[Chem. Pharm. Bull. 31(12)4524—4529(1983)]

Comparative Study of the Ventral and Retro-peritoneal Approach Procedures for Ureteral Catheterization in the Rat. Changes of Renal Functions and Hemodynamics

JUN-ICHI SUDO,* ATSUSHI ISHIHARA and TSUNEYOSHI TANABE

Department of Toxicology, Faculty of Pharmaceutical Sciences, Higashi-Nippon-Gakuen University, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061-02, Japan

(Received April 25, 1983)

Mean blood pressure, renal clearances of electrolytes and urinary volume, in addition to renal hemodynamics and functions, were comparatively investigated in rats subjected to ureteral catheterization by the ventral and the retro-peritoneal approach procedures in order to determine the validity of each of these procedures. From 60 min after the operation, the mean blood pressure in the case of ventral approach gradually decreased, while the pressure in the case of retro-peritoneal approach remained constant. The urinary volume and the urinary sodium excretion in the case of the ventral approach decreased about a half in comparison with those in the retro-peritoneal one, although the plasma sodium concentration was well maintained in both cases. The renal hemodynamics and functions remained normal in both cases. The decrease of urinary volume and urinary sodium excretion observed in the case of the ventral approach is considered to be due to the homeostatic protection against loss of body fluid as well as against the decrease of pressure in the abdominal cavity, both of which are caused by the opening of the abdominal cavity. It is accordingly considered that the retro-peritoneal approach procedure should be adopted for pharmacological and pharmacokinetical studies on the clearances of electrolytes and water in comparison with the clearance(s) of drug(s).

Keywords——ureteral catheterization; blood pressure; urinary volume; urinary sodium excretion; renal hemodynamics; renal function; rat

In experiments on renal pharmacology and pharmacokinetics using rats, urine samples have usually been collected through a ureteral catheter surgically positioned through the ventral approach.[1,2] This approach is technically easy, but has the disadvantage of severity of operative invasion. Another method, the retro-peritoneal approach, for ureteral catheterization is advantageous because the peritoneum remains intact.

The authors recently noticed that in the experiments adopting the retro-peritoneal approach the renal transports of electrolytes and water showed different patterns from those in experiments based on the ventral approach. In previous reports[2,3] on the effects of β-adrenoceptor-blocking agents on diuresis as well as electrolyte excretions, the following tendencies were noticed: the urinary volume and sodium excretion in the case of the ventral approach, irrespective of administration of the agent(s), were less than those in the case of the retro-peritoneal one. If data on renal transports depend on the operating procedure used for catheterization, this raises a serious question regarding the validity of many pharmacological and pharmacokinetical studies.

In this paper, mean blood pressure, renal clearances of electrolytes and urinary volume, in addition to renal hemodynamics and functions, were comparatively investigated in rats subjected to ureteral catheterization by the ventral and the retro-peritoneal procedures, in order to determine the validity of these procedures for pharmacological and pharmacokinetical studies.

Experimental

Materials——Inulin (Koso Chemical Co., Ltd., Tokyo, Japan), *p*-aminohippurate (PAH) (Aldrich Chemical Co., Inc., Milwaukee, U.S.A.), sodium pentobarbital (Nembutal) (Abbott Laboratories, Illinois, U.S.A.), and heparin sodium salt (Wako Pure Chemical Industries Ltd., Tokyo, Japan) were used in this study. All other reagents were commercial products of the highest grade available. Male Wistar rats weighing 200—250 g were used.

General Procedures——The rats were allowed free access to water and fed a standard rat pellet diet prior to the study. They were divided into two groups: one being catheterized by the ventral approach procedure, and the other by the retro-peritoneal one. The rats were anesthetized intraperitoneally with 50 mg/kg body weight of sodium pentobarbital, and were intubated for free respiration, after which a left jugular vein was catheterized with a polyethylene tube (PE-50). Two ml of saline containing 1.2% inulin was injected as a prime, followed by infusion of saline containing 0.24% inulin and 0.15% PAH at a rate of 0.5 ml/kg body weight/min: an equilibration period of 60 min was allowed. The left ureter was then catheterized through the ventral or retro-peritoneal approach, as represented below. Urine was collected every 10 min through the ureteral catheter, with blood samples being taken from the tail vein. The blood volume collected was restricted to 0.2 ml, and the blood was sampled only at 120, 150 and 180 min after the start of operation. Urine collection was begun at 120 min after the start of operation.

