A Signaling Role of Glutamine in Insulin Secretion*

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Children with hypoglycemia due to recessive loss of function mutations of the β-cell ATP-sensitive potassium (KATP) channel can develop hypoglycemia in response to protein feeding. We hypothesized that amino acids might stimulate insulin secretion by unknown mechanisms, because the KATP channel-dependent pathway of insulin secretion is defective. We therefore investigated the effects of amino acids on insulin secretion and intracellular calcium in islets from normal and sulfonylurea receptor 1 knockout (SUR1/−/−) mice. Even though SUR1/−/− mice are euglycemic, their islets are considered a suitable model for studies of the human genetic defect. SUR1/−/− islets, but not normal islets, released insulin in response to an amino acid mixture ramp. This response to amino acids was decreased by 60% when glutamine was omitted. Insulin release by SUR1/−/− islets was also stimulated by a ramp of glutamine alone. Glutamine was more potent than leucine or dimethyl glutamate. Basal intracellular calcium was elevated in SUR1/−/− islets and was increased further by glutamine. In normal islets, methionine sulfoximine, a glutamine synthetase inhibitor, suppressed insulin release in response to a glucose ramp. This inhibition was reversed by glutamine or by 6-diazo-5-oxo-l-norleucine, a non-metabolizable glutamine analogue. High glucose doubled glutamine levels of islets. Methionine sulfoximine inhibition of glucose stimulated insulin secretion was associated with accumulation of glutamate and aspartate. We hypothesize that glutamine plays a critical role as a signaling molecule in amino acid- and glucose-stimulated insulin secretion, and that β-cell depolarization and subsequent intracellular calcium elevation are required for this glutamine effect to occur.

The study of inherited disorders of insulin secretion expands our understanding of metabolism and stimulus secretion coupling in pancreatic β-cells. For example, the exploration of glucokinase diseases, which are due to activating or inactivating mutants of β-cell glucokinase, has confirmed basic concepts about this enzyme as the sensor for glucose-stimulated insulin secretion (GSIS) (1, 2). Similarly, the elucidation of a novel hyperinsulinism syndrome associated with mild hyperammonemia and linked to overactivity the glutamate dehydrogenase enzyme (GDH-HI) has led to the identification of a prominent role of glutaminolysis in the regulation of insulin secretion (3, 4, 5). These successful studies in human biochemical genetics of insulin secretion motivated the present investigation to find an explanation for the striking clinical observation that the patients with hyperinsulinism caused by inactivating mutations of the β-cell ATP-sensitive potassium channel (KATP-HI) (6, 7) exhibit hypoglycemia following a protein meal (6, 8, 9), while the β-cell response to glucose is impaired (10). An additional and unexplained observation of relevance in this context is the lack of β-cell responsiveness in KATP-HI to pharmacological stimulation with leucine (6, 10) that contrasts with the remarkable leucine hypersensitivity of patients with GDH-HI (11).

Sulfonylurea receptor 1 knockout (SUR1/−/−) mice were designed to facilitate experimental studies of the human KATP-HI syndrome (12, 13). However, SUR1/−/− mice do not share the hypoglycemia phenotype of the human KATP-HI syndrome. Nevertheless, isolated islets from these animals exhibit all the features expected to result from nonfunctional KATP channels, i.e. β-cell depolarization and elevation of intracellular calcium (12, 13). Thus, islets from SUR1 knockout mice provide a useful in vitro model to explore the mechanism of protein, i.e. amino acid hypersensitivity, and leucine refractoriness observed in KATP-HI. Study of this model also provides an opportunity to extend the understanding of the role of amino acids in modulating physiological glucose-dependent β-cell functions (14). In the course of these studies, a prominent role for glutamine was identified in amino acid stimulated insulin secretion (AASIS) and also in GSIS. This glutamine effect emerges to be central for the understanding of fuel stimulated insulin release from the β-cell.

