Original Article

Nutrient Control of Insulin Secretion in Isolated Normal Human Islets

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Pancreatic islets were isolated from 16 nondiabetic organ donors and, after culture for ~2 days in 5 mmol/l glucose, were perifused to characterize nutrient-induced insulin secretion in human islets. Stepwise increases from 0 to 30 mmol/l glucose (eight 30-min steps) evoked concentration-dependent insulin secretion with a threshold at 3–4 mmol/l glucose, K_m at 6.5 mmol/l glucose, and V_{max} at 15 mmol/l glucose. An increase from 1 to 15 mmol/l glucose induced biphasic insulin secretion with a prominent first phase (peak increase of ~18-fold) and a sustained, flat second phase (~10-fold increase), which were both potentiated by forskolin. The central role of ATP-sensitive K^+ channels in the response to glucose was established by abrogation of insulin secretion by diazoxide and reversible restoration by tolbutamide. Depolarization with tolbutamide or KCl (plus diazoxide) triggered rapid insulin secretion in 1 mmol/l glucose. Subsequent application of 15 mmol/l glucose further increased insulin secretion, showing that the amplifying pathway is operative. In control medium, glutamine alone was ineffective, but its combination with leucine or nonmetabolized 2-amino-bicyclo [2,2,1]-heptane-2-carboxylic acid (BCH) evoked rapid insulin secretion. The effect of BCH was larger in low glucose than in high glucose. In contrast, the insulin secretion response to arginine or a mixture of four amino acids was potentiated by glucose or tolbutamide. Palmitate slightly augmented insulin secretion only at the supraphysiological palmitate-to-albumin ratio of 5. Inosine and membrane-permeant analogs of pyruvate, glutamate, or succinate increased insulin secretion in 3 and 10 mmol/l glucose, whereas lactate and pyruvate had no effect. In conclusion, nutrient-induced insulin secretion in normal human islets is larger than often reported. Its characteristics are globally similar to those of insulin secretion by rodent islets, with both triggering and amplifying pathways. The pattern of the biphasic response to glucose is superimposable on that in mouse islets, but the concentration-response curve is shifted to the left, and various nutrients, in particular amino acids, influence insulin secretion within the physiological range of glucose concentrations. *Diabetes* 55: 3470–3477, 2006

Glucose homeostasis requires precise control of insulin secretion by pancreatic β-cells. This control is primarily ensured by glucose itself, but it also involves other nutrients and numerous neural and hormonal factors. Our understanding of stimulus secretion coupling in β-cells has considerably progressed through the use of in vitro models such as mouse or rat islets and cell lines. Recent work from various laboratories (rev. in 1) has led to the concept that nutrient-induced insulin secretion is controlled by a hierarchical interaction between two pathways that both depend on β-cell metabolism. The triggering, or ATP-sensitive K^+ (K_{ATP}) channel–dependent, pathway ultimately leads to a rise in the concentration of cytosolic Ca^{2+} that induces exocytosis of insulin granules. The amplifying, or K_{ATP} channel–independent, pathway does not cause a further rise in cytosolic Ca^{2+} concentration but augments the secretory response to the triggering Ca^{2+} signal (1).

Studies of a biologically important phenomenon in experimental models have an obvious intrinsic scientific interest, but they are also expected to help elucidate the pathophysiology of human diseases, such as hyperinsulinemic hypoglycemia and diabetes. Therefore, in vitro studies with human islets are indispensable to assess whether the information collected with models is completely relevant and transposable to the human species.

Insulin secretion from isolated human islets was first measured 35 years ago (2). A number of studies have been published since, but, owing to the paucity of available material, they usually were very focused or fragmentary and based on a small number of islet preparations (3–12). More recently, technical progress in the procedures for isolation of human islets for transplantation have increased the possibilities of research with human β-cells. However, very often insulin secretion is only measured as a test of islet functionality, and the results typically show great variability and poorer responsiveness (small stimulation index) than in rodent islets. Other recent studies have used these islets to determine how human β-cells react and adapt to aggression by agents or conditions (β-cytotoxic drugs, cytokines, or high glucose or fatty acid concentrations) thought to be pathogenic in β-cell death or dysfunction occurring in type 1 or type 2 diabetes (13–22).

