

Sulfonylurea drugs are used in type 2 diabetes mellitus therapy to induce release of endogenous insulin from pancreatic β -cells. They act on sulfonylurea receptors, which are the regulatory subunits of adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels, and cause channel closure to trigger exocytosis. Epac2 was identified as an intracellular target for sulfonylurea drugs, providing a potential non-electrogenic signaling component to the mechanism of action for these agents. Commonly used sulfonylureas such as tolbutamide and glibenclamide induced Epac2 activation with distinct kinetic profiles. *Epac2*^{-/-} mice failed to respond to sulfonylureas, suggesting that both sulfonylurea receptors and Epac2 are necessary for the action of these drugs. These data require that the cellular and physiological effects of drugs that alter the open state of the K_{ATP} channel be reassessed.

Diabetes mellitus is a metabolic disease defined by hyperglycemia, and it results from deficiency in insulin secretion or action. According to the Center for Disease Control and Prevention, an estimated 8% of Americans have diabetes and 17.9 million have been diagnosed with the disease. Most patients suffer from type 2 diabetes (T2D) and can be treated by diet and exercise, oral medications (such as sulfonylureas, glinides, and biguanides), or administration of insulin. Approximately 70% of diabetics are treated with oral pharmaceuticals (1), which generally rely on residual insulin in pancreatic β -cells and either stimulate additional insulin secretion or sensitize peripheral tissues to insulin action. A commonly prescribed class of T2D drugs are sulfonylureas, which inhibit potassium conductance through β -cell K^+ channels to trigger cell membrane depolarization and exocytosis through the same machinery that is required for glucose-stimulated insulin release, but in this case independently of metabolic status. Zhang and colleagues (2) provide evidence suggesting a complementary action of sulfonylurea drugs on insulin secretion by activation of the Rap1 guanine nucleotide exchange factor (GEF) Epac2 [exchange proteins directly activated by cyclic AMP 2; also known as cyclic AMP (cAMP)-GEFII].

ATP-sensitive K^+ channels (K_{ATP}) couple cellular metabolic state to membrane potential in various cell types, including pancreatic β -cells and cardiac muscle (3). The classical stimulus-secretion cascade that culminates in insulin secretion from β -cells begins with the rapid influx and metabolism of

glucose. In the basal state, inwardly rectifying K_{ATP} channels are open and maintain the resting membrane potential, but they close in response to the increase in the ratio of ATP to ADP resulting from glucose metabolism (Fig. 1). This triggers membrane depolarization and opening of voltage-dependent Ca^{2+} channels (VDCCs); the Ca^{2+} influx then provokes exocytosis of insulin granules (4) (Fig. 1). K_{ATP} channels are heterooctamers that consist of pore-forming (Kir6.x) subunits in a 1:1 stoichiometry with associated regulatory ATP-binding cassette proteins (ABCC8 or ABCC9). The regulatory subunits are more commonly known as the sulfonylurea receptors: *ABCC8* encodes SUR1, whereas splice variants of *ABCC9* produce SUR2A and SUR2B. The accessory subunits are required for proper plasmalemmal localization of the channel and for gating of the channel by ATP (5-8). Channel assemblies have tissue-specific distributions and differ in their sensitivities to sulfonylurea and glinide drugs, both of which act by binding to the regulatory subunits (3, 6). The SUR subunits of K_{ATP} channels are generally considered to be the site of action for sulfonylureas (Fig. 2), although the existence of a distinct intracellular sulfonylurea receptor has been proposed (9).

The classical nutrient stimulated insulin cascade is also modulated by circulating hormones. Incretins are peptides produced by the gut and secreted into the bloodstream in response to nutrient ingestion, and act through cell surface G-protein coupled receptors to potentiate glucose-stimulated insulin release. The two

hormones believed to contribute to the majority of the incretin effect on insulin secretion are GIP (glucose-dependent insulinotropic polypeptide) and GLP-1 (glucagon-like peptide-1), both of which stimulate the production of cyclic AMP (cAMP) (10, 11). Thus, incretins can accelerate the stimulus-exocytosis cascade, mobilize additional pools of secretory granules for release, or recruit additional responsive (or unresponsive) β -cells to augment insulin secretion, but only in the presence of stimulatory glucose concentrations. cAMP is a positive regulator of β -cell function (12), and for decades, it was thought that the second messenger's effects were mediated by cAMP-dependent protein kinase (PKA) phosphorylation of β -cell proteins (13). However, the existence of additional cAMP effectors in β -cells has challenged this dogma.

