Glucose Regulation of Glutaminolysis and Its Role in Insulin Secretion

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Leucine or the nonmetabolized leucine analog ± 2-amino-2-norbornane-carboxylic acid (BCH) (both at 10 mmol/l) induced biphasic insulin secretion in the presence of 2 mmol/l glutamine (Q2) in cultured mouse islets pretreated for 40 min without glucose but with Q2 present. The β-cell response consisted of an initial peak of 20- to 25-fold above basal and a less marked secondary phase. However, BCH produced only a delayed response, while leucine was totally ineffective when islets were pretreated with 25 mmol/l glucose plus Q2. With Q2, 10 mmol/l BCH or leucine caused a nearly threefold increase, a twofold increase, or had no effect on cytosolic Ca^{2+} levels in islets pretreated for 40 min with 0, 5, or 15 mmol/l glucose, respectively. Thus, pretreatment of islets with high glucose inhibited BCH- and leucine-induced cytosolic Ca^{2+} changes and insulin release. Glucose decreased glutamine oxidation in cultured rat islets when BCH was present at 10 mmol/l, but not in its absence, with a lowest effective level of ~0.1 mmol/l, a maximum of 18–30 mmol/l, and an inhibitory concentration, 50%, of ~3 mmol/l. The data are consistent with the hypothesis that glucose inhibits glutaminolysis in pancreatic β-cells in a concentration-dependent manner and hence blocks leucine-stimulated insulin secretion. We postulate that in the basal interprandial state, glutaminolysis of β-cells is partly turned on because glutamate dehydrogenase (GDH) is activated by a decreased P-potential due to partial fuel depletion and sensitization to endogenous activators such as leucine. Additionally, it may contribute significantly to basal insulin release, which is known to be responsible for about half of the insulin released daily. The data explain "leucine-hypersensitivity" of β-cells during hypoglycemia and contribute to the elucidation of the GDH-linked syndrome of hyperinsulinism associated with elevated serum ammonia levels. Thus, understanding the precise regulation and role of β-cell glutaminolysis is probably central to our concept of normal blood glucose control. *Diabetes* 47:1535–1542, 1999

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ANOVA, analysis of variance; BCH, 2-amino-2-norbornane-carboxylic acid; BCKDH, branched-chain ketoacid dehydrogenase; BSA, bovine serum albumin; EC, effective concentration; EC_{50}, effective concentration, 50%; G, D-glucose; GDH, glutamate dehydrogenase; HI-GDH, glutamate dehydrogenase–linked hyperinsulinism; IC_{50}, inhibitory concentration, 50%; KRBB, Krebs-Ringer bicarbonate buffer; NIH, National Institutes of Health; Q, glutamine.

A recent study reported a rare syndrome of hyperinsulinism associated with hyperammonemia. Subjects with this syndrome present with fasting hypoglycemia with inappropriately elevated insulin levels and an inappropriately large glycemic response to the administration of glucagon. Inheritance of the familiar form is autosomal dominant, although sporadic cases have also been described. It was originally proposed that the underlying defect involves a site that is common to amino acid regulation of both insulin secretion in pancreatic β-cells and urea synthesis in the liver (1). This lesion was subsequently discovered to involve glutamate dehydrogenase (GDH) (L-glutamate: $NAD(P)^+$ oxidoreductase EC1.4.1.3), and many point mutations located in the allosteric regulatory site of the enzyme have been found (e.g., S445L, G446S, S448P, H454Y) (2). The syndrome of GDH-linked hyperinsulinism (HI-GDH) underscores the central role of GDH in β-cell intermediary metabolism and the potential importance of glutaminolysis in physiological insulin secretion.

At least two issues related to glutamate metabolism in pancreatic β-cells require comment here: the first one deals with the phenomenon of leucine hypersensitivity during hypoglycemia (3), and the second one addresses the phenomenon of amino acid–stimulated insulin release. Leucine hypersensitivity of β-cells is observed in patients with an insulinoma or after treatment with sulfonylureas (3). Sener and Malaisse (4) were the first to attribute this effect to the well-established activating action of leucine on GDH. The essential intracellular signaling pathway involved in this phenomenon has been elucidated (4–9). Activation of glutamate metabolism enhances production of NAD(P)H (8,10,11) and fuels the citric acid cycle by providing α-ketoglutarate. Metabolic coupling factors (ATP and possibly malonyl-CoA) are generated (10) or ADP is lowered (12), and these cause depolarization of the β-cells (8,13), enhanced Ca^{2+} influx (10), and direct or indirect augmentation of exocytosis of insulin (8,10,13–17). Yet it is not understood mechanistically how hypoglycemia sensitizes the β-cells to leucine action. MacDonald et al. (18) attributed leucine hypersensitivity in hypoglycemia to induction of branched-chain ketoacid dehydrogenase (BCKDH) and concluded that alteration of GDH expression was not involved, but they did not consider the possibility that allosteric changes of GDH activity might play a critical role. This possibility is attractive because GDH is very precisely regulated. It may be activated by L-leucine, L-isoleucine, L-methionine, succinyl-CoA, and ADP, while it is inhibited by palmitoyl-CoA, pyruvate, malate, succinate, and GTP (19). It seemed plausible that alterations of the intra-

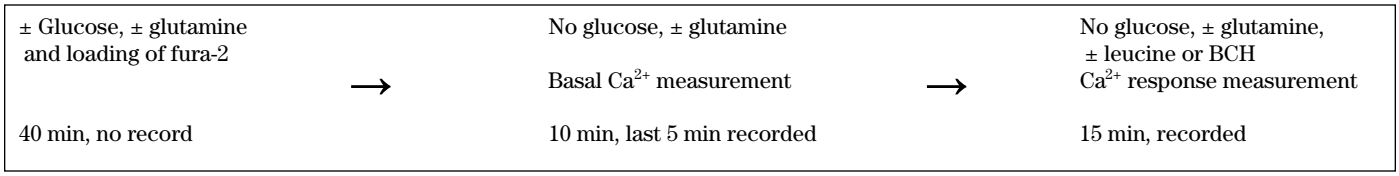

FIG. 1. Experimental design.

cellular concentration of these regulatory metabolites might be the critical event. Suitable experiments needed to be designed to test this possibility.

