The Elevation of Glutamate Content and the Amplification of Insulin Secretion in Glucose-stimulated Pancreatic Islets Are Not Causally Related*

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Glucose increases insulin secretion by raising cytoplasmic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)) in β-cells (triggering pathway) and augmenting the efficacy of Ca\(^{2+}\) on exocytosis (amplifying pathway). It has been suggested that glutamate formed from α-ketoglutarate is a messenger of the amplifying pathway (Maechler, P., and Wollheim, C. B. (1999) Nature 402, 685–689). This hypothesis was tested with mouse islets depolarized with 30 mM KCl (+ diazoxide) or with a saturating concentration of sulfonylurea. Because [Ca\(^{2+}\)]\(_i\) was elevated under these conditions, insulin secretion was stimulated already in 0 mM glucose. The amplification of secretion produced by glucose was accompanied by an increase in islet glutamate. However, glutamine (0.5–2 mM) markedly augmented islet glutamate without affecting insulin secretion, whereas glucose augmented secretion without influencing glutamate levels when these were elevated by glutamine. Allosteric activation of glutamate dehydrogenase by BCH (2-amino 2-norbornane carboxylic acid) lowered islet glutamate but increased insulin secretion. Similar insulin secretion thus occurred at very different cellular glutamate levels. Glutamine did not affect islet [Ca\(^{2+}\)]\(_i\) and pH\(_i\), whereas glucose and BCH slightly raised pH\(_i\), and either slightly decreased (30 mM KCl) or increased (tolbutamide) [Ca\(^{2+}\)]\(_i\). The general dissociation between changes in islet glutamate and insulin secretion refutes a role of β-cell glutamate in the amplification of insulin secretion by glucose.

Tight control of insulin secretion by pancreatic β-cells is critical for glucose homeostasis. This control is exerted by a number of physiological agents, among which circulating nutrients, in particular glucose, play a central role. Glucose regulation of insulin secretion involves two major signaling pathways leading to the production of triggering and amplifying signals respectively (1). The triggering pathway consists in a now well accepted cascade of events. The metabolism of glucose by oxidative glycolysis causes a rise in the ATP/ADP ratio, which closes ATP-sensitive K\(^+\) (K\(^+-\)ATP)\(^3\) channels. The resulting decrease in K\(^+\) conductance leads to membrane depolarization, with subsequent opening of voltage-dependent Ca\(^{2+}\) channels, Ca\(^{2+}\) influx from the extracellular space, and rise in the concentration of free cytoplasmic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\)), which triggers the exocytosis of insulin granules (2–7). However, the triggering action of Ca\(^{2+}\) does not completely explain the stimulation of insulin secretion by glucose. Amplifying signals are also produced in β-cells, and these augment the magnitude of the secretory response, in particular during the sustained phase of stimulation (8–10). Whereas the importance of this pathway is now undisputed (1), the underlying mechanisms remain controversial (1, 11–16).

The present study was prompted by the current controversy surrounding the hypothesis that intracellular glutamate, formed through amination of α-ketoglutarate by glutamate dehydrogenase (GDH), may serve as second messenger in this amplifying pathway (17). The hypothesis was originally based on the observations that glutamate increased insulin release from permeabilized INS-1 cells perfused with elevated fixed concentrations of Ca\(^{2+}\) and ATP, that glucose increased insulinoma and islet cell glutamate content, and that a membrane-permeant ester of glutamate increased insulin secretion from intact cells (17). On the other hand, the hypothesis was contradicted by reports suggesting that glucose does not affect glutamate levels in islets from ob/ob mice (18, 19) or rats (20) and in insulinoma cells (15). In addition, the ability of glutamate dimethyl ester to increase insulin secretion has been attributed to its use as a nutrient by β-cells (21).