cAMP-GEFI and cAMP-GEFII (Epac1 and Epac2, respectively) were first identified in a screen for genes expressed in the striatum that encode proteins with cAMP-binding motifs; these proteins have intrinsic GEF (guanine nucleotide exchange factor) activity and couple the production of cAMP to activation of the guanosine triphosphatase (GTPase) Rap1 (14). Epac1 and Epac2 are highly similar, sharing approximately 43% sequence identity, such that their regulatory and catalytic domains are functionally equivalent; however, Epac2 has an N-terminal cyclic nucleotide binding domain that is not present in Epac1 (15). Subsequently, the mouse homolog of Epac2 was independently cloned from a yeast two-hybrid screen of a mouse insulinoma (MIN6) cDNA library,

through interaction with one of the SUR1 nucleotide-binding folds (NBFs) (16). Epac2 is the predominant isoform in the pancreatic β -cell (17, 18) and mediates the PKA-independent portion of cAMP-induced insulin release by GIP and GLP-1, accounting for as much as half of their stimulatory activity (9, 16, 19).

The localization of Epac2 to the plasma membrane by its N-terminal nucleotide binding domain is necessary for its effects on the secretion of insulin, independent of the ability of this domain to bind to cAMP (20). Furthermore, islets from *Abcc8*-deficient mice do not exhibit cAMP-mediated, PKA-independent potentiation of insulin secretion (9), making it tempting to speculate that SUR1 interaction targets Epac2 to the plasma membrane, or that SUR1 directly transduces the effect of Epac2 on secretion. Indeed, Kang and colleagues found that SUR1 interacts with both Epac1 and Epac2 in transfected cells, and in an elegant series of electrophysiological studies, showed inhibition of K_{ATP} currents in human β -cells and rat INS-1 insulinoma cells by Epac-selective cAMP analogs (18, 21). Epac2 has also been proposed to facilitate calcium-induced calcium release (CICR) from intracellular stores in INS-1 β -cells (22), an effect that likely contributes to release of insulin granules (23). Although the underlying molecular mechanisms are unclear, one possible mechanism for CICR includes the modulation of $Ca_v1.2$ L-type VDCCs by Epac2 (24, 25).

The cAMP-GEF activity of Epac2 in the context of insulin secretion is

mediated by various effectors. Silencing of Rap1 in MIN6 β -cells attenuates insulin release stimulated by 8-bromo-cAMP (a non-selective cAMP analog that activates PKA and Epacs) to a similar degree to that observed with application of antisense oligodeoxynucleotides directed against Epac2 (16, 17). This implicates signaling molecules downstream of Rap1 activation, such as protein kinase B (PKB), extracellular signal-regulated kinase (ERK), or phospholipase C ϵ (PLC- ϵ) (26), as effectors of Epac2 in exocytosis. However, the role of Rap1 in mediating the cellular effects of Epac2, such as K_{ATP} channel regulation or calcium-induced calcium release, has not yet been examined. Additionally, evidence exists for direct interaction between Epac2 and secretory granule-associated proteins that play a role in PKA-independent cAMP-stimulated insulin release, which therefore act as Epac2 effectors independently of Rap1 (23, 25).

Zhang *et al.* (2) have added an additional level of complexity to the mechanism of action of sulfonylurea drugs (Fig. 2). Epac1-based fluorescence resonance energy transfer (FRET) reporters have been used to measure Epac1 activation and to monitor intracellular cAMP dynamics (27-30). Because Epac2 is a mediator of incretin-stimulated insulin secretion (19), and because T2D therapies based on incretin signaling are effective (11, 31), Epac2 appeared to be a potential drug target. Zhang and colleagues screened insulin secretagogues in MIN6 cells transfected with a FRET sensor based on full-length Epac2 and discovered that various

sulfonylurea drugs altered the FRET ratio of this reporter, indicating a change in its molecular conformation, and potentially reflecting Epac2 activation (2). Because Epac2 interacts with SUR1, it was important to determine whether the conformational change was secondary to drug binding to SUR1 or a result of direct drug interaction with the FRET reporter. In cells lacking endogenous K_{ATP} channels, Epac2-FRET reporter changes induced by specific sulfonylureas were still observed (each with different potencies and kinetics). Radioligand competition assays indicated that affinities of tolbutamide and glibenclamide were 1.7- to 3.5-fold greater for SUR1 compared to Epac2. Although SUR1 binds to these two sulfonylureas with greater affinity than Epac2, the steady-state blood concentrations of the drugs (32, 33) suggests that both compounds theoretically would bind to both SUR1 and Epac2 in treated diabetic patients.

