Media-influenced secretory and ^{13}C isotopomer behavior of insulinoma cells

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Introduction:

Understanding how media complexity affects cellular metabolism and function is important to obtain physiologically relevant insight into the mechanisms of various biochemical processes. This is particularly important for dynamic processes such as insulin secretion that are influenced by various metabolic factors, many of which are found in media (amino acids) and additives such as sera (fats). ^{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 'flux' through critical metabolic pathways. Given the complexity of resultant isotopomer patterns, and the need to obtain the best possible spectral resolution to deconvolute these patterns, laboratories commonly perform extract experiments on cells incubated in phosphate buffered saline (PBS) or similar simple solutions containing only the ^{13}C enriched substrate, or media that does not contain sera. It is reasonable to assume that as the incubation media get more complex with the addition of amino acids and sera components, metabolic pathways are altered to accommodate the presence of these nutrients, thus the isotopomer patterns that result from the ^{13}C substrate are altered as well. Therefore, when using isotopomer modeling analysis to correlate energetics with cellular function, it is critical to understand the effect that the incubation media composition has on the metabolic, secretory and isotopomer patterns of cells. Moreover, application of an appropriate model of the bioenergetic pathways for the cell-line being studied is paramount to obtaining relevant correlative information.

Materials and Methods:

Insulinoma cell cultures (INS-1, βTC3, βTC-tet, R7T1) were grown and expanded in vitro. Confluent T-175 flasks of the cells were exposed for 4 hours to media (PBS, incomplete media or media completed with sera) containing 3 or 15 mM uniformly-labeled ^{13}C-glucose. Extractions of the cells were performed as described by Tyagi et al. [2]. The aqueous portion of each extract was lyophilized, resuspended in D_2O, and placed in a 5 mm NMR tube. Spectroscopic ^{1}H and ^{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. Acquisition parameters were as follows: ^{1}H- sweep width = 6.67 kHz at 500.4 MHz, repetition time = 5 s, number of transients = 32; ^{13}C- sweep width = 30 kHz at 125.84 MHz, repetition time = 6 s, number of transients = 10240. Waltz ^{1}H decoupling was applied throughout the ^{13}C acquisitions.

Area calculations of the multiplets of the glutamate C2, C3, C4 and C5 resonances 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) to determine relative metabolic pathways and rates for each extract was performed with the program TCA-calc (UTSW, Dallas, TX). Pearson Product Moment Correlations between GCR, ISR and parameters involved in the metabolic modeling were calculated for each cell line and glucose concentration.

Results and Discussion:

Figure 1 illustrates metabolic and secretory responses that occur in INS-1 cultures exposed to media of varying complexity and secretagogue content. Both the ISR and GCR increase with media complexity, regardless of glucose content. Additionally, analyzing the INS-1 extract data using a metabolic model that contains two pyruvate pools [3] demonstrates decreases in the fraction of acetyl-CoA derived from the label, and increases in pyruvate cycling as the media become more complex as shown in Figure 2. Similar relationships were discovered in all four cell lines tested. However, it is important to note that although the INS-1 cell line was best modeled using the two pyruvate pool model, the βTC cell lines (βTC3 and βTC-tet) had more robust fits using a simpler model that contained only one pyruvate pool. This suggests that the choice of an appropriate model itself can be cell-line dependent.

The data presented here suggest that the composition of the incubation media impacts the metabolic and secretory ability of the cells as well as the isotopomer patterns derived from ^{13}C NMR spectroscopic data. Because the complexity of the incubation media plays a significant role in the performance of the cells under study, when determining physiological relationships between insulin secretion and metabolism it is imperative that 'physiologically' relevant media are selected, and appropriate metabolic models are implemented.

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References:

[1] Malloy, C.R. et al. J. Biol. Chem. **263**: 6964, 1988

[2] Tyagi, R.K., et al. Magn. Reson. Med. **35**: 194, 1996

[3] Lu, D. et al. PNAS. **99**: 2708, 2002

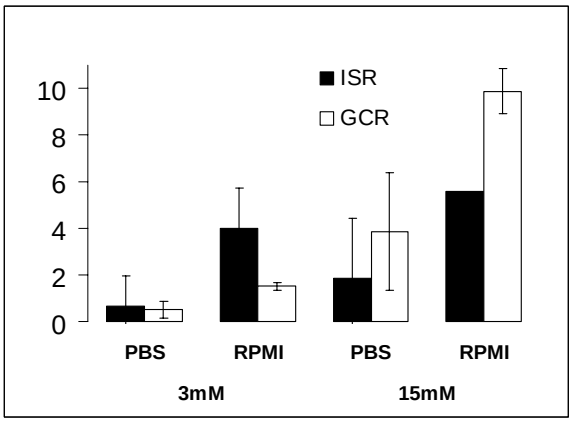

Figure 1: Pre-extraction glucose consumption and insulin secretion rates of INS-1 cells cultured in RPMI (complete) or PBS containing 3 or 15 mM uniformly-labeled ^{13}C-glucose.

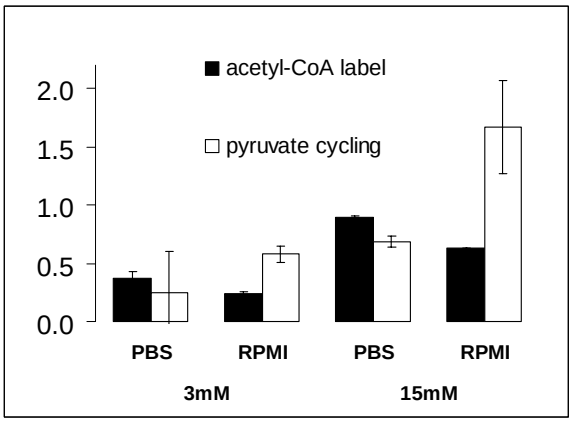

Figure 2: Model results: fraction of acetyl-CoA derived from ^{13}C-glucose; and pyruvate cycling (relative to the TCA cycle turnover) of INS-1 cells cultured in RPMI (complete) or PBS containing 3 or 15 mM uniformly-labeled ^{13}C-glucose.