Ventral Approach Procedure for Ureteral Catheterization——The hairs on the abdominal skin were first removed with electric clippers, and a longitudinal incision of about 3 cm was made about 1 cm to the left of the linea alba, with cauterization of the bleeding portion of the abdominal wall. The abdominal viscera, soaked with saline, were gently moved to the right side, and then the left ureter was catheterized with a polyethylene tube (PE-10). After replacement of the viscera, the abdominal wall was closed by applying clips.

Retro-peritoneal Approach Procedure for Ureteral Catheterization——The back of the rat was positioned toward the operator, and the hairs on the back were removed with electric clippers. A longitudinal incision of about 3 cm in the fascia of the cutaneous maximum muscle was made subcostally about 1 cm to the right of the midline of the vertebral column. The space between the epaxial musclature and the fat under the fascia of the cutaneous maximum muscle was gently opened in order to approach the left ureter. While soaking this space with saline, the left ureter was catheterized with a polyethylene tube (PE-10), after which the space was closed by applying clips.

Analytical Procedures——Inulin and PAH in urine and plasma were analyzed with a Hitachi-320 spectrophotometer (Hitachi Co., Tokyo, Japan) by the anthrone method of Führ *et al.*[4] and by the method of Smith *et al.*,[5] respectively. Hematocrit values were obtained by centrifugation of blood samples using hematocrit tubes coated with heparin sodium. The renal blood flow (*RBF*), renal plasma flow (*RPF*), glomerular filtration rate (*GFR*) and filtration fraction (*FF*) were calculated as shown below from the observed values including hematocrit values (%), urinary volume (ml/min), and concentrations of PAH and inulin (mg/ml) in urine and plasma.[6]

$$RPF\ (\text{ml/min}) = (\text{urinary PAH concentration}) \times (\text{urinary volume}) / (\text{plasma PAH concentration})$$

$$RBF\ (\text{ml/min}) = RPF \times 100 / (100 - \text{hematocrit value})$$

$$GFR\ (\text{ml/min}) = (\text{urinary inulin concentration}) \times (\text{urinary volume}) / (\text{plasma inulin concentration})$$

$$FF = GFR / RPF$$

Sodium and potassium in urine and plasma were measured with a flame photometer (FLAME-30C; JASCO Medical Instruments Inc., Tokyo, Japan). Mean blood pressure was measured through the femoral artery with a pressure transducer and a polygraph (San-Ei Instruments Co., Tokyo, Japan); heparin sodium salt in saline was used to fill the catheter of the transducer.

Results are given as means ± S.E. Statistical significance was assessed by means of Student's *t* test: the value in the ventral approach, at each time point, was compared with that in the retro-peritoneal one. *p* Values of less than 0.05 were considered significant.

Results

Figure 1 shows the changes of mean blood pressure after the two operation procedures. At one hour after the start of continuous measurement of mean blood pressure, ureteral catheterization was done by the ventral approach or the retro-peritoneal one. It was found that the mean blood pressure in the case of the ventral approach significantly decreased 60 min after the start of operation, while the mean blood pressure in the case of the retro-peritoneal aproach remained constant.

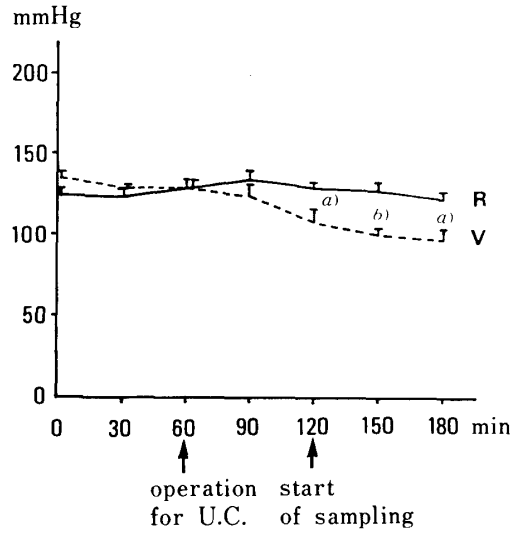

Fig. 1. Changes of Mean Blood Pressure after the Ventral and the Retro-Peritoneal Approach for Ureteral Catheterization

