A Nuclear Magnetic Resonance–Based Demonstration of Substantial Oxidative L-Alanine Metabolism and L-Alanine–Enhanced Glucose Metabolism in a Clonal Pancreatic β-Cell Line

Metabolism of L-Alanine Is Important to the Regulation of Insulin Secretion

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Early experiments indicated that islet β-cells substantially metabolized L-alanine but that insulin secretion was largely unaffected by the amino acid. It was subsequently demonstrated using more intricate studies that L-alanine is a strong stimulus to insulin secretion in the presence of glucose in normal rodent islets and β-cell lines. Using ^{13}C nuclear magnetic resonance (NMR), we have demonstrated substantial oxidative metabolism of L-alanine by the clonal β-cell line BRIN-BD11, with time-dependent increases in production of cellular glutamate and aspartate. Stimulatory effects of L-alanine on insulin secretion were attenuated by the inhibition of β-cell oxidative phosphorylation using oligomycin. Additionally, we detected substantial production of lactate, alanine, and glutamate from glucose (16.7 mmol/l) after 60 min. On addition of 10 mmol/l L-alanine to a stimulus of 16.7 mmol/l glucose, the utilization rate of glucose increased ~2.4-fold. L-Alanine dramatically enhanced NMR-measurable aspects of glucose metabolism (both oxidative and nonoxidative). The enhanced rate of entry of glucose-derived pyruvate into the tricarboxylic acid (TCA) cycle in the presence of alanine may have stimulated rates of generation of key metabolites, including ATP, which affect the insulin secretory process. Thus L-alanine metabolism, in addition to the enhancing effect on glucose metabolism, contributes to the stimulatory effects of this amino acid on insulin secretion in vitro. *Diabetes* 51:1714–1721, 2002

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AIB, α-aminoisobutyric acid; $[Ca^{2+}]_i$, intracellular free Ca^{2+}; NMR, nuclear magnetic resonance; PC, pyruvate carboxylase; PDH, pyruvate dehydrogenase; TCA, tricarboxylic acid.

Despite considerable research effort, the mechanisms by which some amino acids stimulate insulin secretion alone, or synergistically enhance glucose-stimulated insulin secretion, remain unknown. In contrast, the key mechanism by which glucose enhances insulin secretion is known in considerable detail. Glucose enters the cell by facilitated diffusion and is converted by the glycolytic pathway to pyruvate and subsequently via pyruvate dehydrogenase (PDH) to acetyl CoA or via pyruvate carboxylase (PC) to oxaloacetate. Approximately equal amounts of pyruvate are utilized by the latter two enzymes (1–3). After subsequent conversion to citrate, the glucose carbon can be fully oxidized to CO_2 in the tricarboxylic acid (TCA) cycle (4). This results in an increase in the ATP/ADP ratio, closure of ATP-sensitive K^+ channels, membrane depolarization, opening of voltage-activated Ca^{2+} channels, Ca^{2+} influx, a rise in intracellular free Ca^{2+} ($[Ca^{2+}]_i$), and activation of the exocytotic machinery (4). The opening of Ca^{2+} channels is intermittent, oscillating with the membrane potential, and therefore results in oscillations of $[Ca^{2+}]_i$ (5,6) that in turn trigger oscillations of insulin secretion (7).

Amino acids may enter the β-cell via specific transport proteins, which are usually Na^+ dependent (8,9). There are three fundamental mechanisms by which amino acids may stimulate insulin secretion (10,11): *1*) direct depolarization of the plasma membrane by transport of cationic amino acids such as L-arginine, resulting in opening of voltage-activated L-type Ca^{2+} channels; *2*) cotransport with Na^+, resulting in opening of voltage-activated L-type Ca^{2+} channels; and *3*) metabolism of the amino acid (e.g., L-leucine), resulting in an elevation of the ATP/ADP ratio, which results in membrane depolarization and Ca^{2+} influx following closure of the ATP-sensitive K^+ channel as described above. It has been reported that L-alanine induces insulin secretion from β-cell–rich *ob/ob* mouse islets and the clonal RINm5F cell line by the second mechanism described above (12,13). In a recent study, it was demon-

strated that L-alanine induced insulin secretion from the clonal β-cell line BRIN-BD11 at a substantially greater rate than all other amino acids tested, including L-arginine (11). Because a high activity of the first enzyme of L-alanine metabolism, alanine aminotransferase, has been described recently in purified rat islet β-cells (14), we investigated the pathway of L-alanine metabolism in BRIN-BD11 cells to ascertain whether substantial oxidative metabolism occurred. Through elevation of the ATP/ADP ratio, this would then affect early as well as late signaling events in the secretory process. It has been reported additionally that L-alanine synergistically enhances glucose-stimulated insulin secretion from BRIN-BD11 cells (11,15), raising the possibility that β-cell glucose metabolism is enhanced in the presence of this amino acid.

