Roles of ATP-Sensitive K^+ Channels as Metabolic Sensors

Studies of Kir6.x Null Mice

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ATP-sensitive K^+ channels (K_{ATP} channels) are present in various tissues, including pancreatic β-cells, heart, skeletal muscles, vascular smooth muscles, and brain. K_{ATP} channels are hetero-octameric proteins composed of inwardly rectifying K^+ channel (Kir6.x) and sulfonylurea receptor (SUR) subunits. Different combinations of Kir6.x and SUR subunits comprise K_{ATP} channels with distinct electrophysiological and pharmacological properties. Recent studies of genetically engineered mice have provided insight into the physiological and pathophysiological roles of Kir6.x-containing K_{ATP} channels. Analysis of Kir6.2 null mice has shown that Kir6.2/SUR1 channels in pancreatic β-cells and the hypothalamus are essential in glucose-induced insulin secretion and hypoglycemia-induced glucagon secretion, respectively, and that Kir6.2/SUR2 channels are involved in glucose uptake in skeletal muscles. Kir6.2-containing K_{ATP} channels in brain also are involved in protection from hypoxia-induced generalized seizure. In cardiovascular tissues, Kir6.1-containing K_{ATP} channels are involved in regulation of vascular tonus. In addition, the Kir6.1 null mouse is a model of Prinzmetal angina in humans. Our studies of Kir6.2 null and Kir6.1 null mice reveal that K_{ATP} channels are critical metabolic sensors in acute metabolic changes, including hyperglycemia, hypoglycemia, ischemia, and hypoxia. *Diabetes* 53 (Suppl. 3): S176–S180, 2004

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AMPK, cAMP-activated protein kinase; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; GRN, glucose-responsive neuron; IPC, ischemic preconditioning; IRS, insulin receptor substrate; K_{ATP} channel, ATP-sensitive K^+ channel; Kir channel, inwardly rectifying K^+ channel; PI3K, phosphatidylinositol 3-kinase; SUR, sulfonylurea receptor; VDCC, voltage-dependent calcium channel; VMH, ventromedial hypothalamus.

Pharmacological and electrophysiological studies found ATP-sensitive K^+ channels (K_{ATP} channels) in various tissues, including heart, pancreatic β-cells, central neurons, skeletal and smooth muscles, and kidney (1). In 1995, K_{ATP} channels were reconstituted in vitro, and the molecular structure of the channels was determined (2). K_{ATP} channels are hetero-octameric proteins composed of pore-forming Kir6.x subunits, members of the inwardly rectifying K^+ channel (Kir channel) family, and regulatory sulfonylurea receptor (SUR) subunits (2,3). K_{ATP} channels comprising various combinations of Kir6.x and SUR subunits have been observed in several tissues, but their physiological functions have not been fully elucidated.

In pancreatic β-cells, the increased ATP concentration due to increased glucose metabolism closes the K_{ATP} channels, depolarizing the plasma membrane, leading to opening of the voltage-dependent calcium channels (VDCCs), which allows calcium influx. The resultant intracellular calcium concentration ($[Ca^{2+}]_i$) rise triggers exocytosis of the insulin-containing granules. Sulfonylureas such as glibenclamide, widely used in treatment of type 2 diabetes, stimulate insulin release by closing the K_{ATP} channels (1). Thus, the K_{ATP} channels in β-cells have been known to be critical in the regulation of insulin secretion. However, K_{ATP} channel–independent pathways of insulin secretion also were proposed (4–6). In the brain, K_{ATP} channels with different properties were detected in various cell types, including hippocampal neurons (7), glial cells (8), dorsal vagal neurons (9), hypothalamic neurons (10), and substantia nigra (SNr) (11), but their physiological roles remained largely unknown. In the cardiovascular system, K_{ATP} channels play a protective role in metabolic stress, such as ischemia and hypoxia, that decreases the intracellular ATP concentration (12). In heart, K_{ATP} channels are involved in the shortening of action potential duration and the loss of cellular K^+, which occur during metabolic inhibition (13). In the vascular system, K_{ATP} channels are thought to regulate tonus of smooth muscles (14).

The physiological roles of K_{ATP} channels as metabolic sensors learned in our studies of Kir6.x null mice are discussed in this review.