Abbreviations: Operation for U. C., operation for ureteral catheterization; V (---), ventral approach procedure for ureteral catheterization; R (——), retro-peritoneal approach procedure for ureteral catheterization. Vertical bars represent the mean ± S.E. The number of experiments was 6 in each group. The values in the ventral and retro-peritoneal approaches at each time point were statistically analyzed using Student's t test.
a) $p<0.05$. *b*) $p<0.01$.

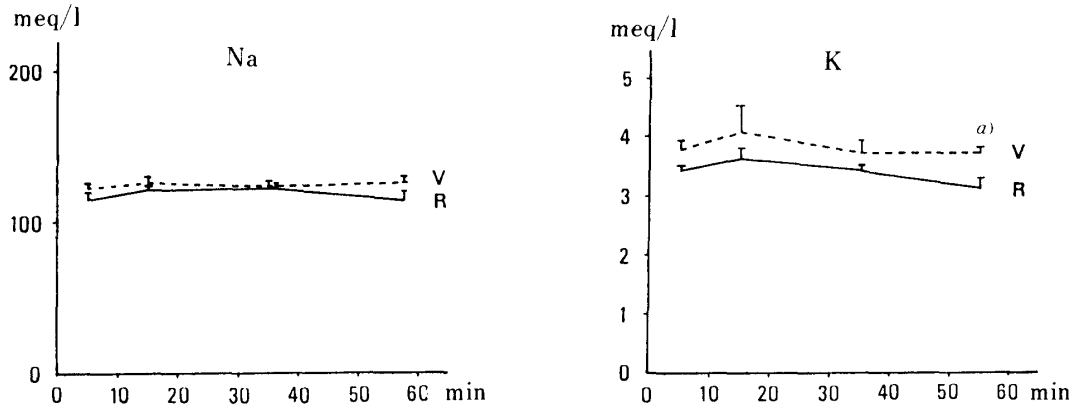

Fig. 2. Changes of Plasma Sodium and Potassium Concentrations after Both Procedures

Abbreviations: V (---), ventral approach procedure for ureteral catheterization; R (——), retro-peritoneal approach procedure for ureteral catheterization. Vertical bars represent the mean ± S.E. The number of experiments was 6 in each group. The values in the ventral and retro-peritoneal approaches at each time point were statistically analyzed using Student's t test.
a) $p<0.05$.

Figure 2 shows the changes of plasma sodium and potassium concentrations in the two cases; the concentrations remained constant and showed no significant differences, except for potassium at 50 to 60 min after the start of sampling.

Figure 3 shows the changes of urinary volume. The urinary volume in the case of the retro-peritoneal approach was consistently significantly greater than that in the case of the ventral one, with the former being about twice the latter.

Figure 4 shows the changes of urinary sodium concentration (corrected for inulin clearance) and of urinary sodium excretion per min per g wet weight of the left kidney. In general, the urinary sodium concentration and excretion in the case of the retro-peritoneal approach were found to be significantly greater than those in the case of the ventral one, with the former being about twice the latter.

Figure 5 shows the changes of urinary potassium concentration and of urinary potassium excretion, represented in the same way as in Fig. 4. They remained constant and showed no significant difference between the two procedures.

Figure 6 shows the changes of renal hemodynamics, *RPF* and *RBF*, in the two cases. No

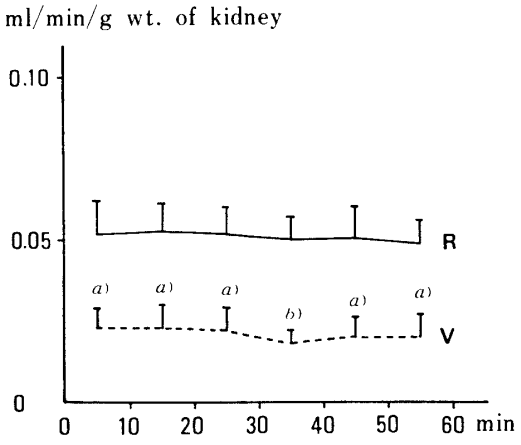

Fig. 3. Changes of Urinary Volume after Both Procedures

The urinary volume per min was calculated per g wet weight of the left kidney. Other details were as in Fig. 2.

a) $p<0.05$. *b*) $p<0.01$.

