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Urine Collection in the Freely Moving Rat: Reliability for Measurement of Short-term Renal Effects

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Studies on short-term renal responses to (pharmacological) intervention require accurate and multiple collection of urine samples. Several invasive techniques have been described for frequent urine collection of the conscious rat, each having their own limitations. No data are available about the feasibility of the spontaneously voiding, freely moving rat for this purpose. In the present study, bladder voidings of six rats were time-registered and collected separately for several days. The data show a considerable 24-h variation coefficient of both the voided volume and the bladder collection time with a poor correlation between the two parameters. Forced diuresis induced by continuous i.v. infusion (2 ml/h) increased the frequency of urine voiding and thus the time-resolution of the urine-production pattern. However, this method failed to reduce the variation coefficient of the voided volume, the collection time, and the correlation between the two parameters. The fact that variations in creatinine excretion paralleled the variation in urinary flow suggests that both phenomena are likely be due to incomplete bladder emptying. Correction for this incomplete bladder collection, using the creatinine excretion, indeed reduced the variation coefficient of sodium excretion successfully from 61 ± 17% to 29 ± 5% during normal diuresis and from 56 ± 19% to 22 ± 6% during forced diuresis. In conclusion, the spontaneously voiding, freely moving rat can be used for short-term renal response studies if the collected urine samples are corrected for incomplete bladder emptying using urinary creatinine concentrations. This procedure allows the detection of changes in a urinary parameter if this exceeds a 40% deviation of the normal value.

Key Words: Rat; Conscious; Unrestrained; Urine collection; Micturition; Renal effects

Introduction

The conscious rat is an attractive model to study renal (patho)physiology and related pharmacological interventions. In contrast to the anesthetized rat, there is no potential interference by anesthesia (Walter et al., 1989) and fewer limitations in the duration of the study. The individual rat may thus serve as its own control if subjected to different pharmacological interventions; however, the conscious animal model may also have its disadvantages. For short-term renal studies, accurate timed urine collection is essential. Under anesthesia, this can be rather easily obtained by cannulation of the bladder or even of the urethras. In order to obtain an accurate urine collection in the conscious rat, things are far more complicated. Several approaches have been taken such as forced micturition and collection through acute or chronic catheters with various implanting devices (Engberg, 1969; Rogenes and Gottschalk, 1982; Burgess et al., 1993; Rosas-Arellano et al., 1988; Harada et al., 1992; Thomsen and Olesen, 1986; Lassen et al., 1986; Shirley et al., 1989). While stress is the major drawback of using forced diuresis and acute implantations, infection and obstruction are the risk factors of the chronic invasive devices. Since the "true" physiological state of the rat is most likely best represented by the conscious, unrestrained rat that micturates at free will,

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this approach is to be preferred, however, no data are available on its feasibility to study short-term renal effects under these experimental conditions. Therefore, in the present study the micturition pattern of the spontaneously voiding, freely moving rat was tested. The accuracy and time-resolution of the model was determined during normal and forced diuresis, and the impact on the renal parameters to be determined was established.

Methods

Materials

Polyphenyl pyrrolidone 25 (PVP) was purchased from Serva (Heidelberg, Germany). Creatinine and dopamine. HCl were obtained from Sigma Chemicals Co. All chemicals used for analytical purpose were of analytical grade. Water was of Millipore quality. Infusion solutions were sterile.

Urine Collection System

A plastic metabolic cage with a wire mesh floor (Tecniplast Gazzada, Buguggiate, Italy) was placed above a fraction collector (model 2110, Bio-Rad Laboratories, Inc). The times of voiding were recorded, and the individual voidings were collected separately using computer communication. The computer connections were made via an I/O interface. The software was written in Borland C/C^{++}.

Experimental Setup

Six male Wistar rats (Harlan, Zeist, The Netherlands) weighing 250 g were kept in a temperature-controlled room with a 12/12 h light/dark cycle. They were given solid chow (Hope Farms Inc., Woerden, The Netherlands) and tap water ad libitum throughout the study.

Study 1 (normal diuresis). Six rats were transferred to individual metabolic cages. After a stabilization period of 14 h, the spontaneously voided urine samples were time recorded and collected separately for 5 days.