Over the last two decades, ^{13}C nuclear magnetic resonance (NMR) has been used successfully to investigate metabolic pathways and determine carbon fluxes in living cells (16–18). Despite the success of this powerful technique, very little attention has been devoted to its application in investigations of the metabolism of glucose and other nutrient fuels in pancreatic β-cells (19–21). Previous research using NMR to monitor metabolism in diabetes has concentrated mainly on studies of the liver (22,23). In this study, we investigated the utilization and metabolism of L-[3-^{13}C]alanine and D-[1-^{13}C]glucose in the insulin-secreting cell line BRIN-BD11. We present ^{13}C NMR data that provide evidence for substantial metabolism of L-alanine by BRIN-BD11 β-cells and enhancement of glucose metabolism by L-alanine. These cells have relatively high rates of amino acid metabolism compared with all other β-cell lines tested, and thus are ideally suited to NMR-based experiments (L.B., P.N., unpublished observations). Previous studies have shown that BRIN-BD11 cells display insulin secretory responses to a wide range of secretogogues, including glucose, amino acids, hormones, neurotransmitters, sulfonylureas, and other insulinotropic drugs (11,24–28)

RESEARCH DESIGN AND METHODS

Reagents. All chemicals were obtained from Sigma-Aldrich Chemical (Poole, Dorset, U.K.) except for L-[3-^{13}C]alanine and D-[3-^{13}C]glucose, which were obtained from Goss Scientific (Great Baddow, Essex, U.K.). Culture media, fetal bovine serum, and plastic were obtained from Gibco (Glasgow, U.K.).

Culture of BRIN-BD11 cells. Clonal insulin-secreting BRIN-BD11 cells were maintained in RPMI-1640 tissue culture medium with 10% (vol/vol) fetal bovine serum, 0.1% antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin), and 11.1 mmol/l D-glucose, pH 7.4. The origin and characteristics of BRIN-BD11 cells are described in detail elsewhere (15,24–28). The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air using a Forma Scientific incubator. The cells were cultured in 50–70 ml tissue culture medium in T175 sterile tissue culture flasks. Cells were preincubated at 37°C in monolayer culture for 20 min at 1.1 mmol/l D-glucose and then incubated at an approximate density of 2.4×10^8 cells per 60 ml Krebs Ringer bicarbonate buffer (115 mmol/l NaCl, 4.7 mmol/l KCl, 1.28 mmol/l $CaCl_2$, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4 \cdot 7H_2O$, 10 mmol/l $NaHCO_3$, 5 g/l BSA, pH 7.4) supplemented with one of the following: 10 mmol/l L-[3-^{13}C]alanine, 16.7 mmol/l D-[1-^{13}C]glucose, 10 mmol/l L-[3-^{13}C]alanine plus 16.7 mmol/l D-glucose, or 16.7 mmol/l D-[1-^{13}C]glucose plus 10 mmol/l L-alanine. Control experiments were also performed with 10 mmol/l L-alanine or 16.7 mmol/l D-glucose. After incubation for 20 min, 1 h, or 2 h, the cells were extracted with 6% PCA, and debris was removed from the flask using a cell scraper. After centrifugation, the supernatant was neutralized with KOH, the pellets were soaked overnight in 0.1 mol/l NaOH, and the protein concentration was determined using the Lowry method. The neutralized supernatant was subsequently treated with Chelex-100 resin (to remove paramagnetic ions) and lyophilized. An NMR sample was prepared (addition of 3 ml of 100 mmol/l potassium phosphate buffer, pH 7.0, containing equimolar quantities of K_2HPO_4 and KH_2PO_4 with 0.33 ml D_2O), and the pH was adjusted to 7.0. A coaxial capillary tube containing 1% vol/vol dioxane in water was used as an external signal intensity reference for quantification of the NMR spectra. Also, a solution of L-alanine, L-glutamate, lactate, and D-glucose, each at a concentration of 100 mmol/l, was prepared and used to quantitate concentrations of metabolites in the ^{13}C spectra.

NMR spectroscopy. Proton-decoupled ^{13}C spectra were acquired on either a Bruker DRX 500 spectrometer or a Varian UnityPlus 400 spectrometer using a 10-mm probe. Typically, spectra were acquired with 32,000 data points using 9.4-μs pulses (90° pulse angle), 260 ppm spectral width, 2-s relaxation delay, and 12,000 scans. Spectra were recorded at 25°C. Chemical shifts in aqueous media were referenced to tetramethylsilane at 0 ppm as previously described (29). Exponential multiplications with 2 Hz line broadening were perfomed using WIN-NMR software. The assignments of the intermediate metabolites were made by comparison with chemical shift tables in the literature (30) or by addition of 100 mmol/l unlabeled amino acids to the NMR sample. The amount of ^{13}C in each resonance was evaluated by integration of the extract peaks and the corresponding peaks in the standard sample relative to the dioxane signal. Corrections for the natural abundance signal were made. The absolute enrichments of the glutamate and lactate peaks were related to the glutamate and lactate concentrations in the extracts, determined by enzymatic methods, to give the specific enrichments (31).

Insulin secretion. For evaluation of insulin secretion, BRIN BD11 cells were seeded in 24-well multiplates (1.0×10^5 cells/well). After overnight culture, the medium was removed gently and replaced with 1 ml Krebs Ringer bicarbonate buffer containing 1.1 mmol/l D-glucose. After 40 min preincubation at 37°C in the presence or absence of oligomycin (1.8 μg/ml), the buffer was removed, and the cell monolayers were incubated in Krebs Ringer bicarbonate buffer with or without oligomycin and containing either 1.1 mmol/l D-glucose or 1.1 mmol/l D-glucose supplemented with 10 mmol/l L-alanine. After a further 20-min incubation, an aliquot of buffer (900 μl) was removed from each well and centrifuged at 500*g* for 5 min at 4°C. The supernatant was stored at −20°C for subsequent measurement of insulin by Mercodia Ultrasensitive rat insulin enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

It was checked that cell density and insulin content were not significantly different under any of the incubation conditions.