MATERIALS AND METHODS

Mouse Islets Preparation and Insulin Secretion—SUR1 knockout mice (SUR1/−/−) were obtained from Dr. Mark A. Magnuson. The knockout procedure and genotyping were described by Shio et al. (12). Both SUR1/−/− mice and control mice (B6D2H1) were fed a standard rodent chow diet, maintained on a 12-hour light/dark cycle. Islets were

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‡ These abbreviations used are: GSIS, glucose-stimulated insulin secretion; AASIS, amino acid-stimulated insulin secretion; SUR1, sulfonylurea receptor 1; KATP, channel, ATP-sensitive potassium channel; GDH, glutamate dehydrogenase; GS, glutamine synthetase; AST, aspartate aminotransferase; PDG, phosphate-dependent glutaminase; DON, 6-diazo-5-oxo-norleucine; MSO, methionine sulfoximine; BSO, bithiouride sulfoximine; APE, amino acid excess; GABA, γ-aminobutyric acid.
isolated by collagenase digestion and cultured for 3 days in RPMI 1640 medium containing 10 mM glucose. The culture medium was supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 50 μg/ml streptomycin, and the islets were incubated at 37 °C in a 5% CO2, 95% air-humidified incubator. Batches of 100 cultured mouse islets were loaded onto a nylon filter in a chamber and perfused with Krebs-Ringer bicarbonate buffer (115 mM NaCl, 24 mM NaHCO3, 5 mM KH2PO4, 1 mM Na2HPO4, 1.2 mM CaCl2, 10 mM HEPES, pH 7.4) with 0.25% bovine serum albumin at a flow rate of 2 ml/min. Perifusate solutions were gassed with 95% O2, 5% CO2 and maintained at 37 °C. The physiological mixture of 20 amino acids when used at a maximum concentration of 12 mM (about 3 times physiological concentration) had the following composition (in mM): glutamine 2.0, alanine 1.25, arginine 0.55, aspartate 0.11, citrulline 0.27, glutamate 0.35, glycine 0.85, histidine 0.22, isoleucine 0.27, leucine 0.46, lysine 1.06, methionine 0.14, ornithine 0.20, phenylalanine 0.23, proline 1.0, serine 1.62, threonine 0.77, tryptophan 0.21, valine 0.57. Samples were collected every minute for insulin assays. Insulin was measured by radioimmunoassay.

Cytosolic Free Ca2+ Measurements—Mouse islets were isolated and cultured on poly-L-lysine coated glass coverslips under the same condition as described above. The perfusion procedure and cytosolic-free Ca2++ (Ca2++2) measurement were described previously (4). In brief, the coverslip with attached islets was incubated with 15 μM Fura-2 ace-toxymethyl ester (Molecular Probes, Eugene, OR) in Krebs-Ringer bicarbonate buffer with 5 mM glucose for 35 min at 37 °C. Islets were then perfused with Krebs-Ringer bicarbonate buffer with 0.25% bovine serum albumin at 37 °C at a flow rate of 2 ml/min, while various agents were applied. [Ca2++]i was measured with a dual wavelength fluorescence microscope as previously described.

Studies with 13NH4Cl—Control mouse islets were cultured with 10 mM glucose for 3–4 days as described above. Batches of 1,000 islets were first preincubated with glucose free Krebs-Ringer bicarbonate buffer for 60 min at 37 °C with or without 1 mM methionine sulfoximine (MSO), an inhibitor of glutamine synthetase (GS). The islets were then incubated 120 min with either 300 μM 13NH4Cl (Cambridge Isotope Laboratories, Inc., Andover, MA) alone or control, or with additional 25 mM glutamate. The homogenate was centrifuged at 15,000 × g for 5 min and the supernatant was used to determine GS activity by the method of Meister (16). The reaction mixture contained in a final volume of 0.5 ml: 50 mM sodium glutamate, 10 mM ATP, 20 mM MgCl2, 125 mM hydroxy- ylamine, and 25 mM 2-mercaptoethanol. The reaction was initiated by the addition of islet or cell extract, and the assay tube was then incubated for 15 min at 37 °C. After centrifugation at 3,000 × g the absorbance of the supernatant was read at 535 nm. A tube containing 4 mM MSO was considered as blank. ATP Assays—Control mouse islets were cultured with 10 mM glucose for 3 days. Batches of 100 islets were first preincubated with glucose free Krebs-Ringer bicarbonate buffer for 60 min at 37 °C with or without 1 mM methionine sulfoximine (MSO). The reaction was initiated by adding 0.75 ml of FeCl3 solution (2.5% FeCl3 in 1.5 N HCl with 5% trichloroacetic acid). The reaction was stopped by adding 0.75 ml of 15N HCl (Cambridge Isotope Laboratories) as a secondary antibody. A total of 25 μg of protein from cultured islets or from β-HC9 cells was used for Western blotting.