The involvement of GDH in β-cell intermediary metabolism may also provide a plausible explanation for the absolute glucose requirement of normal β-cell responsiveness to physiological mixtures of amino acids. Amino acids are taken up by transport systems probably not too different from those serving most cells and are likely to be channeled into intermediary metabolism by the transdeamination cycle, which involves a family of pyridoxal-P–dependent transaminase enzymes that form glutamate coupled to oxidation by GDH. Glucose may provide acceptor ketoacids (pyruvate, oxaloacetate, and α-ketoglutarate) as substrate for transaminations and may therefore be required as permissive fuel to facilitate metabolic flux.

The present study addresses the first of the two interrelated issues and shows that a precise regulation of glutaminolysis by glucose does indeed exist in pancreatic β-cells. The results help explain the pathological syndrome of HI-GDH and the leucine hypersensitivity in hypoglycemia, and they also suggest to us an important physiological role of glutaminolysis for basal insulin release.

RESEARCH DESIGN AND METHODS

Mouse islet preparation and insulin secretion. Mouse islets were isolated by collagenase digestion and cultured for 3–7 days in RPMI 1640 containing 10 mmol/l glucose, 0.05 mmol/l L-leucine, and 0.2 mmol/l L-glutamine in addition to other amino acids, as specified by the composition of this culture medium (Sigma, St. Louis, MO). The culture medium was supplemented with 10% fetal bovine serum. Cultured islets were then placed in perifusion chambers and perifused with Krebs-Ringer bicarbonate buffer (KRBB) (115 mmol/l NaCl, 24 mmol/l $NaHCO_3$, 5 mmol/l KCl, 1 mmol/l $MgCl_2$, 2.5 mmol/l $CaCl_2$, 25 mmol/l HEPES, pH 7.40, and 1% bovine serum albumin [BSA]) at a flow rate of 2 ml/min at 37°C. KRBB was continuously gassed with 5% CO_2 and 95% O_2 and always contained 2 mmol/l glutamine in the insulin secretion experiments. Cultured islets were first perifused in the absence or presence of 25 mmol/l glucose for 40 min, and then without glucose for 10 min, before stimulation by 10 mmol/l ± 2-amino-2-norbornane-carboxylic acid (BCH) (mixture of the two isomers) or leucine was initiated. Perifusate was collected frequently and insulin content of perifusion samples was determined by radioimmunoassay.

Cytosolic-free Ca^{2+} measurement. Mouse islets were isolated and cultured in the same way as outlined above. They were then loaded with fura-2 during a 40-min pretreatment at 37°C in 2 ml KRBB supplemented with 1 μmol/l fura-2 acetoxymethylester (Molecular Probes, Eugene, OR) and 0.2 mg/ml pluronic F-127 (Molecular Probes), which was used to increase the dye loading. During this pretreatment period, islets were exposed to various glucose concentrations and/or various glutamine concentrations as specified in RESULTS. The loaded islets were then fixed by slight suction on the tip of a micropipette in a perifusion chamber placed on the homeothermic platform of an inverted Zeiss microscope. Islets were perifused with KRBB at 37°C at a flow rate of 1 ml/min, while various treatments were applied to the islets as illustrated schematically in Fig. 1. The microscope was used with a 40× oil immersion objective. Fura-2 was successively excited at 334 and 380 nm by two narrow band-pass filters. The emitted fluorescence was filtered through a 520-nm filter, captured with an Attofluor CCD video camera at a resolution of 512 × 480 pixels, digitized into 256 gray levels, and analyzed with version 6.07 of the Attofluor RatioVision software (Atto Instruments, Rockville, MD). The concentration of Ca^{2+} was calculated by comparing the ratio of fluorescence at each pixel to an in vitro 2-point calibration curve. The Ca^{2+} concentration is presented by averaging the values of all pixels in an islet. Data points were collected at intervals of 20 s (unless otherwise indicated).

Measurement of reduced pyridine nucleotide fluorescence. Cultured mouse islets were first treated for 40 min at 37°C in a manner similar to that used for fura-2 experiments, except for the absence of fura-2 in the KRBB. Islets were then transferred to the same experimental setup as that used for fura-2 fluorescence measurement. The reduced forms of NAD and NADP, referred to as NAD(P)H, were excited at 360 nm, and the fluorescence emitted through a dichroic mirror centered at 400 nm was filtered at 470 nm before it was captured by a CCD camera and digitized into 256 gray levels. Changes in NAD(P)H fluorescence were expressed as a percentage of the control value by dividing the integrated gray levels at a given time by those obtained during the last minute before stimulation.

[^{14}C]glutamine oxidation. Wistar rat islets were isolated by collagenase digestion and cultured similarly to mouse islets. Batches of 100 cultured rat islets were incubated for 1 h at 37°C in 100 μl of KRBB containing various concentrations of nonradioactive glutamine, as indicated in RESULTS, and 1 μCi/tube radioactive L-[U-^{14}C]glutamine (specific activity = 244 mCi/mmol) (NEN–Life Science Products, Boston, MA) in Eppendorf tubes, which were placed in 20-ml scintillation vials. A trap filter was also placed in each tightly sealed scintillation vial to collect the $^{14}CO_2$ produced by the islets, and the amount of radioactivity was determined by liquid scintillation counting.

[^{14}C]glucose oxidation. Batches of 100 cultured rat islets were incubated for 1 h at 37°C in 100 μl of KRBB containing 10 μCi/tube radioactive D-[U-^{14}C]glucose (specific activity = 250 to 360 mCi/mmol) (NEN–Life Science Products) in Eppendorf tubes, which were placed in 20-ml scintillation vials. A trap filter was also placed in each tightly sealed scintillation vial to collect the $^{14}CO_2$ produced by the islets, and the amount of radioactivity was determined by liquid scintillation counting.

[^{3}H]glucose glycolysis. Batches of 100 cultured rat islets were incubated for 1 h at 37°C in 100 μl of KRBB containing 10 μCi/tube radioactive d-[N-5-3H]glucose (specific activity = 10 to 20 Ci/mmol) (NEN–Life Science Products) in Eppendorf tubes, which were placed in 20-ml scintillation vials. The radioactive $^{3}H_2O$ produced by the islets was separated from the substrate by a diffusion step, and the amount of radioactivity was determined by liquid scintillation counting.