Glucose utilization experiments. BRIN-BD11 cells were preincubated at 37°C in Krebs Ringer bicarbonate buffer supplemented with 1.1 mmol/l glucose for 20 min. The cells were subsequently incubated in one of the following for 60 min: 16.7 mmol/l D-glucose, 16.7 mmol/l D-glucose plus 10 mmol/l L-alanine, 16.7 mmol/l glucose plus 10 mmol/l α-aminoisobutyric acid (AIB) methyl ester, or 16.7 mmol/l glucose plus 10 mmol/l AIB. The glucose concentration was determined before and after the 60-min incubation period.

Metabolite determination. Incubation medium glucose concentrations were measured by use of a hexokinase/glucose 6-phosphate dehydrogenase assay kit (Sigma) according to the manufacturer's instructions. Cellular glutamate concentration was quantified using a glutamate dehydrogenase–based assay kit supplied by Roche Diagnostics.

Statistical analysis. Where appropriate, results are presented as means ± SD. Analysis was performed by Student's *t* test. $P < 0.05$ was considered to be statistically significant.

RESULTS

Alanine metabolism and insulin secretion. Perchloric acid extracts of BRIN-BD11 cultures were analyzed by ^1H-decoupled ^{13}C NMR spectroscopy. The chemical shift range of 0–90 ppm displayed was selected to illustrate the ^{13}C metabolites that resulted from L-[3-^{13}C]alanine or D-[1-^{13}C]glucose. [3-^{13}C]alanine metabolites were detectable after 20 min of incubation (Fig. 1*A*) and included [3-^{13}C]lactate (21.3 ppm), [4-^{13}C]glutamate (34.7 ppm), and [2-^{13}C]acetate (24.5 ppm). As the time course of incubation was increased to 60 min, further metabolites became detectable (Fig. 1*B*), including [3-^{13}C]glutamate (28.1 ppm), [2-^{13}C]glutamate (56.2 ppm), and [2-^{13}C]aspartate (53.5 ppm). Increasing the time of incubation to 120 min allowed additional detection of [3-^{13}C]aspartate (37.5 ppm) (Fig. 1*C*).

It is clear from these results that L-alanine is rapidly converted in BRIN-BD11 cells to pyruvate and then to

