CAPSULES
notes and applications from Bioanalytical Systems, Inc.
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Short Interval Monitoring of Glucose in Zucker Diabetic Fatty (ZDF) Rats

Introduction

Zucker diabetic fatty rats (ZDF/Gmi™) [1-3] are a useful animal model for human Type II diabetes mellitus (NIDDM). These rats are available from Genetic Models, Inc. of Indianapolis (P.O. Box 68737, Indianapolis, IN 46268-0737).

Studies on these diabetic rat models frequently involve monitoring glucose levels. Traditional methods of obtaining samples are bleeding from the tail or cannulation of a jugular vein. Both of these methods limit the number of samples and the amount and frequency of data that can be collected.

Research in NIDDM using Zucker rats and research in animal models of disease in general would benefit from sample collection methods that allow for frequent sampling without stressing the animal or causing anemia.

Microdialysis (MD) and ultrafiltration (UF) are two techniques that use long membrane probes to sample the extracellular space [4].

In MD, an isotonic solution is pumped through the membrane. Low molecular weight molecules diffuse across the membrane in response to a concentration gradient.

In UF, a negative pressure is applied to the probe while extracellular fluid is drawn through the membrane under the driving force of a pressure gradient. Both of these methods are useful in studying glucose dynamics in the Zucker diabetic rat.

Each technique has its advantages. In MD, it is possible to precisely control the flow rate, and therefore the sample volume. However, in MD, the recovery of the analyte (the concentration in sample/concentration in extracellular fluid (ECF)) is < 100%. In UF, recovery of low molecular weight hydrophilic molecules is approximately 100%. The UF glucose concentration therefore is the same as the ECF glucose concentration [5]. The simultaneous use of MD and UF offers the benefits of each technique. Membrane probe sampling used with the BAS method of glucose determination requires small sample volumes and permits sampling intervals of 3 to 5 minutes.

Methods

Probe implantation: The ideal probe implantation site for glucose measurement in the rat is the subcutaneous tissue. This provides a large, easily accessible space for long membrane probes which optimize recovery for MD and sample volume for UF.

The best UF probe for this application is the UF-3-12 (PN MF-7023). The large surface area of this particular probe yields the highest flow rate, allowing for frequent sampling.

For MD, the long membrane DL-5 (PN MF-7051), maximizes recovery. Detailed instructions on probe implantation are included in BAS Capsule #241 [6].

Awake animal sampling: Sampling takes place in the BAS Awake Animal System (PN MD-1575), equipped with a swivel and tether system to allow normal rodent behavior while sample collection is in progress. Before placing the animal in the Awake Animal System, the tubing and swivel components are sterilized by perfusion for one hour with a cold sterilant. This is followed by one hour of sterile Ringers' solution to wash out the sterilant. This procedure prevents introduction of bacteria into the samples which could consume the glucose and yield erroneous data.

When the rat is secured in the Awake Animal System, the microdialysis probe is connected to the liquid swivel and the probe is perfused with sterile Ringers' solution using the modular BAS microdialysis pump (PN MD-1000, PN MD-1001).

Automated sample collection is performed by connecting the outflow of the MD probe to the swivel and collecting samples with a fraction collector. For simultaneous UF collection, a hub assembly (PN MF-7021) is attached to the UF probe and inserted into a Vacutainer®. The Vacutainer can be attached to the flag on the swivel. For automated UF fraction collection, the UF probe can be connected to the swivel. A minipump (PN MF-5200) can be used to generate the negative pressure.

Glucose analysis: Glucose is analyzed on a BAS LC system by flow injection analysis using a BAS glucose kit (PN MF-6152). This method uses an immobilized enzyme reactor coupled with a "wired" peroxidase electrode [7,8].

Results and Discussion

This system can be used for many different types of studies. The following illustrates the results obtained in individual rats under different sets of conditions.

F1 illustrates variations in fasting glucose levels using MD probes in 2-month-old control and obese rats and in a 6-month-old obese rat. Flow rates were 5 µL/minute. Sample collection was at 5-minute intervals. The average microdialysate glucose in a 2-month-old lean rat was 2.1 mM. The average glucose in the obese litter mate of this rat at the same age was 1.7 mM. An obese 6-month-old rat had an average fasting glucose of 9.6 mM. Since recovery was < 100%, these were relative concentrations.

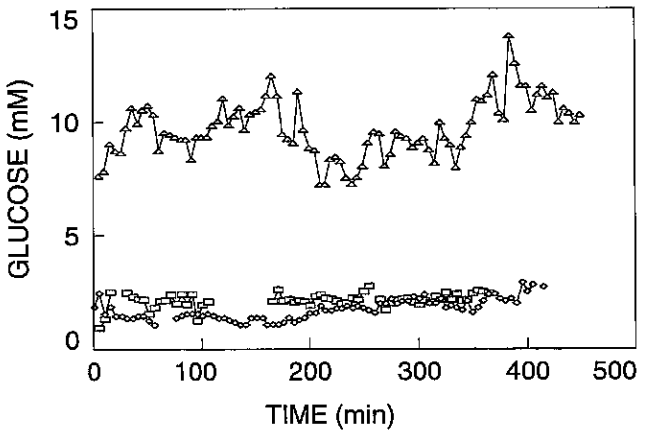

Figure 1. Subcutaneous microdialysate glucose in fasting lean (□) and obese (◇) 2-month old and 6-month-old obese (△) Zucker rats.

For absolute ECF glucose concentrations, UF is recommended. F2 illustrates the relationship of glucose in a 6-month-old diabetic rat and a 2-month-old lean rat. In this study, the rats were allowed food *ad libitum*. It has been demonstrated in previous studies that UF and blood glucose levels are the same [5], therefore the glucose values also represent blood glucose concentrations.

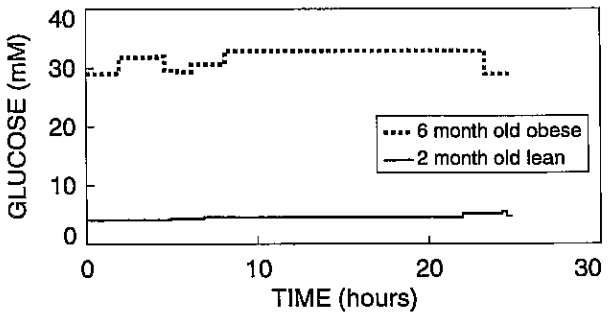

Figure 2. Subcutaneous ultrafiltrate glucose in 2-month-old lean (—) and 6-month-old obese (····) Zucker rats with food *ad libitum*.

The 100% *in vivo* recovery rate for UF probes can also be used to determine an *in vivo* recovery for MD probes. Both MD and UF probes are implanted and samples are collected simultaneously. Glucose is determined in both UF and MD samples and an *in vivo* recovery for MD is calculated.

The ability to conduct frequent sampling makes it possible to monitor glucose dynamics under a variety of conditions.

References

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