

α -Ketoisocaproate-induced hypersecretion of insulin by islets from diabetes-susceptible mice

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Rabaglia, Mary E., Mark P. Gray-Keller, Brian L. Frey, Michael R. Shortreed, Lloyd M. Smith, and Alan D. Attie. α -Ketoisocaproate-induced hypersecretion of insulin by islets from diabetes-susceptible mice. *Am J Physiol Endocrinol Metab* 289: E218–E224, 2005. First published March 1, 2005; doi:10.1152/ajpendo.00573.2004.—Most patients at risk for developing type 2 diabetes are hyperinsulinemic. Hyperinsulinemia may be a response to insulin resistance, but another possible abnormality is insulin hypersecretion. BTBR mice are insulin resistant and hyperinsulinemic. When the *leptin*^{ob} mutation is introgressed into BTBR mice, they develop severe diabetes. We compared the responsiveness of lean B6 and BTBR mouse islets to various insulin secretagogues. The transamination product of leucine, α -ketoisocaproate (KIC), elicited a dramatic insulin secretory response in BTBR islets. The KIC response was blocked by methyl-leucine or aminooxyacetate, inhibitors of branched-chain amino transferase. When dimethylglutamate was combined with KIC, the fractional insulin secretion was identical in islets from both mouse strains, predicting that the amine donor is rate-limiting for KIC-induced insulin secretion. Consistent with this prediction, glutamate levels were higher in BTBR than in B6 islets. The transamination product of glutamate, α -ketoglutarate, elicited insulin secretion equally from B6 and BTBR islets. Thus formation of α -ketoglutarate is a requisite step in the response of mouse islets to KIC. α -Ketoglutarate can be oxidized to succinate. However, succinate does not stimulate insulin secretion in mouse islets. Our data suggest that α -ketoglutarate may directly stimulate insulin secretion and that increased formation of α -ketoglutarate leads to hyperinsulinemia.

β -cells; hyperinsulinemia; glutamate dehydrogenase; branched-chain aminotransferase

INCREASED INSULIN SECRETION is often found in association with insulin resistance. The simplest explanation is that a normal level of insulin secretion is unable to stimulate normal glucose clearance. Thus the residual glucose triggers additional insulin secretion. However, biochemical studies of islets indicate that there are numerous changes in the expression of metabolic enzymes and other genes that affect the responsiveness of islets to insulin secretagogues (35). Insulin hypersecretion does not always represent a compensatory reaction to insulin resistance; it can occur in the absence of insulin resistance and in some instances may represent a primary metabolic defect (22). Basal insulin hypersecretion is associated with obesity. The prevalence of insulin hypersecretion is higher than the prevalence of insulin resistance in obese people (8).

Insulin hypersecretion is prevalent in populations thought to carry “thrift genes,” which are genes that promote efficient energy storage (35). Paradoxically, despite their hyperinsulinemia, these populations have an unusually high prevalence of

diabetes mellitus (33). In fact, in one such population, the Pima Indians, a longitudinal study showed that nondiabetic individuals with the highest level of fasting insulin (controlled for insulin sensitivity) were six times more likely to develop diabetes six years later than nondiabetic subjects with the lowest levels of fasting insulin (35). Similar results were obtained in another population carrying thrift genes (27). The progression to diabetes was associated with a loss of insulin secretory capacity rather than a loss of insulin sensitivity. In short, it appears that, in certain individuals, insulin hypersecretion correlates with subsequent loss of insulin secretory capacity.

We have developed a model of type 2 diabetes, whose phenotype resembles that of humans carrying thrift genes. When the *leptin*^{ob} mutation is bred into the BTBR mouse strain, the animals develop severe hyperglycemia and loss of β -cell mass (30). By contrast, as first reported by Coleman and Hummel (6), the C57BL/6J mouse strain is relatively diabetes resistant when it carries the *leptin*^{ob} mutation. Indeed, islets from C57BL/6J *leptin*^{ob/ob} mice are hyperplastic (4), and the animals have insulin levels 50–100 times normal, whereas islets from the diabetic BTBR *leptin*^{ob/ob} mice are not hyperplastic and are fewer in number, consistent with loss of islet cell mass (30).

Lean BTBR mice are insulin resistant because of adipose tissue insulin resistance (21). When bred to C57BL/6J mice, the F₁ offspring are severely insulin resistant because of insulin resistance in both adipose tissue and muscle (23). The BTBR mice, like the diabetes-susceptible Pima Indians, are hyperinsulinemic. We therefore compared the responses of isolated B6 and BTBR islets with various insulin secretagogues. The BTBR islets were hyperresponsive to some secretagogues, with the greatest response seen with α -ketoisocaproic acid (KIC). By studying the response of C57BL/6J and BTBR islets to a variety of secretagogues and inhibitors, we conclude that the formation of α -ketoglutarate (α -KG), catalyzed by branched-chain amino transferase (BCAT), is the basis for the hypersensitivity of the islets from the diabetes-susceptible BTBR mice.