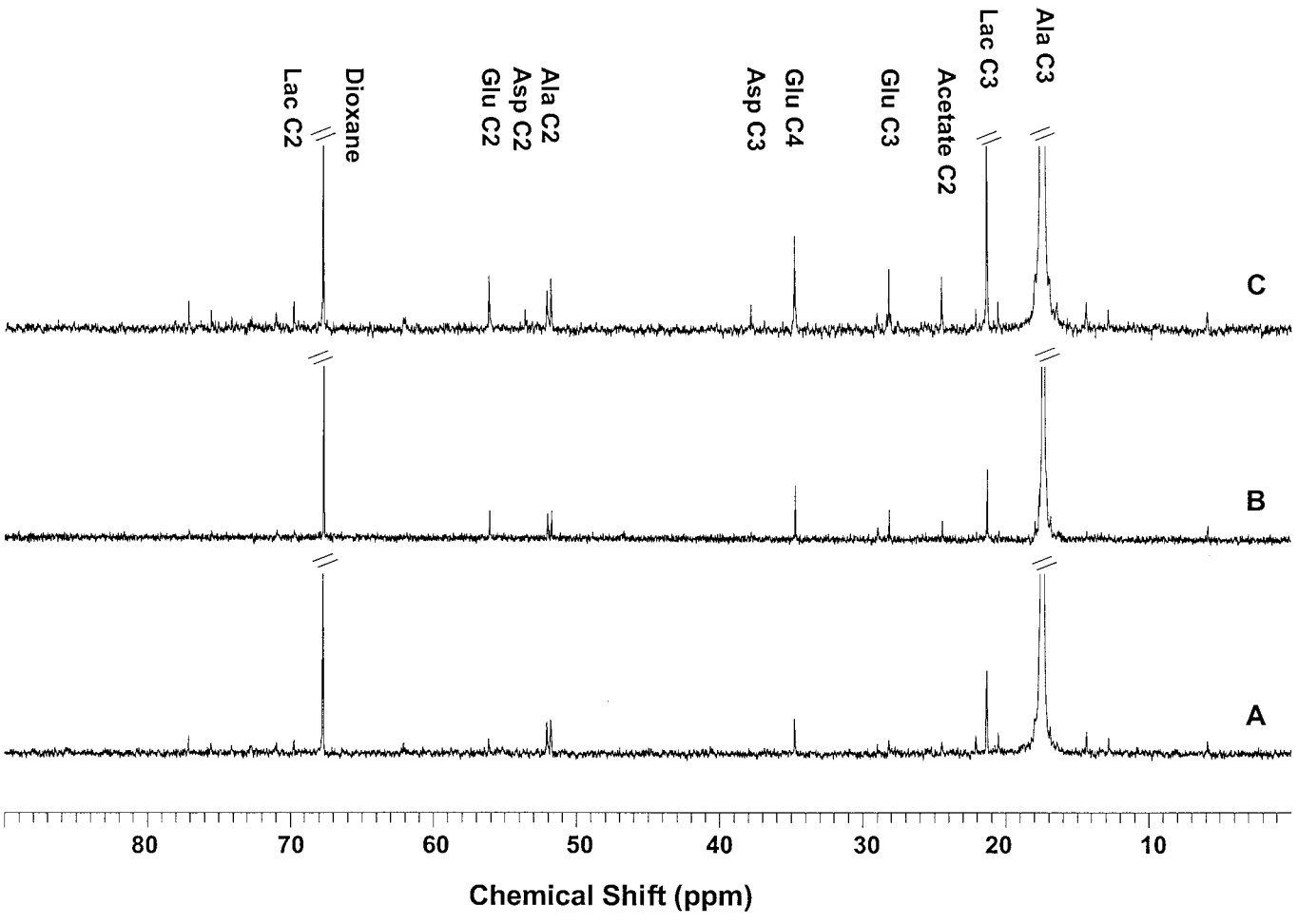

FIG. 1. ^{13}C-NMR spectra of perchloric acid extracts of BRIN-BD11 cells incubated with 10 mmol/l L-[3-^{13}C]alanine for 20 min (*A*), 60 min (*B*), and 120 min (*C*). Lac, lactate.

measurable end products of metabolism, e.g., lactate, glutamate (via the TCA cycle intermediate 2-oxoglutarate), and aspartate (via the TCA cycle intermediate oxaloacetate). To determine whether oxidative L-alanine metabolism was important for the process of alanine-induced stimulation of insulin secretion, an experiment was performed whereby the respiratory poison oligomycin was added into the incubation with L-alanine. The addition of 10 mmol/l L-alanine alone induced a 5.3-fold increase of insulin secretion (Table 1), which was reduced to a 1.8-fold increase with the addition of oligomycin (Table 1). The basal rate of insulin secretion at 1.1 mmol/l D-glucose alone was 0.68–0.79 ng/10^6 cells per 20 min. As demonstrated in Table 1, increasing the glucose concentration to 16.7 mmol/l significantly enhanced insulin release in the absence and particularly in the presence of 10 mmol/l L-alanine ($P < 0.001$). The stimulatory effects of equimolar AIB were modest compared with those of L-alanine.

Effect of glucose on L-alanine metabolism. The synergistic enhancement of insulin secretion from BRIN-BD11 cells previously observed on addition of 10 mmol/l L-alanine to a 16.7 mmol/l D-glucose stimulus (11) might have been due to glucose enhancing the rate of metabolism of L-alanine. To test this hypothesis, BRIN-BD11 cells were incubated for 60 min with [3-^{13}C]alanine in the absence (Fig. 2*A*) or presence (Fig. 2*B*) of glucose. It is clear from the spectra obtained that L-alanine metabolism was not enhanced by glucose over the 60-min incubation period. In fact, there was a slight decrease in the peak

TABLE 1
Effect of 16.7 mmol/l glucose, 10 mmol/l L-alanine, or 10 mmol/l AIB in the presence or absence of the respiratory poison oligomycin on insulin secretion from BRIN-BD11 cells

Incubation condition	Insulin secretion (normalized)
1.1 mmol/l D-glucose	100
1.1 mmol/l D-glucose + 10 mmol/l L-alanine	533 ± 28*
1.1 mmol/l D-glucose + 10 mmol/l L-alanine + 1.8 μg/ml oligomycin	178 ± 88†
1.1 mmol/l D-glucose + 10 mmol/l AIB	147 ± 15*
16.7 mmol/l D-glucose	279 ± 24‡
16.7 mmol/l D-glucose + 10 mmol/l L-alanine	1,940 ± 87‡§
16.7 mmol/l D-glucose + 10 mmol/l L-alanine + 1.8 μg/ml oligomycin	567 ± 42‖
16.7 mmol/l D-glucose + 10 mmol/l AIB	368 ± 37‡¶

Data are means ± SD (n = 2-6). Insulin concentration was determined as described in RESEARCH DESIGN AND METHODS. The basal rate of insulin secretion was determined as 0.68 − 0.79 ng/10^6 cells per 20 min. *$P < 0.01$, ‡$P < 0.001$ vs. basal insulin secretion; §$P < 0.001$, ¶$P < 0.05$ vs. 16.7 mmol/l glucose alone; †$P < 0.05$, ‖$P < 0.001$ vs. absence of oligomycin.