GS Activity Measurements—Batches of at least 1,000 cultured islets were collected and washed with Hank’s buffer. Islets were then homogenized with 0.1% potassium-HCI buffer (pH 7.1, containing 0.01 mM EDTA). The homogenate was centrifuged at 18,000 × g for 5 min, and the supernatant was used to determine GS activity by the method of Meister (16). The reaction mixture contained in a final volume of 0.5 ml: 50 mM sodium glutamate, 10 mM ATP, 20 mM MgCl2, 125 mM hydroxy- ylamine, and 25 mM 2-mercaptoethanol. The reaction was initiated by the addition of islet or cell extract, and the assay tube was then incubated for 15 min at 37 °C. The reaction was stopped by adding 0.75 ml of FeCl3 solution (2.5% FeCl3, in 1.5 N HCl with 5% trichloroacetic acid). After centrifugation at 3,000 × g the absorbance of the supernatant was read at 535 nm. A tube containing 4 mM MSO was considered as blank. One unit of GS activity was defined as 1 μmol of l-glutamate γ-mono-

Data Analysis—All the data are presented as mean ± S.E. The Student’s t test was performed when two groups were compared. One way analysis of variances were used when multiple groups were com-

RESULTS

AA SIS of SUR1−/− and Control Islets—We studied the molecular basis of pancreatic β-cell hypersensitivity to amino acids that had been observed in patients with loss of function mutations of the KATP channel by perfusing isolated and cultured islets of SUR1−/− and control mice with an amino acid mixture. As shown in Fig. 1 (panel A), SUR1−/− islets responded to ramp stimulation by a physiological mixture of 20 amino acids (using an increment of 0.04 mM/min for glutamine and 0.2 mM/min for the other 19 amino acids). Baseline insulin secretion in SUR1−/− islets was twice that of control islets. The amino acid mixture increased insulin release nearly 3-fold in SUR1−/− islets, while normal islets were not affected by this stimulus. The threshold concentrations at which amino acids induced insulin secretion in SUR1−/− islets were about 0.5 mM for glutamine and 3 mM for the 19 other amino acids, i.e.
close to the physiological concentrations. The presence of 10 mM glucose did not change the response of SUR1−/− islets to the amino acid mixture (data not shown). The effects of the amino acid were also tested in the absence of glutamine, because glutaminolysis is known to increase insulin secretion. Omitting glutamine reduced the effect of the mixture by 60% (Fig. 1, panel A).

As shown in panel B of Fig. 1, in the presence of 10 mM glucose, control islets showed a very sensitive concentration dependent response when stimulated with a ramp of amino acids. The threshold concentrations of amino acids were about 0.2 mM for glutamine and 1.0 mM for the other 19 amino acids when infused as a complete mixture. When glutamine was omitted, the secretory response to the remaining amino acid mixture was delayed by 10 min, and the maximum insulin release was reduced by 60% or more. These results in SUR1−/− and control mouse islets suggested a predominant role for glutamine in both AASIS and GSIS.