Despite the undisputable individual importance of all these contributions, the picture of nutrient control of insulin secretion in human islets is still built on data from heterogenous sources and remains incomplete. Such a basic reference is, however, necessary at a time when
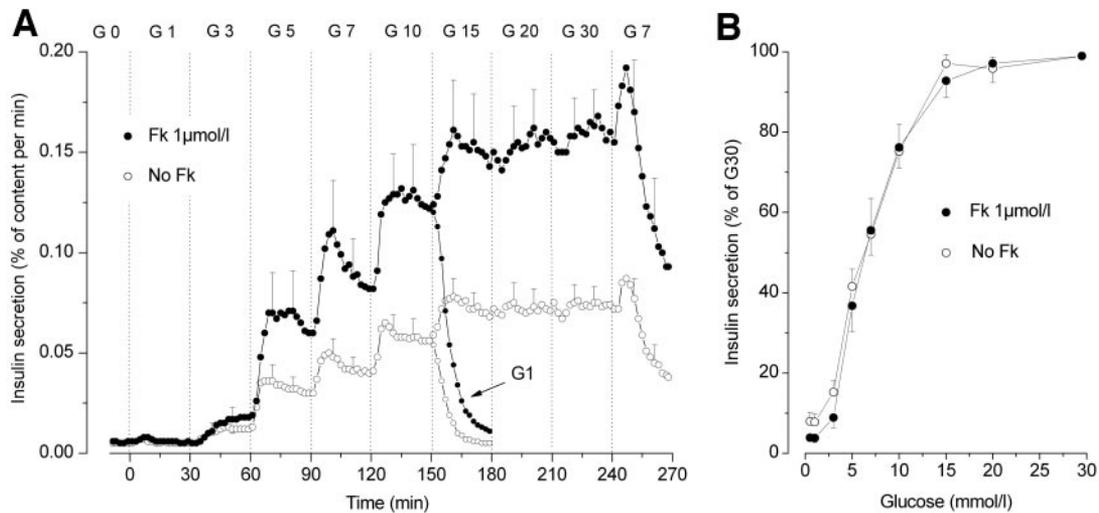
FIG. 1. Concentration dependency of glucose-induced insulin secretion in perifused human islets. *A*: The concentration of glucose (G; in mmol/l) was increased and decreased, as indicated, in a medium containing 1 μmol/l forskolin (Fk) or not. The islets were not exposed to the full range of glucose concentrations. One series started in 0 mmol/l glucose before the glucose concentration was increased stepwise to 10 mmol/l and eventually decreased to 1 mmol/l (lines with small symbols). The other series started in 7 mmol/l glucose before the glucose concentration was increased stepwise to 30 mmol/l and eventually decreased to 7 mmol/l. Insulin secretion rates in 7 and 10 mmol/l glucose from the two series, run in parallel with islets from the same preparation, were then averaged to obtain the complete dose curve for each islet preparation. *B*: Concentration-dependency curve expressed as a percentage of insulin secretion rate in 30 mmol/l glucose. Values are the means ± SE for eight islet preparations (all experiments with and without forskolin were paired).

selective aspects of β-cell (dys)function in islets of type 2 diabetic patients start to be investigated (23–25). The aim of the current study was to define the major characteristics of insulin secretion induced by glucose and other nutrients in normal human islets studied in vitro in a dynamic system of perifusion.

RESEARCH DESIGN AND METHODS

The study was conducted within the framework of institutional programs of basic research and transplantation of human islets, which were approved by the local ethics committee (protocol UCL-HIA-001, authorization 2001/79) in accordance with the principles of the Declaration of Helsinki of 2000 and the guidelines defined by the Belgian authorities.

Organ procurement and islet isolation. This study was performed with islets isolated from 16 human pancreas obtained (between April 2002 and May 2006) from multiorgan donors through the Eurotransplant Network (Leiden, the Netherlands). Abdominal organs were perfused in situ with University of Wisconsin solution (4°C). The whole pancreas still attached to the duodenum (10 of 16) or the isthmus and tail only (6 of 16) were received in our isolation center within 8 h. The characteristics of the donors, of whom none was known to have diabetes, were: male-to-female sex ratio 10:6, mean age 43 years (range 16–60), mean BMI 24.4 kg/m^2 (18.0–29.4).

On arrival, the pancreas was digested with Liberase HI in a polymethylpenten chamber, and the islets were purified on a discontinuous Ficoll-EuroCollins gradient. The whole procedure has been described in detail recently (26). The final purity (assessed by dithizone staining) and viability (assessed by trypan blue exclusion) of the islets (26) averaged 68% (range 30–95) and 87% (70–93), respectively. The fraction of islets dedicated to studies of insulin secretion was cultured at 37°C in RPMI medium containing 10% heat-inactivated FCS, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 5 mmol/l glucose, in an atmosphere of 95% air/5% CO$_2$.

Measurements of insulin secretion. Depending on the amount of islets made available to in vitro studies, the experiments could be performed during 1 day, 2 days (6 of 16), or 3 consecutive days (4 of 16). The mean time elapsed between the start of islet culture and the start of the experiments was 46 h (range 28–88).

The medium used for measurement of insulin secretion contained (in mmol/l): 120 NaCl, 4.8 KCl, 2.5 CaCl$_2$, 1.2 MgCl$_2$, and 24 NaHCO$_3$. It was continuously gassed with a mixture of O$_2$/CO$_2$ (94:6% ratio) and was supplemented with 1 mg/ml BSA (fraction V; Roche, Basel, Switzerland) and appropriate concentrations of glucose and test substances. When the concentration of KCl was increased to 30 mmol/l, that of NaCl was decreased accordingly. Ca^{2+}-free solutions were prepared by substitution of CaCl$_2$ with MgCl$_2$ and the addition of 100 μmol/l EGTA. Palmitate (100 mmol/l in 0.1 mol/l

NaOH) was added at different concentrations to a medium containing different concentrations of fatty acid–free BSA (Roche), as previously reported in detail (27).

At the end of the culture period, the islets were placed in the chambers of the same perifusion system as that used for studying insulin secretion in mouse islets (28). All experiments began with a 60-min equilibration period, usually followed by a 120-min period of stimulation with various test agents (small variations are indicated in the figure legends). Changes in the medium composition are shown at the top of each figure.

The islets used in each experiment were not counted but, at the end, were recovered from the chambers and transferred into acid-ethanol for insulin extraction. Insulin, in the effluent fractions (collected at 2-min intervals) and the diluted extracts, was measured by double-antibody radioimmunoassay using human insulin as a standard. The fractional insulin secretion rate was calculated as the percent of islet insulin content that was secreted per minute. Results are thus presented as the fractional insulin secretion rates (means ± SE) for perifusions performed with the indicated number of different islet preparations. Owing to the limited number of chambers and amount of tissue, it was not always possible to compare the effects of test agents at different glucose concentrations with islets from the same preparations. The number of paired experiments is given in the figure legends.