K_{ATP} channels are essential for insulin secretion in pancreatic β-cells. Kir6.2 and SUR1 both are expressed in all endocrine cells in pancreatic islets (15,16). Because

Kir6.2 subunits form the K^+ ion–selective pore of the channel (3), mice lacking K_{ATP} channel function can be generated by genetic disruption of Kir6.2 (17). Both whole-cell and single-cell recordings show K_{ATP} channel activity to be completely absent in the pancreatic β-cells of Kir6.2 null mice, demonstrating that the Kir6.2 subunits are essential for β-cell K_{ATP} channel function. Although Kir6.2 null mice show transient hypoglycemia as neonates, blood glucose levels of Kir6.2 null mice as adults are not significantly different from those of wild-type mice in the nonfasting state. The insulin response to glucose in vivo is impaired in Kir6.2 null mice, as evaluated by an intraperitoneal glucose tolerance test. The resting membrane potential of Kir6.2 null β-cells is significantly higher than in wild-type β-cells. In the presence of low glucose (2.8 mmol/l), repetitive bursts of action potential were frequently found in Kir6.2 null β-cells. In contrast to control β-cells, high glucose did not alter the membrane potential of Kir6.2 null β-cells. The basal levels of $[Ca^{2+}]_i$ in single β-cells were significantly elevated in Kir6.2 null β-cells. However, neither high glucose nor tolbutamide elicited any change in $[Ca^{2+}]_i$ in Kir6.2 null β-cells. Thus, the rise in normal pancreatic β-cells of $[Ca^{2+}]_i$ elicited by both glucose and tolbutamide requires closure of the K_{ATP} channels, and the K_{ATP} channels in pancreatic β-cells are crucial in determining both membrane potential and Ca^{2+} influx. In contrast, acetylcholine or high K^+ stimulation increased $[Ca^{2+}]_i$ in Kir6.2 null β-cells to levels comparable to those in control β-cells, suggesting that intracellular calcium mobilization from inositol 1,4,5-triphosphate–sensitive Ca^{2+} stores and Ca^{2+} influx through VDCCs are independent of K_{ATP} channel activity. The insulin secretory responses to glucose and tolbutamide were determined using both batch incubation and perifusion of pancreatic islets of adult mice (17). Neither glucose nor tolbutamide elicited significant insulin secretion in Kir6.2 null mice, demonstrating that the K_{ATP} channels in β-cells are required in both glucose- and sulfonylurea-induced insulin secretion.

K_{ATP} CHANNELS IN THE HYPOTHALAMUS ARE CRITICAL IN HYPOGLYCEMIA-INDUCED GLUCAGON SECRETION

Kir6.2 null mice show markedly delayed recovery from insulin-induced systemic hypoglycemia, suggesting impaired secretion of counterregulatory hormones such as glucagon and catecholamines. While epinephrine secretion in response to insulin-induced hypoglycemia in Kir6.2 null mice is similar to that in wild-type in vivo, glucagon secretion is markedly reduced (18). Glucagon secretion from isolated pancreatic islets in response to change from high to low glucose concentration is similar in Kir6.2 null and wild-type mice. In addition, glucagon response to carbachol, a synthetic choline ester, is somewhat enhanced in Kir6.2 null mice compared with wild-type mice. These findings indicate that the primary defect in glucagon secretion in Kir6.2 null mice is upstream of the α-cells. Neuroglycopenia is known to stimulate glucagon secretion through activation of autonomic neurons in the brain. 2-Deoxyglucose (2DG) induces neuroglycopenia in the hypothalamus, thereby stimulating glucagon secretion (19). In contrast to wild-type mice, there is almost no glucagon secretion in response to intracerebroventricularly injected 2DG in Kir6.2 null mice. In the ventromedial hypothalamus (VMH), a subset of neurons known as glucose-responsive neurons (GRNs) increase their firing rate in response to elevated extracellular glucose (20). Wild-type VMH neurons show a twofold increase in spontaneous discharge rate in response to high glucose, while Kir6.2 null VMH neurons display no change in spontaneous discharge rate. Kir6.2 null VMH neurons show higher discharge rate at low glucose than wild-type neurons. In addition, dialysis with an ATP-free solution does not activate K^+ currents in Kir6.2 null VMH neurons. These findings show that Kir6.2-containing K_{ATP} channels are required for glucose responsiveness of GRNs. Single-cell RT-PCR analysis demonstrates that VMH K_{ATP} channels comprise Kir6.2 and SUR1, the same composition as the β-cell K_{ATP} channel. Accordingly, Kir6.2/SUR1 K_{ATP} channels in both pancreatic β-cell and hypothalamus participate in the control of blood glucose level (18). As the blood glucose level rises, inhibition of the K_{ATP} channels in pancreatic β-cells induces insulin secretion, thereby lowering the glucose level. As the blood glucose level falls, activation of the K_{ATP} channels in the GRNs of the hypothalamus triggers glucagon secretion, most likely through stimulation of autonomic input to the pancreatic α-cells (21), thereby raising the glucose level. In addition to their involvement in glucagon secretion, hypothalamic K_{ATP} channels also are involved in acute food intake (18).

