Adenosine Triphosphate-Sensitive Potassium (K<sub>ATP</sub>) Channel Activity Is Coupled with Insulin Resistance in Obesity and Type 2 Diabetes Mellitus

Taro WASADA

Abstract

ATP sensitive potassium (K<sub>ATP</sub>) channels reside in the plasma membrane of many excitable cells such as pancreatic β-cells, heart, skeletal muscle and brain, where they link cellular metabolic energy to membrane electrical activity. They are composed of two subunits, K<sup>+</sup> ion selective pore (Kir) and sulfonylurea receptor (SUR). In addition to the central role of pancreatic β-cell K<sub>ATP</sub> channels in glucose-mediated insulin secretion, several lines of evidence support the hypothesis that K<sub>ATP</sub> channels modulate glucose transport in the insulin target tissues. Inhibition of K<sub>ATP</sub> channels by glibenclamide or gliclazide or an increase in intracellular ATP during hyperglycemia (glucose effect) or exercise facilitates glucose utilization, while activation of the channels by potassium channel openers, hypothermia (cardiac surgery), or ischemic damage (myocardial and brain infarction) reduces glucose uptake induced by insulin or hyperglycemia. Because insulin action has been known to depend on the energy level of the target cells, K<sub>ATP</sub> channels are composed of the physical association of four inwardly rectifying K<sup>+</sup> channel (Kir6.2) subunits with four sulfonylurea receptor (SUR) subunits (1–3). Kir6.2 serves as a K<sup>+</sup> ion selective pore. SUR is a regulatory subunit that modulates the channel gating property, and acts as the target for sulfonylureas (SUs), K<sup>+</sup> channel openers and Mg-nucleotide, all modulate Kir6.2 subunit sensitivity to ATP (4–8). It is currently believed that Kir6.2/SUR1 forms the pancreatic β-cell and the hypothalamic glucose-responsive neuron K<sub>ATP</sub> channel, Kir6.2/SUR2A forms the cardiac and skeletal muscle K<sub>ATP</sub> channel, Kir6.2/SUR2B forms the smooth muscle K<sub>ATP</sub> channel, and Kir6.1/SUR2B forms the vascular smooth muscle counterpart (9). K<sub>ATP</sub> channels are found in a variety of excitable cells, where they serve to couple the metabolic state of the cell to its electrical activity (10). Thus, they primarily set the resting membrane potential. Its closure elicits membrane depolarization, activation of voltage-dependent Ca<sup>2+</sup> channels, and a rise in Ca<sup>2+</sup> influx which regulates many cellular functions including insulin exocytosis from β-cells, vascular contraction, and neurotransmitter release from nerve terminals. Conversely, its opening elicits membrane hyperpolarization which brings the cells to a resting state that allows the minimal energy requirement, thereby protecting cells against damage from energy depletion as seen in cardiac and brain ischemia (11, 12).

Here I review the previous studies including ours on the role of extrapancreatic K<sub>ATP</sub> channels in glucose homeostasis, and I propose the hypothesis that a sustained opening of K<sub>ATP</sub> channels in insulin target tissues may be a unifying adaptive mechanism for insulin resistance in obesity and type 2 diabetes mellitus.
Evidence for the Role of Extrapancreatic $K_{ATP}$ Channels in Glucose Metabolism

A number of previous studies have shown that hypoglycemic SUs have insulin-mimetic actions on glucose metabolism in the extrapancreatic tissues. In vitro studies with SUs seem to yield two patterns of response; insulin-independent stimulation of glucose uptake (13, 14) and potentiation of insulin-stimulated glucose uptake with no effect on the basal rate (15, 16). Probably it depends on the tissue difference in the $K_{ATP}$ channel sensitivity or selectivity to SUs: for example, $K_{ATP}$ channels in skeletal muscle are approximately 30 to 300-fold less sensitive to inhibition by glibenclamide compared with those in pancreatic ß-cells (17, 18).

Tolbutamide stimulated glucose oxidation in the isolated perfused rat heart, and the contribution of glucose oxidation to overall ATP production rose with increasing concentration of tolbutamide from 8% in the absence of drug to 30% (19). Infusion of the therapeutic level of tolbutamide (7×10⁻⁴ M) acutely stimulated glucose uptake in the perfused rat hindlimb (20).

Glyburide and gliclazide have been shown to enhance glucose transport in a time- and concentration-dependent manner in L6 skeletal muscle cells independently of insulin (16, 21). This action was associated with an increase in the plasma membrane level of the glucose transporters.