Manipulations of the genes coding for glutamate decarboxylase (GAD) and for GDH have also yielded conflicting results. Overexpression of GAD65 in INS-1E cells increased the enzyme activity 26-fold, lowered cell glutamate content by ~40%, but inhibited insulin secretion (40%) at 15 mM glucose only, being without effect at 2.5 and 7.5 mM glucose (22). Overexpression of GAD65 in rat islet cells did not affect insulin secretion in response to 8.3 mM glucose but inhibited the sustained response to 16.7 mM glucose (22). These results were considered to support the role of glutamate in glucose-induced insulin secretion. In contrast, transgenic mice overexpressing GAD65 in β-cells, normally released insulin in response to high glucose; the only defect was an inhibition of first phase insulin release induced by 7 mM glucose. The study was considered not

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§ This abbreviation used is: K\(^+\)-ATP channel, ATP-sensitive K\(^+\) channel; [Ca\(^{2+}\)]\(_i\), cytoplasmic Ca\(^{2+}\) concentration; GDH, glutamate dehydrogenase; GAD, glutamate decarboxylase; pH\(_i\), cytoplasmic pH; BCH, 2-amino 2-norbornane carboxylic acid.
to support the glutamate hypothesis (23). Overexpression of GDH in INS-1E cells increased human growth hormone release (reporter of insulin release by transfected cells) induced by high glucose without affecting basal release. It was assumed, not verified, that the overexpressed enzyme was working in the direction of α-ketoglutarate to glutamate and thus increasing the concentration of glutamate (24). Conversely, overexpression of a mutated, constitutively active GDH in MIN6 cells increased insulin release at low glucose without affecting the response to high glucose. It was now assumed that the enzyme worked in the opposite direction and thus lowered cell glutamate content (25).

Glutamine markedly increases islet glutamate content (20, 26, 27) but does not induce insulin secretion unless GDH is concomitantly activated by leucine or its non-metabolized analogue BCH (28, 29). This lack of effect on insulin secretion is explained by the inability of glutamine alone to produce enough ATP to close K⁺ channels, again, if GDH, is activated (9, 32). This poor effect of glutamine alone has tentatively been ascribed to an alleged alkalinization of cell alkalinization (33).

In the present study, mouse islets were thus incubated or perfused under depolarizing conditions (high K⁺ or sulfonylurea) to study the effects of glucose, glutamine, and BCH to activate GDH, on the amplifying pathway of insulin secretion. Glutamate was measured in the same islets, and possible changes in [Ca²⁺], and pH, were checked for in parallel experiments. A control series of tests was also performed with rat islets to validate our conclusions in a second species.

EXPERIMENTAL PROCEDURES

Solutions and Reagents—The control medium was a bicarbonate-buffered solution containing (mM): NaCl, 120; KCl, 4.8; CaCl₂, 2.5; MgCl₂, 1.2; and NaHCO₃, 24. It was maintained under O₂/CO₂ (94:6) to support the glutamate hypothesis (23). Overexpression of GDH in INS-1E cells increased human growth hormone release (reporter of insulin release by transfected cells) induced by high glucose without affecting basal release. It was assumed, not verified, that the overexpressed enzyme was working in the direction of α-ketoglutarate to glutamate and thus increasing the concentration of glutamate (24). Conversely, overexpression of a mutated, constitutively active GDH in MIN6 cells increased insulin release at low glucose without affecting the response to high glucose. It was now assumed that the enzyme worked in the opposite direction and thus lowered cell glutamate content (25).

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Glutamine (Microselect) was from Fluka (Buchs, Switzerland), diazoxide was a gift of Schering-Plough Avondale (Rathdrum, Ireland), glibenclamide was from Merck AG (Darmstadt, Germany), and NaHCO₃ was from Merck AG (Darmstadt, Germany).