EXPERIMENTAL PROCEDURES

Materials. Collagenase type XI, α -KIC, RIA-grade BSA, glutamine, glutamic acid dimethyl ester, leucine, 2-amino-2-norbornane-carboxylic acid (BCH), aminooxyacetate, and diazoxide (DZX) were purchased from Sigma. Dimethyl 2-oxoglutarate (α -KG) was obtained from Aldrich. Methyl-leucine was obtained from Fisher Chemicals

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(Arcros). Hanks' balanced salt solution (HBSS) was from GIBCO. The U- $^{13}\text{C}_5$ versions of both L-glutamine and L-glutamic acid were obtained from Cambridge Isotope Laboratories. HPLC-grade water and acetonitrile were purchased from Burdick & Jackson, and the formic acid was from EM Science.

Animals. C57BL/6J and BTBR mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were maintained at the University of Wisconsin-Madison animal care facility on a 12:12-h light-dark cycle. Mice were fed Purina Formulab Chow 5008 and water ad libitum.

Islet isolation. Intact pancreatic islets were isolated from mice using a collagenase digestion procedure. Mice were killed by CO_2 asphyxiation, and the pancreata were immediately inflated with 5 ml HBSS supplemented with 0.02% BSA and collagenase (0.45 mg/ml). The pancreata were removed from the animals, placed in 25 ml of HBSS/BSA/collagenase, and incubated for 16 min at 37°C , with intermittent agitation. A ficoll gradient was used to partially purify islets from the digested pancreata. As a further purification, islets were hand-picked at 18 – 20°C using a stereomicroscope while maintained in Krebs-Ringer bicarbonate buffer (KRB) containing (in mM): 118.41 NaCl, 4.69 KCl, 2.52 CaCl_2 , 1.18 MgSO_4 , 1.18 KH_2PO_4 , 25 NaHCO_3 , and 5 HEPES supplemented with 0.2% BSA and 16.7 mM glucose. The use of the 16.7 mM glucose as a supplement in the picking media did not alter the islet insulin response to high glucose, since the picking process is performed at room temperature.

Insulin secretion. Five islets of equivalent size were placed in 12×75 -mm glass tubes, where the bottom of the tube was formed by a 62 - μm mesh (Tetko). The 12×75 -mm tubes were transferred to 16×100 -mm tubes containing 1 ml of KRB with 1.7 mM glucose and 0.5% BSA and preincubated at 37°C for 45 min. After the preincubation, the 12×75 -mm tubes were transferred to a fresh 16×100 -mm tube containing 1 ml KRB supplemented with 1.7 or 16.7 mM glucose with and without the indicated test compound. For experiments utilizing methyl-leucine inhibition of BCAT, 10 mM methyl-leucine was included in the preincubation medium. After a 45-min incubation period at 37°C , the 12×75 -mm tubes were transferred to a fresh tube containing 1 ml of HCl-ethanol-water (1:50:14) to extract cellular insulin from the islets. The incubation media left in the 16×100 -mm tube was collected and frozen for insulin determination by RIA (Linco).

Amino acid analysis by LC-MS. Samples of 50 islets were extracted into 51 μl of 80:20 acetonitrile- H_2O containing two isotopically labeled internal standards (9.4 μM [U- $^{13}\text{C}_5$]glutamine and 18.8 μM [U- $^{13}\text{C}_5$]glutamic acid). After sonication for 15 min and centrifugation for 10 min, the supernatant was evaporated to dryness in a vacuum centrifuge followed by resuspension in 10 μl of 5 mM formic acid in 80:20 acetonitrile- H_2O . This sample (1 μl) was injected in an LC Packings HPLC system (Dionex) with a poly(hydroxyethyl)aspartamide capillary column. Samples were eluted using a gradient profile that began with 5 min of 20% of 25 mM aqueous formic acid-80% acetonitrile, followed by a 25-min transition to 75% of 25 mM aqueous formic acid-25% acetonitrile, and ending with a 5-min hold at that ratio. The LC effluent was directed to a Mariner time-of-flight mass spectrometer (Applied Biosystems). Electrospray ionization was performed in positive ion mode utilizing a spray tip voltage of +2.8 kV and N_2 nebulizer gas.