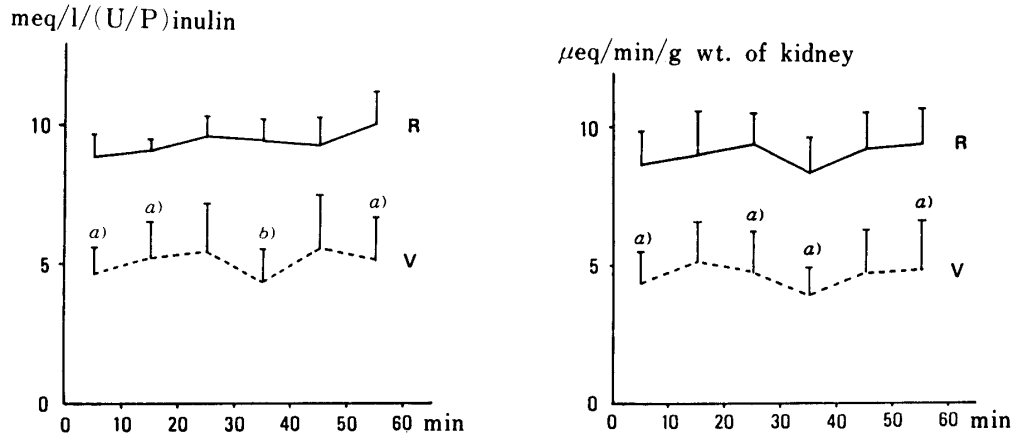

Fig. 4. Changes of Urinary Sodium Concentration and of Urinary Sodium Excretion after Both Procedures

In the left figure, the urinary concentration was corrected for inulin clearance. In the right figure, the urinary excretion per min is shown per g wet weight of the left kidney. Other details were as in Fig. 2.

a) $p<0.05$. *b*) $p<0.01$.

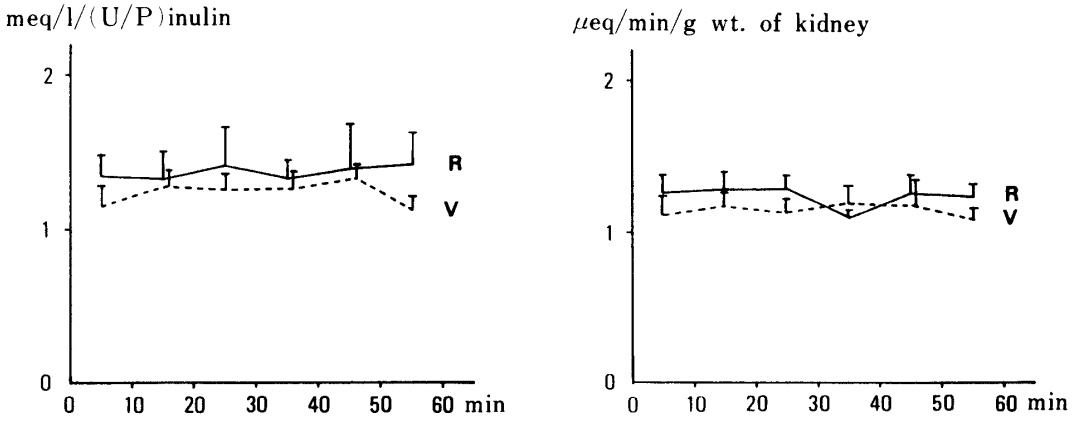

Fig. 5. Changes of Urinary Potassium Concentration and of Urinary Potassium Excretion after Both Procedures

In the left figure, the urinary concentration was corrected for inulin clearance. In the right figure, the urinary excretion per min is shown per g wet weight of the left kidney. Other details were as in Fig. 2.