**Effects of Glutamine on Insulin Secretion of SUR1−/− and Control Islets—**SUR1−/− and control islets showed striking qualitative and quantitative differences in fuel responsiveness (Fig. 2, A–D). SUR1−/−, but not control islets responded to glutamine ramp stimulation (increasing 0.5 mM/min) with a threshold of about 5 mM (Fig. 2A). A glucose ramp (increasing 0.5 mM/min) was totally ineffective in the SUR1−/− islets, but produced the expected stimulation in control islets with a threshold of 5–6 mM (Fig. 2B). A leucine ramp (increasing 0.5 mM/min) superimposed on 2 mM glutamine caused a 10-fold increase of insulin secretion in control islets, but much less pronounced response in SUR1−/− islets (Fig. 2C). The secretion profile showed an initial dip, i.e. insulin secretion first declined but then recovered back to or slightly above baseline (difference not significant statistically). It is noteworthy that baseline insulin secretion of SUR1−/− islets was elevated in all experiments and that 2 mM glutamine augmented basal insulin release even further (2.3 ± 0.06 versus 1.7 ± 0.07 ng/100 islets/min, p < 0.01), consistent with the results of glutamine ramp stimulation. These observations were interpreted to indicate that the apparent activation of SUR1−/− islets did not reflect increased glutamine metabolism, but was a direct response to glutamine itself. This conclusion is supported by the greatly reduced leucine sensitivity. This interpretation was further strengthened by the results with a dimethyl-glutamate ramp (increasing 0.5 mM/min) as we noted an initial shallow dip of insulin secretion followed by a slight increase over baseline in SUR1−/− islets, but virtually no response in control islets (Fig. 2D). The insulin release profile of dimethyl-glutamate stimulation in SUR1−/− islets was comparable to that seen during leucine stimulation: both showed the “dip” phenomenon, apparently an expression of inhibited secretion. These results with glutamine, complemented by the lack of responsiveness to the other fuels suggested that the remarkable effects of glutamine in SUR1−/− islets were mediated neither by enhanced glutaminolysis nor via increased glutamate. Instead, the effect appeared to be the direct result of a glutamine-induced signal.

**A Role for Elevated [Ca$$^{2+}$$], in Glutamine-stimulated Insulin Secretion—**SUR1−/− islets characteristically showed mark-
edly elevated basal [Ca^{2+}]i, compared with controls (Fig. 3, panels A–D). Glutamine (10 mM) caused a transient [Ca^{2+}]i rise in SUR1−/− but not in control islets (Fig. 3A). Glucose induced a persistent [Ca^{2+}]i rise in control islets, and a dip in [Ca^{2+}]i, in SUR1−/− islets (Fig. 3B). Leucine (10 mM), superimposed on 10 mM basal glutamine similar with glucose caused a sustained [Ca^{2+}]i rise in control, but not in SUR1−/− islets (data not shown). Instead, leucine caused a transient dip of the [Ca^{2+}]i levels in SUR1−/− islets, analogous to the dip in the insulin secretion profile produced by leucine plus glutamine (Fig. 2C). These results again suggested that the glutamine effect might not depend on its metabolism. In order to further test this concept we performed studies with 6-diazo-5-oxo-1-norleucine (DON), an inhibitor of glutaminase. DON (40 μM) did not affect the transient [Ca^{2+}]i rise induced by glutamine in SUR1−/− islets (Fig. 3C). As shown in panel D of Fig. 3, in the absence of DON, the combination of glutamine and the leucine analogue 2-aminobicyclo-(2,2,1)heptane-2-carboxylic acid (BCH) caused a sustained [Ca^{2+}]i increase in control islets. This effect was completely abolished by 40 μM DON, indicating that this concentration of the inhibitor was sufficient to block the flux from glutamine to glutamate and, thus, limited the substrate for the GDH reaction. These results support the concept that glutamate can directly affect insulin release without being metabolized via glutamate.

**Possible Mechanism of Glutamine Effects**—Further experiments were performed to understand the molecular basis of the apparently direct glutamine effect on SUR1−/− islets. Fig. 4 (panel A) shows that the mitochondrial poison, sodium azide (4 mM), did not block the glutamine induced elevation of [Ca^{2+}]i, suggesting that the action of glutamine is independent of ATP and does not require glutamine metabolism. Thapsigargin (1 μM), which inhibits sarco/endoplasmic reticulum Ca^{2+}-ATPases (SERCA), increased the basal [Ca^{2+}]i levels in SUR1−/− islets even further, but did not interfere with the glutamine effect (Fig. 4, panel B). The calcium channel blocker verapamil (50 μM) lowered [Ca^{2+}]i in SUR1−/− islets and inhibited the glutamine effect on [Ca^{2+}]i (Fig. 4, panel C). The glutamine effect is thus conditional: it requires functional calcium channels as well as depolarization of the β-cell.