Pulido et al (22) reported that in perfused rat hindquarter muscle preparations, gliclazide produced dose-dependent stimulation of glucose uptake in the absence of insulin, and its effect was totally reversed by diazoxide, a potent $K_{ATP}$ channel opener, indicating that the acute effect of gliclazide on glucose uptake by skeletal muscle is produced through a possible inhibition of $K_{ATP}$ channels.

To investigate the physiological significance of $K_{ATP}$ channels in glucose homeostasis in vivo, Miki et al (23) generated mice lacking $K_{ATP}$ channels by disruption of the Kir6.2 gene through homologous recombination. In these animals $K_{ATP}$ channel activity was found to be completely absent in pancreatic ß-cells, skeletal muscle, and heart. The K⁺ conductance of the ß-cells of these mice could not be increased by the K⁺ channel openers diazoxide and pinacidil. The basal concentration of intracellular Ca²⁺ ion in ß-cells was significantly elevated compared with control ß-cells, and spiking activities were recorded at 2.8 mM glucose. These findings indicate that the gating of $K_{ATP}$ channels in these mutant mice is shifted toward the closed state. Interestingly, these homozygously deficient mice (Kir6.2⁻/⁻) demonstrated only mild glucose intolerance, despite the marked defect in insulin secretion. The insulin tolerance test (0.1 U/kg) revealed higher insulin sensitivity in Kir6.2⁻/⁻ mice than control mice. Thus, the authors speculated that $K_{ATP}$ channels in skeletal muscle might be involved in insulin-stimulated whole-body glucose disposal.

Recently, SUR1 knockout mice (SUR1⁻/⁻) have also been generated. ß-cells from SUR1⁻/⁻ mice showed similar electrophysiological behaviors to those found in Kir6.2⁻/⁻ mice. Intraperitoneal injection of glucose caused no insulin response in mutant mice, but they maintained a near normoglycemic state, suggesting increased whole-body insulin sensitivity (24).

In humans, an α₂-adrenergic blocker midaglizole, which was later found to have $K_{ATP}$ channel blocking activity in the imidazoline moiety of this compound (25), was shown to promote not only insulin secretion but also glucose disposal determined by a euglycemic hyperinsulinenic clamp method in NIDDM patients (26). Thus, the inhibition of $K_{ATP}$ channels in peripheral tissues (primarily skeletal muscle) likely improves a whole-body insulin sensitivity in humans. In addition, Hansen et al (27) searched for mutations in the Kir6.2 gene in 346 young healthy subjects. The amino acid polymorphisms were associated neither with altered insulin secretion after intravenous administration of glucose or tolbutamide nor with altered glucose effectiveness by the minimal model analysis. However, carriers of the maximal load of amino acid variants had on average a 62% higher insulin sensitivity index (p=0.006) compared with noncarriers. They suggested that a combination of common Kir6.2 amino acid variants may contribute to the genetic background behind the large variation of the insulin sensitivity in the general population.

We have also addressed this issue at both the clinical and cellular levels. Our initial interest was the following observation that anti-anginal drug nicorandil, a $K_{ATP}$ channel opener, was found to impair glycemic control in type 2 diabetic patients without interfering with insulin secretion (28). A 7-day trial of nicorandil (15 mg/day) in three healthy male volunteers caused on average a 37.3% (26.9–42.9) reduction of glucose infusion rate estimated by a euglycemic hyperinsulinenic clamp technique (Fig. 1). To avoid a potential hemodynamic effect of nicorandil, a series of in vitro studies were performed (29). We examined the effects of $K_{ATP}$ channel openers (PCO-400 and nicorandil) alone or in combination with channel blockers (glibenclamide and gliclazide) on insulin- or high glucose concentration-stimulated glucose transport in cultured human skeletal muscle cells. PCO-400 alone reduced the basal glucose uptake in the absence of insulin. PCO-400 and nicorandil dose-dependently inhibited insulin-stimulated glucose uptake, and their inhibition was reversed by the pretreatment with glibenclamide or gliclazide in dose-dependent manner (Fig. 2). PCO-400 inhibited 25 mM glucose-facilitated glucose transport in the absence of insulin, and this effect was also antagonized by both SUs (Fig. 3). We have further shown that a phorbol ester (PMA), an activator of PKC, dose-dependently reversed the PCO-400 inhibition of insulin-stimulated glucose uptake; this finding was consistent with the previous observation in rat aortic rings (30), but not with other reports in insulinoma cells (31, 32). In our experiments, membrane-associated PKC activity showed no change following incubation of cells with insulin and PCO-400 or PCO-400 and PMA, suggesting no significant contribution of PKC to the alteration in glucose transport induced by these $K_{ATP}$ channel modulators. These results, therefore, indicate that $K_{ATP}$ channel opening per se impairs glucose transport induced by both physiological stimuli (insulin and high glucose), and hence, might contribute to insulin resistance in vivo. Because the signaling...
pathway induced by insulin and high glucose is believed to be separate (33), $K_{\text{ATP}}$ channel seems to be a common effector responsible for both processes.