RESULTS

Insulin secretion in response to glucose is maintained in BTBR islets. BTBR mice have higher fasting insulin levels than B6 mice and are insulin resistant (21, 23). BTBR islets secrete more insulin in the presence of 1.7 mM (basal) or 16.7 mM (high) glucose (Fig. 1A). In addition, BTBR islets have higher insulin content than B6 islets (Fig. 1B, inset). Thus the frac-

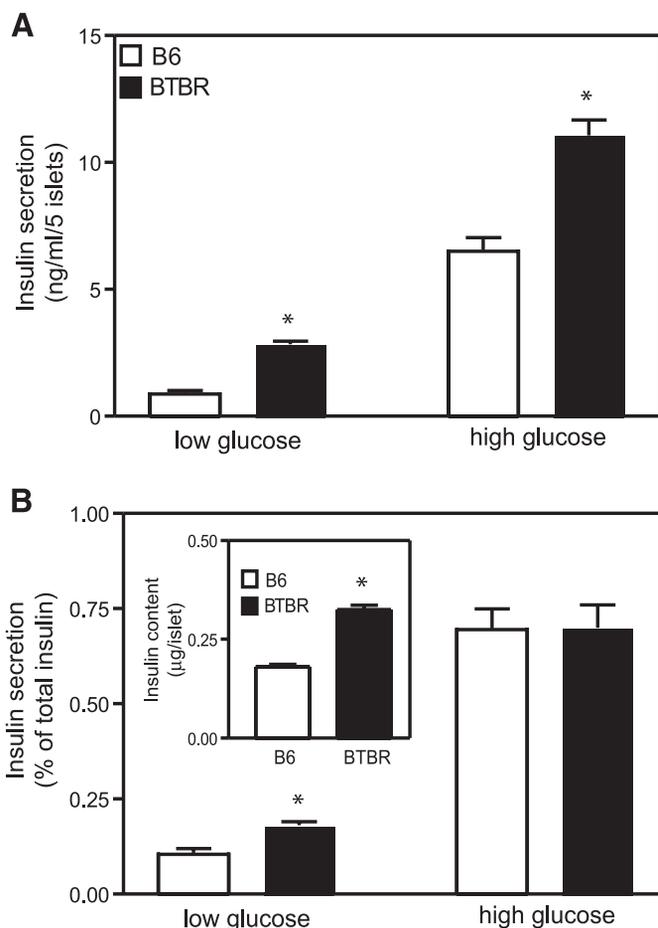


Fig. 1. Secretagogues in the glycolytic pathway elicit insulin secretion from B6 and BTBR islets. Insulin secretion in response to low (1.7 mM) and high (16.7 mM) glucose is plotted as either the absolute amount of insulin in the medium (A) or as a percentage of total present in the medium plus that remaining in the islets (B). Inset shows that BTBR islets contain ~ 2 -fold more insulin than B6 islets and explains why the differences in secretion observed in A are not present when expressed as %total insulin (cellular + secreted insulin) in B. * P values < 0.0001 for BTBR vs. B6.

tional insulin secretion in response to high glucose is identical in islets from both mouse strains (Fig. 1B). However, because of the increase in basal insulin secretion in the BTBR islets (~ 3 -fold), the response to high glucose is reduced ($\sim 45\%$). Fractional insulin secretion elicited by 20 mM methylpyruvate, another secretagogue in the glycolytic pathway, was identical in both strains (B6 $0.58 \pm 0.01\%$; BTBR $0.63 \pm 0.04\%$).

BTBR and BTBR \times B6 F_1 islets are hyperresponsive to α -KIC. In addition to glucose, amino acids, some of their α -ketoacid counterparts, and substrates for mitochondrial metabolism are potent insulin secretagogues. These secretagogues are responsible for maintaining basal insulin secretion, which accounts for $\sim 50\%$ of all insulin secretion. KIC, the transamination product of leucine, stimulates insulin secretion (20). At basal glucose, 15 mM KIC stimulated insulin secretion by approximately ninefold over basal in BTBR islets vs. threefold in B6 islets (Fig. 2). This hyperresponsiveness is also seen in the F_1 islets, indicating a BTBR-dominant phenotype (Fig. 2). KIC-mediated insulin secretion is twofold higher than that observed with high glucose in BTBR islets. Either DZX (0.25 mM) or pinacidil (0.5 mM), agonists that target the ATP-

