Papillary Plasma Flow in Rats

I. Relation to Urine Osmolality in Normal and Brattleboro Rats with Hereditary Diabetes Insipidus

François Bayle, Laure Eloy, Marie-Marcelle Trinh-Trang-Tan, Jean-Pierre Grünfeld, and Lise Bankir
INSERM Unité 90, Clinique Néphrologique, Hôpital Necker, F-75730 Paris Cedex 15, France

Abstract. Papillary plasma flow (PPF) was measured by the albumin accumulation technique in rats of the Brattleboro strain with or without diabetes insipidus (DI and HZ respectively) and in Wistar rats. Measurements were also performed in DI rats receiving antidiuretic hormone for 30 min or 5 days and in dehydrated Wistar rats. PPF in HZ control and Wistar control rats was similar to previously published measurements. In contrast PPF was significantly higher in DI rats (461 ± 26 μl/min · g versus 263 ± 28 in HZ) and decreased significantly after acute ADH administration. It returned to control values after prolonged ADH administration (262 ± 40). Plasma flow entering the papilla was inversely correlated with urine osmolality up to 1000 mosmol/kg H2O. Further increases in urine concentration (dehydration of Wistar rats) did not modify further PPF (255 ± 28 versus 270 ± 16 in non dehydrated Wistar). PPF might be influenced indirectly by ADH or prostaglandins and seems to depend on the osmotic environment of the papilla up to a certain limit. The factors which maintain PPF at a given minimum level with further increases in urine concentration are not known.

Key words: Papillary plasma flow — Brattleboro rats — Diabetes insipidus — Urine osmolality — Urine concentrating ability — Antidiuretic hormone

Introduction

Twenty years ago, Thurau and coworkers (1960) showed that, in the dog, the reversal from antidiuresis to diuresis was accompanied by a marked decrease in dye transit time, i.e., an increase in flow rate in the papilla, and thus introduced the concept that medullary blood flow could affect the urinary concentrating process. Subsequently, numerous work devoted to the effects of antidiuretic hormone on intrarenal (or intracortical) blood flow distribution, or on medullary blood flow (Gussis et al. 1979; Aukland 1976) have led to controversial results. As underlined by Rasmussen (1978), the term "renal medullary blood flow" is ambiguous. Due to the arrangement of the vessels in the outer and inner medulla, to the countercurrent exchanges and to the uptake of tubular reabsorbate, the results obtained by different techniques may not represent the same "flow". It is thus important to define clearly which kidney zone and which flow (afferent or efferent) are considered in each study.

In the present study we attempted to evaluate the afferent papillary perfusion in relation to urine concentration. The "papillary plasma flow" (PPF) was measured by the albumin accumulation technique in rats of the Brattleboro strain with or without diabetes insipidus, and in Wistar rats under various conditions. Our findings show that the plasma flow entering the papilla is inversely proportional to the urine osmolality in the range 100—1000 mosmol/kg H2O. In the 1000—2500 mosmol/kg H2O range, this correlation is no longer observed.

Material and Methods

Animals

Brattleboro rats were bred in our laboratory in a humidified atmosphere (70%) at a temperature of 24—26°C and a 12/12 h light/dark cycle. Homozygous Brattleboro rats (Valtin and Schroeder 1964) exhibit hereditary diabetes insipidus (DI) due to a complete absence of antidiuretic hormone (ADH) secretion. Heterozygotes (HZ), although not able to respond to dehydration as well as do normal rats, have a normal urine osmolality when normally hydrated. Wistar rats were bought from Lesieux (France). All rats were fed a normal rat pellet diet and given water ad libitum. Only male rats, 3—5 months old, were used.

Papillary plasma flow (PPF) was assessed in the following groups of rats (number of rats in each group is given in Table 1):

- group I: Wistar rats, under normal conditions;
- group II: Wistar rats after 48 h water deprivation;
- groups III and IV: Brattleboro heterozygous (III) and homozygous (IV) rats under normal conditions;
- groups V and VI: Brattleboro homozygous rats receiving an acute infusion of AVP (V) or dDAVP (VI). AVP was administered as a priming dose of 800 IU followed by a sustaining infusion of 160 μU/min for the 30 min preceding the PPF measurement. dDAVP, a long-lasting analogue of AVP (Sawyer et al. 1974), was given as a single dose of 10 ng, 30 min before the PPF measurement;
- group VII: Brattleboro homozygous rats which received dDAVP during the 5 days preceding the experiment. Alzet minipumps (Alza Corp. Los Angeles, Model 1701) were implanted intraperitoneally under light ether anesthesia and delivered a continuous infusion of dDAVP (42 ng/mg) at a rate of 1 μl/h (0.7 ng per min).
Experimental Procedure

Rats were anesthetized with Nembutal (Abbot) 5 mg/100 g BW i.p., placed on a temperature-controlled table and tracheotomized. Polyethylene catheters (Clay Adams) were inserted into the left jugular vein (Pe 50), the right carotid (Pe 10), both femoral arteries (Pe 50) and into the bladder (Pe 90) by a small suprapubic incision. A midline abdominal incision was made and a loose ligature placed around each renal pedicle. In groups I, II, III, IV and VII, PPF measurement was performed about half an hour after the end of surgery. During this period blood hematocrit was measured and urine output and osmolality (5100 B Vapor pressure osmometer, Wescor, Inc.) were assessed in timed-collected samples. The urine remaining in the vesical catheter at the end of the experiment was also collected in order to measure the osmolality of the urine formed in the last minutes before the PPF determination. The same procedure for PPF measurement was applied to groups V and VI after the 30 min ADH administration.