When not indicated otherwise, reagents were from Sigma (St. Louis, MO) or Merck (Darmstadt, Germany). Diazoxide (a gift from Schering-Plough, Brussels, Belgium) and tolbutamide were added from freshly prepared stock solutions in 0.1 mol/l NaOH. Forskolin (Calbiochem-Behring, San Diego, CA) was added from a 1 mmol/l stock solution in DMSO.

RESULTS

Stimulation of insulin secretion by glucose. After 60 min of perifusion in the absence of glucose, human islets were stimulated by stepwise increases in the glucose concentration (Fig. 1). This resulted in a concentration-dependent increase in insulin secretion between 3 and 15 mmol/l glucose, with no consistent further stimulation at higher glucose levels. Supplementation of the medium with 1 μmol/l forskolin, to increase islet cAMP levels, did not alter the glucose dependency but approximately doubled the secretory response at each glucose concentration above 1 mmol/l (Fig. 1*A*). The secretory rate kept increasing slightly above 15 mmol/l glucose in a few preparations. Without and with forskolin, one islet preparation showed a small, transient response to 1 mmol/l glucose, and four to
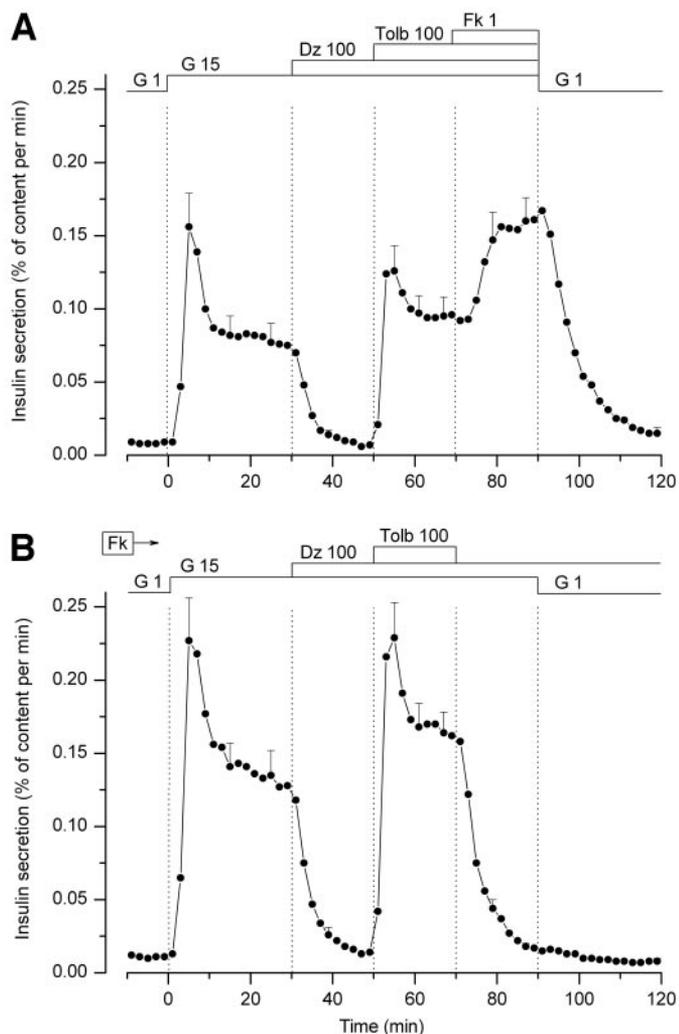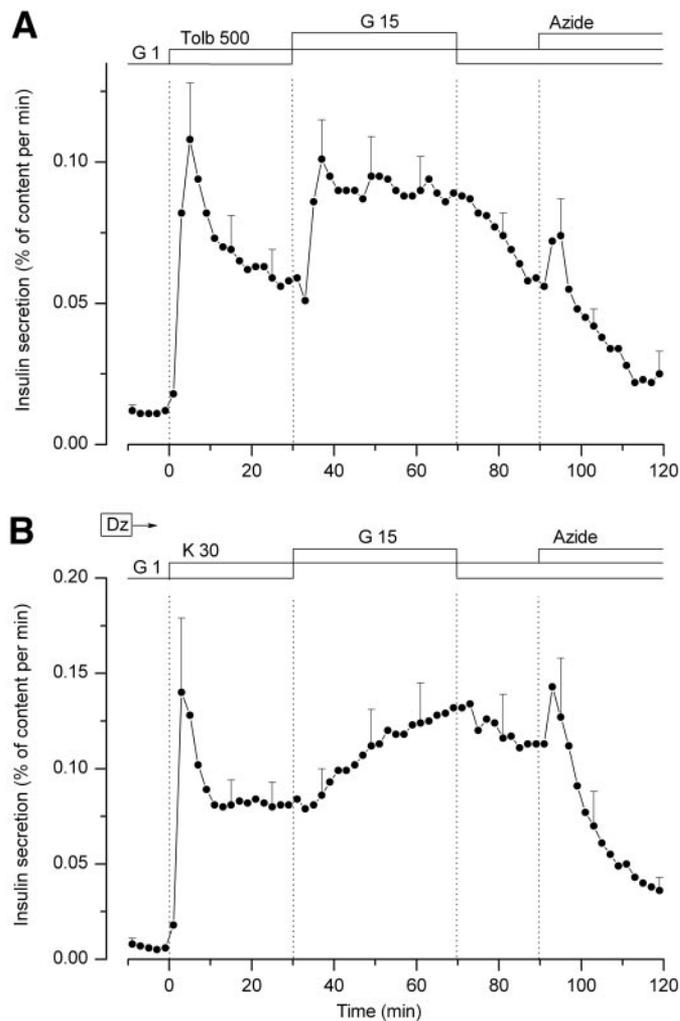
FIG. 2. Kinetics of glucose-induced insulin secretion and effects of drugs acting on K_{ATP} channels in perifused human islets. *A*: The concentration of glucose (G; in mmol/l) was changed between 1 and 15 mmol/l, and diazoxide (Dz; 100 μmol/l), tolbutamide (Tolb; 100 μmol/l), and forskolin (Fk; 1 μmol/l) were added and withdrawn as indicated. *B*: The whole experiment was performed in the presence of 1 μmol/l forskolin (Fk). Values are means ± SE for 14 and 16 islet preparations.