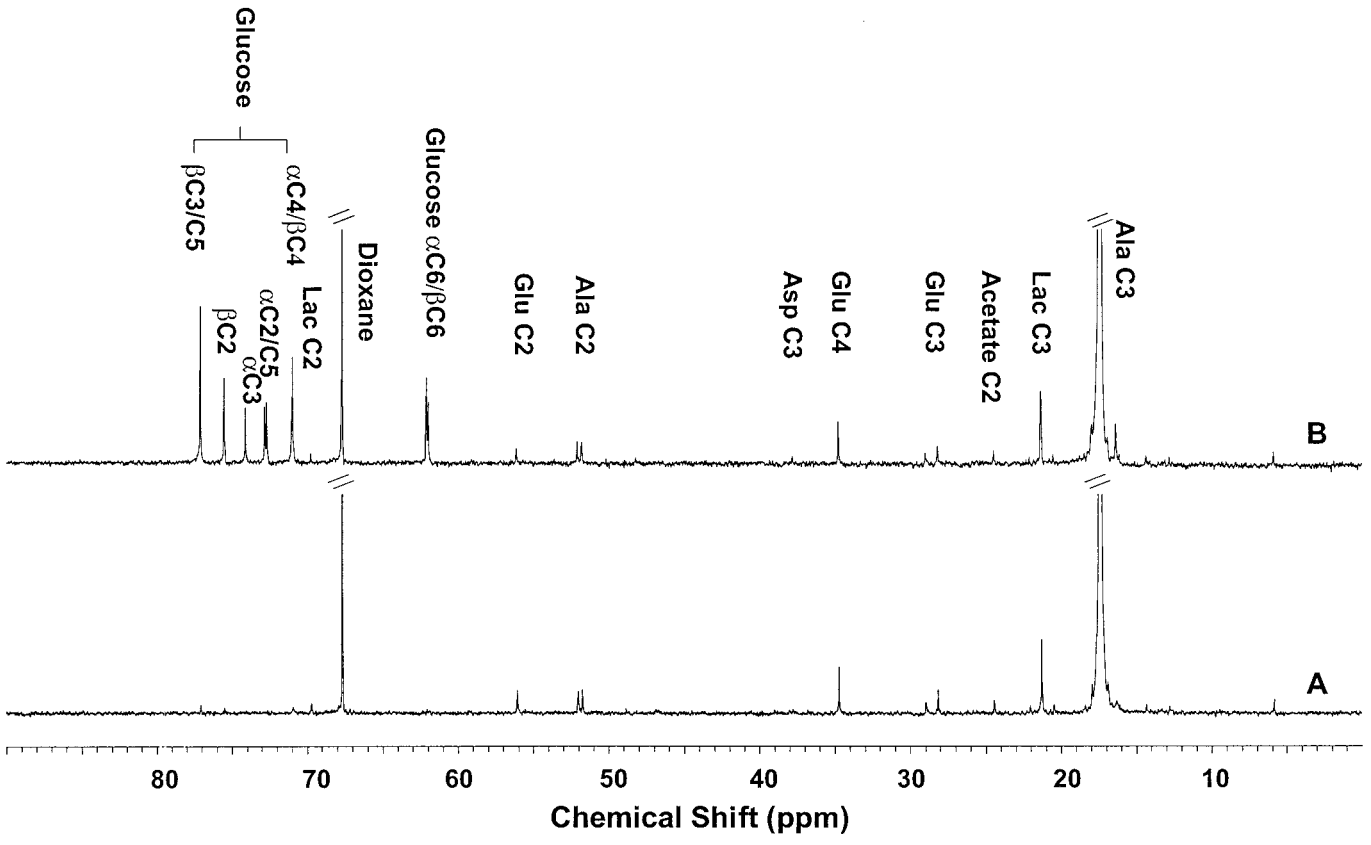

FIG. 2. ^{13}C-NMR spectra of BRIN-BD11 cell extracts obtained after incubation for 60 min with 10 mmol/l L-[3-^{13}C]alanine (*A*) and 10 mmol/l L-[3-^{13}C]alanine and 16.7 mmol/l D-glucose (*B*). Lac, lactate.

integrations corresponding to glutamate C2, C3, and C4. The new signals at 70–80 ppm (Fig. 2*B*) were from the added glucose.

Effect of L-alanine on glucose metabolism. As an alternative to an effect on L-alanine metabolism, it is possible that the synergistic enhancement of insulin secretion by the combination of 10 mmol/l L-alanine and 16.7 mmol/l D-glucose might reflect an enhanced rate of metabolism of glucose in the presence of L-alanine. To evaluate this possibility, BRIN-BD11 cells were incubated for 60 min with D-[1-^{13}C]glucose in the absence (Fig. 3*A*) or presence (Fig. 3*B*) of L-alanine. It is clear from the spectra obtained that aspects of glucose metabolism were enhanced in the presence of L-alanine—note peak amplitude increases associated with [3-^{13}C]lactate (21.3 ppm), [4-^{13}C]glutamate (34.7 ppm), [3-^{13}C]glutamate (28.1 ppm), [2-^{13}C]glutamate (56.2 ppm), and [2-^{13}C]acetate (24.5 ppm). Thus the data presented in Fig. 3 indicate an increase in the rate of production of lactate and glutamate from glucose. This observation is supported by the metabolic pool concentrations obtained by enzymatic methods. When alanine was added to the incubation medium containing labeled glucose, the glutamate pool size increased from 0.25 to 0.49 μmol/mg of protein (Table 2).

To quantify the increase in BRIN-BD11 glucose utilization rate in the presence of L-alanine, the glucose concentration in the buffer was determined after 60 min incubation with 16.7 mmol/l D-glucose or 16.7 mmol/l D-glucose plus 10 mmol/l L-alanine. In the presence of L-alanine, the utilization rate of glucose increased 2.4-fold (Table 3). Such an increase was not found when the cells were incubated in the presence of the nonmetabolizable alanine analog AIB or its methylated derivative (Table 3).

DISCUSSION

There has been considerable debate (10,32–35) as to the mechanism by which some amino acids can enhance insulin secretion from islet cells or β-cell lines. L-Arginine, for example, because of its positive charge at neutral pH, can directly depolarize the β-cell plasma membrane. L-Leucine, by virtue of rapid conversion to acetyl-CoA via branched-chain keto-acid dehydrogenase and related enzymes, is ultimately oxidized in the TCA cycle. L-Glutamine, by virtue of anaplerosis, can increase the rate of the TCA cycle and thus can synergistically enhance glucose-stimulated insulin secretion, but is a poor enhancer of insulin secretion on its own.