Data analysis. Student's *t* test was performed when two groups were compared. Analysis of variance (ANOVA) was used, followed by Dunnett's test, when multiple groups were compared. Differences were considered significant for $P < 0.05$.

Materials. All chemicals were from Sigma except as otherwise indicated: EGTA, EDTA, $CaCl_2$, KCl, HEPES, glucose, BCH, L-leucine, L-glutamine, potassium phosphates, and BSA.

RESULTS

BCH and leucine effects on insulin secretion of cultured mouse islets. After culture for 3–7 days, mouse islets were perifused for 50 min with KRBB containing 2 mmol/l glutamine (Q2) and no glucose. Insulin secretion decreased rapidly and reached a low baseline of 0.17 ± 0.04 ng · min^{-1} · 100 $islets^{-1}$. Addition of 10 mmol/l glucose (G10) induced a biphasic insulin secretion, with a rapid initial peak at 4.03 ± 0.70 ng · min^{-1} · 100 $islets^{-1}$ and a plateau of ~1–2 ng · min^{-1} · 100 $islets^{-1}$. When islets were first perifused for 40 min with KRBB containing 25 mmol/l glucose (G25) and Q2, insulin secretion was high at ~3–6 ng · min^{-1} · 100 $islets^{-1}$. The glucose concentration was then decreased to 0 mmol/l for 10 min, which caused insulin secretion to drop to 0.5–1.0 ng · min^{-1} · 100 $islets^{-1}$. Subsequent addition of G10 caused a rapid biphasic insulin secretion with a peak of 3.52 ± 0.84 ng · min^{-1} · 100 $islets^{-1}$ and a plateau of ~2 ng · min^{-1} · 100 $islets^{-1}$. Thus the G10-induced insulin secretion in G25-primed islets was practically the same as that of islets deprived of glucose for 50 min (20).

Addition of 10 mmol/l BCH in the presence of Q2 caused a biphasic increase of insulin secretion in islets perifused for

50 min in the absence of glucose. Insulin secretion was increased to a peak rate of 4.27 ± 0.14 ng · min^{-1} · 100 $islets^{-1}$ within 5 min and then returned to 0.34 ± 0.11 ng · min^{-1} · 100 $islets^{-1}$ before a second phase gradually appeared. Addition of 10 mmol/l leucine in the presence of Q2 produced a similar biphasic increase of insulin secretion in fuel-depleted islets.

In striking contrast, addition of BCH or leucine in the presence of Q2 produced a much smaller insulin secretion in G25-primed islets. BCH caused a delayed and progressive increase of insulin secretion lacking an initial peak ($P < 0.05$), while leucine had hardly any effect on the insulin secretion of these islets ($P < 0.05$) (Fig. 2).

BCH and leucine effects on cytosolic Ca^{2+} of islets pretreated with high glucose. In pilot experiments, not illustrated here, cytosolic Ca^{2+} of cultured mouse islets was 177 ± 13 nmol/l in the presence of Q2 tested without glucose. Addition of 15 mmol/l glucose increased the Ca^{2+} level to 456 ± 83 nmol/l. The mitochondrial oxidation inhibitor azide (2 mmol/l) caused a significant and reversible lowering of cytosolic Ca^{2+} (270 ± 33 nmol/l) in the presence of 15 mmol/l glucose. In the absence of glucose, the NAD(P)H fluorescence signal decreased gradually with time. In contrast to an ~2-min delay of the Ca^{2+} response, addition of 15 mmol/l glucose instantly increased islet NAD(P)H fluorescence (137 ± 7% at 12 min vs. 100 ± 1% at 5 min before glucose stimulation, $P < 0.05$). Azide caused a rapid increase of islet NAD(P)H fluorescence (183 ± 5%, $P < 0.05$) that was reversible on the removal of the inhibitor (150 ± 5%, $P < 0.05$). Glucose enhancement of NAD(P)H fluorescence was also reversible on the removal of sugar (118 ± 5%). These data thus illustrate that the imaging system operates reliably and that islets responded in a typical manner.

Cytosolic Ca^{2+} measurements were performed with Q2 present throughout the entire experiment. Islets were pretreated with 0, 5, 15, or 30 mmol/l glucose (Pre-G0, Pre-G5, Pre-G15, and Pre-G30, respectively) for 40 min and were then perifused for 10 min in the absence of glucose before they were stimulated by BCH or leucine for 15 min in the absence of glucose. In Pre-G0 islets, basal Ca^{2+} was 154 ± 24 nmol/l, and the BCH-induced initial decrease of 13 nmol/l occurred within 2 min, while the BCH-induced Ca^{2+} increase of 438 ± 36 nmol/l at the plateau ($P < 0.05$) occurred much later. The delay in Ca^{2+} increase compared with the increase of insulin release could be caused in part by the slower flow rate and hence the slower mixing of buffers in the Ca^{2+}-measuring chamber. Pre-G5 islets demonstrated a slightly higher basal Ca^{2+} of 186 ± 41 nmol/l and a significantly ($P < 0.05$) smaller BCH-induced increase of Ca^{2+} with a plateau of ~326 ± 30 nmol/l. In Pre-G15 islets, basal Ca^{2+} was high at 242 ± 28 nmol/l, and there was no response to BCH stimulation (254 ± 27 nmol/l, $P > 0.05$), as shown in Fig. 3*A*. The Pre-G30 islet time course was similar to that of Pre-G15 islets. The ΔCa^{2+} increase induced by BCH in the presence of Q2 was calculated by subtracting the averaged basal Ca^{2+} level from the stimulated Ca^{2+} level averaged over the entire 15-min BCH stimulation and is plotted as a function of the glucose concentrations at which the islets were pretreated (Fig. 4). Similarly, the effects of 10 mmol/l leucine (Leucine10) was tested in Pre-G0, Pre-G5, Pre-G15, and Pre-G30 (time course not shown) islets, as shown in Fig. 3*B*. Leucine produced effects similar to those of BCH in Pre-G0 islets (135 ± 15 at basal vs. 438 ± 25 nmol/l at the plateau of stimulation, $P < 0.05$). Pre-G5 partially, but significantly ($P < 0.05$), inhibited the leucine-induced Ca^{2+} response (124 ± 15 at basal vs. 214 ± 19 nmol/l at the plateau of stimulation, $P < 0.05$), while Pre-G15 inhibited it totally (158 ± 9 at basal vs. 134 ± 12 nmol/l at the plateau of stimulation, $P > 0.05$). The dose-response curve of glucose pretreatment on the leucine-induced ΔCa^{2+} above baseline was calculated similarly to that of BCH and is also plotted in Fig. 4. The leucine curve was very similar to the BCH curve, with an apparent inhibitory concentration, 50% (IC_{50}) of ~5 mmol/l and a maximal effective concentration (EC) of 15 mmol/l glucose for both amino acids.