During surgery and up to the time of PPF measurement isotonic saline was infused i.v. at 10 μl/min/100 g BW in Wistar, HZ and 5 day-ADH-restored-DI rats (groups I, II, III, and VII). In other DI rats (groups IV, V and VI) the infusion rate was adjusted to 70% of the urine flow rate observed in the preceding 24 h. During acute AVP or dDAVP administration, this infusion was reduced by 3/4 for the first 10 min, then adjusted to the urine flow of the previous 10 min for the last two 10 min periods.

Papillary Plasma Flow Measurements

The method used for papillary plasma flow measurement is based on the fact that albumin accumulates in the papilla during the first seconds after a peripheral intravascular infusion. This accumulation is due to the relatively long transit time of the plasma in the inner medulla [about 40 s in the normal rat (Solez et al. 1974a)]. Thus, during the first 15–20 s of albumin infusion, virtually none of the albumin that has reached the papilla will leave it. If renal circulation is suddenly stopped by clamping the renal pedicle ~20 s after the beginning of the albumin infusion, the amount of albumin found in the papilla will be proportional to the amount of plasma which entered the papilla during this time i.e. to the afferent papillary plasma flow. This plasma flow can be calculated if the mean albumin concentration in the plasma during the infusion is known. This technique was first described by Lilienfeld et al. (1961) and subsequently modified by Ganguli and Tobian (1974) and by Solez et al. (1974a). These authors infused radiolabelled albumin and collected arterial blood during an exactly timed period and calculated PPF according to the following equation:

PPF \( \mu l/min \cdot g = \frac{cpm/g \ \text{papilla}}{cpm/\mu l \ \text{plasma}} \times (1 - \text{Hematocrit}) \times \frac{60 \ \text{s}}{t} \times \text{flow rate of} \ \text{blood collection} \times \text{cpm of collected blood} \)

where \( t \) is the exact duration of albumin infusion. It is necessary to determine accurately the time of arrival of the albumin in the kidney and to begin the blood collection exactly at this time. We simplified this protocol, reasoning that, as in the “reference sample” of the microsphere method (Aukland 1976), it was not necessary to know the beginning and the duration of the tracer infusion period. The femoral arterial catheter collection is considered as a “reference organ” with a known blood flow (imposed by a withdrawal pump). The blood sampling can start before the beginning of the tracer infusion. It needs only to the ascertained 1) that the tracer reached the kidney and the blood collection catheter simultaneously (this is certainly the case since the transit time in the aorta between renal and femoral arteries is much less than a second); 2) that the flow is stopped in both the kidneys and the collection catheter simultaneously. This is achieved by clamping the kidneys and the catheter at the same time. As the kidney and the “reference organ” have received the albumin at the same arterial concentration during exactly the same time (even if this time is not known) it is not necessary to know the actual concentration of the radiolabelled albumin in the plasma but only the total amount of albumin corresponding to a known blood withdrawal rate. The papillary plasma flow can be calculated from the following formula:

PPF \( \mu l/min \cdot g = \frac{cpm/g \ \text{papilla}}{cpm/\mu l \ \text{plasma}} \times (1 - \text{Hematocrit}) \times \frac{60 \ \text{s}}{t} \times \text{flow rate of} \ \text{blood collection} \times \text{cpm of collected blood} \)

We chose to express the PPF in \( \mu l/min \cdot g \) papilla, a unit better adapted to the rat papillary plasma flow than the generally used ml/min/100 g, probably after the initial work of Lilienfeld et al. (1961) which was performed in dogs.

In our experimental protocol, \(^{125}\text{I}-\text{radiolabelled albumin} \) (CNTS, France, 25 μCi/ml) was infused into the root of the aorta (right carotid catheter) or into the jugular vein at 0.4 ml/min for about 20 s (not precisely measured, but one drop of 3% Lissamine green was added to the albumin, in order to see approximately when the dead volume of the catheter was filled). A few seconds before the start of albumin infusion, the withdrawal of blood from the femoral artery was begun, at a constant rate of 0.4 ml/min (Sage pump, model 351). After 18–22 s, circulation was stopped abruptly and simultaneously in both kidneys and in the arterial sampling catheter, by ligating the two renal pedicles and clamping the catheter. Both kidneys were removed, weighed and quickly cut in half longitudinally. The white papilla (= inner medulla) was separated from the reddish outer medulla with small scissors and placed in a pre-weighed tube containing 1 ml of isotonic saline (to avoid dessication of the tissue). The tubules were weighed again to determine papillary wet weight. After radioactive counting, papillas were desiccated for 48 h at 60°C and weighed again to determine papillary dry weight.

The syringe with the attached blood collection catheter was emptied into a tube and rinsed 2 times with 2 ml saline. The \(^{125}\text{I}-\text{albumin radioactivity of the papillas and of the blood (+ rinsing saline) was counted in a Packard } y \text{ counter (5320) for a time sufficient to give a 1% counting accuracy.} \)

The PPF was calculated for each kidney according to the formula given above and the mean of right and left kidney values was considered as the mean PPF for a given rat. As mentioned earlier, albumin was infused into the carotid artery in a few animals (4 Wistar controls, 5 HZ and 4 DI rats). In subsequent experiments we infused the albumin into the jugular vein, a route which ensures better mixing. But, as a carotid had been catherized in some animals, all others underwent carotid ligation. After intra-venous albumin infusion, PPF in left and right kidneys never differed by more than 10%. In rats receiving albumin intra-arterially this difference was sometimes greater, and rats with more than a