**Glyburide Increases Glutamine Sensitivity of Normal β-Cells**—In order to test the role of glutamine after acute depolarization, glyburide (300 nM) was used to mimic the SUR1−/− condition (Fig. 5). Glyburide depolarized β-cells and caused elevation of [Ca^{2+}]i in control islets, as expected. Following glyburide treatment, the effects on [Ca^{2+}]i of glutamine, the glutamine/leucine combination, and also glucose were similar to those observed in SUR1−/− islets (Fig. 5, panels A and B). Insulin secretion studies confirmed these observations (Fig. 5, panel C): glutamine augmented insulin release, whereas glucose caused a transient dip in the release profile as seen in Ca^{2+} studies. The glutamine effects in glyburide-depolarized islets were inhibited by the calcium channel blocker nimodipine (5 μM). These results confirmed that β-cell depolarization and elevation of [Ca^{2+}]i, are required for the direct effects of glutamine on insulin secretion to occur.

**Role of Glutamine in GSIS**—Since elevated intracellular calcium appeared to be the critical permissive factor, sensitizing the β-cell to glutamine stimulation, exposure of islets to high glucose should also induce a glutamine responsive state. Fig. 6 shows the response of normal islets to a glutamine ramp superimposed on a sustained 10 mM glucose stimulus. Glutamine amplified the second phase of glucose stimulated insulin release about 3-fold. The threshold concentration for glutamine-
induced insulin secretion was about 2 mM, which was higher than the threshold for glutamine when the other 19 amino acids were present (compare Fig. 1B and Fig. 6).

We speculated that glutamine, generated endogenously in the \( \beta \)-cell, might play a role as a physiological co-factor in GSIS, because it could be synthesized by the enzyme GS under conditions where high glucose provides ample glutamate and ATP as substrates. To test this hypothesis we used the GS inhibitor MSO. As shown in Fig. 7 (panel A), 1 mM MSO decreased the maximal islet responsiveness to a glucose ramp (increasing 0.5 mM/min) by 70%. Butyriion sulfoximine (BSO), a noninhibitory analogue of MSO, caused no inhibition of insulin secretion. The threshold concentration for glucose stimulation was the same for MSO treated and control islets. If the inhibitory effect of MSO on GSIS was due to decreased generation of glutamine, glutamine added back to the perifusate should reverse this inhibition. As shown in Fig. 7 (panel B), 2 and 5 mM glutamine completely reversed the MSO induced inhibition. Finally, an experiment was performed to test whether the non-metabolizable glutamine analogue DON could substitute for glutamine in reversing the MSO inhibition of GSIS. DON (40 \( \mu \)M) was indeed at least as effective as 5 mM glutamine. MSO did not appear to interfere with glucose metabolism, because the islet ATP content was identical after 1 h incubation with glucose (10 mM) and with or without MSO (1 mM) (Table I). However, insulin release was inhibited about 40% by MSO.

Islets Contain Glutamine Synthetase—These studies suggested that the enzyme GS might be important for GSIS. Western blot analysis and measurement of catalytic activity of GS were performed in cultured control mouse islets, as well as \( \beta \)-HC9 cells. GS was clearly expressed in mouse islets and \( \beta \)-HC9 cells (Fig. 8). The enzyme activity in cultured mouse islets was 1.9 ± 0.2 units/mg islet protein and 3.3 ± 0.5 units/mg protein in \( \beta \)-HC9 cells (\( p < 0.01 \)). For comparison, using the same assay, mouse brain had GS activity of 5.4 ± 0.5 units/mg protein.