Preparations—The experiments were performed with overnight-cultured mouse islets and freshly isolated rat islets. Mouse islets were aseptically isolated by collagenase digestion of the pancreas of female NMRI mice (25–30 g) followed by hand selection. The islets were then cultured for about 18 h in RPMI 1640 medium containing 10 mM glucose, 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Rat islets were isolated by collagenase digestion of the pancreas of male Wistar rats (300–320 g). They were used immediately after isolation.

Measurements of Insulin Secretion and Islet Glutamate Content—Cultured mouse islets and freshly isolated rat islets were preincubated for 60 min at 37 °C in control medium containing 10 mM glucose. They were then distributed in batches of 12, at room temperature, in control medium containing 3 mM glucose. Each batch was transferred into an Eppendorf conical tube containing 750 μl of test medium and incubated for 30 min at 37 °C. At the end of the incubation, the tubes were gently (10 s) centrifuged, 700 μl of medium were removed and saved for insulin measurement, and 700 μl of cold control medium were added. After gentle shaking to ensure good rinsing of the islets, the tubes were again briefly centrifuged, and 700 μl of medium were removed and discarded. On the islets and the remaining 50 μl of medium, 200 μl of an acid-ethanol mixture, the samples were processed as above.

Insulin and Glutamate Assays—Insulin in the incubation or perfusion medium was measured by radioimmunoassay using rat insulin as a standard. Glutamate in the islet extracts was measured by high performance liquid chromatography system (Beckman) using orthophthalaldialdehyde (OPA) derivatization. 40 μl of islet extract were diluted 5-fold with water, mixed with 70 μl of an OPA-2-mercaptoethanol derivative solution, and incubated 1 min later under anaerobic conditions. The sample was analyzed on a Gold system (Beckman) with which [Ca²⁺±]i was determined with the Gold system (Beckman), and the glutamate content was measured by radioimmunoassay using rat insulin as a standard. Glutamate in the islet extracts was measured by high performance liquid chromatography system (Beckman) using orthophthalaldialdehyde (OPA) derivatization. 40 μl of islet extract were diluted 5-fold with water, mixed with 70 μl of an OPA-2-mercaptoethanol derivative solution, and incubated 1 min later under anaerobic conditions. The sample was analyzed on a Gold system (Beckman) with which [Ca²⁺±]i was determined with the Gold system (Beckman), and the glutamate content was measured by radioimmunoassay using rat insulin as a standard. Glutamate in the islet extracts was measured by high performance liquid chromatography system (Beckman) using orthophthalaldialdehyde (OPA) derivatization. 40 μl of islet extract were diluted 5-fold with water, mixed with 70 μl of an OPA-2-mercaptoethanol derivative solution, and incubated 1 min later under anaerobic conditions. The sample was analyzed on a Gold system (Beckman) with which [Ca²⁺±]i was determined with the Gold system (Beckman), and the glutamate content was measured by radioimmunoassay using rat insulin as a standard.
containing 2 μM glibenclamide, a concentration-dependent increase in islet glutamate content was observed (Fig. 1). As compared with control islets, this increase was particularly large (3- to 5-fold) in 0 and 3 mM glucose, whereas insulin secretion was at the most doubled by 2 mM glutamine in 3 mM glucose (Fig. 1). There was no significant effect of glutamine on insulin secretion in the other conditions. For example, the 2-fold increase in glutamate content induced by 0.5 mM glutamine in 0 or 3 mM glucose, or by 2 mM glutamine in 20 mM glucose was not accompanied by significant changes in insulin secretion (Fig. 1). The situation was strikingly different in the presence of BCH, an activator of glutamate dehydrogenase (28, 42). BCH lowered the islet glutamate content by about 50% in 0 and 3 mM glucose while increasing insulin secretion 2-fold (Fig. 1). When BCH and glutamine were combined, the elevation of islet glutamate normally produced by glutamine was attenuated, but insulin secretion was amplified except in 20 mM glucose, where BCH lowered islet glutamate without affecting insulin secretion (Fig. 1).

