Video Article

Physiology Lab Demonstration: Glomerular Filtration Rate in a Rat

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Abstract

Measurements of glomerular filtration rate (GFR), and the fractional excretion of sodium (Na) and potassium (K) are critical in assessing renal function in health and disease. GFR is measured as the steady state renal clearance of inulin which is filtered at the glomerulus, but not secreted or reabsorbed along the nephron. The fractional excretion of Na and K can be determined from the concentration of Na and K in plasma and urine. The renal clearance of inulin can be demonstrated in an anesthetized animal which has catheters in the femoral artery, femoral vein and bladder. The equipment and supplies used for this procedure are those commonly available in a research core facility, and thus makes this procedure a practical means for measuring renal function. The purpose of this video is to demonstrate the procedures required to perform a lab demonstration in which renal function is assessed before and after a diuretic drug. The presented technique can be utilized to assess renal function in rat models of renal disease.

Video Link

The video component of this article can be found at https://www.jove.com/video/52425/

Introduction

The most important function of the kidney is the homeostatic regulation of extracellular water and electrolyte content. The kidneys closely regulate extracellular water, sodium (Na) and potassium (K) to maintain normal physiological levels. Disturbances in renal function can result in serious metabolic disorders which can be fatal. The basic renal process occurs in the nephron and begins with the filtration of plasma at the glomerulus and ends with the excretion of urine. Other processes that determine the final concentration of water, Na and K in the urine are secretion and reabsorption within the nephron. Measurements of glomerular filtration rate (GFR) and the fractional excretion of Na and K are critical in assessing renal function in health and disease. The reader is referred to previously published review articles and textbooks for a more thorough discussion of kidney function[1-4].

GFR can be measured as the steady state renal clearance of inulin which is filtered at the glomerulus, but not secreted or reabsorbed along the nephron[5]. While this technique requires anesthesia, surgical preparation, and a terminal experiment, it is considered the gold standard of GFR measurement. Using inulin that is tagged with fluorescein-isothiocyanate (FITC), plasma and urine concentration of FITC-inulin can be easily measured in small volumes and used to calculate GFR during multiple time points of an experiment. The fractional excretion of Na and K can be determined from the concentration of Na and K in plasma and urine.

The conceptual understanding of how to measure renal function can easily be demonstrated in a short lab designed to allow students to actively participate in some aspects of the experiment. This video depicts the pre-lab preparation, the renal function demonstration, and the post-lab evaluation of results. The surgical techniques necessary for making measurements of GFR are demonstrated in an anesthetized rat. In addition, example calculations for GFR, and the fractional excretion of Na and K are shown before and after administration of a diuretic drug.

Protocol

Prior to any animal procedure, the institutional animal care and use committee (IACUC) must approve the protocol. This protocol was approved by the Michigan State University IACUC.

1. Pre-lab Preparation of FITC-inulin Solution

1. Warm 20 ml of saline to 70 °C and slowly stir in 100 mg of FITC-inulin (5 mg/ml FITC-inulin) until all inulin is dissolved.

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2. Cool solution to RT and add 800 mg of bovine serum albumin (40 mg/ml BSA, lyophilized powder, essentially globulin free, low endotoxin, ≥98% purity by agarose gel electrophoresis).
3. Filter the inulin-BSA solution with filter paper (grade 1). Place the filtered solution in a 20 ml syringe with a syringe-tip filter (0.2 µm) and cover with foil to protect from light.

2. Anesthesia and Surgery

1. Place the rat in an induction chamber filled with 5% isoflurane to induce anesthesia. Record body weight (250-350 g) and place the rat on a heated surgical platform designed to maintain 37 °C body temperature throughout the experiment. Gently secure the rat to the platform with laboratory tape over the paws. Maintain anesthesia with 1-2% isoflurane with medical grade 100% O_2 at airflow rate of 0.8-1.0 L/min.
2. Insert a tapered catheter (intravascular tip O.D., 2.7F) into the femoral artery for blood pressure and heart rate monitoring, and blood sampling.
3. Insert a catheter (PE-50) into the femoral vein for inulin infusion. Secure the catheter to surrounding tissue with 5-O braided silk surgical suture[6].
4. Attach the arterial catheter to a strain gauge pressure transducer. Record blood pressure and heart rate using data acquisition software and display on a computer screen in real-time. This technique is demonstrated in detail on video[6].
5. Expose the bladder via a suprapubic incision. Cut a small hole in the tip of the bladder and insert a cannula (PE-190) with a heat flared tip inside the bladder for urine collection. Secure the cannula to the bladder with a purse-string suture.