K_{ATP} CHANNELS ARE INVOLVED IN GLUCOSE UPTAKE IN SKELETAL MUSCLES

K_{ATP} channels also are present in skeletal muscles (22). The pharmacological and electrophysiological properties of the channel have been examined in native skeletal muscles isolated from several vertebrates (23–25). While the unitary conductance in these muscles is similar, the electrophysiological properties of the K_{ATP} channels recorded in native skeletal muscles vary to some extent in terms of sensitivity to ATP, glibenclamide, and potassium channel openers (PCOs) (25,26). These differences could be due in part to differences in experimental conditions, species, and splice variations of SUR2 (27). Although the electrophysiological properties of the channels reconstituted by Kir6.2 and SUR2A (3) are similar to those of K_{ATP} channels recorded in native skeletal muscles, the molecular composition of skeletal muscle K_{ATP} channels in native tissues has not been determined. Some of the sulfonylureas have been shown to improve glycemic control in patients with type 2 diabetes by acting on extrapancreatic tissues to reduce insulin resistance (28). Although there is no direct evidence of the involvement of skeletal muscle K_{ATP} channels in extrapancreatic effects of sulfonylureas, these findings suggest that the K_{ATP} channel in skeletal muscle is involved in glucose uptake. Interestingly, the glucose-lowering effect of insulin is enhanced in Kir6.2 null mice (18), indicating that insulin sensitivity is increased. Basal and insulin-stimulated glucose uptake in gastrocnemius of Kir6.2 null mice were both significantly increased in vivo and in vitro compared with wild type, although the effects of genetic disruption of the K_{ATP} channel vary in the different types of muscle (29). Because there is no K_{ATP} channel activity in the skeletal muscles of Kir6.2 null mice, these findings demonstrate that the K_{ATP} channel is in-

TABLE 1
Features of Kir6.2 null, IRS-1 null, and Kir6.2/IRS-1 double null mice

	Kir6.2 KO	IRS-1 KO	Kir6.2/IRS-1 DKO
Blood glucose (nonfasting)	Normal	Normal	Normal
Blood glucose (fasted)	Low	Normal	Low
Serum insulin levels (nonfasting)	Low	High	High
Glucose tolerance	Slightly impaired	Normal	Almost normal
Glucose-induced insulin secretion	Lost	Enhanced	Reduced
Insulin action	Increased	Reduced	Increased

volved in glucose uptake. The involvement of these K_{ATP} channels also has been demonstrated in SUR2 null mice (30). SUR2 null mice show lower fasting and nonfasting glucose levels, improved glucose tolerance during glucose tolerance test, and more rapid and severe hypoglycemia after administration of insulin. Enhanced glucose utilization was confirmed by in vivo hyperinsulinemic-euglycemic clamp studies in which SUR2 null mice required a greater glucose infusion rate to maintain the blood glucose level. Such enhancement of insulin action seems to be characteristic of the skeletal muscles, as in vitro insulin-stimulated glucose uptake was 1.5-fold greater in SUR2 null muscle than in wild-type muscle. Together, these findings clearly show that the K_{ATP} channels in skeletal muscle are involved in glucose uptake.