A second approach to studying the amplifying action of glucose on insulin secretion consists in holding K⁺/ATP channels open with diazoxide and elevating β-cell [Ca²⁺], by depolarizing the membrane with 30 mM K⁺ (8). Under these conditions, insulin secretion was stimulated even in the absence of glucose (2.29 ± 0.17 ng·h⁻¹·islet⁻¹) and was further increased by 3 mM glucose (3.31 ± 0.25 ng·h⁻¹·islet⁻¹, p < 0.05) and 20 mM glucose (8.26 ± 0.43 ng·h⁻¹·islet⁻¹, p < 0.001) (Fig. 2). The glutamate content of the islets incubated in 0 mM glucose and 30 mM K⁺ averaged 6.15 ± 0.53 pmol·islet⁻¹. It increased 35% (p < 0.05 by Student's t test only) and 132% (p < 0.001) in the presence of 3 and 20 mM glucose, respectively (Fig. 2).

Glutamate consistently elevated islet glutamate content above controls (from 35% by 0.5 mM glutamine in 20 mM glucose to 400% by 2 mM glutamine in 0 mM glucose). However, the only significant effect on insulin secretion was a 50% increase produced by 2 mM glutamine in 0 mM glucose (Fig. 2). Again, the contrast with BCH was striking. BCH alone amplified insulin secretion (50–70%) at 0 and 3 mM glucose while lowering islet glutamate by 65%. When BCH was combined with glutamine the rise in islet glutamate was attenuated, but the increase in insulin secretion was even larger (Fig. 2).

When insulin secretion and the islet glutamate content were compared in the different experimental conditions tested at each glucose concentration (each panel of Figs. 1 and 2), no correlation was ever found. However, when the analysis focused on the differences between 0, 3, and 20 mM glucose, a significant correlation was found for control islets and for islets incubated in the presence of BCH (Fig. 3). There was no correlation in the presence of 0.5 (Fig. 3) or 2 mM glucose alone (not shown). Correlative arguments for and against the glutamate hypothesis can thus be obtained with this commonly used technique of incubation. The weakness of the approach is that the correlation is made between insulin secreted during the whole incubation and the concentration of an islet metabolite at one single late time point. We therefore also used a dynamic system of perfusion to permit closer comparison of the islet glutamate content with the actual rate of insulin secretion.

Study of the Amplifying Pathway in Perifused Mouse Islets—The islets were perifused with a medium containing 7 mM glucose and a concentration of tolbutamide (500 μM) that also completely blocks K⁺/ATP channels (3, 40, 41). Under these conditions the rate of insulin secretion slightly declined with time (Fig. 4A) but remained well above (5–7-fold) the basal rate in the presence of 3 mM glucose alone. Addition of 0.5 mM glutamine to the medium increased the islet glutamate content 2.2-fold without influencing insulin secretion (Fig. 4, A and B). In contrast, BCH lowered islet glutamate and increased insulin secretion ~4-fold. The combination of glutamine and BCH increased insulin secretion 5-fold without changing islet glutamate as compared with controls. Raising the concentration of glucose from 7 to 20 mM also strongly amplified insulin secretion without changing islet glutamate. Thus, there was no correlation between islet glutamate content and the actual rate of insulin secretion at the same moment (Fig. 4B).

We also compared the influence of the different test agents...
on islet \([Ca^{2+}]_i\) and \(pH_i\) (Fig. 5). During continuous depolarization with either a high concentration of sulfonylurea or a high concentration of extracellular \(K^+\), apparent islet \([Ca^{2+}]_i\) slowly but steadily increases at a rate of approximately 2 nM/min (43). This trend must be taken into account when assessing possible effects of test agents. A similar increase was observed in this series during perifusion of control islets with 7 mM glucose and 500 \(\mu M\) tolbutamide (Fig. 5, A and B). For all islets tested under these conditions \((n = 65)\), i.e., during the 5-min period preceding application of test substances, average \([Ca^{2+}]_i\) was 214 ± 2 nM, well above basal \([Ca^{2+}]_i\) in 3 mM glucose alone (70–90 nM, data not shown). Glutamine (0.5 mM) did not affect \([Ca^{2+}]_i\), whereas BCH alone, BCH combined with glutamine, and a rise in the glucose concentration to 20 mM all produced similar changes, described previously for glucose (43) and characterized by a small transient decrease followed by an increase slightly above control values (Fig. 5).