Additionally, evidence indicated sulfonylureas stimulated Rap1 activation in the absence of changes in cAMP concentrations. One possible explanation is an alternative binding site that activates Epac2 (2), although it seems more likely that sulfonylureas could mimic cAMP by binding to the cAMP-binding domains of Epac2 and inducing conformational changes to permit Rap1 binding (34). Further radioligand binding assays and structural studies will be needed to determine the mechanism of sulfonylurea binding to Epac2. The half-maximal effect of tolbutamide-stimulated Rap1 activation was between 30 to 50 μM (2), a concentration below the blood

concentration of tolbutamide measured in diabetic patients over a wide range of dosage regimens (32). Glibenclamide-stimulated Rap1 activity did not follow a sigmoidal concentration-response relationship: Lower concentrations (10 to 50 nM) stimulated substantial Rap1 activation, whereas >100 nM glibenclamide was ineffective (2). The blood concentrations of glibenclamide observed in treated diabetic patients generally exceeds 100 nM (33); hence, although it is predicted to bind to Epac2 in vivo, it is unlikely that glibenclamide will activate Rap1 therapeutically. Because no selective Epac antagonists are available to assess the contribution of Epac2 to the efficacy of sulfonylurea drugs in T2D patients, the authors used *Epac2*^{-/-} mice that they had generated (17) to begin to address this question. Although islets of Langerhans from *Epac2*^{-/-} mice secreted insulin in response to glucose similarly to those of wild-type mice, they did not release insulin in response to sulfonylureas. These observations were replicated during in vivo glucose tolerance tests that measured blood glucose and plasma insulin concentrations (2). The requirement for Epac2 in sulfonylurea-stimulated exocytosis from MIN6 β -cells was first reported by Eliasson and coworkers (9), who used antisense oligodeoxynucleotides and a dominant-negative Epac2 with mutations in the cyclic nucleotide-binding domains. The latter observation supports the hypothesis that sulfonylureas may bind directly to the cAMP-binding domains of Epac2.

The discovery that Epac2 may be another intracellular sulfonylurea

receptor raises a number of questions. Epac2 is highly homologous to Epac1, but they have substantially different patterns of tissue distribution (14, 16). Nonselective sulfonylureas may cause adverse cardiac effects through SUR2 in myocardium (35). Thus, in order to fully understand the potential side effects of sulfonylureas, it will be necessary to assess if Epac1 can also be activated by these drugs, and whether sulfonylureas can activate Epac1 or Epac2 signaling in the presence of SUR2 isoforms. It may be beneficial to tailor next generation sulfonylureas to not only selectively target β -cell SUR1, but also Epac2, due to its more limited tissue distribution. Furthermore, activators of K_{ATP} channels, such as diazoxide and related compounds, also modulate K^+ channel activity by binding to the sulfonylurea receptors; might they also activate or inhibit Rap1 signaling through Epacs? If so, the beneficial effects of diazoxide analogs on sensitivity of β -cells to glucose (36) may also stem from maintenance of β -cell function through Epac2.

Sulfonylurea drugs require the presence of both SUR1 and Epac2 to stimulate insulin exocytosis. The evidence of direct activation of Epac2 by sulfonylureas will prompt the re-evaluation of the cellular effects of sulfonylurea drugs, particularly in the context of the pathways and biological processes known to be mediated by Epac2. In particular, this finding adds the broad spectrum of Rap1 targets to the cellular effects of sulfonylureas. For instance, the anti-apoptotic effect of Epac2 (37), which is likely mediated by Rap1 stimulation of PKB and ERK,

must be reconciled with the pro-apoptotic effects of sulfonylurea drugs (38, 39). The generation of conditional *Rap1* knockout mice (40) means that it should be possible to generate β -cell-specific knockout animals and to more precisely quantitate the contribution of Epac2 signaling to the efficacy and mechanism of action of sulfonylurea drugs. Although at this point sulfonylureas have only been shown to stimulate Rap1 through Epac2 activation, they may also target the insulin granule-associated binding partners of Epac2 to cause secretory effects (23). The findings of Zhang *et al.* provide insight that can be used to maximize effectiveness while minimizing side effects of the next generation of sulfonylureas, and they give food for thought regarding the combination of sulfonylureas with emerging diabetic therapies based on enhancing or mimicking the cAMP-mediated incretin pathway.

Fig. 1. K_{ATP} channels (which consist of Kir6.2 and SUR1 subunits) close in response to sulfonylurea binding or the increase in the ratio of ATP to ADP resulting from glucose metabolism. This triggers membrane depolarization and opening of voltage-dependent Ca^{2+} channels (VDCCs). Ca^{2+} influx then elicits exocytosis of insulin granules.

Fig. 2. (A) Physiologically, the K_{ATP} channel is regulated by the binding of ATP and ADP to nucleotide binding folds (NBF1 and NBF2), and it can also be modulated by cAMP-stimulated Epac2 activity. Pharmacologically, K_{ATP} channels can be modulated by channel closers (such as glinides and

sulfonylureas) and channel openers (diazoxide and NN414). (B) Zhang *et al.* (2) found that certain sulfonylureas may also bind directly to Epac2 and can stimulate Rap1 activity. Thus, additional cellular effects of sulfonylureas may be Rap1-dependent and may contribute to their antidiabetic action.

References and Notes

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