^{13}C ISOTOPOMER STUDIES OF INSULIN SECRETING CELLS: THE ROLE OF ANAPLEROSIS

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Introduction

Understanding the enzymatic paths critical to insulin 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 and can determine information regarding carbon entry to the TCA cycle (e.g., 'flux'). Recently, Lu et al. (*1*) 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. Though there are possible mechanisms for such a separation, this two-pool concept is controversial, difficult to envision physiologically, and has not been robustly demonstrated in β-cells. In this report we present an alternative model that fits the data equally well and it is physiologically more relevant.

Experimental

Insulinomas (INS-1, βTC3, βTC-tet, R7T1) were grown and expanded in vitro as previously described (*2*). Cells were examined under basal and stimulating conditions of secretion using uniformly-labeled ^{13}C-glucose. Adult porcine islets were also examined within 24 hours of isolation only under stimulating conditions. Cell extracts were performed based on the dual phase method (*3*). ^{13}C NMR spectra were acquired using a 500 MHz vertical bore magnet equipped with a Bruker Avance console located at the AMRIS facility of UF. Isotopomer analysis was performed based on the public domain program TCA-calc (UTSW, Dallas, TX).

Results and Discussion

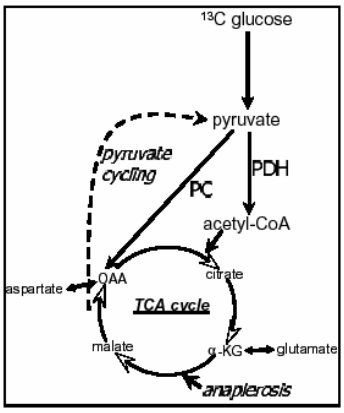

Figure 1: Proposed model of glucose consumption.

Visual inspection of ^{13}C spectra shows an increase in labeling of amino acids, glycolytic intermediates, and TCA cycle intermediates as glucose levels increases, with the notable exception of aspartate, which is higher under low glucose conditions than under high glucose conditions. The glutamate isotopomeric fraction data shows that our proposed model (Fig. 1) fit all data equally well as the two-pool model proposed by Lu et al. Also our data show that there is a strong correlation between the anaplerotic entrance and insulin secretion.

Conclusions

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 insulin secretion, as demonstrated by four insulinoma cell lines, and adult porcine islets.

Acknowledgements

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References

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