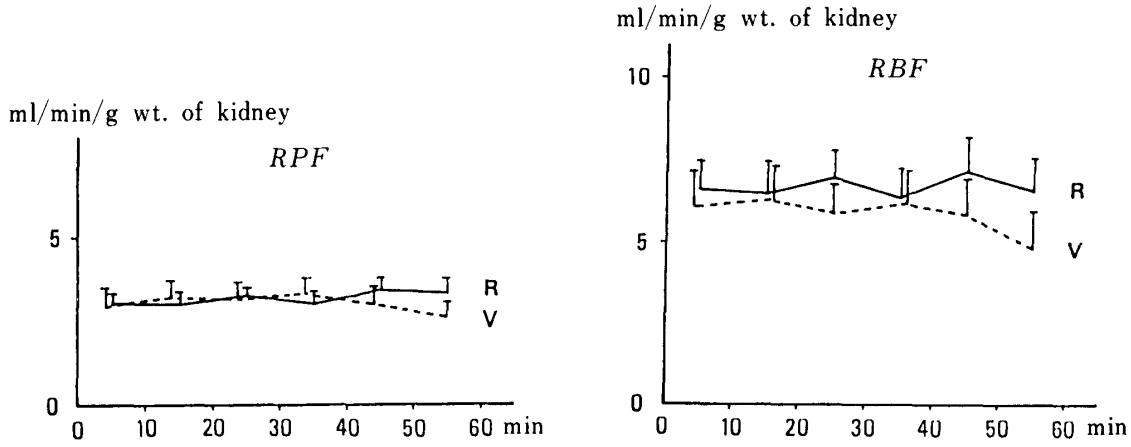

Fig. 6. Changes of Renal Plasma Flow and of Renal Blood Flow after Both Procedures

Details were as in Fig. 2.

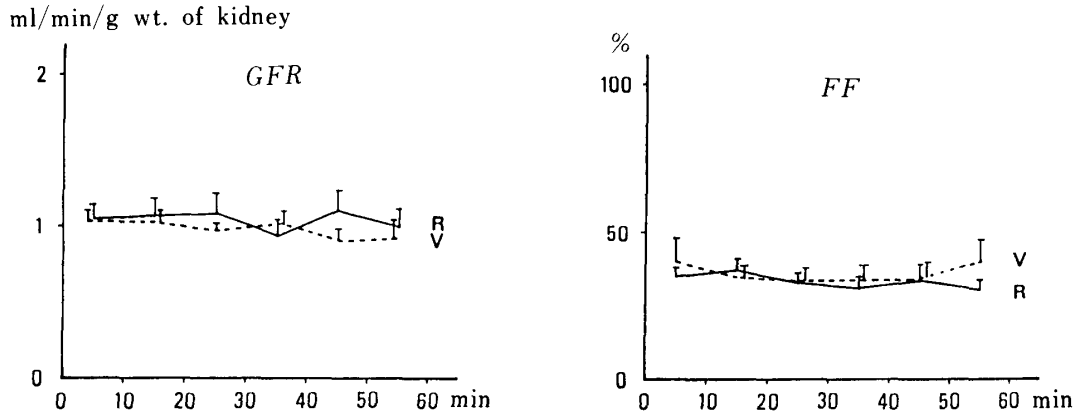

Fig. 7. Changes of Glomerular Filtration Rate and of Filtration Fraction after Both Procedures

Details were as in Fig. 2.

significant difference between the two procedures were observed in *RPF* and *RBF*.

Figure 7 shows the changes of renal functions including *GFR* and *FF* in the two procedures. No significant difference between the two procedures was observed in *GFR* or *FF*.

The results in Figs. 6 and 7 indicate that the renal hemodynamics and functions remained normal in both cases.

Discussion

There is controversy as to whether the ventral approach for ureteral catheterization is better than the retro-peritoneal one in the rat. The former does not require as much manipulation of the kidney as the latter. However, in the latter the abdominal incision is smaller, and the visceral displacement is less. The retro-peritoneal approach is therefore less traumatic. In addition, the peritoneum in the rat is not damaged in this procedure, because the kidney is completely extraperitoneal in position. Accordingly, decrease of abdominal pressure and loss of ascitic fluid are completely avoided in the retro-peritoneal approach.