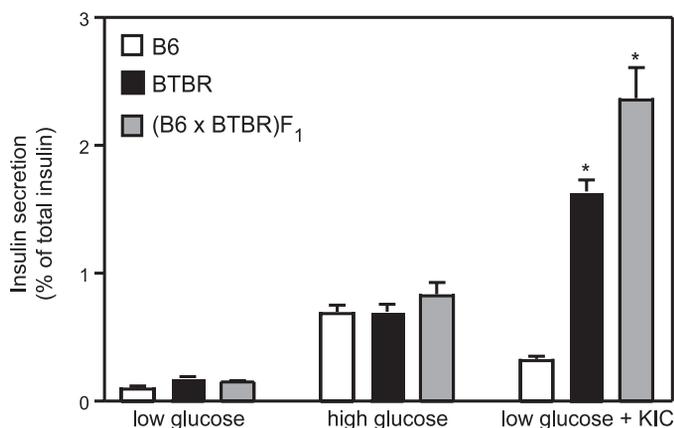


Fig. 2. Islets from BTBR or (B6 \times BTBR) F_1 mice are hyperresponsive to α -ketoisocaproate (KIC). Insulin secretion in response to low glucose (1.7 mM), high glucose (16.7 mM), or low glucose + KIC (15 mM) was measured in islets from B6, BTBR, or (B6 \times BTBR) F_1 mice. Insulin secretion is expressed as %total insulin content. * $P < 0.0001$ for BTBR or F_1 vs. B6.

sensitive (K_{ATP}) and voltage-sensitive K^+ channels, respectively, abolished the KIC response (Table 1). Similarly, verapamil (0.1 mM), an L-type Ca^{2+} channel blocker, or Ca^{2+} -free medium (no added Ca^{2+} + 1 mM EGTA) completely blocked the KIC response (Table 1). Collectively, these results indicate that the hypersensitivity of BTBR islets to KIC-dependent insulin secretion involves two well-known steps in the normal insulin secretory pathway, K_{ATP} channel activity and the regulation of Ca^{2+} influx.

The KIC effect on insulin secretion is not because of activation of glutamate dehydrogenase. KIC can either be oxidized to acetyl-CoA and acetoacetate, or it yields leucine through transamination with glutamate, forming α -KG. Direct oxidation of KIC does not occur at a significant level in β -cells (15). Leucine and its nonmetabolizable analog (BCH) are insulin secretagogues (25). However, oxidation of leucine does not appear to contribute significantly to insulin secretion (16). Rather, leucine is thought to mediate its effects through allosteric activation of glutamate dehydrogenase (GDH), an enzyme that catalyzes the oxidative deamination of glutamate (25). To determine if the leucine produced by transamination of KIC was involved in the hypersecretion seen in the BTBR islets, we asked whether leucine or BCH is sufficient to mimic the hyperresponsiveness to KIC of BTBR islets. Neither leucine (15 mM) nor BCH (15 mM) elicited an insulin secretion comparable to that observed with KIC when added at basal glucose concentrations. The ratios of the response observed with BTBR vs. B6 islets (BTBR-to-B6 ratio) for leucine, BCH, and KIC were 1.3, 1.8, and 5.4, respectively (Table 2). These

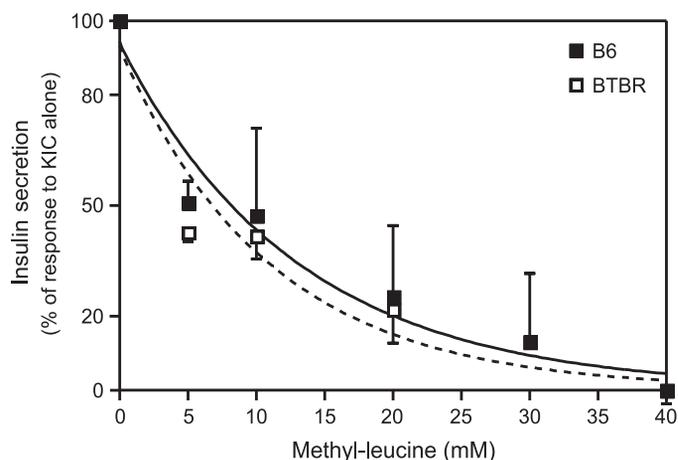


Fig. 3. Inhibition of BCAT-mediated transamination by methyl-leucine suppresses KIC-induced insulin secretion equally in B6 and BTBR islets. Islets from B6 or BTBR mice were exposed to 1.7 mM glucose and varying concentrations of methyl-leucine for 90 min. During the final 45 min, 10 mM KIC was added to induce insulin secretion. The data are plotted as percent of the amount of insulin secreted in the absence of methyl-leucine in each strain. The apparent IC_{50} of methyl-leucine inhibition was ~ 10 mM in islets from both strains. Data plotted are the means \pm SE of 3 measurements. BCAT inhibitors had no effect on basal insulin release.

results suggest that the hyperresponsiveness of BTBR islets to KIC is not a consequence of increased activation of GDH.