3. Urine and Blood Collection

1. Place the syringe of FITC-inulin in a syringe pump with flow rate set of 1 ml/hr per 100 g of body weight (3 ml/hr for a rat weighing 300 g). Attach the syringe to the femoral vein catheter. Start the inulin infusion and allow a 1-2 hr equilibration period. Keep syringe covered with foil to protect from light.
2. Determine if urine flow rate is stable and adequate for sample analysis (20 µl/min) by collecting a urine sample in a pre-weighed collection vial for a period of 10 min. Determine urine volume gravimetrically with a digital scale. An adequate urine volume for a 10 min collection period is 0.2 ml. Continue to collect urine samples until two consecutive collections indicate a urine flow rate of 20 µl/min or more.
3. Pre-Drug Samples
    1. Collect a urine sample during a 20 min period. Collect a blood sample (0.5 ml) from the arterial catheter at the midpoint of the urine collection period. Be careful to completely clear the arterial catheter of saline before collecting a blood sample in a collection vial containing 1 U heparin. Use collection vials with volume markings to facilitate the collection of 0.5 ml of arterial blood.
    2. Flush the arterial catheter with heparin-saline (20 U/ml) to clear the catheter of blood (approx. 0.1 ml). The length of the arterial catheter should be as short as possible to limit the volume of heparin-saline required to flush.
    Note: Diluted blood samples produce inaccurate calculations of GFR and fractional excretion of Na and K.
    3. Wait 10 min, and repeat the collection of a second Pre-drug urine and blood sample.
4. Following the collection of two Pre-drug samples, administer a diuretic drug, furosemide (10 mg/kg), via the arterial catheter. Flush the arterial catheter with heparinized saline to clear the catheter of drug. Take care to prevent the injection of air through the arterial catheter. Record the time of the furosemide injection.
5. Post-drug Samples: At each of the 3 time points below, collect a urine sample during a 10 min collection period, and a blood sample (0.5 ml) at the midpoint of the urine collection period.
    1. For Post-Drug Sample 1 – collect five min after furosemide.
    2. For Post-Drug Sample 2 – collect ten min after furosemide.
    3. For Post-Drug Sample 3 – collect fifteen min after furosemide.
6. After all samples have been collected, euthanize the rat in accordance with institutional procedures by thoracotomy and removal of the heart. Remove both kidneys. Decapsulate (remove the surrounding membrane) and blot the kidneys to remove excess blood. Weigh the kidneys.

4. Sample Analysis

1. Measure all urine sample volumes gravimetrically with a digital scale, and record weights.
2. Centrifuge whole blood samples with a table-top centrifuge (1,800 x g) to separate plasma. Transfer plasma samples to small labeled vials.
3. Analyze Na and K concentrations in urine and plasma samples with a sodium/potassium analyzer.
4. Measurement of FITC-inulin in plasma and urine
    1. Dilute pre-drug urine (from 1:200 to 1:400), and post-drug urine (1:10) with HEPES buffer (500 mM, pH 7.4).
    2. Add 40 µl of standard or sample and 60 µl of HEPES buffer in a 96 well plate (one sample per well) and allow to mix for 10 min while covered with aluminum foil.
    3. Generate a standard curve for FITC-inulin for concentrations of 6.25, 12.5, 25, 50, 100, 200, 400 µg/ml (**Figure 1**). Determine FITC-inulin fluorescence in samples and standards using a microplate reader with excitation and emission wavelengths of 485 and 538 nm, respectively.
    4. Fit the fluorescent values for the standards to a 4-paramter logistic function regression analysis. The regression function parameters are used to calculate FITC-inulin concentration in plasma and urine samples (**Table 1**).