Glutamine dose dependence of BCH and leucine-induced Ca^{2+} changes. The dose dependence of glutamine

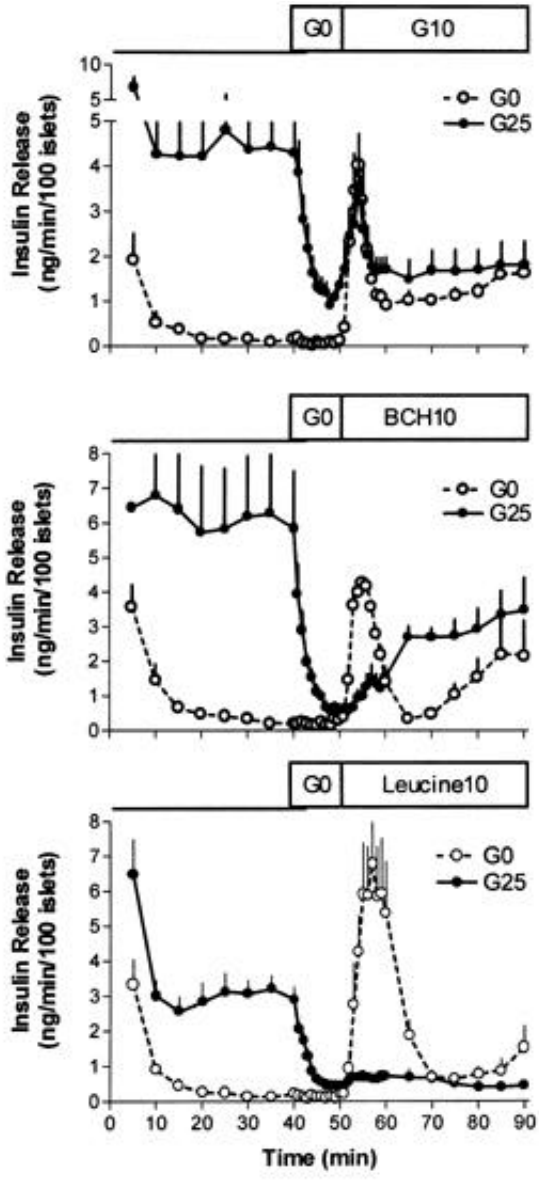

FIG. 2. Effects of glucose pretreatment on glucose-, BCH-, or leucine-induced insulin secretion from cultured mouse islets in the presence of 2 mmol/l glutamine. Cultured mouse islets were perifused without (G0) or with (G25) 25 mmol/l glucose for 40 min, followed by a perifusion without glucose for 10 min, before they were stimulated by 10 mmol/l glucose (G10), BCH (BCH10), or leucine (Leucine10) for 40 min. The 2 mmol/l glutamine was present during the entire experiment. Each trace is the mean ± SE of three to five experiments.

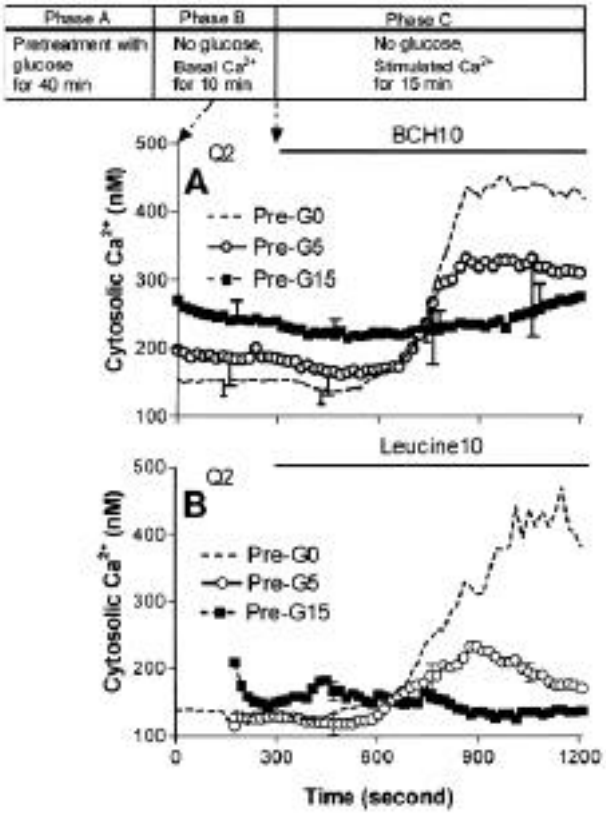

FIG. 3. Effects of 10 mmol/l BCH (*A*) or leucine (*B*) on cytosolic Ca^{2+} levels of cultured mouse islets pretreated with various concentrations of glucose in the presence of 2 mmol/l glutamine. Cultured islets were loaded with 1 µmol/l fura-2 and pretreated with 2 mmol/l glutamine (Q2) in the absence (Pre-G0) or presence of 5 or 15 mmol/l glucose (Pre-G5 and Pre-G15, respectively) during a 40-min incubation in 2 ml KRBB at 37°C (phase A). During phases B and C, islets were perifused with the same KRBB containing Q2, but no glucose. After a 10-min stabilization period (phase B), islets were exposed to 10 mmol/l BCH (BCH10) or 10 mmol/l leucine (Leucine10) for 15 min (phase C). The timing of the three phases is shown above the figure. Data are means ± SE of 9–15 islets tested in four to six separate islet preparations.