The KIC effect on insulin secretion is suppressed by inhibition of transamination. Neither high glucose nor methylpyruvate, both fuels for ATP and NADH production, stimulate greater insulin secretion in BTBR than B6 islets. Therefore, it is unlikely that KIC exerts its strain-specific effect on insulin secretion through oxidative metabolism; an alternative mechanism is the transamination to form α -KG. BTBR islets were incubated with KIC, along with a competitive inhibitor of the BCAT, methyl-leucine. Methyl-leucine suppressed the KIC-stimulated insulin secretion in a dose-dependent manner equally in both B6 and BTBR islets (Fig. 3). Suppression was also observed with another BCAT inhibitor, aminooxyacetate; at 10 mM, the inhibitor caused a 100% inhibition of KIC-mediated insulin secretion (data not shown). Neither methyl-leucine nor aminooxyacetate affected basal insulin release in B6 or BTBR islets. The KIC effect on insulin secretion therefore requires its transamination by BCAT.

Glutamate determines the magnitude of the KIC effect on insulin secretion. Glutamate is the preferred amine donor in the transamination of KIC to leucine (7). Because leucine and its analog BCH failed to mimic the KIC effect on insulin secretion in the BTBR islets (Table 2), we ruled out activation of GDH as a potential mechanism. Because BCAT is required for the

Table 1. Effect of different inhibitors on insulin release in response to 15 mM KIC

Inhibitor	B6	<i>n</i>	BTBR	<i>n</i>
DZX (250 μ M)	86 \pm 0.5	(7)	92 \pm 0.7	(8)
Verapamil (100 μ M)	100	(1)	91	(1)
Pinacidil (500 μ M)	100	(1)	92	(1)
Ca^{2+} -free medium + 1 mM EGTA	100	(1)	92	(1)

Values are represented as the %suppression of α -ketoisocaproate (KIC)-induced insulin secretion observed without inhibitor present for each strain; *n*, no. of replicate experiments. Data for diazoxide (DZX) are means \pm SE.

Table 2. Effect of allosteric glutamate dehydrogenase activators, leucine, BCH, and KIC on insulin secretion in B6 and BTBR islets

Strain	Control	n	Leucine	n	BCH	n	KIC	n
B6	0.11 \pm 0.01	(4)	0.20 \pm 0.04*	(6)	0.16 \pm 0.04	(3)	0.30 \pm 0.03†	(5)
BTBR	0.21 \pm 0.03	(7)	0.29 \pm 0.02*	(5)	0.26 \pm 0.04	(4)	1.72 \pm 0.26†	(6)
BTBR-to-B6 ratio			0.89		1.0		7.9	

Values shown are mean \pm SE fractional insulin secretion in the presence of basal glucose alone (control) or with 15 mM leucine, 2-amino-2-norbornone-carboxylic acid (BCH), or KIC; n, no. of replicate experiments. * $P < 0.02$ and † $P < 0.0001$ relative to control for each strain. The BTBR-to-B6 ratio was calculated for each segregant after subtraction of the insulin secretion observed under control conditions.

KIC effect, we predicted that glutamate limits the magnitude of the KIC response. To test this prediction, we incubated islets with KIC (15 mM) in the presence of dimethylglutamate (DMG, 15 mM) or glutamine (15 mM). In the presence of either exogenous glutamate or glutamine, the secretion of insulin was the same in islets from both mouse strains (Fig. 4A). The addition of either BCH or DMG alone does not mimic the hypersecretion observed with KIC. However, when BCH and DMG are combined, yielding maximum GDH activity, insulin secretion was the same from B6 and BTBR islets; it was equal in magnitude to the KIC effect observed in BTBR and \sim 50% that achievable when KIC is combined with either amine donor.

To ask if the hyperresponsiveness of BTBR islets to KIC correlates with increased levels of glutamate, we measured the levels of seven amino acids, including glutamate, by LC-MS in B6 and BTBR islets. Glutamate levels in BTBR islets were twofold higher than in B6 islets ($P < 0.0001$; Fig. 4B, inset). There was no strain difference in the six other amino acids measured in the same islet samples. RNA expression levels of BCAT in islets from both strains were measured. Although expression levels were low, there was a slightly higher BCAT expression level in the BTBR islets than in B6 islets (data not shown). These results suggest that a strain difference in the concentration or flux through the amine donor utilized by BCAT explains the difference in the response to KIC.

B6 and BTBR islets are equally responsive to α -KG. If the magnitude of formation of α -KG from transamination of KIC is the basis for the difference in responsiveness of B6 and BTBR islets, then an excess of exogenously provided α -KG should abolish this difference. When incubated with 15 mM α -KG (as its dimethyl ester), insulin secretion was the same in B6 and BTBR islets and equal to that observed in BTBR islets incubated with 15 mM KIC (Fig. 5). These results suggest that the difference in response to KIC is in the ability to form α -KG, rather than their responsiveness to α -KG.