When perifused mouse islets are stimulated with 30 mM KCl in the presence of 3 mM glucose and diazoxide, an initial large peak of insulin secretion is followed by a progressive decline of the secretory rate (9). Only this decline is shown in Fig. 6. Raising the glucose concentration to 20 mM stopped this spontaneous evolution and amplified insulin secretion while augmenting islet glutamate levels. Addition of 0.5 or 2 mM glutamine to the medium failed to influence insulin secretion, although islet glutamate was increased severalfold. In contrast, BCH alone or combined with 0.5 mM glutamine markedly increased insulin secretion while lowering islet glutamate content (Fig. 6). Thus, there was no correlation between islet glutamate and the rate of insulin secretion at the same moment (Fig. 6).

During steady state depolarization with 30 mM KCl in the presence of 3 mM glucose and 250 \(\mu M\) diazoxide, \([Ca^{2+}]_i\) was...
elevated, averaging 286 ± 4 μmol/l for the period of 25–30 min in all islets (n = 84). As already mentioned above, [Ca^{2+}]_{i} slowly increased with time under control conditions (Fig. 7). Raising the concentration of glucose to 20 mM caused a rapid decrease in [Ca^{2+}]_{i}, followed by an incomplete recovery so that [Ca^{2+}]_{i} remained below control values during the period of 40–45 min (Fig. 7, A and B). A similar biphasic decrease in [Ca^{2+}]_{i} occurred upon addition of BCH alone or together with 0.5 mM glutamine. In contrast, glutamine alone, at 0.5 or 2 mM, was without effect on [Ca^{2+}]_{i} (Fig. 7, A and B).

In these depolarized islets, pH_{i} averaged 6.87 ± 0.01 (n = 66) during the period of 25–30 min (Fig. 7). Raising the glucose concentration from 3 to 20 mM increased pH_{i} as reported previously (37). Similar changes were produced by BCH alone and the combination of BCH and 0.5 mM glutamine, whereas glutamine alone had no significant effect (Fig. 7, C and D).

Although 0.5 mM glutamine was generally found not to amplify insulin secretion, a significant effect was disclosed when the islets were subjected to the influence of the different test agents throughout the experiment, and the stimulation with 30 mM K^{+} was applied after 50 min only (Fig. 8). The initial rate of insulin secretion was low and independent of the test agent because of...
the presence of diazoxide (Fig. 8A). Under these conditions, the presence of 0.5 mM glutamine in the medium containing 3 mM glucose resulted in a 2-fold increase in K^+/-induced insulin secretion, whereas islet glutamate was tripled (Fig. 8, A and B). However, 10 mM glucose alone tripled insulin secretion while increasing islet glutamate by 1.5-fold only. At 10 mM glucose, 0.5 mM glutamine doubled islet glutamate without influencing insulin secretion. Under the same experimental conditions, BCH lowered glutamate content in 3 mM glucose but did not increase insulin secretion unless it was combined with exogenous glutamine. In 10 mM glucose the decrease in islet glutamate induced by BCH had no impact on insulin secretion (Fig. 8, A and B).