Early experiments initially indicated that islet β-cells substantially metabolized L-alanine (36) but were at variance with the apparent ineffectiveness of the amino acid as an insulin secretogogue in isolated islets (37). Subsequently, using more sensitive assays and shorter-term incubations, it has been established that L-alanine is a strong stimulus to insulin secretion in the presence of glucose in normal rodent islets and cell lines, also provoking large increases in intracellular Ca^{2+} (12,38–40). These effects of L-alanine have been attributed in large part to cotransport with Na^+, thereby depolarizing the cell in the presence of glucose and provoking Ca^{2+} influx (35,36). The present demonstration that the respiratory poison, oligomycin, dramatically decreased L-alanine–stimulated

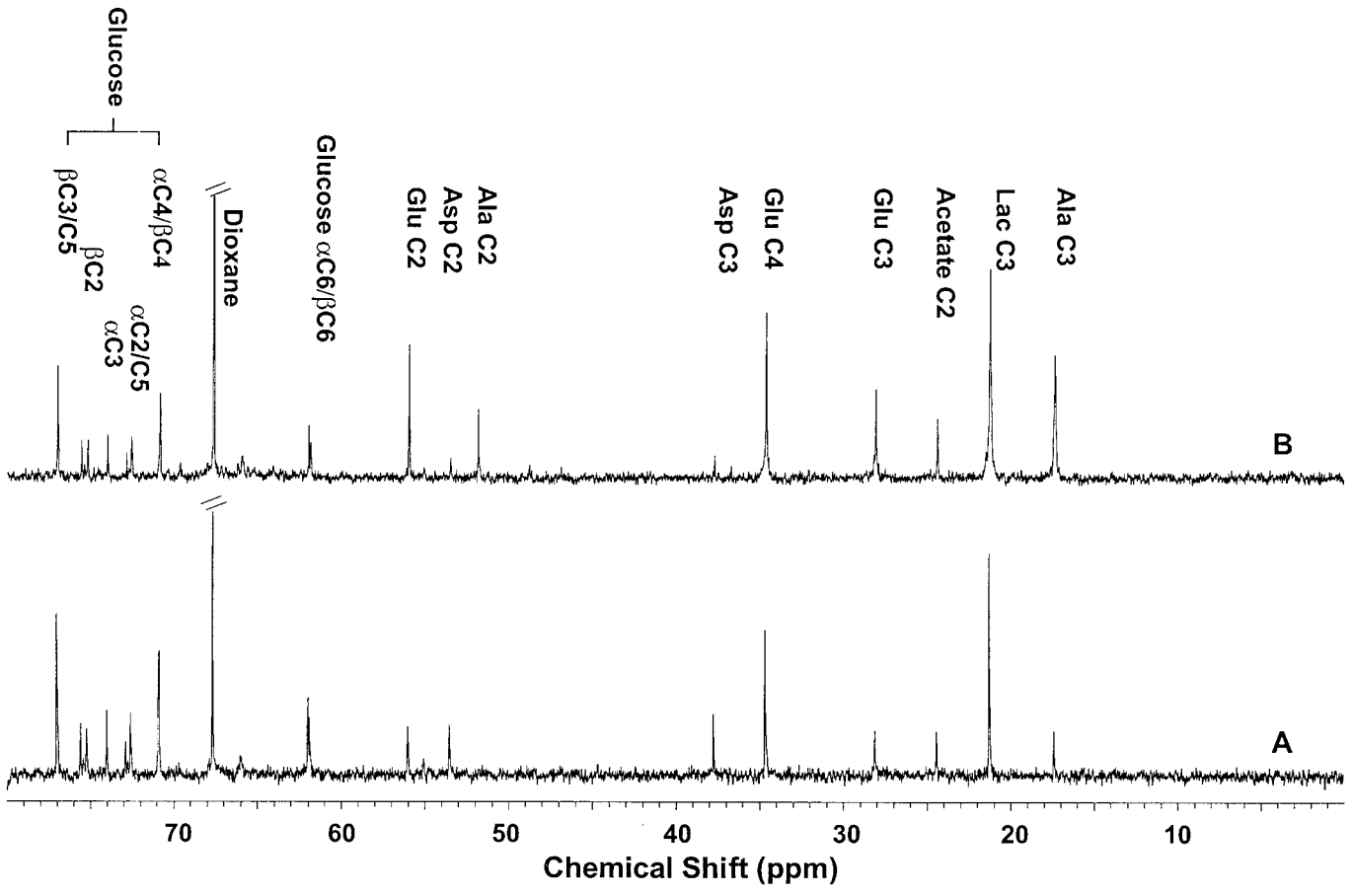

FIG. 3. ^{13}C-NMR spectra of BRIN-BD11 cell extracts obtained after incubation for 60 min with 16.7 mmol/l L-[1-^{13}C]glucose (*A*) and 16.7 mmol/l L-[1-^{13}C]glucose and 10 mmol/l L-alanine (*B*). Lac, lactate.

insulin secretion from BRIN-BD11 cells (Table 1) further underlines the involvement of oxidative metabolism in the mechanism of action. Together with parallel observations made using NMR to probe the metabolic fate of L-alanine and the comparatively weak effects of the nonmetabolizable analog AIB (Table 1), these data lead us to suggest that metabolism of L-alanine is important for the stimulatory effects of the amino acid on insulin secretion. In addition, strong evidence that cotransport with Na^+ accounts for only a minor proportion (10–20%) of L-alanine–stimulated insulin secretion is provided in Table 1. Thus the nonmetabolizable analog of L-alanine, AIB, increased insulin secretion to an extent similar to that obtained in the presence of both L-alanine and oligomycin (10–20% of the increase obtained in the presence of L-alanine only). These data, taken together, suggest that metabolism of L-alanine provides key stimulus-secretion coupling factors that are important for promotion of insulin secretion. The production of such stimulus-secretion coupling factors may be amplified in the presence of elevated intracellular (and mitochondrial) Ca^{2+}, as a number of mitochondrial metabolic steps are Ca^{2+} dependent, such as PDH, 2-oxoglutarate dehydrogenase, and the malate-aspartate shuttle (41,42).

