Hyperpolarized Butyrate: a Novel Substrate for the Assessment of Cardiac Fatty Acid Metabolism

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Introduction: Since the advent of liquid state dynamic nuclear polarization (DNP), pyruvate has provided the major focus of research because of its ease of preparation, high polarization levels and rapid metabolism[1]. However, as the terminal molecule of glycolysis, pyruvate can only yield information about carbohydrate metabolism and provides no direct information about the metabolism of fats. The aim of this study was to explore the potential for hyperpolarized butyrate, a 4-carbon short chain fatty acid, to probe cardiac fat metabolism. Butyrate is produced in the colon of both humans and animals and can act as a fuel source for the heart. Its metabolic pathway is via the production of 3-β-hydroxybutyrate, acetoacetyl-CoA and acetyl-CoA, after which it can proceed via the TCA cycle [2].

Materials and Methods:

Sample preparation: Aliquots (21.9 mg) of [1-^{13}C]butyric acid (Sigma, UK) were mixed with the trityl radical OXO63, 15 mM (GE Healthcare, Amersham, UK), DMSO (4 µl) and a trace amount of Dotarem (Guerbet, France) then placed in a HyperSense hyperpolarizer (Oxford Instruments, Abingdon, UK). To determine the optimum frequency for polarization, the solid-state polarization achieved after 5 minutes of microwave irradiation (100 mW) was measured at a range of frequencies (94.10 to 94.18 GHz in 4 MHz steps). Following this, the samples were polarized at the optimal frequency for an hour and the maximum solid-state polarization level and time constant of polarization enhancement was recorded.

Heart perfusion: Hearts from 4 male Wistar rats (Harlan, UK) were perfused in the Langendorff mode with Krebs-Henseleit buffer containing 10 mM glucose and oxygenated with 95% O_2/5% CO_2. The hearts were placed in the bore of an 11.7 T MRI system (Bruker, Germany) for spectral assessment (Figure 1a).

Spectroscopy: Following polarization, the butyric acid was dissolved with 6 ml of a NaOH/EDTA/Tris buffer, to neutralise the pH, and subsequently added to 50 ml of Krebs-Henseleit buffer to give a 4 mM hyperpolarized sodium butyrate solution. This solution was then delivered to the heart over 120 seconds. A series of 120 carbon spectra were acquired using a pulse-acquire sequence (TR=1 s, FA=30°, BW=180 ppm, 8192 pts, centred at 186.67 ppm).

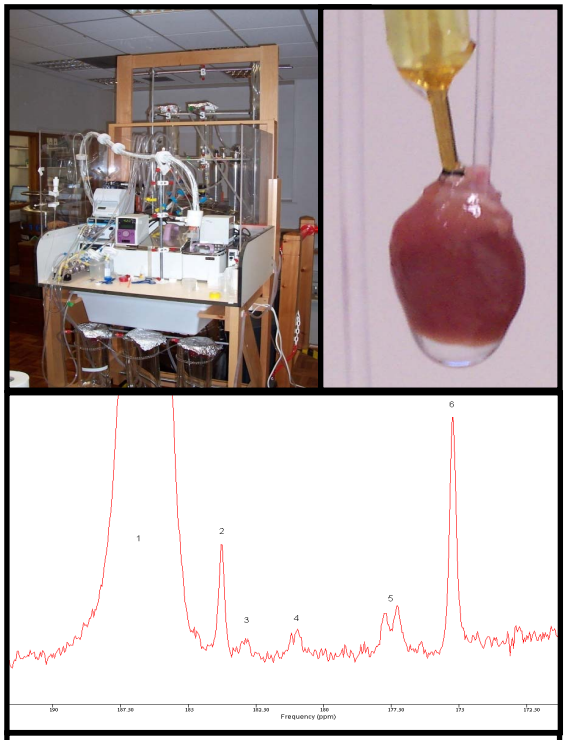

Figure 1 (a) The apparatus used to monitor the metabolism of hyperpolarized butyrate in the perfused heart. (b) An example butyrate spectrum (1: butyrate, 2: glutamate, 3: β-hydroxybutyrate, 4: citrate, 5: acetoacetate, 6: acetylcarnitine)

Metabolite extraction: Following perfusion, one of the hearts was freeze clamped and a chloroform/methanol extraction performed to isolate aqueous metabolites. After freeze-drying, the sample was redissolved in D_2O and a 2D HMBC spectrum run on a Bruker 700 MHz NMR system.

Results and Discussion: The solid-state polarization of butyric acid as a function of microwave frequency reveals optimal polarization was achieved at 94.168 GHz. The solid-state polarization levels achieved were about 2/3 those achieved by [1-^{13}C]pyruvic acid indicating a liquid state polarization of approximately 20-25%. The average polarization time-constant was 1700 s. Figure 1b shows a representative spectrum acquired after dissolution of hyperpolarized butyrate into the perfused heart. As well as the large resonance from the hyperpolarized butyrate, several other resonances were also visible with sufficient SNR for quantification. Provisional peak assignment, from the acquired 2D NMR spectrum, suggests that these resonances are from glutamate (184 ppm), β-hydroxybutyrate (183 ppm), citrate (181 ppm), acetoacetate (177 ppm) and acetylcarnitine (175 ppm). This would indicate that hyperpolarized butyrate is capable of probing both the initial stages of short chain fatty acid metabolism and subsequent incorporation of the acetyl-CoA produced into the TCA cycle. Perfusion with butyrate was also shown to have no effect on the energetic state of the heart, as assessed by ^{31}P MR spectroscopy.

Conclusion: Hyperpolarized butyrate is a promising new substrate for use in the assessment of short chain fatty acid metabolism in the heart. It provides a good level of polarization and its uptake and metabolism are sufficiently rapid to allow the ^{13}C label to be followed over multiple metabolic steps. Future work will focus on confirming the assignment of the observed resonances, investigation of the potential for *in vivo* utilization of hyperpolarized butyrate and subsequent application of hyperpolarized butyrate in the assessment of pathologies linked with disordered fatty acid metabolism.

[1] Golman, K., et al. Proc Natl Acad Sci U S A, 2006. 103(30): p.11270-5.
[2] http://pathman.smpdb.ca/pathways/SMP00073/pathway?level=1 *Butyrate Metabolism*

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