Succinate does not mimic the effect of α -KG on insulin secretion. If α -KG oxidation via α -KG dehydrogenase is necessary for α -KG to stimulate insulin secretion, then succinate (delivered as succinic acid methyl ester) should mimic the effects of α -KG on insulin secretion. Confirming the studies of MacDonald (17), we show that mouse islets do not secrete insulin in response to succinate (Fig. 5). In contrast, we have observed that succinate elicits insulin secretion from rat insulinoma cells (data not shown). Thus α -KG stimulates insulin secretion through a mechanism that does not depend on its oxidation through α -KG dehydrogenase.

KIC, like glucose, stimulates insulin secretion through K_{ATP} -dependent and K_{ATP} -independent pathways in both mouse strains. A major mechanism for nutrient-stimulated insulin secretion involves oxidative metabolism, an elevation in ATP, and closure of the K_{ATP} . The resulting depolarization of the

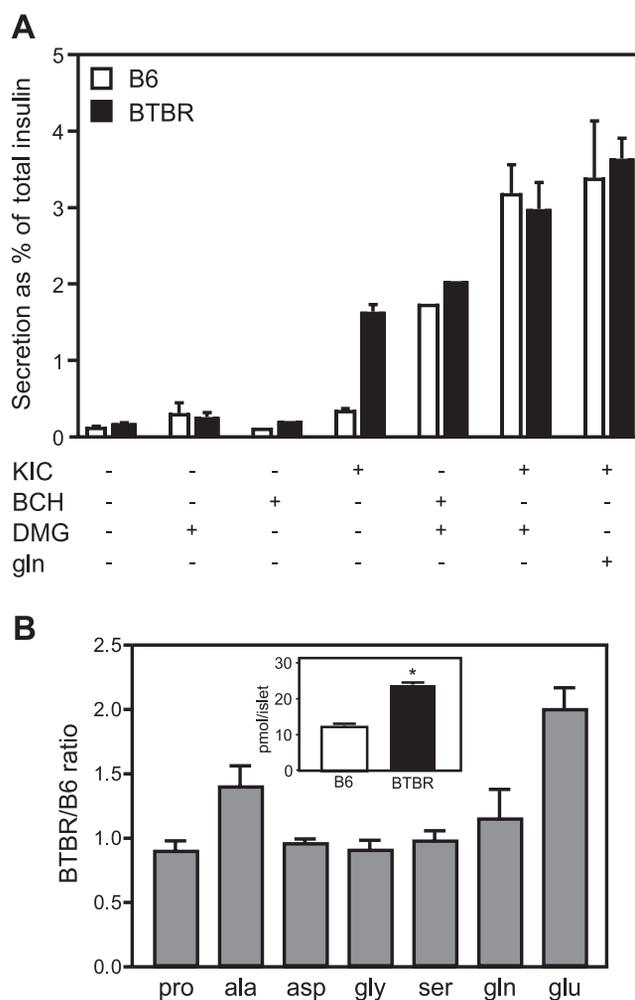


Fig. 4. Increasing the amine donor concentration equalizes KIC-induced insulin secretion in B6 and BTBR islets. A: insulin secretion, plotted as fractional secretion, was measured in B6 and BTBR islets with basal glucose (1.7 mM) and exposed to (15 mM each) dimethylglutamate (DMG), 2-amino-2-norbornone-carboxylic acid (BCH), KIC, BCH + DMG, KIC + DMG, or KIC + glutamine (gln). B: LC-MS was used to determine endogenous levels of 7 amino acids in B6 and BTBR islets and plotted as the BTBR-to-B6 ratio of peak area for each amino acid. Glutamate was the only amino acid to show a strain difference. Inset: glutamate levels for B6 and BTBR islets when plotted as pmol glutamate/islet. * $P < 0.0001$ for BTBR vs. B6 islets.

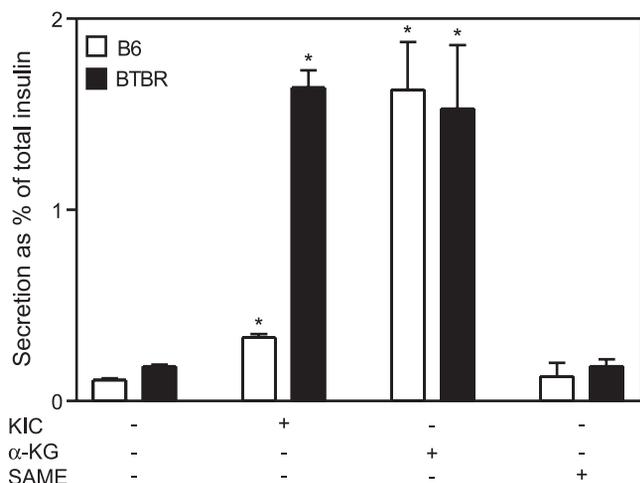


Fig. 5. α -Ketoglutarate (α -KG) is a direct and equally effective insulin secretagogue in B6 and BTBR islets. Insulin secretion, plotted as fractional secretion, was measured in B6 and BTBR islets with basal glucose (1.7 mM) alone or with KIC (15 mM), dimethyl ester of α -KG (15 mM), or succinic acid methyl ester (SAME; 20 mM). * $P < 0.0001$ for KIC or α -KG relative to basal conditions for B6 and BTBR islets.