Metabolism of L-alanine in β-cells could proceed by one or both of two pathways. Anaplerotic metabolism via

TABLE 2
Glutamate pool size in BRIN-BD11 cells after incubation for 60 min in the presence of various combinations of nutrients

Experiment	Pool size (μmol/mg of protein)
1.1 mmol/l D-[1-^{13}C]glucose	0.08 ± 0.02
16.7 mmol/l D-[1-^{13}C]glucose	0.25 ± 0.04
16.7 mmol/l D-[1-^{13}C]glucose + 10 mmol/l L-alanine	0.49 ± 0.10
10 mmol/l L-[3-^{13}C]alanine	0.32 ± 0.05
10 mmol/l L-[3-^{13}C]alanine + 16.7 mmol/l D-glucose	0.46 ± 0.06

Data are means ± SD (n = 3). Glutamate concentrations were determined as described in RESEARCH DESIGN AND METHODS.

TABLE 3
Glucose utilization rates of BRIN-BD11 cells in the presence or absence of 10 mmol/l L-alanine or its nonmetabolizable analogs AIB or methylated AIB

Incubation condition	Glucose utilization (μmol/10^6 cells per 60 min)
16.7 mmol/l D-glucose	1.01 ± 0.21
16.7 mmol/l D-glucose + 10 mmol/l L-alanine	2.44 ± 0.59*
16.7 mmol/l D-glucose + 10 mmol/l methylated AIB	0.70 ± 0.10
16.7 mmol/l D-glucose + 10 mmol/l AIB	0.60 ± 0.15

Data are means ± SD (n = 2-4). Glucose concentrations were determined as described in RESEARCH DESIGN AND METHODS. *$P < 0.01$ vs. glucose alone.