\( ^{15}\text{NH}_4\text{Cl} \) Flux Studies in Control Mouse Islets—Flux through GS was measured using \( ^{15}\text{NH}_4\text{Cl} \) as tracer. Batches of 1,000 control mouse islets were cultured with 10 mM glucose for 3 days and then incubated with \( ^{15}\text{NH}_4\text{Cl} \) (300 \( \mu \)M) alone or with 25 mM glucose in the presence or absence of 1 mM MSO. Insulin secretion was inhibited by about 40% with 1 mM MSO present, in agreement with the perifusion studies (Table II). After 2 h of incubation, ammonia in the medium was slightly but significantly (\( p < 0.05 \)) decreased by glucose, and this decrement was abolished by MSO. These results implied that during GSIS islets consumed ammonia, which probably was incorporated into glutamine. Measurements of critical amino acids in islets supported this interpretation (Table III). In the presence of high glucose cellular aspartate was decreased by 60%. In contrast, glutamine and alanine were elevated, and \( \gamma \)-aminobutyric acid (GABA) was lowered. Cellular glutamate remained constant. The cellular glutamine pool was small, only 1–3% of the total amino acid pool.

MSO dramatically changed the concentrations of islets amino acids. The accumulation of glutamate as result of MSO treatment suggested that flux from glutamate to glutamine was inhibited. MSO also prevented the drop of aspartate that...
was induced by high glucose. Glutamine measurement was confounded because glutamine had the same retention time as MSO in the standard HPLC assay. The collective data support, however, the interpretation that a glucose-induced rise of glutamine was abolished by the GS inhibitor MSO. The data indicate that high glucose primarily increased the production of glutamine and alanine. Under control conditions, glutamine contributed about 16% to the incubation medium amino acid pool, and this percentage increased to 25% in the present of 25 mM glucose. Glucose changed the amino acid pools in both the islets and the incubation medium, but did not substantially change the total amino acid pool size. MSO increased the concentrations of individual amino acids like glutamate and aspartate without affecting the total amino acid pool. Islet GABA was decreased by high glucose and MSO did not interfere with this effect. Glycine, another large fraction of the total amino acid pool, remained unchanged under all the conditions.

The islet 15N amino acid enrichments were less than 5 APE (atom % excess), the detection limit of the GC-MS method for both [15N]glutamate and [15N]aspartate (actual data are therefore not shown). This result indicates that reductive amination of α-ketoglutarate by GDH was unlikely to be quantitatively

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**Fig. 5. Effects of glutamine on glyburide-treated control islets.** Experiments were carried out as described in Fig. 3. The sequence of concentrations and additions are shown in the figure. Representative experiments are shown. All studies were repeated at least three times and showed comparable results. Insulin secretion by 100 perifused islets is recorded in panel C. Islets were first exposed to 300 nM glyburide, and then stimulated by 10 mM glutamine (solid circles) or 10 mM glucose (open triangles), and finally 5 μM nimodipine was added.
Fig. 6. Effects of glutamine on GSIS in normal islets. Isolated control mouse islets were cultured with 10 mM glucose for 3 days and then perifused with 10 mM glucose prior to stimulation with (solid circles) or without (open diamonds) a ramp of glutamine (0–25 mM at 0.5 mM/min). Results are presented as means ± S.E. for 100 islets from 3 to 6 separate perifusions for each condition.

Fig. 7. Effects of inhibition of GS by MSO on GSIS in normal islets. Isolated control mouse islets were cultured with 10 mM glucose for 3 days and then perifused without (solid squares) or with (solid diamonds) 1 mM MSO or with 1 mM BSO (open triangles) prior to starting the glucose ramp (panel A, 0–25 mM at 0.5 mM/min). Results are presented as means ± S.E. for 100 islets from 3 to 6 separate perifusions. Panel B, islets were perifused in the absence of glutamine (solid diamonds) or in presence of 2 (open circles), 5 (open diamonds) mM glutamine, or 40 μM DON (open squares) with 1 mM MSO added prior to stimulating by a glucose ramp.

significant even with high glucose present. Because most of the glutamine was lost to the medium, islet glutamine was undetectable by GC-MS and 15N enrichment could not be determined.