To further examine the involvement of K_{ATP} channels in glucose uptake, we generated double null mice lacking both Kir6.2 and insulin receptor substrate (IRS)-1 (31). IRS-1 null mice show severe insulin resistance in skeletal muscles but exhibit normal glucose tolerance (32,33), apparently because β-cell hyperplasia and the resultant hyperinsulinemia can compensate for the increased peripheral insulin demand (34). In contrast, Kir6.2 null mice show severely impaired glucose-induced insulin secretion but also normoglycemia (18). As mentioned, Kir6.2 null mice exhibit an enhanced glucose-lowering response by exogenous insulin, probably due to the increased insulin-stimulated glucose uptake in skeletal muscles (29) that contributes to the preservation of normoglycemia. Because insulin-stimulated glucose uptake is mediated by IRS-1/phosphatidylinositol 3-kinase (PI3K) signaling, the major pathway of insulin signal transduction, disruption of the IRS-1 gene should impair glucose uptake in Kir6.2 null mice, and the double null mice were expected to develop overt diabetes. However, Kir6.2/IRS-1 double null mice had nonfasting blood glucose levels similar to those of wild-type mice but had lower fasting blood glucose levels like Kir6.2 null mice (Table 1). The glucose-lowering effect by exogenous insulin is similarly enhanced in double null mice compared with Kir6.2 null mice. Moreover, glucose uptake in skeletal muscles is significantly increased in double null mice similarly to Kir6.2 null mice. In terms of blood glucose levels and glucose uptake in skeletal muscles, the phenotype of Kir6.2 null mice is unchanged by absence of the IRS-1 gene (Table 1), demonstrating that K_{ATP} channel–associated change in glucose uptake is independent of the IRS-1–mediated signaling pathway.

IRS-1–mediated glucose uptake requires activation of PI3K followed by activation of downstream signals. Accordingly, disruption of the IRS-1 gene causes reduction of insulin-stimulated activation of PI3K (31–33). However, lack of the Kir6.2 gene does not increase PI3K activity in either the presence or absence of the IRS-1 gene, indicating that the K_{ATP} channel–associated increase in glucose uptake is independent of PI3K activity. Furthermore, phosphorylation of Akt/protein kinase B (PKB), a signaling molecule downstream of PI3K, is not affected by lack of the Kir6.2 gene, indicating that the Akt/PKB signal is not involved in K_{ATP} channel–associated increase in glucose uptake. Glucose uptake in skeletal muscles also is stimulated by an insulin-independent, cAMP-activated protein kinase (AMPK)-dependent mechanism (35,36). Upon phosphorylation, AMPK stimulates glucose uptake that is additive to insulin action, suggesting that if the AMPK signal is enhanced, glucose uptake is increased. However, this is not the case in Kir6.2 null mice, as the phosphorylated form of AMPK was not increased but was decreased in mice lacking the Kir6.2 gene. Although the pathways involved in K_{ATP} channel–associated glucose uptake remain to be clarified, these findings establish that they are independent of AMPK and the IRS-1/PI3K signal.

Kir6.2/IRS-1 double null mice provide other interesting avenues to be investigated. While disruption of the Kir6.2 gene results in loss of glucose-induced insulin secretion, lack of the IRS-1 gene causes insulin hypersecretion. Kir6.2/IRS-1 double null mice have some but poor insulin response after glucose challenge compared with Kir6.2 null mice (31), indicating that disruption of the IRS-1 gene partially restores glucose-induced insulin secretion even in the absence of the pancreatic β-cell K_{ATP} channels. Moreover, in the nonfasting state, Kir6.2 null mice exhibit serum insulin levels similar to those in wild-type mice, while Kir6.2/IRS-1 double null mice exhibit marked hyperinsulinemia (31). These findings show that insulin secretion stimulated by secretagogues other than glucose is preserved or even increased in the absence of the pancreatic β-cell K_{ATP} channels when IRS-1 is lacking. Recently, Doliba et al. (37) reported that insulin secretion from SUR1 null islets is hypersensitive to the muscarine receptor signal and the cAMP signal. The insulin response to mixed-meal ingestion is also observed in Kir6.2 null mice (38). While Kir6.2/IRS-1 double null mice show nonfasting hyperinsulinemia, they exhibit somewhat reduced islet insulin content compared with wild-type mice (31), like IRS-1 null mice (34). In IRS-1 null mice, the extensive β-cell hyperplasia might explain the increased serum insulin concentration despite the reduced islet insulin content (34). β-Cell hyperplasia and the resultant hyperinsulinemia are thought to be a compensatory response to insulin resistance. Indeed, IRS-1 null mice show severe insulin resistance (32,33). However, although there is no evidence of insulin resistance in Kir6.2/IRS-1 double null mice, they exhibit hyperinsulinemia (31), most likely due to β-cell hyperplasia. The molecular basis of hyperinsulin-