FIG. 3. Increase of insulin secretion by glucose (G; in mmol/l) in perifused human islets depolarized with 500 μmol/l tolbutamide (Tolb) (*A*) or 30 mmol/l KCl (*B*) in the presence of 100 μmol/l diazoxide (Dz). In both series, the glucose concentration was changed between 1 and 15 mmol/l, and 5 mmol/l azide was added as indicated. Values are the means ± SE for seven islet preparations.

five of the eight preparations were stimulated by 3 mmol/l glucose. Glucose-induced insulin secretion was readily reversible when the glucose concentration was lowered from 10 to 1 mmol/l, but sometimes it exhibited a transient further increase when glucose was decreased from 30 to 7 mmol/l (Fig. 1*A*).

These experiments thus show that the glucose-dependency curve of insulin secretion by human islets in vitro is sigmoidal but shifted to the left compared with rodent islets, with a threshold at ~3–4 mmol/l, half-maximum stimulation at ~6.5 mmol/l, and maximum stimulation at ~15 mmol/l (Fig. 1*B*). Basal (no glucose) and maximal (30 mmol/l glucose) rates of insulin secretion averaged 0.005 ± 0.001 and 0.074 ± 0.008% per min (~15-fold increase) in the absence of forskolin and 0.006 ± 0.001 and 0.161 ± 0.019% per min (~27-fold increase) in the presence of forskolin.

Stimulation of human islets by a rapid increase in the glucose concentration from 1 to 15 mmol/l induced biphasic insulin secretion characterized by a sharp and marked first phase and a lower but sustained second phase (Fig. 2*A*). Basal, peak first-phase (5–8 min), and second-phase

(21–30 min) insulin secretion averaged 0.008 ± 0.001, 0.147 ± 0.020 (~18-fold increase), and 0.078 ± 0.013% per min (~10-fold increase). The addition of diazoxide, an effective opener of K_{ATP} channels in human β-cells (29), suppressed glucose-induced insulin secretion, whereas subsequent closure of the channels by tolbutamide (29,30) completely reversed the inhibition. The addition of forskolin augmented the secretory rate within 5 min. Finally, withdrawal of all drugs and return to a nonstimulatory medium lowered the insulin secretion rate without delay (Fig. 2*A*). The presence of forskolin during the whole experiment augmented the amplitude of first-phase (~50%) and second-phase (~70%) glucose-induced insulin secretion, but it did not alter the kinetics nor affect the inhibitory and stimulatory effects of diazoxide and tolbutamide (Fig. 2*B*). No significant alteration of glucose-induced insulin secretion was observed over the short (2–3 days) period of islet culture.
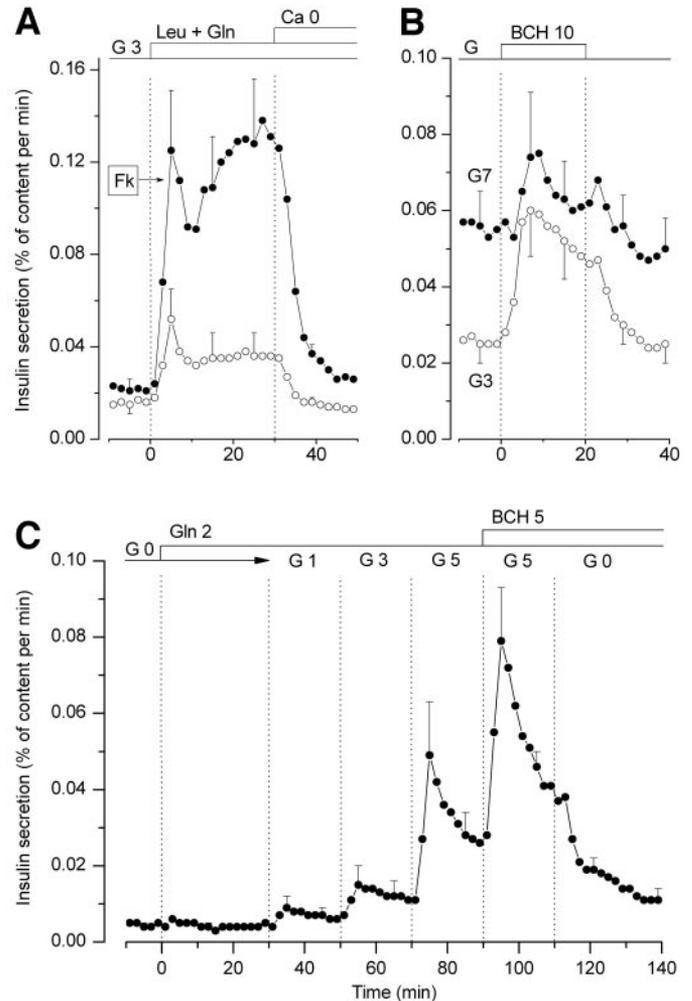
We next tested whether glucose can increase insulin secretion via a K_{ATP} channel–independent or amplifying pathway. Figure 3 shows that tolbutamide, purportedly used at the high concentration of 500 μmol/l to block all K_{ATP} channels (29,30), and 30 mmol/l KCl, used to depo-
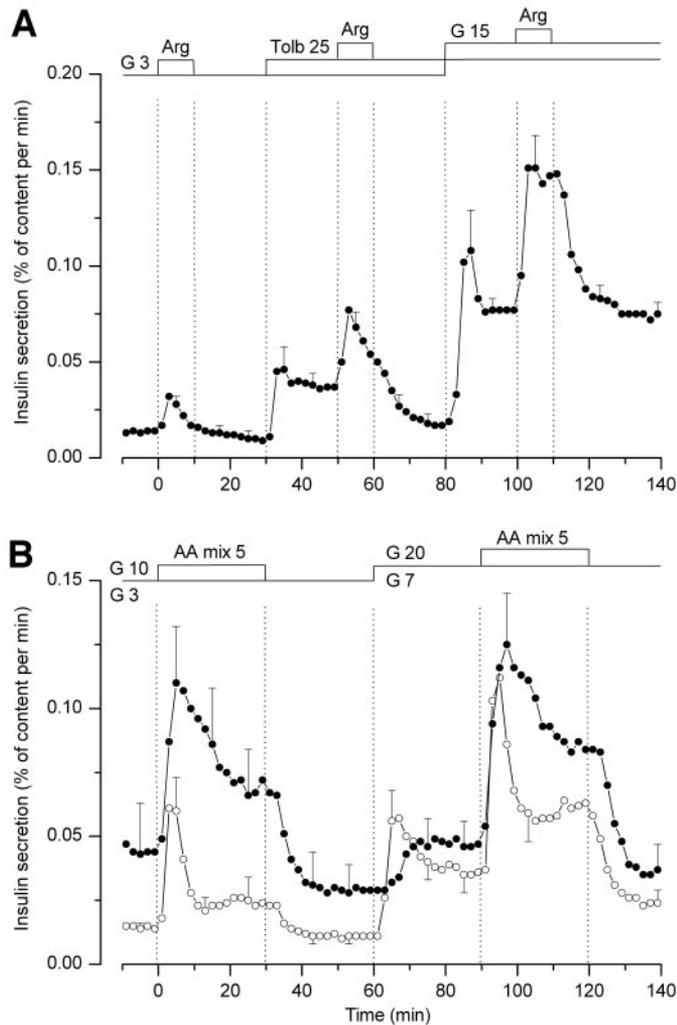
FIG. 4. Influence of glucose (G; in mmol/l) on insulin secretion induced by arginine (Arg) or a mixture of amino acids (AA mix) in perifused human islets. *A*: Three pulses of 10 mmol/l arginine were applied in 3 mmol/l glucose, 3 mmol/l glucose + 25 µmol/l tolbutamide (Tolb), or 15 mmol/l glucose + 25 µmol/l tolbutamide as indicated. Values are the means ± SE for six islet preparations. *B*: Two stimulations with a 5 mmol/l mixture of amino acids (1 mmol/l alanine, 1.5 mmol/l glutamine, 1 mmol/l leucine, and 1.5 mmol/l lysine) were applied in 3 and 7 mmol/l glucose or in 10 and 20 mmol/l glucose as indicated. Values are the means ± SE for five islet preparations (three were paired). ○, 3 and 7 mmol/l glucose; ●, 10 and 20 mmol/l glucose.

