Insulin Release and Energetic Changes in INS-1 Cell Bioreactors in Response to Glucose Challenge

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INTRODUCTION: Glucose homeostasis is a vital physiological mechanism ensuring adequate cellular energy supply with both mitochondria and K^+_{ATP} channels of central importance in the glucose induced insulin secretion (GSIS) of pancreatic beta cells (1). However, the exact nature of mitochondrial signals coupled to K^+_{ATP} channel function is more controversial with adenine nucleotides (ATP, ADP) at the center of this controversy (2). In this study phosphorus nuclear magnetic resonance spectroscopy (^{31}P NMR) was used to measure energetic changes in response to a glucose challenge in miniature INS-1 cell bioreactors to test the hypothesis that insulin secretion is mediated by [ADP]. The results demonstrate that GSIS is associated with large changes in free ADP, but not ATP. In addition treatment with glucose also induced a decrease in inorganic phosphate and intracellular pH. The time course of these energetic changes suggest mitochondrial oxidative phosphorylation mediates insulin release and that a single metabolic regulator (ADP) is the signal coupling mitochondrial function to release of insulin in this insulin secreting pancreatic beta cell model.

METHODS AND MATERIALS: Rat insulinoma (INS-1) cells were plated at an initial density of $10x10^6$ cells in T-75 flasks under standard culture conditions for 5 days in RPMI-1640 containing 11.1 mM glucose, 1 mM sodium pyruvate, 10 mM HEPES (pH 7.4), 50 µM ß-mercaptoethanol, 10% fetal bovine serum, 100 units penicillin, and 100 µg streptomycin. Cells were creatine loaded by incubation in medium supplemented with either 40 mM creatine or no supplement for 48 hrs before being loaded into customized 1.0 mm dia permeable bioreactor membrane (5×10^6 cells). ^{31}P- NMR spectroscopy experiments were performed on a 9.4 T widebore Bruker AM400 with 4096 points, a sweep width of 4000 Hz and 3 sec pre-delay. Summed FIDs (300 transients) were processed by zero filling once then apodized with a 25 Hz exponential filter prior to the Fourier transform. The experimental protocol used serial acquisition of a total of 12 spectra (6 low glucose and 6 high glucose) while superfusate fractions were collected to assay for insulin release. Following the completion of each experiment, the portion of the bioreactor in the sensitive volume of the NMR coil was frozen in liquid nitrogen for subsequent analysis of metabolite content and protein quantification. Insulin was assayed in lyophilized fractions of superfusate and detected by radioimmunoassay (RIA, LINCO Research) (rat insulin) and normalized to cellular protein.

RESULTS AND DISCUSSION: ^{31}P-NMR spectra summed from steady-state exposure to a glucose challenge for creatine free (A,B) and creatine loaded (C,D) bioreactors are presented in Fig. 1. Creatine loading altered the PCr content but did not change total adenylate or inorganic phosphate content relative to the internal standard (phenylphosphonic acid), however switching from low to high glucose caused PCr to increase with a stoichiometric decrease in inorganic phosphate. There was no change in ATP. These changes were fully reversible on return to low glucose superfusion (data not shown). Basal insulin release was decreased slightly in creatine loaded cells from 100 to ~50 ng/mg cell protein. Interestingly, increasing glucose from 2 to 16.7 mM resulted in a 3.5-4-fold increase in insulin secretion in both untreated (350 ng/mg) and creatine treated (150 ng/mg) respectively. Thus the fold-increase for each remains constant and proportionate to the glucose challenge however creatine loading appears to reset the amount of insulin released at each [glucose]. The glucose induced decrease in intracellular pH was not altered by creatine treatment. Fig. 2 contains a plot of the calculated ADP versus insulin in untreated and creatine loaded INS1 cells and shows a first order inverse function relating these two parameters. The inset shows a plot of the ATP free energy versus insulin. These data show three important findings. First, ATP control of K_{ATP} channel activation triggering insulin release is not within the physiologic domain of INS-1 cell function as substantial changes in insulin release occur in the complete absence of ATP changes in the NMR spectra. Second, creatine loading suppresses insulin release both in basal and glucose stimulated metabolic states supporting the role of adenylates in insulin secretion. Finally, the first order relation of ADP and insulin suggests that regulation of insulin release resides in ADP as the coupling metabolite between mitochondrial function, insulin release and substrate oxidation.

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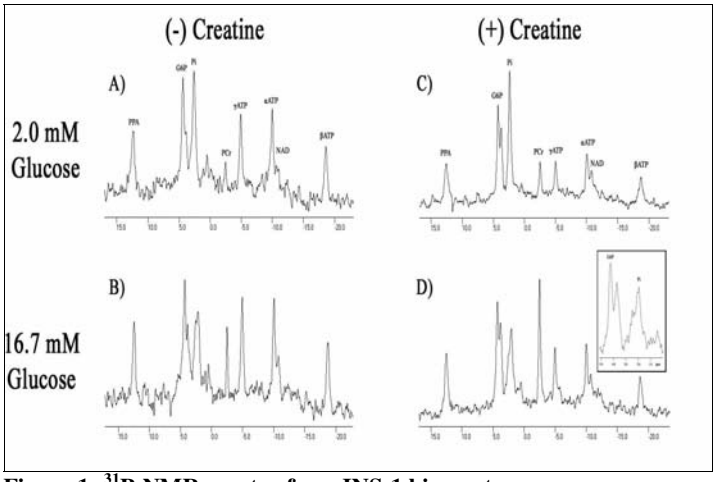

Figure 1: ^{31}P-NMR spectra from INS-1 bioreactors.

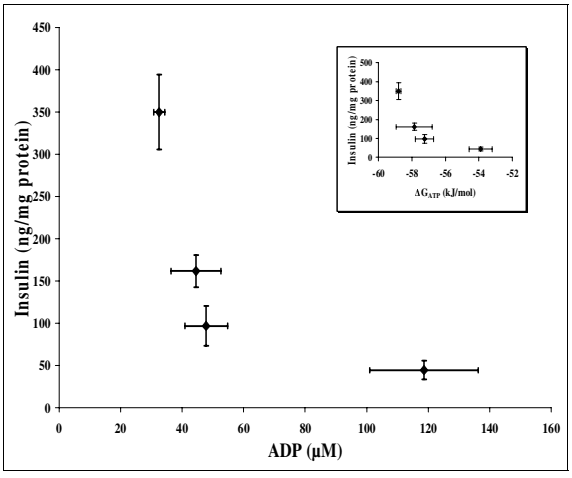

Figure 2: ADP-Insulin in INS-1 cells. Inset is ΔG_{ATP}.