High Glucose Levels do not Stimulate TCA Cycle Flux in ßHC9 Insulinomas Detected with ^{13}C NMR

Anthony Mancuso[1], Christophe Desbarats[1], Susanne Wehrli[2], Franz M Matschinsky[3], Jerry Glickson[1]
[1]University of Pennsylvania, Department of Radiology, Philadelphia, PA USA; [2]Children's Hospital of Pennsylvania, Philadelphia, PA USA;
[3]University of Pennsylvania, Department of Biochemistry & Biophysics, Philadelphia, PA USA;

Introduction

PCell based therapies for Type I Diabetes Mellitus are attractive since they provide a complete solution –both glucose sensing and insulin secretion-- to the problem of blood glucose regulation. However, because diabetes is so prevalent, it is impractical to treat the disease with beta cells from Islet of Langerhans of deceased donors. One potential solution to this problem is to use immortilized, insulin-secreting, beta cells. A model cell line for this purpose is the ßHC9 insulinoma. Preliminary characterization of this line has shown that its insulin response to glucose is similar to that of healthy beta cells (1). However, a detailed undertanding of how insulin secretion is controlled by external glucose levels will be essential before this or any insulinoma line is successfully used in an artificial pancreas. In normal beta cells, external glucose levels are "sensed" when the glucose is actually catabolized. The primary regulatory enzyme in this process is glucokinase, which controls the first committed step in glycolysis (phosphorylation of glucose to glucose-6-phosphate) (2). This enzyme has a high K_m for glucose, which allows it to control blood glucose levels in the range of ~5 to 7 mM. However, the exact mechanism by which glucokinase controls insulin secretion is not fully understood (2), because insulin secretion is also affected by catabolism of other substrates such as amino acids and fatty acids. Sweet et al. recently hypothesized that the high level of control by glucokinase could be explained if ATP produced in the glycolytic pathway had much stronger control over insulin secretion than did ATP produced in the TCA cycle (3). The goal of this work was to examine this hypothesis for ßHC9 cells with ^{13}C NMR. Cells were fed with [1-^{13}C] glucose at two different concentrations and labeling patterns in glutamate were analyzed to determine if the level of glucose impacted the relative rates of primary metabolic pathways. Labeling in the 2, 3 and 4 carbons of glutamate, which is believed to reflect the labelings in 2, 3 and 4 alpha-ketoglutarate, has been used extensively to determine flux rates (4). A high ratio of [4-^{13}C] glutamate to [2 and 3-^{13}C] glutamate is indicative of a low TCA cycle rate, relative to the rate of the competing pathways, glutaminolysis and anapleuronic exchange. With increased TCA cycle flux, labeling in glutamate-2 and 3 should increase relative to labeling in glutamate-4 (5).

Methods

ßHC9 cells were grown in DMEM medium with either high (30 mM) or low (6 mM) glucose. The medium also contained: 25 mM HEPES, 10% fetal bovine serum, and 4 mM glutamine. The cells were grown in T-flasks (225 cm^2), with continuous feeding, to allow the ^{13}C labeling to occur at steady state. Peristaltic pumps were used to feed fresh medium and remove spent medium at a rate of 6 ml/min. The T-flasks were gently shaken in a 37 C, 5% CO_2 incubator on an orbital shaker to keep the liquid phase well mixed. The steady state volume in each flask was approximately 50 ml. Confluent flask cultures were fed with medium for 2 days prior to the addition of ^{13}C label to bring all metabolite levels to steady state. Subsequently, [1-^{13}C] glucose was fed to the flasks at either 30 mM or 6 mM for 24 hours to allow complete labeling of all metabolite pools. At the end of the labeling period, the cultures were washed quickly with ice cold PBS and extracted with perchloroacetic acid. The extracts were neutralized with KOH and analyzed in 5 mm NMR tubes on a high resolution, 400 MHz Bruker spectrometer. ^{13}C spectra were acquired with a DEPT 45 sequence, with a TR of 5 sec. Total glucose and lactate (^{13}C and ^{12}C) concentrations were determined with a YSI analyzer and protein levels were determined with the Lowry method. Three experiments were performed at high glucose levels (~30 mM feed) and three were conducted at low glucose levels (~6mM feed).

Results

^{13}C spectra obtained for ßHC9 cells grown at high and low glucose levels are shown in Figures 1A and 1B. The steady state concentrations of glucose in the perfused flasks and the glucose consumption and lactate production rates are summarized in Table I. The expected rates of insulin secretion for the glucose levels were based on previous results with this cell line (1). With a 30 mM glucose feed, the level of intracellular glucose was approximately 3 times higher than it was with a feed of 6 mM glucose (not shown). Intracellular levels of lactate and alanine were also moderately higher with the higher glucose feed. The rate of glucose consumption was approximately 3 times higher and the rate of lactate production was 2 times higher at 30 mM relative to 6 mM. However, the ^{13}C spectra indicate that the relative labeling in glutamate 2, 3 and 4 was not significantly affected by the external glucose concentration. The relative labeling in glutamate 4/3/2 was 10.0/3.4/3.8 at high glucose and 10.0/3.3/3.5 at low glucose. In addition, the level of ^{13}C-^{13}C coupling between glutamate-4 and 3 (which is difficult to see in the spectra in Figure 1) was not significantly different at the two glucose levels.

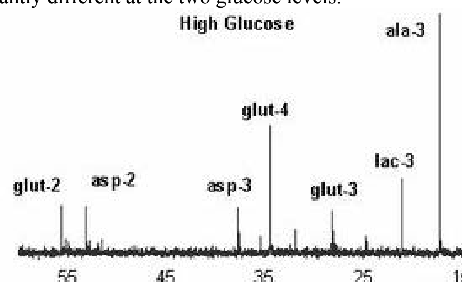

Figure 1A. 13C spectrum (4,500 scans) with high glucose (30 mM feed).

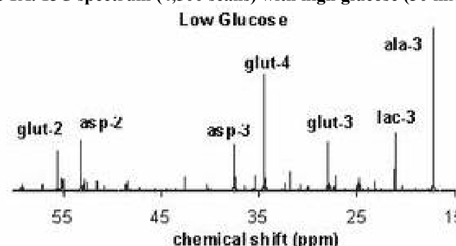

Figure 1B. 13C spectrum (10,000 scans) with low glucose (6 mM feed).

Discussion

These results suggest that the higher level of glucose did not affect the flux of the TCA cycle significantly, relative to other fluxes (e.g. anapleuronic exchange). Weiss et al. demonstrated previously that with increased TCA cycle flux, the labeling in the 2 and 3 positions should increase relative to the labeling in glutamate-4 (5). In ßHC9 cells, higher glucose consumption rates result in higher rates of lactate and alanine (data not shown) production and hence a higher rate of energy produced by glycolysis. This is consistent with the recent findings of Papas et al. (6) with this cell line, who noted that increased environmental levels of glucose do not result in an increase in oxygen consumption. The findings of this work are consistent with the hypothesis that the flux through the glycolytic pathway has a much stronger impact than does TCA cycle flux in controlling insulin secretion. This may be the mechanism by which glucokinase exerts such strong control over insulin secretion in ßHC9 insulinomas.

References

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Table I. Mean steady-state glucose levels and glucose consumption and lactate production rates (umol/mg protein/hr). Expected insulin values were estimated from reference 1 in (mg/L-cell/hr).

	Glucose		Glucose	Lactate	Insulin
	Feed (mM)	Flask (mM)	Cons. Rate	Prod. Rate	Rate
High	30.5	26.7	0.51	0.26	90
Low	5.8	4.7	0.17	0.12	21