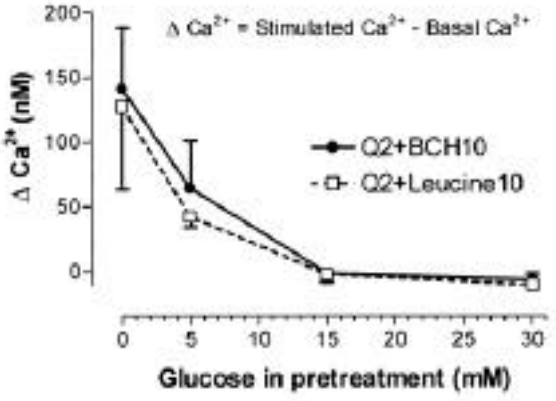

FIG. 4. Concentration dependency of the effects of glucose pretreatment on BCH- or leucine-induced cytosolic Ca^{2+} changes in islets in the presence of 2 mmol/l glutamine. This figure was prepared in part from data presented in Fig. 3. ΔCa^{2+} is calculated by subtraction of averaged basal Ca^{2+} during phase B from averaged stimulated Ca^{2+} during phase C. This represents the net increase of cytosolic Ca^{2+} induced by the addition of 10 mmol/l BCH (BCH10) or 10 mmol/l leucine (Leucine10) in the presence of 2 mmol/l glutamine (Q2). Data are means ± SE of 9–15 islets in four to six experiments from separate islet preparations.

on mouse islet cytosolic Ca^{2+} was determined in the absence of glucose. Islets were pretreated for 40 min and then perifused while the Ca^{2+} signal was recorded. In the absence of glutamine (Q0), cytosolic Ca^{2+} remained stable (165 ± 12 nmol/l) and was unchanged upon addition of 10 mmol/l BCH (BCH10) (162 ± 10 nmol/l, $P > 0.05$), as shown by the broken line in Fig. 5*A*. When 0.5 mmol/l glutamine (Q0.5, triangle in Fig. 5*A*) was present, the basal Ca^{2+} without BCH was 185 ± 20 nmol/l. Addition of 10 mmol/l BCH caused a small increase of Ca^{2+} within ~5 min, which reached a plateau of 225 ± 18 nmol/l ($P < 0.05$). Basal Ca^{2+} was similar (174 ± 15 nmol/l, $P > 0.05$) when compared with the absence of glutamine, while the response to BCH10 was much larger (450 ± 119 nmol/l at the plateau) in the presence of Q2 than in the presence of Q0.5 ($P < 0.05$), as shown in Fig. 5*B*. A further increase of glutamine to 10 mmol/l (Q10, in Fig. 5*B* and *D*) paradoxically produced a smaller stimulation on Ca^{2+} (323 ± 36 nmol/l at the plateau), while 20 (497 ± 16 nmol/l at the plateau) to 30 mmol/l glutamine (Fig. 5*D*) produced the maximal effect. Basal Ca^{2+} before BCH addition was not significantly affected by glutamine, as tested by ANOVA ($P > 0.05$, open circles in Fig. 5*D*). The averaged Ca^{2+} levels over the entire 15-min period of BCH stimulation (closed circles) in the presence of 0–30 mmol/l glutamine are shown in Fig. 5*D*. Those of 0–2 mmol/l glutamine are highlighted in Fig. 5*C* because of their physiological relevance. The dose-response curve of glutamine consisted of two peaks, one at 2–5 mmol/l and the other at 20–30 mmol/l. The apparent effective concentration, 50% (EC_{50}), of the high-affinity component (0–5 mmol/l) was ~1.5 mmol/l.

BCH and leucine dose dependence of cytosolic Ca^{2+} changes. The dose dependence of BCH and leucine on mouse islet cytosolic Ca^{2+} was performed in the presence of Q2. The lowest EC of BCH was between 5 and 10 mmol/l, while 20 mmol/l was almost maximally effective. The apparent EC_{50} of BCH was ~8 mmol/l in the presence of Q2. As shown by the time course experiment in Fig. 6*C*, the lowest EC of leucine was 1 mmol/l. The maximal EC of leucine was ~5 mmol/l, as illustrated in Fig. 6*B*. The apparent EC_{50} of ~2 mmol/l and the threshold of leucine were lower than those of BCH. Leucine was more potent than BCH even when the 50% noneffective isoform of ± BCH is taken into account, which gives an EC_{50} of 4 mmol/l for the active BCH isoform.

Glutamine oxidation and glycolysis in cultured rat islets. All metabolism experiments were performed with cultured rat islets because a greater number of islets were needed (Figs. 7 and 8). In the absence of glucose and BCH, glutamine oxidation increased as a function of glutamine concentration, with a saturation concentration of ~3 mmol/l (16.37 ± 5.16 pmol · islet^{-1} · h^{-1}). The apparent K_m was 0.42 mmol/l using a double plot ($1/v$ vs. 1/[S]). In the presence of 9 mmol/l ± BCH and in the absence of glucose, the magnitude of an increase in oxidation was about two- to threefold higher compared with that without BCH. This resulted in a near-maximal oxidation rate of ~77.36 ± 24.17 pmol · islet^{-1} · h^{-1} at 30 mmol/l of glutamine. The EC_{50} was ~3 mmol/l, and the apparent K_m was 0.76 mmol/l in the presence of BCH. The effects of glucose on glutamine oxidation were thus tested in the presence of 3 mmol/l glutamine and with 10 mmol/l BCH or in the absence of the amino acid. In the presence of 25 µmol/l glucose, the oxidation rate was 19.06 ± 1.53 pmol · islet^{-1} · h^{-1},