5. Post-lab Analysis of Results: Calculations

1. Calculate Urine Flow Rate (UV; ml/min): [volume of urine collected (ml)] ÷ [time of collection (min)]
2. Calculate Glomerular Filtration Rate (GFR; ml/min): [Urine inulin concentration (µg/ml) x UV (ml/min)] ÷ [Plasma inulin conc. (µg/ml)]

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3. Calculate Filtered Sodium Load (µmol/min): Plasma sodium concentration (µmol/ml) x GFR (ml/min)
4. Calculate Sodium Excretion Rate ($U_{Na}V$; µmol/min): Urine sodium concentration (µmol/ml) x UV (ml/min)
5. Calculate Fractional Excretion of Sodium (FE Na; %): [$U_{Na}V$ (µmol/min)] ÷ [Filtered Sodium Load (µmol/min)] x 100
6. Calculate Filtered Potassium Load (µmol/min): Plasma potassium concentration (µmol/ml) x GFR (ml/min)
7. Calculate Potassium Excretion Rate (U_KV; µmol/min): Urine potassium concentration (µmol/ml) x UV (ml/min)
8. Calculate Fractional Excretion of Potassium (FE K; %): [U_KV (µmol/min)] ÷ [Filtered Potassium Load (µmol/min)] x 100

Representative Results

The diuretic used in the lab demonstration was furosemide which very quickly inhibits the reabsorption of Na and K filtered by the kidney resulting in increased Na, K, and water excretion within minutes of drug administration. By its primary mechanism, furosemide should have minimal effects on GFR and the filtered load of Na and K, but will increase urine flow, and fractional excretion of Na and K.

The representative results in **Table 3** show that in an anesthetized rat, the average of the pre-drug values for GFR was 3.2 ml/min, Na excretion was 0.58 µmol/min (0.1% of the filtered load), and K excretion was 4.4 µmol/min (27% of the filtered load). Five minutes after furosemide (post-drug 1), GFR and the filtered load of Na and K were unaffected. However, the fractional excretion of Na increased to 11.5%, and the fractional excretion of K increased to 63% of the respective filtered loads. The measurements of MAP and HR indicate that furosemide had minimal effects on MAP and HR (**Table 2**).

The indices of renal function assessed in the laboratory demonstration were the GFR, defined as the rate by which plasma is filtered by the kidney; the filtered Na and K, defined as the rate by which Na and K are filtered by the kidney; the Na and K Excretion Rate, defined as the rate by which Na and K are excreted by the kidney; and the fractional Excretion of Na and K, defined as the percentage of filtered Na and K that is excreted by the kidney