DISCUSSION

The marked hypersensitivity to protein or amino acid stimulation of insulin release in children with SUR1/Kir6.2 mutations had posed the question how this phenomenon could be explained mechanistically. The present study shows that isolated pancreatic islets from SUR1 knockout mice respond briskly to a physiological mixture of 20 amino acids even though these islets cannot be stimulated by glucose or by leucine. These findings in islets of this mouse model duplicate precisely the clinical observation in KATP-HI patients, showing the impairments of glucose and leucine stimulated insulin release, but enhanced responsiveness to amino acids. Although SUR1−/− mice are not hypoglycemic (12, 13), isolated islets from these animals manifest the features of KATP channel deletion and offer an ideal opportunity to investigate the molecular basis of clinical observation in children with KATP-HI.

Glutamine plays a prominent role in mediating amino acid stimulation of insulin release by SUR1 knockout β-cells. About 60% of the insulin response can be attributed to glutamine, even though it contributes only 16% to the total amino acids load. The importance of glutamine was also demonstrable when normal β-cells were stimulated by a physiological amino acid mixture in presence of glucose. The amino acid threshold for potentiation of GSIS was increased from 1.2 to 2.6 mM when glutamine was omitted. It is worth noting that the glutamine concentration needed for this effect is very low (0.2 mM). In addition, isolated perifused islets of SUR1−/− mice or normal islets treated with glyburide were sensitized to stimulation by a glutamine ramp, whereas normal untreated islets did not respond at all. This phenomenon did not require enhanced glutaminolysis, which was previously reported to be the mechanism of glutamine induced insulin release (4, 5). Thus we hypothesize that glutamine per se plays a signaling role in insulin section that has not been recognized previously. This
newly proposed role does not require deamidation by phosphate dependent glutaminase (PDG) or oxidative deamination of glutamate by GDH, processes that ultimately result in increased ATP production (4, 5). Furthermore, this glutamine effect manifests itself only if intracellular calcium is elevated, and it also requires L-type calcium channels to remain operative. Elevation of intracellular calcium is a prerequisite for this glutamine signaling effect to occur. SUR1 knockout and normal islets treated with sulfonylurea or diazoxide plus KCl all result in β-cell depolarization and elevation of [Ca^{2+}], (17, 18) and thus permit glutamine action. Note that the glutamine signal may result either from an extracellular source or may arise from enhanced intracellular synthesis via the enzyme GS.

The precise mechanism by which glutamine exerts its effects remains to be determined. As noted above, an unlikely factor is glutamate as proposed by Maechler and Wollheim (19). Dimethyl-glutamate is not an effective substitute and MSO, an inhibitor of GS, reduced GSIS even though internal glutamate accumulated significantly and ATP levels were not influenced when the inhibitor was present. The cytosolic glutamate level increased through export of glutamate and/or increased glutamate synthesis. Inhibition of the two steps of glutamine oxidation may be of interest. MSO (5) is a competitive inhibitor of GS, reduced GSIS even though internal glutamate accumulated significantly and ATP levels were not influenced when the inhibitor was present. The cytosolic glutamate level increased through export of glutamate and/or increased glutamate synthesis.

**Summary**

- The precise mechanism by which glutamine exerts its effects remains to be determined.
- An unlikely factor is glutamate as proposed by Maechler and Wollheim (19).
- Dimethyl-glutamate is not an effective substitute and MSO, an inhibitor of GS, reduced GSIS even though internal glutamate accumulated significantly and ATP levels were not influenced when the inhibitor was present.
- The cytosolic glutamate level increased through export of glutamate and/or increased glutamate synthesis.