An advantage of the ventral approach, however, is better access to the renal vein: this is important if the renal blood flow has to be measured electrically and if the renal venous blood has to be collected by the puncture method. The left renal vein, in fact, emerges from the

hilum of the left kidney behind the branches of the renal artery, with the ureter behind it. With the retro-peritoneal approach, the ureter and the renal artery, as well as the hilar fat, hide the vein. Therefore, each approach has advantages and disadvantages.

In this study, the following results were obtained: (A) The mean blood pressure in the case of the ventral approach procedure gradually decreased, while it remained constant in the retro-peritoneal case. (B) The urinary volume and the urinary sodium concentration and excretion in the case of the ventral approach decreased to about a half of the values in the retro-peritoneal case, although the plasma sodium concentration remained normal in both cases. (C) The renal hemodynamics and functions remained normal in both cases.

We consider that in the ventral approach, some bleeding and loss of ascitic fluid are inevitable. In addition, a decrease of pressure in the abdominal cavity is caused by opening of the abdominal cavity. Thus, the decrease of mean blood pressure after this procedure is probably due to the loss of body fluid as well as to the decrease of pressure in the abdominal cavity, as mentioned above.

In spite of the decrease of blood pressure, the renal hemodynamics and functions, including *RPF*, *RBF*, *GFR* and *FF*, remained normal in this study. Shipley and Study,[7] Thurau[8] and others[9,10] reported that the renal circulation was homeostatically autoregulated by the renal intrinsic mechanism(s): *RBF* and *GFR* remained constant when the renal arterial pressure was varied from 70 to 190 mmHg. They increased linearly from 20 to 70 mmHg of renal arterial pressure, and, furthermore, increased linearly beyond 190 mmHg. As a consequence, of course, both *RPF* and *FF* are autoregulated in the same way as *RBF* and *GFR*. Thus, the constancy of renal hemodynamics and functions during the decrease of blood pressure in this study are considered to be ascribable to the above-mentioned mechanism(s).

As regards the urinary volume and the urinary sodium concentration and excretion, the decreases observed in the case of the ventral approach are considered to be due to the homeostatic protection against the loss of body fluid, caused by the opening of the abdominal cavity. This phenomenon in the ventral approach strikingly resembles the state in the rat treated hydropenically. In pharmacological and pharmacokinetic studies investigating the clearances of electrolytes and water in comparison with those of some drug(s), the above-mentioned phenomenon may be crucial, since the transport dynamics of electrolytes, water and drug(s) in the case of the ventral approach may change to resemble those in the hydropenic state. In addition, hormonal levels might be substantially changed for the maintenance of homeostasis. Therefore, the retro-peritoneal approach procedure should be adopted for this kind of research.

References

1) S. G. Rostand, D. Lewis, J. B. Watkins, W. Huang, and L. G. Navar, *Kidney Intern.*, **21**, 330 (1982).
2) N. Yamazaki, Y. Monma, and T. Tanabe, *Folia Pharmacol. Japon.*, **81**, 333 (1983).
3) N. Yamazaki, T. Endo, Y. Monma, and T. Tanabe, *Jpn. J. Pharmacol.* (Suppl.), **32**, 277P (1982).
4) J. Führ, J. Kaczmarczyk, and C. D. Krüttgen, *Klin. Wochenschr.*, **33**, 729 (1955).
5) H. W. Smith, N. Finkelstein, L. Aliminosa, B. Craeford, and M. Graber, *J. Clin. Invest.*, **24**, 388 (1945).
6) M. Levy, "Handbook of Physiology: Renal Physiology," Section 8, ed. by J. Orloff and R. W. Berliner, American Physiological Society, Washington, D.C., 1973, p. 103.
7) R. E. Shipley, and R. S. Study, *Am. J. Physiol.*, **167**, 676 (1951).
8) K. Thurau, *Am. J. Med.*, **36**, 698 (1964).
9) A. J. Vander, *Physiol. Rev.*, **47**, 359 (1967).
10) J. O. Davis, *Circ. Res.*, **28**, 301 (1971).