Single Nephron Filtration, Luminal Flow and Tubular Fluid Reabsorption along the Proximal Convolution and the Pars recta of the Rat Kidney as Influenced by Luminal Pressure Changes

WOLFGANG SEILLER* and KARL-HEINZ GERTZ
Physiologisches Institut der Medizinischen Hochschule Hannover, Karl-Wiechert-Allee 9, D-3000 Hannover-Kleefeld, Federal Republic of Germany

Summary. Intratubular pressures were measured in free flow and after blockade of tubular flow at different distances from the glomerulum in the kidney of Wistar rats. Free flow pressure was \( ffp = 13.3 \pm 2.5 \) Torr and stop flow pressure \( sfp = 41.7 \pm 3.8 \) Torr. With increasing distance of the blockade from the glomerulum the intratubular pressure decreased being \( 22.4 \pm 2.1 \) Torr, when the tube was blocked at the end of the pars recta. In a second series single nephron filtration rate (gfr) and late proximal flow rates \( \dot{V}_r \) were measured at different intratubular pressures. Free flow gfr was \( 26.5 \pm 5.9 \) nl/min and \( \dot{V}_r = 14.7 \pm 4.0 \) nl/min. The difference of these flow rates divided by tubular length results in a local reabsorption rate of \( C = 2.9 \pm 0.9 \) nl/min \( \cdot \) mm in the proximal convolution. In the pars recta local reabsorption rate was \( 1.0 \pm 0.3 \) nl/min \( \cdot \) mm. In the proximal convolution \( C \) increased with increasing intratubular pressure: \( \Delta C/\Delta \text{pip} = (2.7 \pm 1.2) \cdot 10^{-2} \) nl/min \( \cdot \) mm \( \cdot \) Torr.

Filtration was in disequilibrium in these animals under all conditions examined, hydraulic filtration conductance was \( K = 1.2 \pm 0.4 \) nl/min \( \cdot \) Torr.

Modified methods have been used for intratubular pressure and for flow rate measurements in order to reduce experimental procedure. It is shown, that fractional reabsorption, calculated on the basis of pressure measurements, is a good approximation to results usually obtained by inulin measurements.

Key words: Single nephron filtration rate — Effective filtration pressure — Filtration disequilibrium — Proximal fluid reabsorption.

INTRODUCTION

Single nephron filtration rate and proximal fluid reabsorption in the rat kidney have been studied extensively in micropuncture experiments. General accepted explanations, however, for the relative constancy of glomerular filtration and proximal reabsorption fraction have not been developed so far. The intention of the present study, therefore, was to prove, if a filtration equilibrium is of importance for the regulation of the filtration fraction and how intratubular pressures influences filtration rate.

METHODS

Male Wistar rats of 180—230 g were used, having free access to a rat pellet diet until 15 h preceding the experiment and to water ad libitum. The animal was anesthetized by intraperitoneal injection of inactin, 110 mg/kg body weight, and subsequently tracheotomized. The arterial blood pressure was recorded continuously during each experiment by cannulation of the left carotid artery with a polyethylene tube connected to a strain gauge transducer. Two catheters were inserted into the left jugular vein, one for permanent infusion, 1.2 ml/h, of isotonic saline, and one for injection of an 8.5% lissamine green solution. The animal was kept in right side position on a heated micropuncture table. Body temperature was measured rectally by an electrical thermometer and maintained at 37.5 °C. The left kidney was exposed by flank incision and leaving the capsule intact placed into a lucite holder. It was immersed in paraffin oil of body temperature and immobilized for micropuncture.

In blood samples withdrawn from the carotid artery, plasma protein concentration was determined with the Folin phenol reagent as described by Lowry et al. [30]. In superficial loops identified [38] as early or late proximal loops, respectively, by intravenous injection of lissamine green solution, intratubular pressures and flow rates were measured using the methods described below.

Pressure Measurements. The intratubular pressure was measured according to the compensation method described by Landis et al. [28] using 10 µm tipped glass pipette containing Sudan black stained castor oil instead of the usually used saline with lissamine green. Prior to micropuncture the "asymmetry" of every pipette was determined. The tip of the with oil filled pipette was dipped into Ringer's solution in a little cup close to the animal. Due to the sur-
face tension Ringer's solution was sucked into the pipette. The counter-pressure necessary to stop the fluid movement decreased strongly with increasing diameter of the pipette and hence with increasing distance between the tip opening and the interface between Ringer's solution and oil (Fig. 1). This distance was measured with an ocular micrometer.