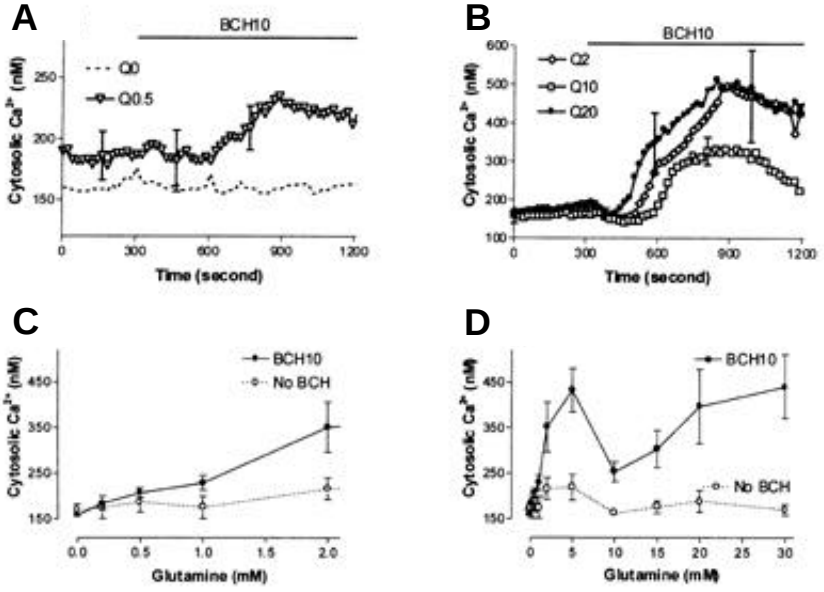

FIG. 5. BCH effects on cytosolic Ca^{2+} level of cultured mouse islets in the presence of various glutamine concentrations. *A* and *B*: Cultured islets were loaded with fura-2 in the absence (Q0) or presence of 0.5, 2, 10, or 20 mmol/l glutamine (Q0.5, Q2, Q10, and Q20, respectively) without glucose (G0) for 40 min at 37°C. They were then perifused with KRBB containing the same glutamine concentration and G0 at 37°C. After a 10-min stabilization period, islets were exposed to 10 mmol/l BCH (BCH10) for 15 min. *C* and *D*: The averaged basal Ca^{2+} without BCH () and the averaged BCH-stimulated Ca^{2+} () in the presence of various glutamine concentrations (0–30 mmol/l) were plotted. Note that the *y*-axes are different between *A* and *B*. Data are means ± SE of 9–15 islets from three to five experiments.

and glucose (up to 30 mmol/l) did not significantly change the rate of glutamine oxidation ($P > 0.05$) (Fig. 8). With 10 mmol/l BCH and 25 µmol/l glucose in the medium, glutamine oxidation was increased to 42.70 ± 2.74 pmol · islet^{-1} · h^{-1} ($P < 0.05$). Glucose decreased glutamine oxidation concentration dependently with a maximal EC of 18–30 mmol/l. Analysis of the data revealed an IC_{50} of ~3 mmol/l (Fig. 8).

In the absence of glutamine and BCH, the glucose oxidation rate was 36.22 ± 2.86 pmol · islet^{-1} · h^{-1} when present at 10 mmol/l. Glutamine, as high as 30 mmol/l, did not significantly affect the glucose oxidation rate ($P > 0.05$). When glucose oxidation was measured in the presence of 10 mmol/l BCH and with various concentrations of glutamine, a similar lack of glutamine action was noted ($P > 0.05$).

Rat islet glycolysis was examined by measuring the ^3H_2O produced with 10 mmol/l [^{3}H]glucose. In the absence of glutamine, the glycolysis rate was 116.46 ± 23.14 when BCH was absent, but it was 82.07 ± 14.59 pmol · islet^{-1} · h^{-1} with 10 mmol/l BCH. The apparent decrease due to BCH was not statistically significant ($P > 0.05$). Glutamine (up to 30 mmol/l) with or without BCH did not affect the glycolytic rate in islets ($P > 0.05$).

DISCUSSION

The recent recognition and biochemical genetic elucidation of the HI-GDH syndrome (1,2) has rekindled an interest in the role of glutaminolysis in β-cell function. This new knowledge requires a reassessment of related previously published material and prompted the present study.

This study was designed to test the working hypothesis that glutaminolysis of β-cells is precisely regulated by glucose and that it may be critical for maintaining basal insulin release because flux through the pathway is inversely related to physiological blood glucose levels (Fig. 9). The results clearly demonstrate in a first step of hypothesis testing that glucose does indeed inhibit glutaminolysis activated by BCH or leucine in a manner that implicates glucokinase as a determinant of the physiological concentration dependency of the effect. Glucose inhibition of glutaminolysis is paralleled by decreased leucine responsiveness of the β-cells as demonstrated by Ca^{2+} imaging and insulin secretion. Both effects find a plausible explanation in the allosteric inhibition of GDH by an increased GTP:ADP ratio known to result from enhanced glucose metabolism (21–23). A quantitative rather than qualitative assessment of the role of glutaminolysis in basal insulin release is more difficult to accomplish because many factors, in addition to those explored here (namely, glutamine, leucine, and glucose), need to be considered. The contributions of fatty acids, the remaining 18 amino acids, ketone bodies, lactate, and pyruvate need to be carefully assessed, and the mechanisms of metabolic coupling need to be elucidated. The present data are, however, consistent with the hypothesis that glutaminolysis might be a critical process supporting basal insulin release.

The present experiments occasion a series of additional comments related to theoretical or technical aspects of the study and to pertinent reports in the literature.

Glucose regulation of β-cell glutaminolysis. The novel finding in this study is that the BCH or leucine stimulation of β-cell insulin secretion, cytosolic Ca^{2+} signals, and glutamine oxidation were all under precise glucose control. At threshold concentrations of 1–3 mmol/l, glucose inhibited leucine or BCH effects on glutaminolysis and cytosolic Ca^{2+}. A near complete inhibition of leucine or BCH-induced glutaminolysis and of its physiological effects (i.e., insulin release and Ca^{2+} uptake) was achieved by 15–30 mmol/l glucose, and this inhibition lasted for at least 20 min after the withdrawal of glucose.

It has been demonstrated previously that BCH is a selective activator of GDH, and it is believed that this is the main or only mechanism by which BCH stimulates β-cell insulin secretion (4–9). BCH stimulation of first-phase insulin secretion, the cytosolic Ca^{2+} response, and glutamine oxidation was strongly inhibited or completely abolished by pretreatment with 15–30 mmol/l glucose. It is postulated that glucose inhibits glutaminolysis and glutamine-induced insulin secretion by inhibiting GDH indirectly by increasing the mitochondrial P-potential.