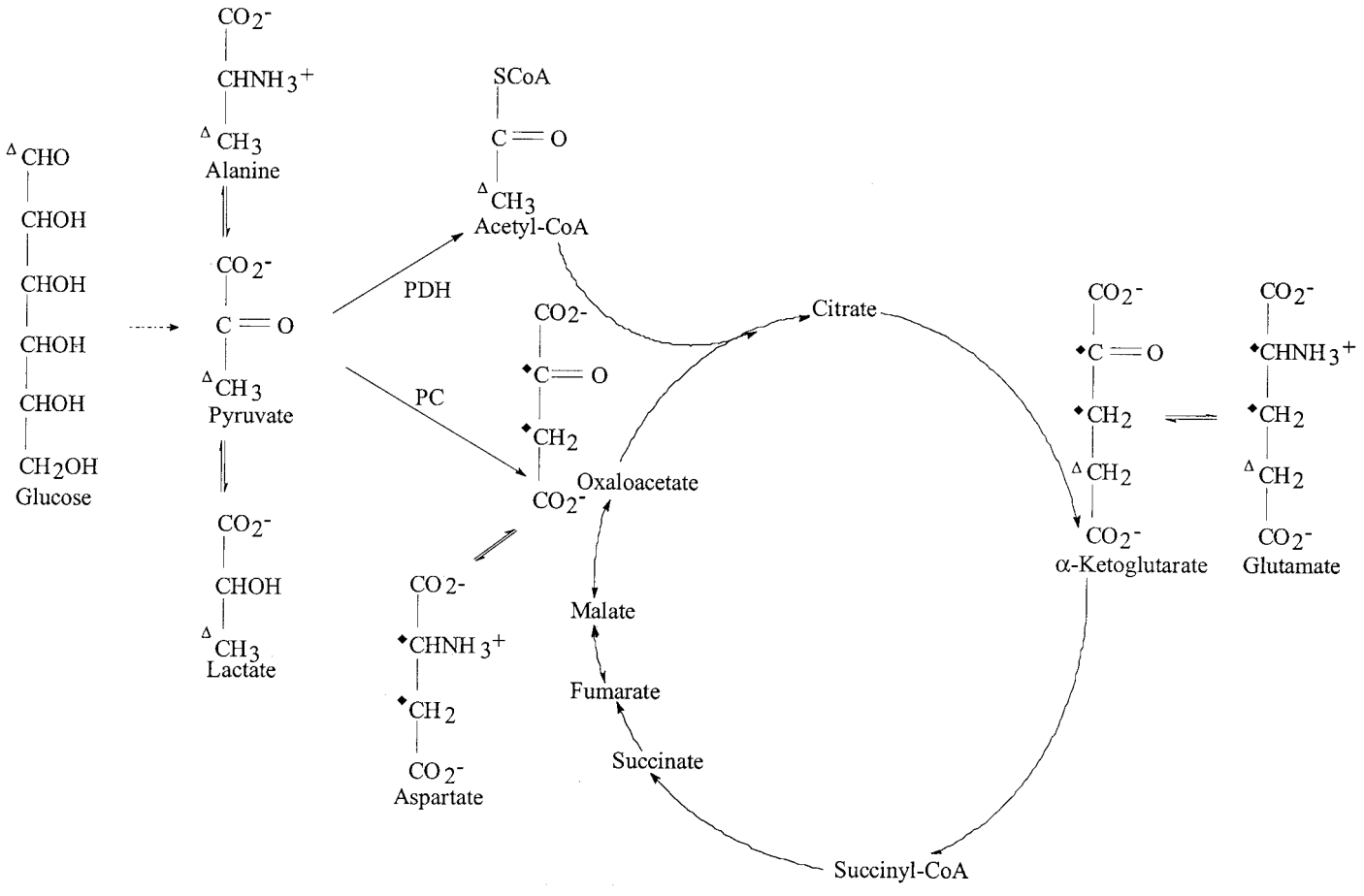

FIG. 4. A simplified scheme of the distribution of the label following [1-^{13}C]glucose metabolism or [3-^{13}C]alanine metabolism. [3-^{13}C]pyruvate formed from both can follow either the PDH or the PC pathway. As a result of the equilibrium between oxaloacetate, malate, and fumarate, the label originally on C3 of pyruvate is distributed between C2 and C3 of oxaloacetate. The labeling shown here is limited to that obtained after one turn of the cycle.

pyruvate carboxylase would result in labeling of the C2 position of oxaloacetate (Fig. 4; the ^{13}C label originating in alanine is identified by a filled symbol). This label is scrambled at the level of succinate, resulting in ^{13}C labeling of the C2 and C3 positions (Fig. 4). Subsequently, due to the action of glutamate dehydrogenase or aspartate aminotransferase, the TCA cycle intermediate 2-oxoglutarate would be converted to glutamate, labeled in positions C2 and C3. Alternatively, if L-alanine was oxidatively metabolized in β-cells via pyruvate dehydrogenase, then this would result in labeling of citrate in position C4. Ultimately, via the action of glutamate dehydrogenase or aspartate aminotransferase, glutamate would be enriched in position C4. However, a degree of label scrambling will occur after more than one turn of the cycle, which will result in complex patterns of metabolite enrichment (43). Our results clearly demonstrated an increase in specific enrichment of C4-labeled glutamate that was time dependent, effectively doubling over the 20- to 60-min incubation period (Table 4). In contrast, early increases in the specific enrichment of C2- and C3-labeled glutamate with respect to time were more difficult to determine because of the low intensity of their signals after 20 min. Thus we speculate that oxidative, PDH-mediated L-alanine metabolism is quantitatively important to the BRIN-BD11 β-cell.

Although L-alanine metabolism was not influenced greatly by increasing the glucose concentration, a substantial increase in β-cell glucose metabolism was induced by this amino acid. The specific enrichments of lactate and glutamate did not decrease dramatically upon addition of

TABLE 4
[^{13}C]enrichment (%) of metabolites in BRIN-BD11 cells after incubation in different experimental conditions

Metabolite	[^{13}C]Ala, 20 min	[^{13}C]Ala, 60 min	[^{13}C]Ala, 120 min	[^{13}C]Ala + Glc, 60 min	[^{13}C]Glc, 60 min	[^{13}C]Glc + Ala, 60 min
Lactate C3	6.5	13.2 ± 1.8	10.5	11.20 ± 0.01	27.9 ± 5.2	24.5 ± 5.2
Glutamate C2	ND	8.5 ± 0.7	9.8	6.5 ± 0.3	10.50 ± 0.01	9.8 ± 2.3
Glutamate C3	ND	6.1 ± 1.3	10.3	5.4 ± 1.0	9.0 ± 0.9	7.8 ± 2.7
Glutamate C4	8.1	18.4 ± 2.5	17.5	16.8 ± 2.1	30.4 ± 1.6	23.8 ± 3.7

Data are the averages of the duplicates obtained in two independent experiments ± SD. Nutrients were added at 16.7 mmol/l (D-glucose) or 10 mmol/l (L-alanine).

L-alanine to β-cells incubated with labeled glucose, indicating that metabolic utilization of unlabeled L-alanine did not give significant amounts of unlabeled pyruvate (Table 4). Likewise, upon addition of glucose to cells incubated with labeled L-alanine, the change in the specific enrichments was also not large. Previous studies indicate that a cotransport of Na^+ with L-alanine will increase β-cell cytoplasmic Ca^{2+} (12,38–40,44). As PDH is known to be activated by Ca^{2+} (45), then oxidation of pyruvate derived from glucose would be anticipated to be enhanced. In contrast, L-alanine metabolism is unlikely to be increased under these conditions due to competition for NAD^+ between the glycolytic glyceraldehyde 3-phosphate dehydrogenase and the alanine aminotransferase enzymes.

In summary L-alanine metabolism is important for stimulation of insulin secretion from a clonal β-cell line. The oxidative route of L-alanine metabolism appears to be especially important in the β-cell, based on insulin secretion data reported here in the presence and absence of oligomycin or in the presence of the nonmetabolizable analog of L-alanine, AIB. In addition, the enhancement of glucose metabolism by L-alanine probably explains the synergistic action of this amino acid on glucose-induced insulin secretion. We speculate that the metabolism of L-alanine and glucose will contribute to a rise in the ATP/ADP ratio, thus inactivating the ATP-sensitive K^+ channel and depolarizing the plasma membrane (4). This may further explain the key role ascribed to L-alanine in the physiological regulation of β-cell electrical activity (46). The mitochondrial metabolite (1,47–48) generated from L-alanine and/or glucose metabolism, which is additionally responsible for the enhancement of insulin secretion, has yet to be identified. Two recent articles, however, have provided evidence that cytosolic glutamate concentration is an important factor for the regulation of insulin secretion (49,50). This article would add weight to such a hypothesis.

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