A proximal tubule was punctured and the ocular scale adapted to the puncture site. The pressure readings were done at a distance from the capillary tip, when the asymmetry curve (see Fig. 1) began to be horizontal, but where changes in the distance of the interface oil/fluid still could be observed easily. The intratubular pressure equals the manometer reading minus the "asymmetry" at this distance.

Following determination of the free flow pressure an oil column with a length of about 3 - 5 times the tubular diameter was injected into the tubule by the same pipette. While the oildroplet was carried away by tubular flow, the intratubular pressure changed but reached a constancy within 1 min when the oil had disappeared from the last visible loop at the kidney surface. This pressure was measured subsequently. As controlled by microdissection, this pressure occurs, when the tubule is blocked at its end ("bottleneck" of proximal tubule). In some cases the oil passed the thin loop of Henle, which could be identified easily by a sudden drop in tubular pressure down to free flow values. Finally the tubule distal to the puncture site was filled with oil by repeated injections and a third pressure measurement in this tubule was performed. The site of puncture was located by microdissection following the experiment.

**Flow Rate Measurements.** In two other sets of experiments the intratubular flow rate was measured in the first and in the last visible loop of the proximal convolution.

As Figure 2 shows, a hair capillary with an inner diameter of about 80 μm, supplied near the one end with a small piece of dental wax, was installed into a pipette prepared for micropuncture. By gently warming of the tip with a heated platin wire the wax melted and the capillary could be fixed into the pipette without clotting. Subsequently the space between capillary and pipette was filled retrogradly with highly fluid silicone oil (AK 10, Wacker Chemie, München) by means of a small polyethylene tube carefully pushed forward towards the waxlayer to avoid air bubbles. The hair capillary finally was filled by capillarity.

Following this procedure lissamine green solution was sucked through the tip into the capillary. Lissamine green was chosen for better visibility of the interface between oil and solution. The amount of lissamine green solution depended on the site of the micropuncture. For measurements of early proximal flow rates the tip of the pipette was entirely filled with lissamine green solution, whereas for measurements of late proximal flow rates the pipette was filled with Ringer's solution except a small region within the hair capillary near the interface, which contained lissamine green. For measurements of late flow rates no lissamine green should enter the blocked tubule, whereas prior to the measurements of early flow rates the tubule intentionally was filled with lissamine green by means of the pipette to reduce tubular reabsorption. The situation is shown diagrammatically in Figure 2.

The hair capillary was previously calibrated by weighing the amount of mercury or by liquid scintillation counting method using a C-14-inulin solution. Both calibration methods resulted in the same volumes of about 5 nl/mm. It was furthermore ascertained by the scintillation counting, that aspiration of fluid into the dry capillary and into the capillary previously filled with silicone oil, didn't result in systematic errors in measured volumes. In order to correlate the flow rates to proper pressures, the pressure correction for every pipette was determined at different flow rates in a cup filled with Ringer's solution placed close to the experimental animal, by changing the counter-pressure (= manometer reading). The pressure correction equals the asymmetry minus the pressure drop in the tip of the pipette. The asymmetry of the pipette was 3 - 5 Torr, the flow resistance 0.2 - 0.3 Torr · mm/nl. The intratubular pressure equals manometer reading minus pressure correction dependent on the measured flow rate.

Following micropuncture of early or late proximal tubule with a flow rate pipette, the tubular flow was blocked using a second pipette to fill the tubule with castor oil distal to the flow rate puncture site. The constant site of the oil block was controlled before and after every measurement. During micropuncture with the flow rate pipette intratubular pressure was balanced by counter-pressure to avoid that the oil-lissamine green interface was pressed out of the pipette. The flow rate results from the number of scale marks in the ocularmicrometer passed within the time measured with a stopwatch, taking into consideration the tilt angle of the pipette. Several values at different pressures could be obtained with the same pipette by repeated ejection of aspirated tubular fluid of about 1.5 - 4 nl. The time required was 10 - 30 s.

Following the last micropuncture, but before sacrificing the rat, the kidney was removed, macerated in 6 N hydrochloric acid at 37° C for 140 min and then kept in distilled water at room temperature for 24 h. The punctured tubules were microdissected to measure the length of the segments between glomerulum and oil block.