FIG. 5. Effects of various amino acids on insulin secretion in perifused human islets. *A*: Leucine and glutamine (5 mmol/l each) were added to a medium containing 3 mmol/l glucose (G; in mmol/l) alone or with 1 µmol/l forskolin (Fk). At the end, $CaCl_2$ was omitted from the medium and 100 µmol/l EGTA added. Values are the means ± SE for five islet preparations (two were paired). *B*: BCH (10 mmol/l) was added to a medium containing 3 or 7 mmol/l glucose. Values are the means ± SE for four islet preparations (three were paired). *C*: Glutamine (Gln; 2 mmol/l) and BCH (5 mmol/l) were added, and glucose was increased or decreased stepwise (between 0 and 5 mmol/l) as indicated. Values are the means ± SE for six islet preparations.

larize β-cells despite opening of K_{ATP} channels by diazoxide (31), rapidly increased insulin secretion in 1 mmol/l glucose. Subsequent stimulation with 15 mmol/l glucose further increased the secretory rate. This effect was faster and more completely reversible in the presence of tolbutamide (Fig. 3*A*) compared with KCl plus diazoxide (Fig. 3*B*). In both cases, inhibition of ATP production by azide markedly inhibited insulin secretion.

Stimulation of insulin secretion by amino acids. Arginine (10 mmol/l) induced a small monophasic secretion of insulin in human islets perifused with a medium containing 3 mmol/l glucose (Fig. 4*A*). The response to arginine was larger in the presence of 25 µmol/l tolbutamide, which itself caused a sustained increase in secretion, and even more so in the presence of 15 mmol/l glucose (Fig. 4*A*). The secretory responses to arginine (areas above prestimulatory values) averaged (% of content in 10 min): 0.11 ± 0.01 in 3 mmol/l glucose, 0.23 ± 0.02 in 3 mmol/l

glucose plus tolbutamide, and 0.61 ± 0.08 in 15 mmol/l glucose plus tolbutamide.

We then studied the effects of a mixture of four amino acids (1 mmol/l alanine, 1 mmol/l leucine, 1.5 mmol/l lysine, and 1.5 mmol/l glutamine) chosen as representative of the major classes of amino acids and used at a total concentration of 5 mmol/l (approximately twofold higher than the total concentration of amino acids in human plasma) (32). As shown in Fig. 4*B*, the amino acid mixture was very efficient in inducing insulin secretion, and its effect increased with the concentration of glucose, at least up to 10 mmol/l. Alanine alone (10 mmol/l) rapidly and reversibly increased insulin secretion by human islets, and its effect was larger in 7 than 3 mmol/l glucose: 0.54 ± 0.10 vs. 0.16 ± 0.04% of content in 20 min (data not shown).