**Study of the Amplifying Pathway in Incubated Rat Islets**—It has been argued that glutamate metabolism and effects might be different in mouse and rat β-cells (16). The potential role of glutamate in the amplification of insulin secretion was therefore tested with rat islets during depolarization with 30 mM K^+ in the presence of diazoxide (Fig. 9). K^+/-induced insulin secretion in 0 mM glucose amounted to 1.34 ± 0.08 ng·h^{-1}·islet^{-1}. It was increased 45 (p < 0.05) and 310% (p < 0.001) by 3 and 20 mM glucose, respectively. Simultaneously, the islet glutamate content (7.19 ± 0.34 pmol·islet^{-1}) was augmented 45 and 95% (p < 0.001), respectively. Glutamine (2 mM) markedly (4- to 7-fold) elevated islet glutamate without influencing insulin secretion.
secretion. In 0 and 3 mM glucose, BCH alone lowered islet glutamate by 35–40% (p < 0.001 by Student’s t test) and increased insulin secretion. The amplification of insulin secretion was not significantly larger when BCH was combined with glutamine, although the islet glutamate content was 5- to 7-fold higher (Fig. 9). In 20 mM glucose, BCH lowered islet glutamate without changing insulin secretion. These results obtained in rat islets are thus essentially similar to those in mouse islets, with no correlation between islet glutamate and insulin secretion.

**DISCUSSION**

When the medium did not contain glutamine or BCH, the glutamate levels of our mouse or rat islets ranged from 5 to 15 pmol/islet, i.e. 1.7 to 3 mM for an intracellular space of 3 nl/islet (26, 44). These values correspond well with those measured by others in rodent islets (19, 20, 27). They are slightly higher than those in human islets (17) and INS-1E cells (22) and much higher than those in INS-1 cells (17).

We confirm that glutamine markedly increases islet glutamate levels in rat islets (20, 26, 27, 45), and we show that it has the same effect in mouse islets, which can be explained by the activity of the glutaminase (27). We also establish that activation of GDH by BCH lowers islet glutamate content not only in the presence of exogenous glutamine (20, 45) but also in its absence. Because this decrease in islet glutamate by BCH was seen at all glucose concentrations it appears that the flux controlled by GDH consistently goes from glutamate to a-ketoglutarate. Yet, in contrast with several reports (18), the islet glutamate content was 5- to 7-fold higher (Fig. 9). In 20 mM glucose, BCH lowered islet glutamate without changing insulin secretion. These results obtained in rat islets are thus essentially similar to those in mouse islets, with no correlation between islet glutamate and insulin secretion (47). In the presence of exogenous glutamine, reduction of the flux through GDH has no impact on the already high glutamate levels, perhaps because of a decrease in glutaminase activity (45). This interpretation is fully compatible with recent studies of leucine-induced insulin secretion in isolated mouse islets (31) and in patients with an activating mutation of GDH (48).

We emphasize again that our experiments were carried out under conditions selected to study the amplifying action of glucose and other agents on insulin secretion. The paradigm is that β-cell [Ca^{2+}], is steadily elevated independently from the tested agents and is little affected by these agents (1). Because the latter condition is difficult to achieve direct control is important, in particular when novel conditions are being tested. The slight decrease in [Ca^{2+}], produced by high glucose in the presence of high K⁺ and diazoxide and the small increase produced in the presence of a maximally effective concentration of sulfonylurea are similar to those described and discussed previously (43). This study shows that BCH alone or with glutamine had a similar or slightly smaller impact on [Ca^{2+}], than that of glucose, whereas glutamine alone did not change [Ca^{2+}]. One can thus be confident that the amplifying pathway of insulin secretion, as opposed to the triggering pathway that involves a stimulus-induced large increase in [Ca^{2+}], from basal values, was being studied in the present experiments (1). It is also clear that differences in the effects of the tested agents on insulin secretion cannot be attributed to markedly divergent actions on [Ca^{2+}].

