^{13}C isotopomer studies of insulin-secreting cells: anaplerosis plays a role

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Introduction:

One goal for insulin-dependent diabetes research is to define mechanisms of fuel-regulated insulin secretion (ISR) in surrogate cell sources. Understanding the enzymatic paths critical to secretion would enable cellular engineers to tailor superior cell sources. ^{13}C NMR spectroscopy and isotopomer analysis are excellent tools that provide a quantitative assessment of glucose metabolism through intermediate labeling [1] and can determine information regarding carbon entry to the TCA cycle (e.g., 'flux' through critical metabolic pathways). Recently, Lu et al. [2] performed such a study on INS-1 insulinoma cells and reported that the ^{13}C data suggests a metabolic model containing two separate pyruvate pools: one feeding acetyl-CoA to the TCA cycle (and back to OAA via pyruvate dehydrogenase: PDH), and the other cycling between TCA intermediates and pyruvate (via pyruvate carboxylase: PC), termed pyruvate cycling. A benefit of the two-pool approach was that the isotopomer data fit well to the model, and a strong correlation between ISR and pyruvate cycling was found. However, though there are possible mechanisms for such a separation [3, 4], this two-pool concept is controversial, difficult to envision physiologically, and has not been robustly demonstrated in β-cells. Certainly, it is critical to determine appropriate metabolic models to analyze isotopomer data, particularly when correlating energetics with cellular function. Towards this end, we broadly studied the effects of applying different metabolic models to ^{13}C data from insulin-secreting cell lines, four insulinoma lines (extracted under a variety of media complexity conditions that alter the metabolic, secretory and isotopomer patterns of cells [5]) and one set of pig islets (extracted under stimulatory conditions).

Materials and Methods:

Insulinomas (INS-1, βTC3, βTC-tet, R7T1) were grown and expanded in vitro. Confluent flasks of the cells were preincubated in glucose-free PBS for 1.5 hours, then exposed for 4 hours to media (PBS, incomplete media or media completed with sera) containing 3 or 15 mM uniformly-labeled ^{13}C-glucose. These media created a variety of conditions to address a potential link to insulin secretion regardless of media complexity. Porcine islets (~100,000) were obtained and similarly preincubated in PBS before exposure to complete media containing 15 mM uniformly-labeled ^{13}C-glucose. Extractions of all cells were performed as described by Tyagi et al. [6]. The aqueous portion of each extract was lyophilized, resuspended in D_2O, and placed in a 5 mm NMR tube. Spectroscopic ^{13}C NMR data of the extracts were acquired using a 5 mm broadband receiving coil in a 500 MHz vertical bore magnet equipped with a Bruker Avance console. ^{13}C acquisition parameters were as follows: sweep width = 30 kHz at 125.84 MHz, repetition time = 6 s, number of transients = 10240. Waltz ^{1}H decoupling was applied throughout.

Area calculations of glutamate multiplets (C2, C3, C4 and C5) in the ^{13}C NMR spectra were performed by line-fit analysis using the 'Nuts' program (Acorn, Fremont, CA). Relaxation and Nuclear Overhauser effects were determined for glutamate signals, and appropriate correction factors were applied to the resultant areas. An isotopomeric modeling analysis of the glutamate patterns (C2, C3, C4, and C5) and C3/C4 ratio was performed with the program TCA-calc (UTSW, Dallas, TX) to determine relative metabolic pathways and rates for each extract. **One**-pool and **two**-pool pyruvate models without ('*simple*') and with ('*modified*') anaplerotic entrance were considered. Pearson Product Moment Correlations between ISR and parameters derived from the metabolic modeling were calculated for each cell line.

Results and Discussion:

Visual inspection of ^{13}C spectra shows an increase in labeling of amino acids, glycolytic intermediates, and TCA cycle intermediates as glucose levels or media complexity increases, with the notable exception of aspartate, which is higher under low glucose conditions than under high glucose conditions. The glutamate isotopomeric fraction data of βTC3 & βTC-tet cells can be well analyzed by TCAcalc with a '*simple*' **one**-pool model (i.e., good correspondence between the input data and the modeled results), but the INS-1, R7T1 and pig islet data have a fitting anomaly with the '*simple*' **one**-pool model and require a '*simple*' **two**-pool model, corroborating the conclusions by Lu, et al. However, by application of the '*modified*' **one**-pool model [Fig.1], all data under all conditions for all cell lines tested can be well analyzed, eliminating the need for a separate pyruvate pool. Moreover, using the '*modified*' **one**-pool model, INS-1 and R7T1 lines (which have the '*simple*' **one**-pool model fitting anomaly) maintain a correlation between ISR and pyruvate cycling (R=0.58 & 0.57, respectively) but exhibit a much stronger correlation between ISR and the anaplerotic entrance to the TCA cycle (R=0.74 & 0.88, respectively), shown in Figure 2. Additionally, porcine islet data is included and supports this link between secretion and the anaplerotic entrance. Exactly by what path this anaplerotic label re-enters the TCA cycle is unclear, but glutamate and aspartate are the most likely candidates: the reduction of aspartate label under high glucose conditions indicates a rapid utilization of this amino acid under stimulatory conditions. The data presented here suggest that effective modeling of insulin-secreting cell lines can be accomplished using a '*modified*' **one**-pyruvate pool model, and that anaplerotic entrance to the TCA cycle, rather than only pyruvate cycling, may play an important role in secretion, demonstrable not only in insulinomas, but in native islets as well.

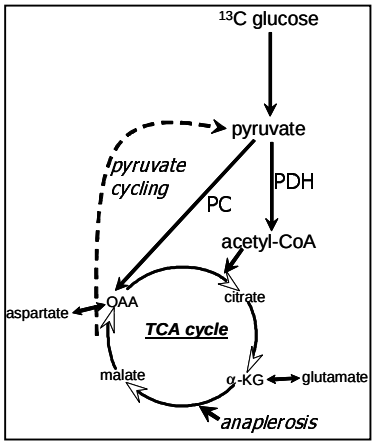

Figure 1: "Modified" one-pool model used to calculate relative rates of PDH, PC, anaplerosis and pyruvate cycling. Anaplerotic substances that feed back into the TCA cycle may include glutamate or aspartate.

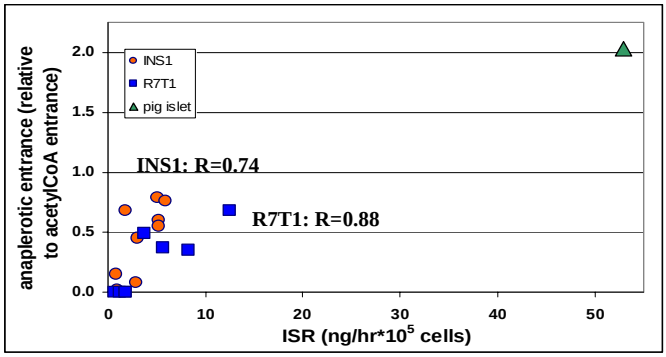

Figure 2: Model results: relationship between insulin secretion (ISR) and anaplerotic entrance to the TCA cycle of INS-1, R7T1 and porcine islet cells. The Pearson Correlation is included for INS1 & R7T1 cells.

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