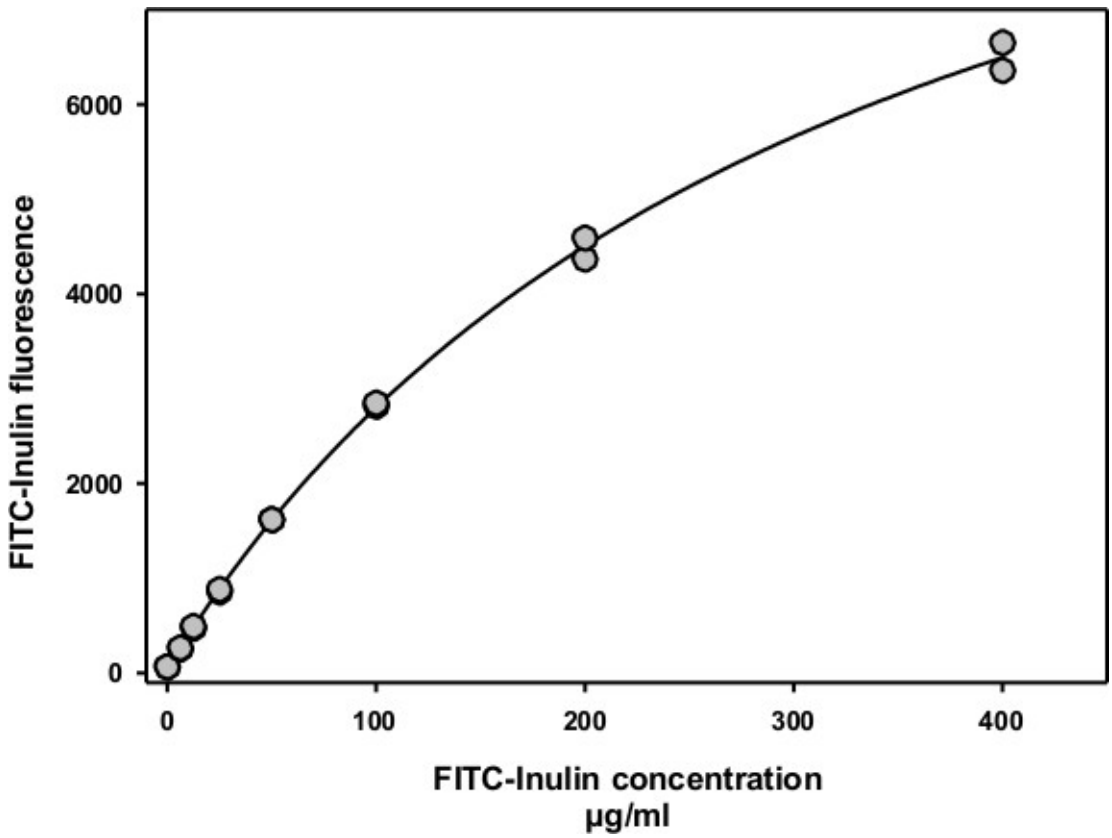

Figure 1: Inulin Standard Curve. FITC fluorescence values are shown for standards containing 6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml inulin. A 4-paramter logistic function regression analysis generates the best-fit curve. The regression function parameters from this curve were used to calculate FITC-inulin concentration in plasma and urine samples.

	FITC-Inulin fluorescence			Concentration		Result
Standard	replicate 1	replicate 2	Mean	µg/ml	Dilution	µg/ml
Blank	63.9	64.8	64.4	0.4	1	0.4
6.25	253.2	264.1	258.7	5.9	1	5.9
12.5	474.0	491.3	482.7	12.5	1	12.5

25	854.8	881.3	868.1	24.4	1	24.4
50	1617.1	1618.0	1617.6	50.3	1	50.3
100	2813.1	2846.1	2829.6	101.3	1	101.3
200	4367.3	4588.7	4478.0	198.2	1	198.2
400	6258.0	6650.0	6454.0	401.6	1	401.6
Urine Sample						
Pre-drug 1	2443.9	2062.3	2253.1	88.5	200	17700
Pre-drug 2	2266.5	1707.0	1986.8	76.3	200	15250
Post-drug 1	1208.9	1391.2	1300.1	44.7	10	447
Post-drug 2	2753.4	2120.5	2437.0	97.0	10	970
Post-drug 3	2888.3	3178.0	3033.2	124.4	10	1244

Table 1: Sample Results of Inulin Assay. FITC-Inulin fluorescence values are shown for the reagent blank, 7 standards, and 5 urine samples. Standards and samples were assayed in duplicate and diluted as needed. The average fluorescence for each sample was used to calculate the concentration of inulin. The inulin concentrations in these urine samples are included in the table of measurements (**Table 2**).

Experiment Time Point	MAP (mm Hg)	HR (beats /min)	Duration of Sample Collection (min)	Urine Vol. (ml)	Plasma Na (µmol/ml)	Urine Na (µmol/ml)	Plasma K (µmol/ml)	Urine K (µmol/ml)	Plasma Inulin (µg/ml)	Urine Inulin (µg/ml)	right and left kidney weight (g)
Pre-drug 1	98	376	20	0.20	141	49	5.1	375.0	63.4	17700	2.1
Pre-drug 2	100	376	20	0.30	142	45	5.0	341.0	62.9	15250	
Post-drug 1	107	376	10	5.80	143	110	4.6	19.5	66.9	447	
Post-drug 2	109	387	10	3.10	142	117	4.0	26.4	62.6	970	
Post-drug 3	108	372	10	2.00	143	117	3.7	33.5	64.0	1244	

Table 2: Measurements Recorded during the Renal Function Lab Demonstration. The variables recorded during five time periods (two Pre-drug and three Post–drug) of the renal function lab demonstration are right and left kidney weight, mean arterial pressure (MAP), heart rate (HR), sample time, urine volume, plasma and urine sodium (Na), potassium (K), and inulin concentrations. The urine inulin concentrations were determined from the inulin assay shown in **Table 1**.

	Urine Flow (ml/min)	GFR (ml/min)	GFR/g Kidney wt (ml/min/g)	Na Excretion (µmol/min)	Filtered Na (µmol/min)	Fractional Excretion of Na (%)	K Excretion (µmol/min)	Filtered K (µmol/min)	Fractional Excretion of K (%)
Pre-drug 1	0.01	2.8	1.3	0.49	393.6	0.12	3.75	14.24	26.34
Pre-drug 2	0.02	3.6	1.7	0.68	518.1	0.13	5.12	18.24	28.04
Post-drug 1	0.58	3.9	1.8	63.80	554.2	11.51	11.31	17.83	63.44
Post-drug 2	0.31	4.8	2.3	36.27	682.1	5.32	8.18	19.21	42.59
Post-drug 3	0.20	3.9	1.9	23.40	555.9	4.21	6.70	14.38	46.58

Table 3: Renal Function Parameters Calculated from Recorded Measurements. Using the formulas shown in Protocol Section 5, the recorded variables (**Table 2**) are used to calculate urine flow rate, glomerular filtration rate (GFR), GFR/g kidney weight, excretion rate, filtered load, and fractional excretion of sodium (Na) and potassium (K) during the two Pre-drug and three Post–drug periods.