Three days after the first study, the rats were anesthetized with 2% Forene/O_2 (500 ml/min) and the jugular vein cannulated [Silastic, medical grade tubing (.020 in. ID × 0.037 in. OD) from Dow Corning (Midland, Michigan, U.S.A.)]. The cannula was subcutaneously pulled under the skin to the head and immobilized with screws and dentist-cement (Simplex (Rapid), Associated Dental Products, Kemdent Works, Purton, Swindon, England). To protect the cannula from blood clotting, the cannula was filled with 50% PVP/500 IE/ml heparin in 0.9% NaCl when not in use (Steffens, 1969). The rats were allowed to recover from surgery for 1 week, after which the second study was performed.

Study 2 (forced diuresis). Six rats were transferred to their metabolic cages and constantly infused with 0.45% NaCl/2.5% glucose (2 ml/h). Again, after a stabilization period of 14 h, urine samples were collected for 5 days.

Study 3 (dopamine). A rat was challenged with dopamine. After a stabilization period of 18 h, 20 μg/min/kg dopamine was infused for 1 h through the permanent jugular cannula. Urine samples were collected starting 5 h before and finishing 10 h after the start of the dopamine infusion.

Analytical Methods

The urine volume was measured by weighing the collected samples (accuracy of 0.02 g). Urinary creatinine was measured by HPLC as described by Nishimaki et al. (1986). In short, the mobile phase consisted of 96.5% 20 mM KH_2PO_4/K_2HPO_4, pH 5.1 containing 80 mg/l sodium lauryl sulphate, and 3.5% acetonitrile. The separation column was a μBondapak C18 (30 cm × 3.9 mm I.D.; Millipore Waters, Milford, MA) guarded with a μBondapak C18 Guard-pak precolumn (Millipore Waters). The injection volume was 20 μl (Jasco autosampler, model 851-AS, Tokyo, Japan), the flow rate 1 ml/min (Jasco pump model 880 PU), and the detection wavelength 230 nm (Jasco UV-detector, model 875-UV). HPLC peak integration was performed using the integration package JCL6000 (Jones Chromatography, Littleton, CO). Urine samples were diluted 50 to 100 times in water and injected on the column without a prepurification. Pure creatinine dissolved in water was used as standard. Urine sodium concentrations were measured by flame photometry (Perkin Elmer 3030B Atomic Absorption Spectrophotometer).

Creatinine Correction

Na excretion (μmol/min) *corrected* = Na excretion (μmol/min)/creatinine excretion (μmol/min) × average creatinine excretion in 24 h (μmol/min).

Results

Micturition Characteristics

The individual voided urine volumes and corresponding bladder collection times (the time between two bladder emptyings) of six rats on day 4 under normal diuresis (study 1) are given in Figure 1A. The variation coefficient of both the voided volume and collection time was large (Table 1), and the correlation between the two parameters appeared to be poor (day 4; $r = 0.69$, $n = 83$). The variation in voided volume did not change in the course of the study as indicated by an average individual variation coefficient of 61, 62, 56, 60, and 50% on days 1, 2, 3, 4, and 5, respectively. Similarly, the

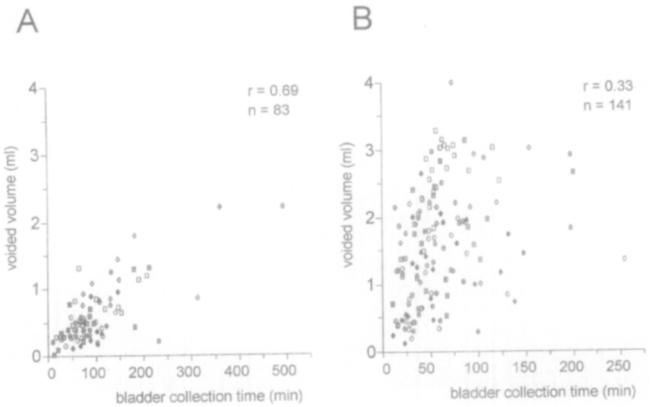

Figure 1. Correlation between the individual voided volumes and collection times of six rats on day 4 of the study. **(A)** During normal diuresis. **(B)** During forced diuresis. The six symbols represent the six rats.

bladder collection time varied to the same degree over the 5 days of the study with an average individual variation coefficient of 80, 61, 70, 61, and 58%, respectively.