In rodent islets, the combination of leucine and glutamine potently increases insulin secretion through allosteric stimulation of glutamate dehydrogenase (by leucine) and provision of carbons to the Krebs cycle (by
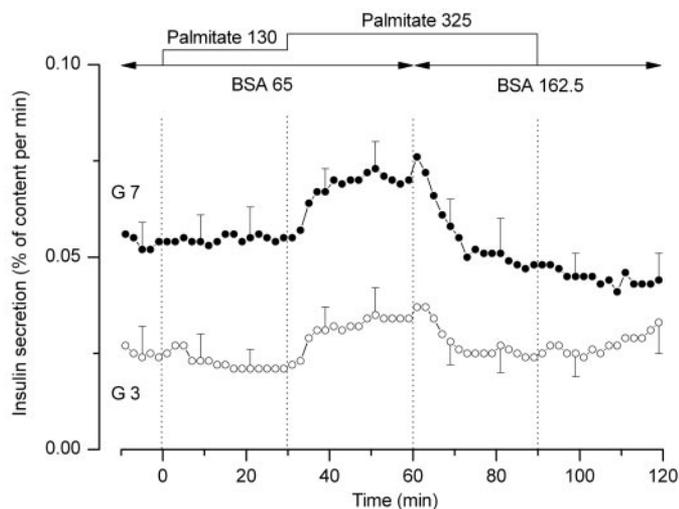
FIG. 6. Effects of palmitate on insulin secretion in perifused human islets. The medium contained 3 or 7 mmol/l glucose (G) throughout and 65 or 162.5 μmol/l fatty acid–free BSA as indicated. Palmitate was added at 130 or 325 μmol/l as shown, resulting in fatty acid–to-BSA ratios of 2, 5, and 2. Values are the means ± SE for six islet preparations (all were paired).

both leucine and glutamine) (33,34). As shown in Fig. 5*A*, the two amino acids (5 mmol/l each) induced biphasic insulin secretion from human islets in the presence of 3 mmol/l glucose. Their effect was markedly potentiated by forskolin and abolished by omission of extracellular Ca^{2+}. The nonmetabolized leucine analog 2-amino-bicyclo [2,2,1]-heptane-2-carboxylic acid (BCH), which also activates glutamate dehydrogenase (34), was very efficient alone (at 10 mmol/l), but its effect was smaller in 7 than 3 mmol/l glucose (Fig. 5*B*), possibly because glutamate dehydrogenase activity decreases at higher glucose levels (33).

Alone, glutamine does not increase insulin secretion in rodent islets (33,35). As shown in Fig. 5*C*, supplementation of a glucose-free medium with 2 mmol/l glutamine did not influence basal insulin secretion in human islets or obviously alter their response to increasing glucose concentrations. Only one of six islet preparations was stimulated by 1 mmol/l glucose, and four of six responded to 3 mmol/l glucose. Subsequent addition of 5 mmol/l BCH rapidly evoked insulin secretion, which immediately decreased on removal of glucose (Fig. 5*C*).

Stimulation of insulin secretion by fatty acids. The acute effects of fatty acids on insulin secretion from rodent islets depend on the concentration of their unbound fraction rather than on their total concentration (27,36). We therefore tested two concentrations of palmitate in the presence of two concentrations of BSA. As shown in Fig. 6, in the presence of 65 μmol/l BSA (4.4 mg/ml), palmitate was ineffective at 130 μmol/l and caused a 35–50% increase in insulin secretion at 325 μmol/l. This stimulation was rapidly abolished by increasing the albumin concentration 2.5-fold, while keeping constant the concentration of palmitate; thus, the ratio of palmitate to BSA was lowered from 5 to 2. The effects were similar in 3 and 7 mmol/l glucose. The insulin-secreting action of palmitate was thus clearly dependent on its unbound fraction.

Stimulation of insulin secretion by various metabolites. Unlike rat islets, mouse islets do not secrete insulin when stimulated by membrane-permeant dimethylsuccinate. This species difference has been attributed to the
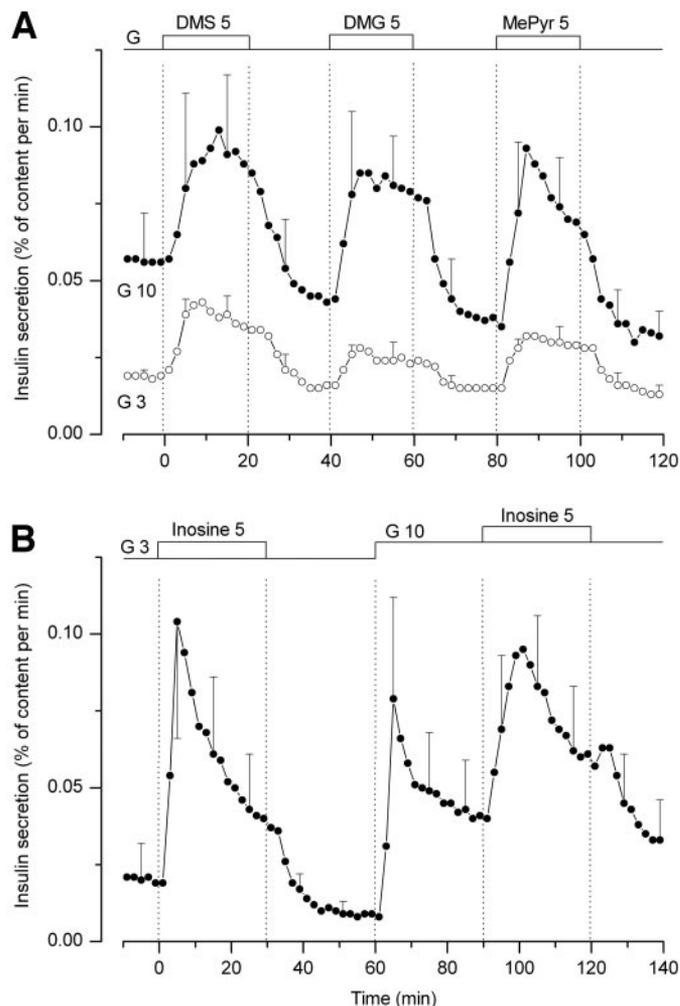
FIG. 7. Effects of various substrates on insulin secretion in perifused human islets. *A*: Dimethylsuccinate (DMS), dimethylglutamate (DMG), and methylpyruvate (MePyr) were successively added (5 mmol/l) to a medium containing 3 or 10 mmol/l glucose (G) as indicated. Values are the means ± SE for five and four islet preparations (two were paired). *B*: Two stimulations with 5 mmol/l inosine were applied in 3 and 10 mmol/l glucose as indicated. Values are the means ± SE for four islet preparations.