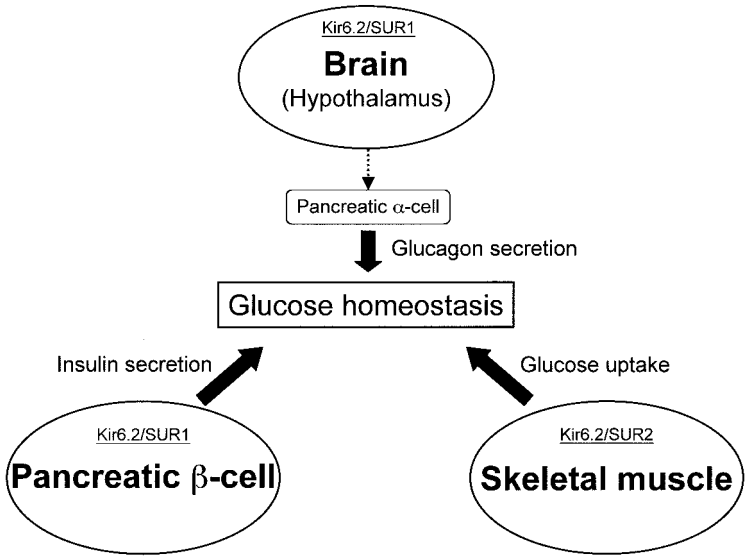

FIG. 1. Kir6.2-containing K_{ATP} channels in glucose homeostasis. In pancreatic β-cells, Kir6.2/SUR1 K_{ATP} channels are critical in insulin secretion in response to glucose and sulfonylureas. In skeletal muscles, Kir6.2/SUR2 channels are involved in IRS-1/PI3K-independent glucose uptake. In the hypothalamus, Kir6.2/SUR1 channels participate in glucagon secretion from pancreatic α-cells in response to hypoglycemia. Thus, K_{ATP} channels coordinate the responses of these three tissues in the maintenance of glucose homeostasis.

emia in Kir6.2/IRS-1 double null mice in the absence of insulin resistance remains to be clarified.

Analyses of the pancreatic islets, hypothalamus, and skeletal muscles of Kir6.2 null mice reveal that the Kir6.2-containing K_{ATP} channels in these tissues act in concert in the maintenance of glucose homeostasis (Fig. 1).

K_{ATP} CHANNELS HAVE A PROTECTIVE ROLE IN ACUTE METABOLIC STRESS

K_{ATP} channels also play a role in acute metabolic stress. In the cardiovascular system, K_{ATP} channels are found in both cardiomyocytes and vascular smooth muscles. Brief and intermittent ischemia paradoxically protects myocardium against prolonged ischemic insult, producing a marked reduction in infarct size, a phenomenon known as ischemic preconditioning (IPC) (39) in which K_{ATP} channels are thought to participate. Infarct size in wild-type mice with IPC is less than without IPC, while there is no significant difference in infarct size with or without IPC in Kir6.2 null mice (40), demonstrating the involvement of Kir6.2-containing K_{ATP} channels in IPC. A reconstitution study has suggested that the K_{ATP} channels in vascular smooth muscle are composed of Kir6.1 and SUR2B (41).

TABLE 2
Physiological roles of K_{ATP} channels learned from Kir6.x null mice

	Ref.
Kir6.2-containing K_{ATP} channels	
Pancreatic β-cells	
Regulation of glucose- and sulfonylurea-induced insulin secretion	17
Skeletal muscles	
Protection against fatigue	47
Glucose uptake	31
Heart	
Ischemic preconditioning	40
Brain	
Hypoglycemia-induced glucagon secretion	18
Prevention of hypoxia-induced generalized seizure	46
Kir6.1-containing K_{ATP} channels	
Regulation of vascular tonus	42

Kir6.1 null mice are prone to sudden, premature death. The mice exhibit spontaneous elevation of ST segments followed by atrioventricular blocks of various degrees, indicating that death is due to myocardial ischemia (42). This phenotype closely resembles Prinzmetal angina (vasospastic angina) in humans, a form of unstable angina associated with hypercontractility of coronary arteries (43). In accord with this finding, it has been found that disruption of SUR2 also produces a phenotype similar to Prinzmetal angina (44). These findings indicate that Kir6.1/SUR2B K_{ATP} channels play a critical role in the regulation of vascular tonus of coronary arteries. Because sulfonylureas are widely used in the treatment of type 2 diabetes, it is important to ascertain the effects of the various sulfonylureas on the Kir6.1/SUR2B channels.

