Phosphorous and Carbon Spectroscopy of Porcine Islet Extracts : Comparison of Effects of Normoxic and Hypoxic Culture Conditions

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INTRODUCTION

Pancreatic islet transplantation is a highly promising treatment for restoring normoglycemia in a subpopulation of patients with Type-1 diabetes. However, islet death induced by hypoxia limits viable islet yield and ultimately the success rate of transplantations. To address the metabolic consequences of islet exposure to hypoxia, we compared anaerobic and oxidative carbohydrate metabolism (as measured by ^{13}C label incorporation from ^{13}C-glucose to lactate and amino acids in exchange with TCA cycle intermediates) and energy status (as measured by ATP-to-ADP ratio via ^{31}P NMR) of highly purified porcine islets. Porcine islets are currently being investigated in xenotransplantation in non-human primates. As the supply of human islets from organ donors is quite small and of variable and often poor quality, and with the improvements in both porcine islet isolation and in immunosuppression protocols, porcine islet xenotransplantation in humans could become a reality in the near future. We took advantage of methods developed in our lab to isolate high numbers of pure porcine islets to initiate NMR studies of islet metabolism.

METHODS

Porcine islets (isolated from n=4 pigs, 30,000 islet equivalents per condition) were incubated in RPMI media supplemented with 11 mM [1, 6- ^{13}C]glucose for 6 hours under 2 conditions: normoxia (95% Air, 5% CO_2) and hypoxia (95% N_2, 5% CO_2). Cultures were extracted with perchloric acid, lyophilized, re-suspended in D_2O containing TMSP and trisodium trimetaphosphate (TTP) as reference compounds for ^{13}C and ^{31}P NMR, and were pH adjusted to 7.2-7.4[1]. ^{31}P and proton decoupled ^{13}C NMR spectra were acquired at 25°C using a 500 MHz vertical bore magnet (Varian) and a pulse and acquire sequence (60° pulse, 2.2 s repetition time, 24,576 transients for ^{13}C, and 45° pulse, 3 s repetition time, 13,440 transients for ^{31}P). Spectra were line broadened by 3 Hz and peak integrals obtained using the spectrometer software. For statistical comparisons two-tailed student's t-tests were performed.

RESULTS

The ATP/ADP ratio(+/- S.D.) significantly decreased from 1.88(+/-0.56) in normoxia to 0.96(+/-0.25) under hypoxia (data not shown). Representative ^{13}C spectra from normoxia (**Figure A, top trace**) and hypoxia (**Figure A, bottom trace**) illustrate changes in between normoxia and hypoxia (Glutamate (Glu), glucose (Glc), gamma-aminobutyric acid (GABA), lactate (Lac), alanine (Ala), aspartate (Asp)). The label flows from [1, 6- ^{13}C]glucose to C4 of glutamate in the first turn of the TCA cycle and C2 and C3 glutamate in the second turn [2]. Glu C2 also receives label via the anaplerotic pyruvate carboxylase pathway[2]. The corresponding GABA carbons are then labeled as glutamate is the precursor of GABA. Changes in the ratio of peak integrals (+/- SD) are shown in **Figure B**, white bars are normoxia, grey bars are hypoxia. Compared to glutamate labeling, GABA, alanine and lactate labeling increased (P=0.017, P=0.067, P=0.042, respectively) in hypoxia, indicating increased anaerobic glycolysis, decreased oxidative metabolism and reduced GABA shunt activity and GABA accumulation in islets. Glutamate isotopomer distributions also demonstrated reduced TCA cycle activity under hypoxia. The glutamate C2 to C3 ratio did not change in hypoxia, indicating the anaplerosis to TCA cycle rate (pyruvate carboxylase to pyruvate dehydrogenase) did not change.

DISCUSSION

This is the first report of NMR spectroscopy of highly purified porcine islet extracts. Because our laboratory has a protocol for isolating an average of 150,000 islets per pig, we have the ability to study islet metabolism from a single donor under several different culture conditions. GABA has been shown to increase in islets when exposed to increased glucose and increased cAMP[3], but this is the first report of increased GABA, lactate and alanine labeling from glucose in porcine islets in response to hypoxic culture conditions. This could be due to increased synthesis or decreased catabolism. The latter is more likely as the reentry of GABA into the TCA cycle at succinate in the GABA shunt pathway is likely decreased with decreased oxidative metabolism. GABA accumulation under hypoxia has been observed before, e.g. in plants[4]. It has been shown in islets that GABA is produced by β-cells, secreted by specialized vesicles, migrates to and is taken in by specialized GABA receptors on α-cells, and glucagon production is suppressed[5]. Interestingly, GABA is also an inhibitory neurotransmitter, and, in intact pancreata GABA containing nerve cell bodies surround islets and even penetrate into the mantle of the islets[6]. Also, the most common autoantigen in Type-1 diabetics is to GAD: the enzyme which converts glutamate into GABA in islets[7]. NMR measurements of carbohydrate metabolism in isolated islets in response to different culture conditions may improve our understanding of glucose metabolism in islets, of the development of Type-1 diabetes, and improve auto-, allo-, and xeno-transplant outcomes.

REFERENCES

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Figure A