lack of malic enzyme in mouse β-cells (37). As shown in Fig. 7*A*, 5 mmol/l dimethylsuccinate reversibly increased insulin secretion in human islets at 3 and 10 mmol/l glucose. This effect is in agreement with the presence of malic enzyme in human islets (37). As in both mouse and rat islets, dimethylglutamate and methylpyruvate (5 mmol/l) were effective insulin secretagogues in human islets (Fig. 7*A*). Whereas the effect of dimethylglutamate is probably entirely attributable to changes in β-cell metabolism (34), that of methylpyruvate may include both changes in metabolism (38) and a direct inhibitory action on K_{ATP} channels (39).

In mouse islets, the purine ribonucleoside inosine is split to hypoxanthine and ribose-1-phosphate, which is then metabolized (40), leading to insulin secretion by mechanisms largely resembling those of glucose-induced secretion (41). As shown in Fig. 7*B*, 5 mmol/l inosine potently and reversibly increased insulin secretion in human islets at both 3 and 10 mmol/l glucose. The effect of inosine was not smaller than that of an equivalent glucose concentration. Pyruvate, lactate, and fructose (10 mmol/l)

did not increase insulin secretion in two islet preparations perifused at 3 and 10 mmol/l glucose (data not shown).

DISCUSSION

In this study, we show that glucose-stimulated insulin secretion in isolated human islets is characterized by a sigmoidal concentration-dependency curve that is shifted to the left compared with mouse or rat islets. This shift is entirely compatible with the lower plasma glucose levels in humans versus rodents. Only one similar, complete concentration-dependency curve has previously been published. It was based on incubation of islets from three subjects and had a small stimulation index (fourfold), but it was also shifted to the left compared with rodent islets (5). Comparing the glucose dependency of insulin secretion in vitro and in vivo is problematic for several reasons, including the confounding influence of various factors on β-cells in situ, the necessity of using deconvolution techniques to estimate secretion rates from changes in plasma insulin or C-peptide concentrations, and the difficulty (impossibility) of imposing a wide range of blood glucose concentrations permitting definition of threshold, K_m, and V_{max}. Nevertheless, there is agreement that the major changes in plasma insulin levels (42,43) or insulin secretion rates (44) occur between 5 and 10 mmol/l glucose, which is in keeping with a K_m at 6.5 mmol/l in vitro. The amplitude of the changes in the insulin secretion rate that we measured in vitro is also commensurate with that of changes observed in vivo (44).

The maximally effective concentration of glucose in vivo is not precisely known because blood glucose levels are only exceptionally raised higher than 15 mmol/l. When this occurred, no saturation of plasma insulin levels was observed (42), or a half-maximum elevation of C-peptide levels was calculated at 14 mmol/l glucose (45). Our observation that insulin secretion by human islets plateaued at ~15 mmol/l glucose in vitro, when the glucose was tested alone, cannot be explained by exhaustion because forskolin doubled the insulin secretory rate to a value corresponding to ~10% of the islet insulin content per hour. Moreover, an amino acid mixture caused a further increase in insulin secretion in 20 mmol/l glucose. The influence of nonglucose stimuli (nutrient and others) could thus explain the difference between in vitro and in vivo observations, if the latter prove to adequately reflect insulin secretion rates.

A threshold glucose concentration at 3–4 mmol/l in isolated human islets is clearly lower than in rodent islets. This observation may explain why in this and other studies, several agents, like arginine (25,46), alanine, an amino acid mixture, and palmitate (12), were effective at this low glucose concentration, whereas they are not effective in rodent islets. It also helps our understanding of how insulin secretion can be controlled in vivo at low physiological blood glucose levels. The threshold has not been defined in vivo, but the insulin secretion rate increases between 3 and 5 mmol/l glucose in fasted subjects (47). Finally, our findings have practical implications for the evaluation of the functionality of human islets. Traditional measurements of insulin secretion at 3 mmol/l glucose do not always identify the basal secretory rate, which contributes to the low stimulation index reported in many studies.