Apart from glucose metabolism, $K_{\text{ATP}}$ channels have been shown to elicit some effects on lipid metabolism. Diazoxide alone fails to exert any effect on fatty acid synthase (FAS) activity in cultured 3T3-L1 adipocytes, but diazoxide completely inhibits insulin-induced activation of FAS activity (34). Glibenclamide stimulates FAS activity, which is inhibited by diazoxide and calcium channel antagonists (35). Therefore, it is interesting to note that there is a cross-talk between the insulin signaling pathway and $K_{\text{ATP}}$ channel activity in adipocytes as well.

**Role of Hexosamine Biosynthetic Pathway in Glucose Toxicity**

Chronic hyperglycemia has been claimed to cause insulin resistance. Among the hypotheses, an accelerated hexosamine biosynthetic pathway has been highlighted as a mechanism for glucose toxicity. Originally, Marshall et al (36) suggested that an increased production of UDP-N-acetyl hexosamine, an ultimate product of this pathway, may be the mechanism by which hyperglycemia leads to insulin resistance. Their conclusion was based on the following evidence: 1) glucose and insulin per se are not sufficient to produce insulin resistance in primary adipocytes, but the presence of glutamine is essential, 2) de-
sensitization to insulin can be prevented by inhibitors of the rate-limiting enzyme of this pathway, glutamine: fructose-6-phosphate amidotransferase (GFAT), and 3) glucosamine, entering the pathway downstream of the rate-limiting step, is 40-times stronger than glucose in mediating desensitization of insulin-stimulated glucose transport. Their results were confirmed by many subsequent studies, in vitro, including studies in primary rat adipocytes (37), 3T3-L1 adipocytes (38), and isolated skeletal muscle (39) and, in vivo, by measuring whole-body glucose disposal after glucosamine clamping (40–42). Similarly, in transgenic mice overexpressing GFAT specifically in muscle and fat, insulin-stimulated glucose uptake was shown to decrease by half (43).

Recently, Hresko et al (44) made the novel observations that allow an alternative explanation for the pathogenesis of insulin resistance induced by glucosamine; 1) insulin desensitization by glucosamine was intimately correlated with the decrease in intracellular ATP content rather than the generation of UDP-N-acetyl hexosamine, 2) reduction of intracellular ATP by sodium azide (inhibitor of mitochondrial electron transport) similarly inhibited GLUT4 translocation to the plasma membrane, and 3) both the reduction in [ATP], and the glucosamine-induced insulin resistance were prevented by the addition of an alternative energy substrate inosine. Based upon these observations, they concluded that any desensitization of the insulin-stimulated glucose transport in 3T3-L1 adipocytes by glucosamine treatment is due entirely to effects on ATP and not to an increase in intracellular UDP-N-acetyl hexosamine levels. They argued that even a minor reduction in ATP (10–20%) is capable of causing insulin desensitization. The decrease in [ATP], by glucosamine was considered to be due to rapid phosphorylation by hexokinase of newly fluxed glucosamine. Like glucosamine, uridine also inhibited insulin-stimulated glucose uptake in 3T3-L1 adipocytes, because this substrate also consumes high energy phosphates upon conversion to UDP-sugar. The authors suggested that any cellular defect that results in a decreased steady-state level of ATP may induce a state of insulin resistance. However, the results by Hresko et al (44) are challenged by several studies to date (45–47).

On the other hand, it has long been known that insulin action depends on the energy levels of the target cells (48–51). For example, when isolated rat epididymal fat cells were incubated with insulin in the presence of uncouplers of oxidative phosphorylation, the resultant decrease in the ATP level coincided with a disappearance of the stimulatory effects of insulin on sugar transport (48). The coupling step between insulin receptors and the glucose transport system, rather than insulin receptor binding and glucose transport itself, was previously shown to have a high activation energy (EA=24 kcal/mol), suggesting an energy requirement for activation (51).

**Role of Intracellular Long Chain Fatty Acyl-CoA or Triglyceride Accumulation in Lipotoxicity**

Recently, Unger and colleagues have extensively explored the relationship between intracellular triglyceride (TG) accumulation in both skeletal muscle and liver and the cellular response of glucose transport to insulin (52–55). They showed a highly significant correlation between insulin resistance and TG content in these insulin target tissues. The concomitant elevation of palmitate-derived ceramide in these tissues has been suggested to lead to insulin resistance by inhibiting PKB pathway, which is a major regulator for glycogen synthase activity.