Leucine may stimulate β-cells by two mechanisms (8,18,24). One is activation of GDH (4,25) and the other is related to its role as a fuel for the β-cells (26). Leucine stim-

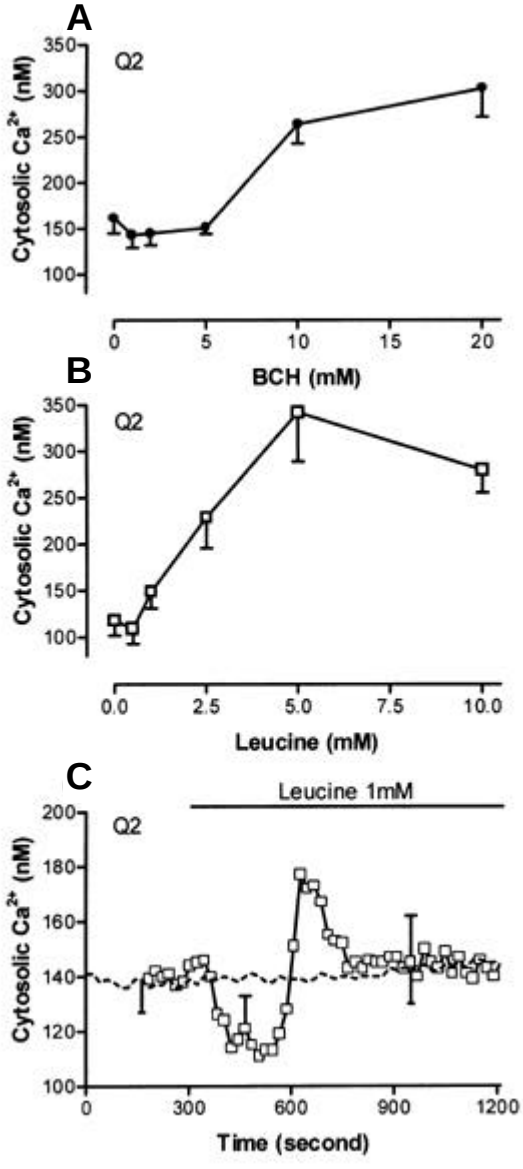

FIG. 6. Comparison of BCH and leucine concentration dependency curves for cytosolic Ca^{2+} of mouse islets in the presence of 2 mmol/l glutamine (Q2). Cultured islets were loaded with fura-2 in the presence of Q2 without glucose (G0) for 40 min. They were then perifused with KRBB containing Q2 and G0. After a 10-min stabilization period, islets were exposed to various concentrations of BCH (*A*) or leucine (*B*) for 15 min in the presence of Q2. *C*: The time course of the effects of 1 mmol/l leucine. Data are means ± SE of three to five experiments.

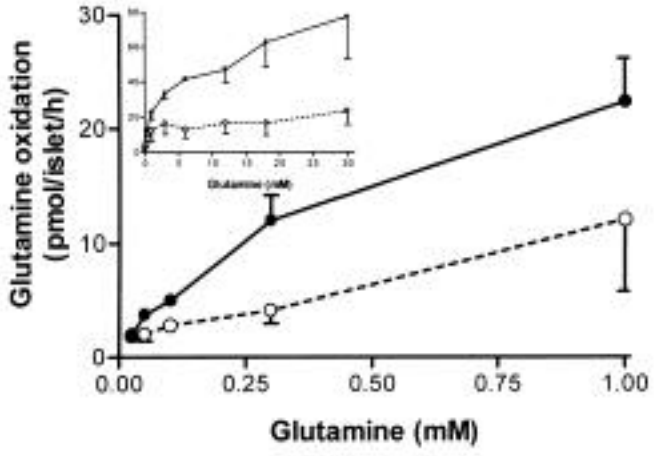

FIG. 7. [^{14}C]glutamine oxidation by cultured rat islets with or without 9 mmol/l BCH. Cultured rat islets were incubated at 37°C in the presence of various concentrations of glutamine plus 1 μCi/tube [^{14}C]glutamine, without ( ) or with ( ) 9 mmol/l BCH for 1 h. The radioactive $^{14}CO_2$ produced by the islets was trapped in a filter placed inside the incubation vial. The amount of radioactivity was determined by liquid scintillation counting. Data are means ± SE of three to five experiments.

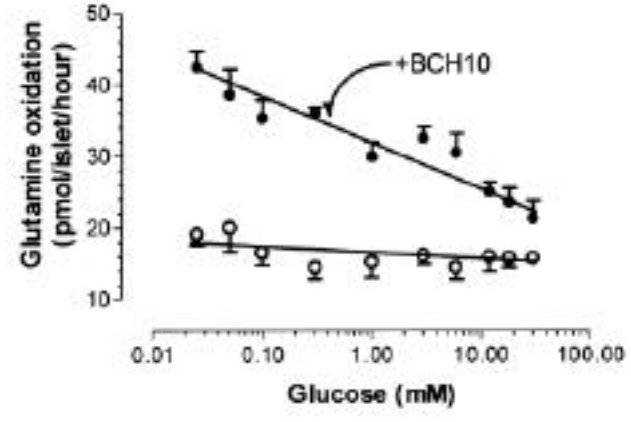

FIG. 8. Glucose inhibition of [^{14}C]glutamine oxidation by cultured rat islets. Cultured rat islets were incubated at 37°C in the presence of various concentrations of glucose plus 3 mmol/l glutamine and 1 μCi/tube [^{14}C]glutamine, with (●) or without (○) 10 mmol/l BCH for 1 h. The radioactive $^{14}CO_2$ produced by the islets was trapped in a filter placed inside the incubation vial. The amount of radioactivity was determined by liquid scintillation counting. The means ± SE of glutamine oxidation from five experiments are plotted as a function of glucose concentrations.

ulation of the mouse islet Ca^{2+} response and of insulin release was completely abolished by 15–30 mmol/l glucose. These results may also have two explanations. First, leucine stimulation may occur solely through the GDH route. Second, leucine stimulation may involve both GDH activation and leucine oxidation, and both might be inhibited by high glucose. Glucose modulation of leucine metabolism via specifically altered expression of BCKDH was reported by MacDonald et al. (18) to explain the effect of high glucose on leucine stimulation of β-cells. This mechanism is unlikely to be involved here considering the short time course. Allosteric effects of nucleotides that could modify BCKDH have not been reported. However, it is unlikely that BCKDH is allosterically inhibited by short-term glucose treatment through changes in nucleotide levels, because the Ca^{2+} response induced by 10 mmol/l α-ketoisocaproate was not inhibited by glucose pretreatment (Z.G., G.L., H.N., B.A.W., F.M.M., unpublished observations).