In the brain generalized seizure can be evoked by metabolic stress such as hypoxia, and the SNr acts as a central gating system in seizure propagation (45). Kir6.2 null mice are especially susceptible to generalized seizure after brief hypoxia (46), probably because the K_{ATP} channel is required to regulate the threshold of seizure.

CONCLUSIONS

K_{ATP} channels belong to the Kir channel family. In distinction from the other inwardly rectifying K^+ channels, in which the pore-forming subunits themselves function as channels, the pore-forming subunit Kir6.x and the regulatory subunit SUR are both required for K_{ATP} channel function. Genetic manipulation of the K_{ATP} channel subunits has clarified many of the physiological and pathophysiological functions of K_{ATP} channels (Table 2). Studies of Kir6.x null mice have revealed that K_{ATP} channels play crucial roles as metabolic sensors in many tissues in acute metabolic changes.

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REFERENCES

1. Ashcroft FM: Adenosine 5′-triphosphate-sensitive potassium channels. *Annu Rev Neurosci* 11:97–118, 1988
2. Inagaki N, Gonoi T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, Aguilar-Bryan L, Seino S, Bryan J: Reconstitution of I_{KATP}: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 270:1166–1170, 1995
3. Inagaki N, Gonoi T, Clement JP, Wang CZ, Aguilar-Bryan L, Bryan J, Seino S: A family of sulfonylurea receptors determines the pharmacological properties of ATP-sensitive K^+ channels. *Neuron* 16:1011–1017, 1996
4. Aizawa T, Sato Y, Komatsu M, Hashizume K: ATP-sensitive K^+ channel-independent, insulinotropic action of glucose in the B-cells. *Endocr Regul* 26:159–162, 1992
5. Okamoto H, Takasawa S, Nata K: The CD38-cyclic ADP-ribose signalling system in insulin secretion: molecular basis and clinical implications. *Diabetologia* 40:1485–1491, 1997
6. Henquin JC: Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 49:1751–1760, 2000
7. Fujimura N, Tanaka E, Yamamoto S, Shigemori M, Higashi H: Contribution of ATP-sensitive potassium channels to hypoxic hyperpolarization in rat hippocampal CA1 neurons in vitro. *J Neurophysiol* 77:378–385, 1997
8. Zawar C, Plant TD, Schirra C, Konnerth A, Neumcke B: Cell-type specific expression of ATP-sensitive potassium channels in the rat hippocampus. *J Physiol* 514:327–341, 1999
9. Trapp S, Ballanyi K: K_{ATP} channel mediation of anoxia-induced outward current in rat dorsal vagal neurons in vitro. *J Physiol* 487:37–50, 1995
10. Ashford ML, Boden PR, Treherne JM: Glucose-induced excitation of hypothalamic neurones is mediated by ATP-sensitive K^+ channels. *Pflugers Arch* 415:479–483, 1990
11. Liss B, Bruns R, Roeper J: Alternative sulfonylurea receptor expression defines metabolic sensitivity of K_{ATP} channels in dopaminergic midbrain neurons. *EMBO J* 18:833–846, 1999
12. Fujita A, Kurachi Y: Molecular aspects of ATP-sensitive K^+ channels in the cardiovascular system and K^+ channel openers. *Pharmacol Ther* 85:39–53, 2000
13. Terzic A, Jahangir A, Kurachi Y: Cardiac ATP-sensitive K^+ channels: regulation by intracellular nucleotides and K^+ channel-opening drugs. *Am J Physiol* 269:C525–C545, 1995
14. Quayle JM, Nelson MT, Standen NB: ATP-sensitive and inwardly rectifying potassium channels in smooth muscle. *Physiol Rev* 77:1165–1232, 1997