A forced diuresis (study 2) was induced with the intention of increasing the time-resolution of the method and increase the accuracy of urine collection. Forced diuresis resulted in a 2.7-fold increase of the voided volume and 1.6-fold reduction of collection time (Table 1). This implied a ±5-fold increase of the urinary flow. Yet, the variation coefficient of voided volume and collection time remained high, and the accuracy of urine collection did not improve, but rather was reduced by forced diuresis as indicated by a lower correlation between voided volume and collection time ($r = 0.33$, $n = 141$, Figure 1B).

Renal Excretion Parameters

As expected from the micturition data, the urinary flow of individual urine voidings fluctuated considerably in time, from less than 1 to more than 20 μl/min. It is interesting to note that the creatinine excretion, which is supposed to be rather constant over the day, showed a fluctuation parallel with the variation in urinary flow. A high correlation was found between the urinary flow and creatinine excretion of individual bladder voidings at day 4 both under normal diuresis ($r = 0.92$, $n = 83$) and forced diuresis ($r = 0.81$, $n = 141$), both $p < 0.0005$ (Figure 2). These data suggest that the observed variations are explained by incomplete bladder emptying of the rat. Correction for creatinine excretion clearly smoothed the 24-h sodium excretion pattern of an individual rat (Figure 3) with an improvement of the variation coefficient of urinary flow and urinary sodium excretion by a factor 2 to 3 (Table 1).

In the typical example (see study 3 in Figure 4), dopamine was infused for 1 h starting directly after the bladder emptying of urine voiding no. 6. The large, short lasting peaks of natriuresis no. 6 and 11 in the uncorrected graph (Figure 4A) were removed using creatinine correction (Figure 4B). This indicates that those peaks were likely due to incomplete bladder voiding. As a result, after creatinine correction, the natriuresis induced by dopamine infusion was more clearly visualized.

Discussion

The spontaneously voiding, freely moving rat is likely to be a better representative of the "true" physiological state of the animal compared to rats under anesthesia or rats undergoing invasive implantation procedures. However, the present study shows that the feasibility of using this model for short-term renal studies is rather limited due to incomplete bladder voiding. During normal diuresis, the voided volume and collection time appeared to be highly variable and poorly correlated. Territorial behavior or adaptation to the metabolic cage is unlikely to be responsible for this large fluctuation: in the 5 days of the study, the housing part of the metabolic cage was

Table 1. Micturition characteristics and renal parameters of the spontaneously voiding rat

Diuresis	Normal		Forced	
$n = 6$, day 4	Mean	VC (mean ± SD)%	Mean	VC (mean ± SD)%
Voided volume (ml)	0.6	60 ± 23	1.6	47 ± 18
Collection time (min)	96	61 ± 14	60	66 ± 20
U-flow (μl/min)	7.3	51 ± 11	34	64 ± 22
Creatinine excretion (μmol/min)	0.10	55 ± 13	0.09	53 ± 22
U-flow (μl/min) *corrected*	6.4	21 ± 10	27	31 ± 7
Na excretion (μmol/min)	2.2	61 ± 17	5.7	56 ± 19
Na excretion (μmol/min) *corrected*	1.9	29 ± 5	4.6	22 ± 6

Urinary flow (U-flow) and sodium excretion (Na excretion) *corrected* indicate a correction for creatinine excretion: renal parameter/creat excretion × average creat excretion in 24 h.

VC = variation coefficient.

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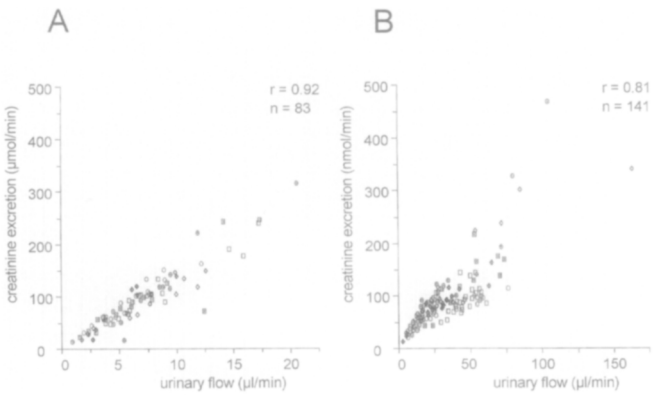

Figure 2. Correlation between the urinary flow and creatinine excretion of the individual voidings of six rats on day 4 of the study. **(A)** During normal diuresis. **(B)** During forced diuresis. The six symbols represent the six rats.