Plasma FFA elevation induced by lipid/heparin infusion during hyperinsulinemic glucose clamps has repeatedly been shown to decrease insulin-dependent whole-body glucose disposal in healthy and diabetic subjects 3–4 hours after the start of lipid infusion (55–60), indicating that FFA exerts a relatively acute effect.

The \(^{13}C/\(^{13}P\) NMR spectrometry studied by Shulman’s group revealed that the rate-controlling step in glucose utilization by skeletal muscle under lipid/heparin infusion is at the glucose transport across the plasma membrane (58, 60).

Han et al demonstrated that a high-fat feeding of 4 weeks in rats reduced the insulin-stimulated skeletal muscle glucose uptake by 25–40% associated with neither decreased GLUT4 content nor increased fat oxidation (61). These findings are against Randle’s glucose-fatty acid cycle hypothesis. High-fat feeding might alter glucose transport by changing the lipid composition of the plasma membrane, which may affect its fluidity/permeability (62). This could in turn affect GLUT4 translocation, or the capacity of GLUT4 vesicles to bind or fuse with the plasma membrane (62). In this regard, it was previously reported that long chain polyunsaturated fatty acids (arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acid: C20–C22) in muscle membrane phospholipids are inversely related to insulin resistance, whereas linoleic acid and trans fatty acids are positively related to insulin resistance (63). One recent study has shown that acyl-CoA synthase is associated with GLUT4-containing vesicles in adipocytes, and fatty acyl-CoAs play a role in budding and fusion in membrane trafficking (61). Therefore, the possibility of lipid-induced abnormalities in the glucose transport system should be seriously evaluated (64).

Recently, there is a growing body of evidence that some FFAs or their metabolically active forms, long chain acyl-CoA esters (LC-CoA), directly activate K\(_{\text{ATP}}\) channels (65, 66).

Larsson et al (67, 68) demonstrated, for the first time, that by using the patch-clamp technique a marked activation of the K\(_{\text{ATP}}\) channel of pancreatic β-cells occurs in response to LC-CoA. The effect was concentration-dependent within the lower physiological concentration range (10 nM-1 μM), and also chain length-dependent: oleoyl-CoA (C18:1), palmitoyl-CoA (C16:0) and myristoyl-CoA (C14:0) had a stimulatory effect but malonyl-CoA (C3:0) did not (Fig. 4). On the molar basis, the LC-CoAs are estimated to be 100–1000 times more potent than ADP in stimulating channel activity. It was shown that 1 μM oleoyl-CoA activates the cloned β-cell K\(_{\text{ATP}}\) channels through an interaction with Kir6.2 subunit, unlike Mg-ADP and diazoxide which interact with SUR1 (69). Thus, it seems reasonable to assume that K\(_{\text{ATP}}\) channels other than the pancreatic...
The pathogenesis of insulin resistance is multietiological. In some physiological and pathophysiological conditions, especially obesity and type 2 diabetes mellitus, insulin resistance seems to link to the activation of K<sub>ATP</sub> channels in insulin target tissues. K<sub>ATP</sub> channels serve as a sensor of cellular energy metabolism in many excitable cells. They might protect the cells when the cells meet the energy shortage (or inversely the cells meet the affluent influx of FFA) by opening the channels, hyperpolarizing plasma membrane, and blocking Ca<sup>2+</sup> entry into the cells. Conversely, when the cells need continuous ATP production as seen during exercise, K<sub>ATP</sub> channels close to facilitate substrate transport. It is now evident that LC-CoA, metabolically active forms of fatty acid, are the most potent and physiologically important regulator of K<sub>ATP</sub> channels. In obesity and type 2 diabetes, LC-CoA concentrations in insulin target tissues should be elevated due to increased availability of FFA, and, in part, inhibition of oxidative phosphorylation by malonyl-CoA derived from increased influx of glucose. Similar but more severe metabolic conditions include starvation, pregnancy, and lipoatrophic diabetes, all known insulin resistant states associated with accelerated lipid catabolism. Exercise raises the intracellular ATP concentration in contracting muscles, and oppositely, a sedentary state, aging and natural hibernation reduce it.