In agreement with a number of studies reviewed recently (1, 13), glucose amplified insulin secretion from rat and mouse islets in a concentration-dependent manner with a significant effect already at 3 mM. In incubated mouse islets, the amplification of insulin secretion by glucose was accompanied by an elevation of islet glutamate content such that a tight, direct correlation was found between the two variables, both in the absence and presence of BCH, i.e. at very different glutamate concentrations. These results, therefore, confirm that insulin secretion may be directly correlated with cellular glutamate under selected conditions (22). However, a correlation between a cumulative event (insulin secretion) and a metabolic situation at a single time point (islet glutamate concentration) has a limited significance. Moreover, correlative evidence is never sufficient to establish a causal link. In fact the arguments against a role of glutamate predominate.
Exogenous glutamine (0.5 and 2 mM) consistently increased islet glutamate levels, generally to a larger extent than did glucose, but amplified insulin secretion weakly under three conditions only. In the majority of situations (14/17), glutamine failed to influence insulin secretion. Importantly, the inefficacy of glutamine on insulin secretion was observed in the presence of 3 or 7 mM glucose, i.e., under optimal conditions where metabolism of the sugar provides basal ATP to sustain Ca\(^{2+}\)-induced exocytosis and where the amplifying pathway is far from being maximally activated (1). We therefore conclude that a rise in islet glutamate content is not a sufficient signal to amplify insulin secretion.

In the presence of exogenous glutamine glucose strongly amplified insulin secretion, although the elevated islet glutamate levels did not change. We therefore conclude that intracellular glutamate is at best a permissive signal and that glucose produces one or several other signals that increase the action of Ca\(^{2+}\) on exocytosis. Because similar insulin secretion sometimes occurred at very different intracellular glutamate levels, we can also exclude the remote possibility that the amplifying action of the amino acid would be restricted to a narrow range of concentrations.

In the absence of exogenous glutamine, BCH lowered islet glutamate to below basal values but amplified insulin secretion (0–7 mM glucose) or did not affect it (10–20 mM glucose). Combination of BCH and glutamine attenuated the elevation of glutamate produced by the latter alone but increased or did not affect (in high glucose) insulin secretion. Changing the glucose concentration from 7 to 20 mM in the presence of tolbutamide also amplified insulin secretion 3-fold without modifying islet glutamate. We therefore conclude that an increase in intracellular glutamate is not a necessary signal for the amplification of insulin secretion.

Exogenous NH\(_4\)Cl is known to impair the amplification of insulin secretion by glucose (9). It has therefore been argued that glutamine is an inadequate tool to test the glutamate hypothesis because NH\(_4\) production by the metabolism of the amino acid in \(\beta\)-cells (26) might also blunt the amplifying pathway through an alkalinization of the cytoplasm or other mechanisms (16). These concerns are completely ungrounded at least when glutamine is used at a close to physiological concentration as in this study. First, under no condition did glutamine impair insulin secretion or impair the amplification by glucose. Second, we have reported previously that raising [Ca\(^{2+}\)] in \(\beta\)-cells by high K\(^+\) or tolbutamide lowers pH\(_i\) at low glucose and that a subsequent rise in glucose or the addition of \(\alpha\)-ketoisocaproate (two conditions causing amplification of insulin secretion) (9), increases pH\(_i\) (37, 49). We now show that BCH and the combination of BCH with glutamine produce a similar alkalinization, whereas glutamine alone has no effect.

Our findings, therefore, contradict the predictions and validate the use of glutamine to test the hypothesis. Another important observation is that only those fuels which increase pH\(_i\) also amplify insulin secretion. This correlation is clearly insufficient to establish any causal relationship, but at least it makes untenable the idea that a decrease in intracellular pH is a major contributor of the amplifying and related actions of glucose and other nutrients (33).

In conclusion, glucose augments glutamate content in mouse and rat islets under conditions where the sugar amplifies insulin secretion. However, this increase is neither sufficient nor necessary. Numerous dissociations between changes in islet glutamate and insulin secretion allow us to refute the idea that an increase in \(\beta\)-cell glutamate is an important messenger in the amplification of insulin secretion by glucose.

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