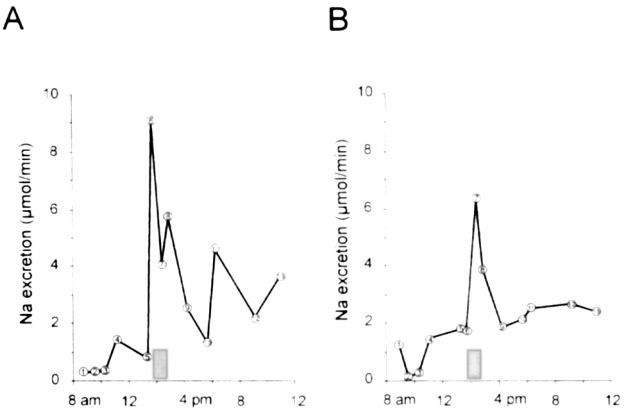

Figure 4. A typical example of the sodium excretion pattern of an individual rat challenged with dopamine. ▨: 1 h i.v. dopamine infusion (20 µg/kg/min). **(A)** No corrections. **(B)** With creatinine correction.

not cleaned, and the rat was not removed from the cage. Evidently, if territorial behavior or adaptation were a major factor, we would have expected the variation in the micturition parameters to decrease in time, which was not the case. Furthermore, female rats, often with less territorial behavior, showed a similar degree of variation in voided volume as their male littermates (Longhurst et al., 1992).

We induced a forced diuresis with the intention of increasing the bladder filling equally over the 24-h cycle to improve the accuracy of urine collection. Unexpectedly, the induced increase in urinary flow reduced rather than improved the accuracy of urine collection. With regard to the time-resolution, a fivefold increase in urinary flow resulted in only a 1.6-fold increase in time-resolution since both the frequency and volume of the bladder voidings were affected. Apparently, the bladder can readily adapt to changes in diuresis by adjustment of capacity without changing the completeness of bladder emptying during voiding.

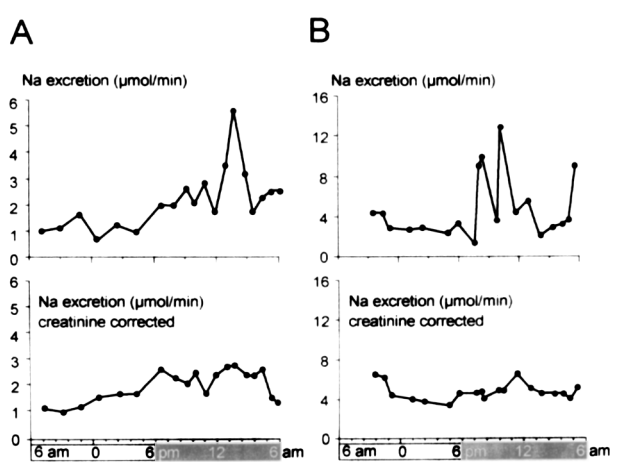

Figure 3. A typical example of a 24-h profile of sodium excretion of an individual rat. On top, the sodium excretion without, and, on the bottom, the sodium excretion with creatinine correction. **(A)** During normal diuresis. **(B)** During forced diuresis.

In contrast with the forced diuresis protocol, creatinine correction was successful in increasing the accuracy of urinary parameters. Creatinine excretion can be used for correction of incomplete bladder emptying since creatinine is constantly produced throughout the body's musculature and subsequently excreted via the kidney (Thomsen and Olesen, 1986; Shirley et al., 1989).

To test the value of the method of creatinine correction in the spontaneously voiding rat model for the detection of short-term changes in renal excretion parameters, the stability of sodium excretion over the day was also measured. Without correction for incomplete bladder voiding, the model seemed not very sensitive. With a coefficient of variation of about 60%, the method only allowed an accurate measurement when at least a twofold change in sodium excretion was present. Creatinine correction, however, resulted in a marked reduction of the coefficient of variation allowing the measurement of a ±40% change in sodium excretion.

In conclusion, creatinine correction makes the spontaneously voiding, freely moving rat an attractive model for short-term renal intervention studies. Without this correction for incomplete bladder voiding only large changes in renal excretion parameters are detectable. If the creatinine correction procedure is used, then the model is also applicable for detection of less-pronounced pharmacological effects.

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