15. Suzuki M, Fujikura K, Inagaki N, Seino S, Takata K: Localization of the ATP-sensitive K^+ channel subunit Kir6.2 in mouse pancreas. *Diabetes* 46: 1440–1444, 1997
16. Suzuki M, Fujikura K, Kotake K, Inagaki N, Seino S, Takata K: Immunolocalization of sulphonylurea receptor 1 in rat pancreas. *Diabetologia* 42:1204–1211, 1999
17. Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, Gonoi T, Iwanaga T, Miyazaki J, Seino S: Defective insulin secretion and enhanced insulin action in K_{ATP} channel-deficient mice. *Proc Natl Acad Sci U S A* 95:10402–10406, 1998
18. Miki T, Liss B, Minami K, Shiuchi T, Saraya A, Kashima Y, Horiuchi M, Ashcroft F, Minokoshi Y, Roeper J, Seino S: ATP-sensitive K^+ channels in the hypothalamus are essential for the maintenance of glucose homeostasis. *Nat Neurosci* 4:507–512, 2001
19. Borg WP, Sherwin RS, During MJ, Borg MA, Shulman GI: Local ventromedial hypothalamus glucopenia triggers counterregulatory hormone release. *Diabetes* 44:180–184, 1995
20. Oomura Y, Ono T, Ooyama H, Wayner MJ: Glucose and osmosensitive neurones of the rat hypothalamus. *Nature* 222:282–284, 1969
21. Taborsky Jr GJ, Ahren B, Havel PJ: Autonomic mediation of glucagon secretion during hypoglycemia: implications for impaired alpha-cell responses in type 1 diabetes. *Diabetes* 47:995–1005, 1998
22. Spruce AE, Standen NB, Stanfield PR: Voltage-dependent ATP-sensitive potassium channels of skeletal muscle membrane. *Nature* 316:736–738, 1985
23. Weik R, Neumcke B: ATP-sensitive potassium channels in adult mouse skeletal muscle: characterization of the ATP-binding site. *J Membr Biol* 110:217–226, 1989
24. Vivaudou MB, Arnoult C, Villaz M: Skeletal muscle ATP-sensitive K^+ channels recorded from sarcolemmal blebs of split fibers: ATP inhibition is reduced by magnesium and ADP. *J Membr Biol* 122:165–175, 1991
25. Allard B, Lazdunski M: Nucleotide diphosphates activate the ATP-sensitive potassium channel in mouse skeletal muscle. *Pflugers Arch* 422:185–192, 1992
26. Davis NW, Standen NB, Stanfield PR: ATP-dependent potassium channels of muscle cells: their properties, regulation, and possible functions. *J Bioenerg Biomembr* 23:509–535, 1991
27. Chutkow WA, Makielski JC, Nelson DJ, Burant CF, Fan Z: Alternative splicing of Sur2 exon 17 regulates nucleotide sensitivity of the ATP-sensitive potassium channel. *J Biol Chem* 274:13656–13665, 1999
28. Wang PH, Moller D, Flier JS, Nayak RC, Smith RJ: Coordinate regulation of glucose transporter function, number, and gene expression by insulin and sulfonylureas in L6 rat skeletal muscle cells. *J Clin Invest* 84:62–67, 1989
29. Miki T, Minami K, Zhang L, Morita M, Gonoi T, Shiuchi T, Minokoshi Y, Renaud JM, Seino S: ATP-sensitive potassium channels participate in glucose uptake in skeletal muscle and adipose tissue. *Am J Physiol Endocrinol Metab* 283:E1178–E1184, 2002
30. Chutkow WA, Samuel V, Hansen PA, Pu J, Valdivia CR, Makielski JC, Burant CF: Disruption of Sur2-containing K_{ATP} channels enhances insulin-stimulated glucose uptake in skeletal muscle. *Proc Natl Acad Sci U S A* 98:11760–11764, 2001
31. Minami K, Morita M, Saraya A, Yano H, Terauchi Y, Miki T, Kuriyama T, Kadowaki T, Seino S: ATP-sensitive K^+ channel-mediated glucose uptake is independent of IRS-1/phosphatidylinositol 3-kinase signaling. *Am J Physiol Endocrinol Metab* 285:E1289–E1296, 2003