By use of NMR spectroscopy, it was demonstrated that the rate-controlling step in insulin-stimulated muscle glycogen

Figure 4. A. The effect of 1 μM oleoyl-CoA on K<sub>ATP</sub> channel activity in an inside-out patch in the continuous presence of 0.1 mM ATP. B. Addition of 1 μM free oleic acid to an inside-out patch did not affect K<sub>ATP</sub> channel activity. Mean currents were estimated to be 0.9 pA (i<sub>a</sub>) in the absence and 0.7 pA (i<sub>b</sub>) in the presence of 1 μM oleic acid. Subsequent addition of oleoyl-CoA to the same patch resulted in an increased mean current of 4.2 pA (i<sub>c</sub>). C. Addition of 100 μM CoASH was without effect on K<sub>ATP</sub> channel activity. The mean current in the absence of CoASH was estimated to be 0.4 pA (i<sub>d</sub>), compared with 0.3 pA (i<sub>e</sub>) in the presence of CoASH. As a control 1 μM oleoyl-CoA was added, increasing mean channel current to 3.1 pA (i<sub>f</sub>). D. No effect on K<sub>ATP</sub> channel activity could be observed when 1 μM malonyl-CoA was added, mean current was estimated to be 1.2 (i<sub>a</sub>) compared to 0.9 (i<sub>b</sub>) pA. E. Addition of palmitoyl-CoA to the intracellular solution also affected channel activity with an estimated 4-fold increase in mean current going from 1.2 (i<sub>e</sub>) to 5.1 (i<sub>f</sub>) pA (Ref. 67).

Summary and Conclusions

The pathogenesis of insulin resistance is multietiological. In some physiological and pathophysiological conditions, especially obesity and type 2 diabetes mellitus, insulin resistance seems to link to the activation of K<sub>ATP</sub> channels in insulin target tissues. K<sub>ATP</sub> channels serve as a sensor of cellular energy metabolism in many excitable cells. They might protect the cells when the cells meet the energy shortage (or inversely the cells meet the affluent influx of FFA) by opening the channels, hyperpolarizing plasma membrane, and blocking Ca<sup>2+</sup> entry into the cells. Conversely, when the cells need continuous ATP production as seen during exercise, K<sub>ATP</sub> channels close to facilitate substrate transport. It is now evident that LC-CoA, metabolically active forms of fatty acid, are the most potent and physiologically important regulator of K<sub>ATP</sub> channels. In obesity and type 2 diabetes, LC-CoA concentrations in insulin target tissues should be elevated due to increased availability of FFA, and, in part, inhibition of oxidative phosphorylation by malonyl-CoA derived from increased influx of glucose. Similar but more severe metabolic conditions include starvation, pregnancy, and lipoatrophic diabetes, all known insulin resistant states associated with accelerated lipid catabolism. Exercise raises the intracellular ATP concentration in contracting muscles, and oppositely, a sedentary state, aging and natural hibernation reduce it.

By use of NMR spectroscopy, it was demonstrated that the rate-controlling step in insulin-stimulated muscle glycogen
synthesis in patients with type 2 diabetes resides in glucose transport rather than glucose phosphorylation by hexokinase or glycogen synthesis. Our findings that pharmacological manipulation of $K_{ATP}$ channels in skeletal muscle cells affects the glucose transport in vitro, supports the hypothesis that $K_{ATP}$ channel activity per se modulates glucose transport through unknown mechanism. In this context, it is of interest to note that the activity of the glucose transporter is increased as the phospholipid headgroup which resides at the surface of the lipid bilayer of the membrane is changed to a more negatively charged species (70). Thus, $K_{ATP}$ channels might elicit some effect on the interaction between the glucose transporter and plasma membrane via altering the electrostatic property of the membrane. Interestingly, gap junctions are known to allow the passage of ions and small molecules, such as second messengers or energetic metabolites from cell to cell to communicate with each other. Recently, $K_{ATP}$ channels in astrocytes have been reported to regulate the permeability of gap junctions: membrane depolarization promotes an increase in gap junctional permeability, whereas membrane hyperpolarization inhibits it (71). Long-term impairment of $K_{ATP}$ channel function could lead to the dual fundamental abnormalities found in diabetes, that is, a defect in insulin secretion and insulin action that occur in the same direction.

Acknowledgements: The author thanks Professors Susumu Seino and Yasuhiro Iwamoto for their valuable comments.

References

34) Barrett-Jolley R, Davies NW. Kinetic analysis of the inhibitory effect of glibenclamide on K<sub 下午 receive a text that, when read naturally, contains several minor errors, such as missing punctuation, incorrect word order, or other grammatical issues. The goal is to carefully read the text and correct these errors to produce a version that accurately conveys the intended meaning. Please provide the corrected text.


