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Tissue targeted metabonomics: Metabolic profiling by microdialysis sampling and microcoil NMR

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Abstract

The concentration of low molecular weight compounds in tissues can yield valuable information about the metabolic state of an organism. Studies of changes in the metabolic state or metabonomics can reflect disease pathways, drug action, or toxicity. This research aims to develop a new approach, tissue targeted metabonomics. Microdialysis sampling and microcoil NMR analysis are employed to compare basal and ischemic metabolic states of various tissues (blood, brain, and heart) of Sprague–Dawley rats. Microdialysis sampling is localized, making the metabolic profile tissue specific. Coupling to NMR analysis is highly advantageous, because a complete metabolic profile is obtained in a single spectrum. However, small sample volumes and low analyte concentrations make analysis of microdialysis samples challenging. Microcoil NMR uses low sample volumes and has improved mass sensitivity, relative to standard 5 mm probes. The coupling of these techniques is a potentially powerful tool for metabonomics analysis.

Keywords: Microcoil NMR; Metabonomics; Ischemia; NMR; Microdialysis

1. Introduction

Metabonomics has emerged as a powerful approach to systems biology, providing unique insights into the physiological effects of disease, drugs, toxicants, or genetic alterations [1–5]. Metabonomics-based investigations typically involve monitoring perturbations in the concentrations of endogenous metabolites in biological fluids, such as urine, plasma, cerebrospinal fluid, bile, or seminal fluid. Common methods of sampling of endogenous metabolites for metabonomics studies vary from biofluid collection to tissue excision. Biofluids, such as urine or plasma, are popular in metabonomics studies because they are fairly simple to collect, contain an abundance of metabolic information, and are minimally invasive to the animal during collection. However, these biofluids represent the average metabolic status of the organism and cannot give tissue-specific metabolic profiles. To address this, some studies have examined whole tissues or tissue extracts. Tissue excision can offer localized metabolic information, but it is conducted post-mortem or through biopsy and cannot give long-term, real-time information on the same animal.

Alternatively, microdialysis sampling allows for the acquisition of localized chemical information in nearly any tissue [6,7]. The technique is minimally invasive, allowing subjects to be awake and mobile during experimentation. Analyte sampling is accomplished by the implantation of a semipermeable membrane in the site of interest. Perfusion of a fluid through the dialysis probe facilitates diffusion of small hydrophilic molecules across the membrane, where they can be collected for analysis. Most dialysate samples are free of the macromolecules that can overshadow small molecules in NMR analysis. Problems arising from enzymatic degradation of the sample are also eliminated.

In this study, microdialysis sampling is applied to a metabonomics study of myocardial ischemia. Ischemia is the cessation of blood flow to a tissue or portion of tissue. Com-

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mon clinical ischemic conditions are stroke and heart attack. Reperfusion is the period following ischemia when blood flow is restored. Both events drastically alter the metabolic state of the tissue in a specific location, making tissue targeted metabonomics studies important to truly understand the condition in vivo. This approach was previously applied to a metabonomics study of rat brain neurochemistry after the administration of tetradotoxin, a neurotoxin, by Khandelwal et al. [8,9].

Metabonomics analysis [5] is most commonly performed with ^{1}H NMR [10,11]. For metabonomics studies of microdialysis samples, the sample volume is insufficient for traditional NMR analysis. Microcoil NMR probes can overcome these sample limitations. The microcoil probe used in these experiments (CapNMR probe produced by Protasis/MRM) is composed of a length of capillary with a bubble cell. The observed volume of this bubble cell is 1.5 μL and total probe volume is approximately 10 μL. Thus, sample volume requirements are reduced significantly when compared to traditional NMR analysis. A further advantage of microcoil NMR analysis is increased mass sensitivity, meaning that given the same mass of analyte detected, the microcoil probe will give a larger response than a traditional 5 mm NMR probe [12]. Therefore, microcoil NMR is advantageous for analysis of mass and volume limited samples.

2. Experimental

2.1. Reagents

All deuterated solvents were obtained from Cambridge Isotope Laboratories, Andover, MA. Ringer's solution was prepared in-house, with the salts obtained from Sigma Chemical Co., St. Louis, MO.

2.2. Animal protocols

For all animal studies, Sprague–Dawley rats (Sasco, Wilmington, MA) were pre-anesthetized with isoflurane, followed by full anesthesia by i.m. injection of a ketamine (100 mg/kg)/xylazine (10 mg/kg) mixture. The animals remained anesthetized and were closely monitored throughout the experimental procedures. Booster doses of one-fourth the original dose of ketamine were used as needed to maintain adequate anesthesia. Heated mats maintained the rats' body temperature. All surgical techniques described below were performed separately on several rats, rather than simultaneously on a single rat. For the data presented in this communication, surgeries were not repeated.

2.3. Brain probe implantation

To insert microdialysis probes into the cerebral cortex of Sprague–Dawley rats, the hair on top of the rat skull was shaved and the region disinfected. The animal was then securely positioned in a stereotaxic apparatus and a midline 1-in. incision was made through the skin at the top of the skull parallel to the saggital suture. Adventitious tissue covering the skull was removed with cotton swabs. To provide a firm location to which dental adhesive could attach, two 1-mm diameter holes were hand-drilled approximately 2 mm anterior to the insertion site of the guide cannula. Two stainless steel anchor screws (1 mm diameter, 2 mm length) (BAS, West Lafayette, IN) were secured in these holes. Next, a 1 mm diameter hole was drilled through the skull at the insertion site and an intracerebral guide cannula was lowered into the brain using a micromanipulator attached to the stereotaxic apparatus. The guide cannula (BAS) was positioned 2 mm above the cerebral cortex, and then affixed to the skull with dental cement. The dummy probe in the guide cannula was replaced with a BAS microdialysis probe (BAS) after the guide cannula was glued securely in place. For all experiments, the molecular weight cut-off of the dialysis membrane was 5000 Da. To collect brain dialysate, Ringer's solution was passed through the probe at 1 μL/min.

2.4. Heart probe implantation

To begin the heart probe implantation, the animal's heart rate was monitored using a Digi-Med sinus rhythm analyzer (Louisville, KY). After the animal's normal heart rate was established, a tracheotomy was performed by first exposing, and then isolating the trachea on a spatula. Cotton swabs removed any moisture from the exposed trachea. A scalpel was used to create an opening between the rings of the trachea, and a tube was inserted into the trachea and secured with sutures. The tube was then connected to a respirator. Artificial ventilation was performed with a constant-volume respirator using room air (Model 683 Rodent Respirator, Harvard Apparatus, Holliston, MA). To prepare for the microdialysis probe insertion into the heart, the thoracic cavity was shaved and cleansed. To expose the heart, with the animal in the lateral position, a left thoracotomy was performed by making an incision between the fifth and sixth ribs approximately 1.5–2 cm in diameter on the left side, and the pericardium was opened. A microdialysis probe (prepared in-house) was implanted into the myocardium through a small incision in the pericardium. Specifically, a needle was used to implant a microdialysis probe into the beating heart muscle adjacent to the left descending coronary artery along the longitudinal axis of the heart. Linear heart probes were made in-house from polyacrylonitrile membrane, and were approximately 2 mm in membrane length. Finally, after the lungs were fully expanded, Ringer's solution was passed through the probe at 1 μL/min to collect heart dialysate.

2.5. Jugular probe implantation

A small midline skin incision was made on the neck and the jugular vein was located. The jugular vein was cleared from fine connective tissue by blunt dissection and cotton