32. Tamemoto H, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S, Sekihara H, Yoshioka S, Horikoshi H, Furuta Y, Ikawa Y, Kasuga M, Yazaki Y, Aizawa S: Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 372:182–186, 1994
33. Araki E, Lipes MA, Patti ME, Bruning JC, Haag B 3rd, Johnson RS, Kahn CR: Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 372:186–190, 1994
34. Kubota N, Tobe K, Terauchi Y, Eto K, Yamauchi T, Suzuki R, Tsubamoto Y, Komeda K, Nakano R, Miki H, Satoh S, Sekihara H, Sciacchitano S, Lesniak M, Aizawa S, Nagai R, Kimura S, Akanuma Y, Taylor SI, Kadowaki T: Disruption of insulin receptor substrate 2 causes type 2 diabetes because of liver insulin resistance and lack of compensatory β-cell hyperplasia. *Diabetes* 49:1880–1889, 2000
35. Mu J, Brozinick JT Jr, Valladares O, Bucan M, Birnbaum MJ: A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. *Mol Cell* 7:1085–1094, 2001
36. Musi N, Fujii N, Hirshman MF, Ekberg I, Froberg S, Ljungqvist O, Thorell A, Goodyear LJ: AMP-activated protein kinase (AMPK) is activated in muscle of subjects with type 2 diabetes during exercise. *Diabetes* 50:921–927, 2001
37. Doliba NM, Qin W, Vatamaniuk MZ, Li C, Zelent D, Najafi H, Buettger CW, Collins HW, Carr RD, Magnuson MA, Matschinsky FM: Restitution of defective glucose-stimulated insulin release of sulfonylurea type 1 receptor knockout mice by acetylcholine. *Am J Physiol Endocrinol Metab* 286: E834–E843, 2004
38. Seino S, Iwanaga T, Nagashima K, Miki T: Diverse roles of K_{ATP} channels learned from Kir6.2 genetically engineered mice. *Diabetes* 49:311–318, 2000
39. Cohen MV, Baines CP, Downey JM: Ischemic preconditioning: from adenosine receptor of K_{ATP} channel. *Annu Rev Physiol* 62:79–109, 2000
40. Suzuki M, Sasaki N, Miki T, Sakamoto N, Ohmoto-Sekine Y, Tamagawa M, Seino S, Marban E, Nakaya H: Role of sarcolemmal K_{ATP} channels in cardioprotection against ischemia/reperfusion injury in mice. *J Clin Invest* 109:509–516, 2002
41. Yamada M, Isomoto S, Matsumoto S, Kondo C, Shindo T, Horio Y, Kurachi Y: Sulphonylurea receptor 2B and Kir6.1 form a sulphonylurea-sensitive but ATP-insensitive K^+ channel. *J Physiol* 499:715–720, 1997
42. Miki T, Suzuki M, Shibasaki T, Uemura H, Sato T, Yamaguchi K, Koseki H, Iwanaga T, Nakaya H, Seino S: Mouse model of Prinzmetal angina by disruption of the inward rectifier Kir6.1. *Nat Med* 8:466–472, 2002
43. Prinzmetal M, Kennamer R, Merliss R, Wada T, Bor N: Angina pectoris. I. A variant form of angina pectoris; preliminary report. *Am J Med* 27:375–388, 1959
44. Chutkow WA, Pu J, Wheeler MT, Wada T, Makielski JC, Burant CF, McNally EM: Episodic coronary artery vasospasm and hypertension develop in the absence of Sur2 K_{ATP} channels. *J Clin Invest* 110:203–208, 2002
45. Iadarola MJ, Gale K: Substantia nigra: site of anticonvulsant activity mediated by gamma-aminobutyric acid. *Science* 218:1237–1240, 1982
46. Yamada K, Ji JJ, Yuan H, Miki T, Sato S, Horimoto N, Shimizu T, Seino S, Inagaki N: Protective role of ATP-sensitive potassium channels in hypoxia-induced generalized seizure. *Science* 292:1543–1546, 2001
47. Gong B, Miki T, Seino S, Renaud JM: A K_{ATP} channel deficiency affects resting tension, not contractile force, during fatigue in skeletal muscle. *Am J Physiol Cell Physiol* 279:C1351–C1358, 2000