Glucose-induced insulin secretion in rodent islets depends on the metabolism of the glucose, which is largely controlled by the high-K_m glucokinase (48). Human β-cells also contain glucokinase (49,50) and metabolize glucose in a concentration-dependent manner between 1 and 15 mmol/l glucose (5,50) to produce an increase in the ATP-to-ADP ratio between 1 and 10 mmol/l glucose (51). The occurrence of insulin secretion at lower glucose concentrations in human β-cells compared with rodent β-cells does not imply the participation of another phosphorylating enzyme. The properties of glucokinase permit control of phosphorylation between 1 and 3 mmol/l glucose (48). It is also important to bear in mind that the threshold of glucose-stimulated insulin secretion is reached at the glucose concentration where the rate of metabolism closes enough K_{ATP} channels to depolarize the membrane up to the threshold potential for activation of voltage-dependent Ca^{2+} channels. Subtle differences in the number or properties of ionic channels might explain the shift between human and rodent β-cells.

Stimulation of human islets by an abrupt increase in the glucose concentration induced biphasic insulin secretion with a prominent first phase followed by a sustained, flat second phase. This time course, also observed in other studies (7,13,21,52), is similar to that of insulin secretion in the perfused mouse pancreas (53,54) or perifused mouse islets (28,55) and contrasts with the ascending second phase seen in rat preparations (53,55,56). It has recently been reported that in mice, as in humans, plasma insulin levels increase with a biphasic time course and an increasing second phase during hyperglycemic clamps (28,57). The pattern of the in vitro and in vivo insulin responses is thus apparently discrepant in both species.

Not only are the kinetics of glucose-stimulated insulin secretion similar in perifused human and mouse islets, but the fractional rates of secretion are also comparable during both the first and second phases (28). The variability of the responses of human islets is often emphasized. In the 14 perifusions studying insulin secretion in response to 15 mmol/l glucose alone (Fig. 2A), the second phase was characterized by a coefficient of variation (CV) of 67%, compared with a CV of 46% in 20 similar perifusions with mouse islets (28). Based on these large numbers of experiments, the difference is not a major one, perhaps because insulin secretion is normalized to the islet insulin content.

Three major features of the glucose control of insulin secretion in rodent islets also characterize human islets. First, the critical role of K_{ATP} channels is convincingly demonstrated by the abrogation of insulin secretion with diazoxide and its reversible restoration with tolbutamide. Patch-clamp studies have shown that K_{ATP} channels in human β-cells are similar to those in rodent β-cells (29,30). Second, the K_{ATP} channel–independent amplifying pathway is also functional. Thus, in agreement with a study using incubated human islets (10), glucose increased insulin secretion when β-cell K_{ATP} channels were completely closed with a sulfonylurea or held open with diazoxide in the presence of depolarizing KCl. Measurements of β-cell cytosolic Ca^{2+} will be necessary to establish whether this amplification reflects an augmentation of secretion without a further rise in the triggering Ca^{2+} signal, as in rodent islets (31). Third, glucose potentiates insulin secretion induced by arginine or a mixture of amino acids. Glucose potentiation of insulin secretion induced by nonmetabolized nutrients, such as arginine, has attracted much attention because the process is impaired in type 2 diabetic patients (58,59). Using mouse islets, we have recently explained the phenomenon by both an augmen-

tation of cytosolic Ca^{2+} and an amplification of the response to Ca^{2+} (60). Our results showing that arginine-induced insulin secretion is also potentiated by tolbutamide, albeit to a lesser extent than by glucose, are in keeping with in vivo studies in humans (61) and in vitro studies with rodent models (details and mechanisms in 60).

Amino acids participate in physiological control of insulin secretion. Regulation of β-cell glutaminolysis at the level of glutamate dehydrogenase is important for that process. Glutamate dehydrogenase can be activated by leucine or its nonmetabolized analog BCH, and it can be inhibited by glucose via the increase in ATP and GTP (33). The combination of leucine and glutamine doubled insulin secretion in 3 mmol/l glucose, but BCH alone was at least as effective, suggesting that the intracellular pool of glutamine is sufficient after islet culture, a conclusion also supported by the lack of effect of 2 mmol/l glutamine on insulin secretion induced by threshold glucose concentrations. One observation points to the preeminence of glucose over glutamine metabolism for insulin secretion. Removal of glucose from a medium containing both BCH and glutamine immediately and markedly decreased insulin secretion, indicating that the positive effect of 5 mmol/l glucose predominates over the inhibitory action that it might exert on glutamate dehydrogenase. However, this observation does not detract from the relevance of amino acids for the control of insulin secretion in vivo. Thus, at the slightly supraphysiological concentration of 5 mmol/l, a mixture of four amino acids was very potent in increasing insulin secretion from a threshold to a maximally effective concentration of glucose (3–20 mmol/l).

The role of fatty acids for the acute regulation of insulin secretion in vivo is controversial, but it is generally considered to be less than that of amino acids. Our in vitro data directly support this view because effects of palmitate on insulin secretion by perifused human islets were only seen at the nonphysiological high palmitate-to-BSA ratio of 5. Palmitate and other fatty acids, used at a fatty acid–to-BSA ratio of 3.4, have been found to increase insulin secretion by human islets incubated in 3.3–16.7 mmol/l glucose (12). In this respect, human islets thus behave like mouse and rat islets (27,36). We acknowledge, however, that some in vivo studies reported an increase in the insulin secretion rate at a stable 5 mmol/l glucose when an infusion of fatty acids plus heparin acutely raised the fatty acid–to-albumin ratio to ~2.0 (62). This ratio, however, rarely exceeds 1 under physiological conditions.

In conclusion, nutrient-induced insulin secretion in normal human islets is larger than often reported. Its characteristics globally resemble those observed in rodent islets, with both triggering and amplifying pathways. The pattern of the biphasic response to glucose is superimposable on that in mouse islets, but the concentration-response curve is shifted to the left, and various nutrients, in particular amino acids, influence insulin secretion at physiological glucose concentrations. Overall, this study provides a reference for the characterization of the secretory defects in islets from type 2 diabetic patients.

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