β -cell triggers Ca^{2+} influx through the voltage-gated Ca^{2+} channel, leading to insulin secretion. DZX, which prevents closure of K_{ATP} , completely blocked KIC-induced insulin secretion in both strains (Fig. 6), confirming a dependence of KIC responsiveness on K_{ATP} (5). However, the closure of K_{ATP} to initiate insulin secretion can be bypassed by exposing islets to 40 mM KCl. The insulin secretion in response to KIC

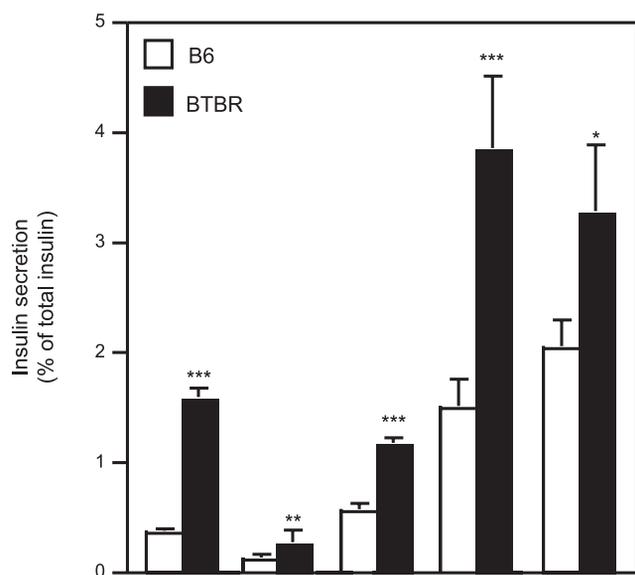


Fig. 6. KIC elicits insulin secretion through ATP-sensitive K^{+} channel (K_{ATP})-dependent and K_{ATP} -independent pathways in B6 and BTBR islets. Insulin secretion was measured in B6 and BTBR islets with 1.7 mM glucose and exposed to KIC, KIC + diazoxide (DZX), KCl, KIC + KCl, or KIC + KCl + DZX. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ for BTBR vs. B6.

and KCl was additive in both strains and not significantly suppressed by DZX in either strain. The failure of DZX to suppress the KIC effect in the presence of KCl suggests that KIC, in addition to its K_{ATP} dependence, stimulates insulin secretion through a K_{ATP} -independent pathway.

DISCUSSION

The aim of this study was to examine the responsiveness of islets to various insulin secretagogues in two mouse strains that differ in their susceptibility to diabetes. The BTBR strain is insulin resistant, has an increase in visceral fat, is mildly hypertriglyceridemic, and is hyperinsulinemic (23). In contrast to the C57BL/6J strain, BTBR mice develop severe diabetes when they carry the *leptin^{ob}* mutation (30). Insulin hypersecretion has also been shown in other animal models of diabetes, including the Zucker (*fal/fa*) rat (24) and DBA/2 mice (13). Studies in obese people without type 2 diabetes have shown that insulin hypersecretion is more prevalent than insulin insensitivity (8) and is a predictor of the development of type 2 diabetes (22).

We report that BTBR islets are hyperresponsive to KIC, but not glucose or α -KG. KIC has two known metabolic fates as follows: it can be oxidized to acetyl-CoA and acetoacetate, or it can be transaminated to generate leucine and α -KG (Fig. 7). We found that KIC-induced insulin secretion could be abolished by inhibitors of BCAT. Furthermore, leucine alone was unable to mimic the hypersecretion observed with KIC. These results suggest that the KIC effect on insulin secretion is mediated by α -KG rather than the other product of KIC transamination, leucine.

Our results address the observation of Sener et al. (26) that, although KIC and ketocaproate are both insulin secretagogues, their corresponding amino acids are not both allosteric activators of GDH; leucine, not "nor"leucine, is an activator. We suggest that both ketoacids are secretagogues because both form α -KG when they are transaminated. Indeed, we have observed that BTBR islets are hyperresponsive to ketocaproate (data not shown).