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**Table III: Effects of glucose and MSO on islets and media amino acid concentrations**

| Amino Acid | Cont | G25 | G25/MSO | Cont | G25 | G25/MSO | Cont | G25 | G25/MSO |
|-----------|------|-----|---------|------|-----|---------|------|-----|---------|
| Glutamine | 0.2 ± 0.02 | 0.4 ± 0.04 | ND | 4.1 ± 0.5 | 7.4 ± 0.7 | ND | 4.3 ± 0.5 | 7.7 ± 0.7 | ND |
| Glutamate | 3.0 ± 0.2 | 3.5 ± 0.2 | 6.4 ± 0.5 | 2.2 ± 0.2 | 3.1 ± 0.8 | 3.3 ± 0.2 | 5.3 ± 0.0 | 6.6 ± 0.6 | 9.7 ± 0.6 |
| Aspartate | 4.6 ± 0.1 | 2.9 ± 0.2 | 5.4 ± 0.4 | 1.2 ± 0.2 | 0.9 ± 0.1 | 1.0 ± 0.04 | 5.8 ± 0.2 | 3.7 ± 0.1 | 6.4 ± 0.4 |
| Serine | 0.2 ± 0.02 | 0.4 ± 0.04 | 0.4 ± 0.1 | 2.1 ± 0.1 | 2.7 ± 0.1 | 4.0 ± 0.3 | 2.3 ± 0.1 | 3.0 ± 0.1 | 4.4 ± 0.2 |
| Glycine | 2.5 ± 0.1 | 3.6 ± 0.7 | 3.3 ± 0.3 | 7.3 ± 1.1 | 7.0 ± 0.4 | 9.3 ± 0.7 | 9.8 ± 1.0 | 10.6 ± 0.4 | 12.6 ± 0.9 |
| Alanine | 0.2 ± 0.03 | 0.7 ± 0.1 | 1.0 ± 0.2 | 3.5 ± 0.6 | 4.9 ± 0.1 | 7.8 ± 0.6 | 3.7 ± 0.6 | 5.7 ± 0.1 | 8.8 ± 0.7 |
| GABA | 1.7 ± 0.1 | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.7 ± 0.6 | 0.7 ± 0.1 | 2.0 ± 0.7 | 3.4 ± 0.7 | 1.8 ± 0.2 | 3.0 ± 0.7 |
| Leucine | 0.04 ± 0.02 | 0.1 ± 0.04 | 0.1 ± 0.01 | 3.7 ± 0.2 | 2.5 ± 0.1 | 4.1 ± 0.2 | 3.7 ± 0.2 | 2.6 ± 0.1 | 4.3 ± 0.2 |

**Notes:**
- p < 0.05, compared with control.
- ND, not determined because measurement of glutamine by HPLC was interfered by MSO.
- p < 0.01, compared with control.
- p < 0.05, compared with G25.
- p < 0.01, compared with G25.
serve as an energy-sensing device to track the changes of the phosphate potential. Glucose metabolism increases ATP and also glutamate that those substrates drive the GS reaction to produce glutamine while hydrolysis of glutamine by PDG and oxidation of glutamate by GDH are inhibited. The glutamate accumulation that is seen when GS is blocked by MSO supports this interpretation. The level of cytosolic glutamine is thus well suited to serve as a sensitive and precise indicator of cellular fuel supply and is a plausible mitochondrial-derived cofactor for fuel stimulated insulin release.

The studies with $^{15}$NH$_4$Cl showed that the formation of glutamate by reductive amination of α-ketoglutarate even in the presence of high glucose is quantitatively insignificant. This is perhaps not surprising considering the fact that the $K_m$ of GDH for NH$_4^+$ is very high ($\sim$ 10 mM) and that the enzyme is probably strongly inhibited when high glucose is present (5). Estimate of GDH flux from α-ketoglutarate to glutamate with $^{15}$NH$_4$Cl as substrate, using the maximum possible $^{15}$N enrichment value of 5 APE of glutamate and aspartate, gives a rate of about 0.4 nmol/1000 islets/2 h. This is about 3% of the flux rate we observed when studying the reverse reaction with [2,15]N glutamine as substrate (5). The effect of high glucose on the islet glutamate level varies greatly with experimental designs (17, 18, 21). It appears that the changes of the glutamate pool are controlled primarily by transamination reactions, by signs (17, 18, 21). It appears that the changes of glutamate level varies greatly with experimental de-

Furthermore, the MSO analogue, BSO inhibits if such an effect were important for its action on GSIS, the MSO

The studies with 15NH$_4$Cl showed that the formation of glutamine seems to be an unlikely mechanism to explain the func-

Signaling Role of Glutamine

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