Only one previous study has tested glucose effects on GDH function in β-cells (18). Rat islets were cultured in 20 or 1 mmol/l glucose for 1 day, and total GDH activity was measured in the islet extracts. GDH activity was not influenced by glucose during the culture. It was concluded that GDH is not regulated by glucose. The experimental design fails to account for the allosteric regulation of GDH.

Even though this study establishes that glucose precisely regulates glutaminolysis and thus leucine-induced insulin

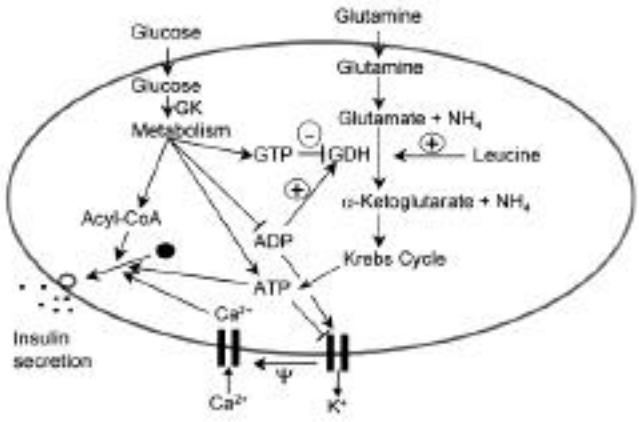

FIG. 9. Glucose regulation of glutaminolysis and insulin secretion. The interaction between the two pathways, i.e., glucose metabolism and glutaminolysis, is schematically depicted. The adenine nucleotide-sensitive K-channel, where ATP functions as inhibitor and ADP as activator, and the voltage (ψ)-sensitive Ca^{2+} channel are indicated. ATP, Ca^{2+}, and long-chain acyl-CoA converge as signals to increase insulin release. Note that glutaminolysis may independently generate long-chain acyl-CoA (not depicted here). GK, glucokinase.

secretion, the exact molecular mechanism remains to be elucidated. Glucose increases GTP levels while it decreases ADP levels in a concentration-dependent manner in isolated pancreatic islets (21–23), and presumably similar changes occur in β-cell mitochondria where GDH is located. The GTP:ADP ratio may control GDH activity, its leucine sensitivity, and may thus regulate glutaminolysis. Glucose oxidation also generates other metabolites that have been shown to be GDH activators, such as succinyl-CoA, NADH, and NADPH, or GDH inhibitors, such as fumarate, malate, succinate, and palmitoyl-CoA (19,24). However, the precise roles of these metabolites, if any, in glucose regulation of GDH and glutaminolysis of pancreatic β-cells remain to be investigated.

Glutaminolysis of β-cells and glucose homeostasis. The concept of precise glucose regulation of glutaminolysis proves useful in understanding the HI-GDH syndrome described by Weinzimer et al. (1) and by Stanley et al. (2). Patients with this syndrome develop hyperinsulinemic hypoglycemia after meals rich in protein but low in carbohydrate. However, carbohydrate ingested before the protein-rich meal prevents the hypoglycemic response (C. Stanley, personal communication). The GTP:ADP ratio in β-cells is probably sufficiently elevated by this precaution in individuals with HI-GDH syndrome.

We extrapolate here that GDH, and thus glutaminolysis, may also play a critical role in interprandial insulin release that accounts for half the daily required insulin secretion from β-cells. This basal insulin release may be fueled by glutaminolysis, since glucose ceases to serve as a β-cell stimulant as it falls below its threshold of 5 mmol/l. Glutaminolysis may be enhanced concomitantly by leucine and synergistically by other GDH activators, such as isoleucine (27,28), valine, and methionine (19) through mechanisms explored in this study. Note that other fuels may also contribute to basal release, e.g., free fatty acids (29,30), which are elevated under fasting conditions.

Hypersensitivity of β-cells to leucine stimulation in hypoglycemic patients with insulinoma and in individuals with hypoglycemia induced by sulfonylurea compounds, which was reported 40 years ago (3,31), can be explained by the mechanism considered here. These patients are hypersensitive to leucine and to protein meals. Because the level of ambient glucose of pancreatic β-cells is reduced in these individuals, the GTP:ADP ratios in their pancreatic β-cells are probably critically low, and thus GDH may be sensitized to leucine and other amino acids (31). It was observed in this laboratory more than 20 years ago that the freshly isolated perfused rat pancreas does not respond to 10 or 20 mmol/l leucine (contrasting with observations made with freshly isolated islets) yet it does respond after prolonged perfusion without fuel (32). The mechanism discussed here provides an explanation for this phenomenon of the time-dependent altered leucine sensitivity of the isolated perfused pancreas.

Type 2 diabetes in rats may be another situation where GDH activation may occur in pancreatic β-cells. In rats with type 2 diabetes induced by a neonatal streptozotocin injection, the pancreatic insulin response to glucose is decreased. However, the insulin response to 10 mmol/l leucine is strikingly 15-fold higher than that in control rats. It is also noteworthy that the responses to 10 mmol/l α-ketoisocaproate, the immediate metabolic product of leucine, are similar in diabetic and control rats (33). The difference in leucine stimulation of insulin secretion is conceivably related to defective regulation of mitochondrial GDH, caused by an impaired glucose oxidation and a reduced GTP:ADP ratio in the mitochondria of β-cells from diabetic rats.

Conclusion. The discussion of the literature and of the present data favors the hypothesis that glucose is the critical inhibitory regulator of glutaminolysis in pancreatic β-cells and that enhanced glutaminolysis may contribute in a quantitatively significant manner to basal insulin release. Lowering of blood glucose below the β-cell threshold of 5 mmol/l will almost certainly lead to a redirection of fuel flow favoring amino acid metabolism such that the essential basal release is maintained.

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