We have shown that the addition of α -KG (as the methyl ester) to mouse islets elicits a very strong insulin response in

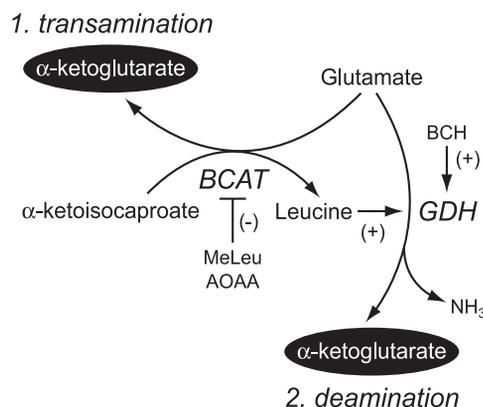


Fig. 7. Two pathways for α -KG production in mouse islets. 1: Glutamate is transaminated to form α -KG through BCAT, converting KIC into leucine. This reaction can be inhibited by methyl-leucine (MeLeu) or aminooxyacetate (AOAA). 2: Glutamate is oxidatively deaminated to form α -KG and ammonia by glutamate dehydrogenase (GDH), which is allosterically activated by leucine or its nonmetabolizable analog BCH. Both reactions are reversible but are only shown in one direction in the diagram.

the presence of basal glucose. α -KG could be oxidized to succinate and thus lead to production of ATP and reducing equivalents. If α -KG oxidation is required to elicit insulin secretion, then succinate, the first product of α -KG oxidation, should be an insulin secretagogue. However, succinate (as the methyl ester) does not promote insulin secretion in mouse islets (Fig. 5 and Ref. 17). These observations suggest that α -KG stimulates insulin secretion through a mechanism that does not require its oxidation.

Our suggestion that α -KG itself stimulates insulin secretion is related to the mechanism by which glutamate stimulates insulin secretion. Maechler and Wollheim (19) proposed that glutamate is a direct insulin secretagogue and that the GDH reaction in β -cells occurs in the reductive direction; i.e., α -KG \rightarrow glutamate. MacDonald and Fahien (18) challenged this proposal by showing that various insulin secretagogues do not increase glutamate levels in β -cells under conditions where they stimulate insulin secretion. Bertrand et al. (2) showed that increasing glutamate concentrations with glutamine does not stimulate insulin secretion unless GDH is allosterically activated with BCH. Leucine and its analog, BCH, stimulate GDH in the direction of deamination while stimulating insulin secretion. This suggests that glutamate oxidation rather than glutamate formation is required for its ability to stimulate insulin secretion.

Genetics provides strong evidence regarding the direction of the GDH reaction that promotes insulin secretion. Mutations in the inhibitory GTP-binding site of GDH lead to hyperinsulinemia and hyperammonemia (29). Overexpression of constitutively active GDH in insulinoma cells also results in increased insulin secretion (1). These results indicate that, when GDH catalyzes the deamination of glutamate, yielding ammonia and α -KG, insulin secretion is stimulated.

Gao et al. (9) showed that glucose treatment of islets decreases their ADP and increases their GTP content. This dampens the stimulatory effect of leucine on insulin secretion by reducing the sensitivity of GDH to allosteric activation by leucine. Conversely, glucose depletion augments the sensitivity of islets to leucine (16). By contrast, glucose does not inhibit KIC-induced insulin secretion, indicating that the KIC effect occurs through a separate mechanism from the leucine effect (9). These data are in accord with our suggestion that KIC-induced insulin secretion occurs through BCAT-catalyzed formation of α -KG, rather than its formation of leucine.

Studies in human subjects and in animal models suggest a relationship between insulin hypersecretion and diabetes susceptibility. Longitudinal studies in humans have shown that hyperinsulinemia, independent of insulin resistance, is a predictor of subsequent development of diabetes (8, 22, 32, 34). Patients with mutations in the sulfonylurea receptor SUR1 and the inward rectifying K⁺ channel Kir6.2 have hyperinsulinemia in childhood and progress to develop diabetes (11, 12). In both humans (3, 10), and rodents (14, 28, 31), treatment with agents that prevent closure of K_{ATP} channels appears to prevent or delay the development of diabetes. These agents, which inhibit insulin secretion, might be expected to exacerbate diabetes. However, reducing the hyperexcitability of the β -cells might enhance their survival. The net effect would be to preserve β -cell mass, a factor that appears to be critical in both type 1 and type 2 diabetes.

In summary, we have shown that the BTBR and B6 mouse strains, which have different susceptibility to islet failure, display different insulin secretory responses to certain insulin secretagogues. The BTBR mouse strain, like other mouse models of type 2 diabetes, has an islet hypersecretion phenotype. These results raise the intriguing possibility that the hypersensitivity of BTBR islets is a contributing factor in the susceptibility of this mouse strain to obesity-induced diabetes.

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