Discussion

An appropriate marker for GFR measurement must meet four criteria: be freely filtered at the glomerulus, be unbound to plasma proteins, and neither be absorbed nor secreted in the nephron. Inulin is a fructose polymer which satisfies these criteria. As a result, the renal clearance of inulin is considered the gold standard for measuring GFR[7]. The demonstrated technique represents the traditional approach of determining the renal clearance of inulin using timed urine collections during a constant infusion of inulin[8,9]. Traditional inulin measurements have been made using the anthrone method to produce a quantitative colorimetric determination of inulin measured by spectrophotometer[10,11]. However, in an attempt to facilitate the measurement of inulin in smaller volumes of urine and plasma, inulin has been tagged with radioactive[12-14], and fluorescent labels[15-17]. The lab demonstration presented in this video used FITC labelled inulin for the measurement of renal function because of the lack of risk of human radiation exposure and the ease of measuring FITC fluorescence[15].

This lab demonstration is intended to provide a conceptual understanding of how to measure renal function to students with minimal laboratory skills. Therefore, the pre-lab preparation of FITC-inulin solution, and surgical preparation of the animals are performed by experienced technicians prior to the start of the demonstration. The students arrive for the demonstration at the end of the 1-2 hr inulin equilibration period. At this time, the students are presented with a Pre-lab overview and informed of the procedures that have been conducted on the animals. Two students are assigned to one animal experiment, and instructed on how to collect blood and urine samples before and after the administration of the diuretic drug. The analysis of blood and urine samples is conducted by experienced technicians and results are delivered to the students for calculations of renal function. Results are presented during a Post-lab discussion which can be scheduled after the demonstration.

There are several critical steps within the protocol to insure valid responses. Firstly, FITC inulin must be completely dissolved and filtered prior to animal administration. Ideally, FITC inulin should be dialyzed in water for 48 hr at RT to remove residual unbound FITC. Secondly, plasma samples must be free of saline. Students are instructed to collect a blood sample only after all of the saline in the arterial catheter has been expelled and only blood is flowing out of the catheter. Blood samples that are diluted with saline will provide inaccurate values for plasma inulin, sodium and potassium. Thirdly, urine flow must be steady and adequate to produce enough sample for analysis. A steady urine flow rate at baseline is critical because it is an indication of a stable experimental preparation. If urine flow is too low, the infusion rate of inulin can be increased prior to sample collections. However, the infusion of inulin must be constant during the course of the experiment, *i.e.*, inulin infusion rate should not be adjusted during the experiment. Finally, the measurement of inulin fluorescence in plasma and urine samples by microplate reader is critical to a successful experiment. Since the specifications of the microplate reader will determine if samples require dilutions, it is recommended that a test run of the inulin assay be conducted prior to the lab demonstration in an effort to optimize the specifications of the microplate reader and ensure that sample fluorescence values are within the mid-range of the standard curve.

While assessing renal function based on the renal clearance of inulin is considered the gold standard, this technique has limitations because the animals must be anesthetized, and instrumented with vascular and bladder catheters. Anesthethetic agents have been shown to affect renal hemodynamics and GFR[18,19]; however isoflurane and inactin are typically used in renal function experiments due to their minimal effects on the kidney[19,20]. The inulin clearance technique also requires a constant infusion of inulin and multiple blood and plasma samples which can be prohibitive in smaller animals such as mice. Modifications of this technique have been developed to allow the measurement of plasma clearance from a single injection of inulin in conscious animals[21]. These modifications also require smaller volumes of blood samples for analysis, and provide an alternate method for assessing renal function in mice.

The measurement of renal function is applicable to studies of physiology, pathology, toxicology, pharmacology and disease states. Students who participate in the Renal Function demonstration will learn the gold standard technique of renal clearance of inulin to assess renal function. By mastering this technique, students will understand the principles of renal function and allow them to apply the technique to their own research and determine if modifications to the technique are appropriate for their studies.

Disclosures

The authors declare that they have no competing financial interests. The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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