*Journal of Physiology* (1990), **427**, *pp.* 421–434
*With 4 figures*
*Printed in Great Britain*



# ATRIAL PEPTIDE NATRIURESIS IN THE RAT WITHOUT GENUINE RISE IN FILTRATION RATE OR WASH-OUT OF MEDULLARY ELECTROLYTES

BY B. BĄDZYŃSKA, J. SADOWSKI AND L. DOBROWOLSKI

*From the Department of Applied Physiology, Medical Research Centre of the Polish Academy of Sciences, 00–730 Warsaw, Poland*

(*Received 17 November 1989*)

SUMMARY

1. Effects of synthetic atrial natriuretic peptide (ANP) on renal excretion, total renal blood flow (RBF), glomerular filtration rate (GFR) and tissue electrical admittance (reciprocal impedance, an estimate of tissue electrolytes) were determined in pentobarbitone-anaesthetized rats. GFR was measured both as inulin clearance ($C_{in}$) and as a product of renal plasma flow (RPF) and inulin extraction ratio ($E_{in}$).

2. With the lowest dose of ANP (0·35 $\mu$g/(kg min) I.V.) a 5-fold increase in sodium excretion occurred without measurable change in $C_{in}$, RPF $\times$ $E_{in}$ nor medullary electrolyte concentration estimated from tissue electrical admittance.

3. With medium and high dosage (2 and 6 $\mu$g/(kg min), respectively), major and rapid increases in sodium excretion and urine flow were associated with an acute increase in $C_{in}$ but not RPF $\times$ $E_{in}$.

4. The RBF increase observed in all groups of rats was not dose-related and did not parallel the natriuresis. Electrolyte concentration in the medullary tissue showed a modest transient decrease in rats given medium and high ANP doses.

5. We conclude that pronounced ANP natriuresis can develop in the absence of a measurable increase of GFR, estimated by a method not subject to urinary dead space error (RPF $\times$ $E_{in}$). The small transient decrease in medullary tissue electrolytes observed with higher peptide doses does not support solute wash-out as an important mechanism of increased sodium excretion.

## INTRODUCTION

In spite of a considerable research effort of the past 6–7 years, the renal mechanisms of action of the recently isolated and synthetized atrial natriuretic peptide (ANP) have not been satisfactorily elucidated (Raine, Firth & Ledingham, 1989). Much of the debate has focused on the role of changes in renal haemodynamics but there have been few direct measurements of total renal blood flow (RBF) in rat kidneys. Regardless of the species investigated, the observed post-ANP increases in the glomerular filtration rate measured as the clearance of inulin or creatinine were interpreted with caution, due to a potential error dependent on the pelvic urinary dead space effect (Davis & Briggs, 1987*a*; Hansell & Ulfendahl, 1987; Banks, 1988).

In the present study of effects of exogenous ANP in the anaesthetized rat, we resolved both the problem of accurate continuous measurement of the total renal blood flow and of the reliable determination of filtration rate. The former was accomplished by a continuous record of the renal vein outflow (Sadowski, Kulczykowska, Kulczykowski & Bądzyńska, 1988), and the latter by estimation of glomerular filtration rate (GFR) from the product of the renal plasma flow (RPF) and the renal extraction ratio of inulin ($E_{in}$), a method that is not subject to the dead space error.

In some experiments ANP action was studied whilst renal artery pressure was maintained constant in order to obviate the influence of changes in renal perfusion pressure, an important variable modulating the natriuretic action of ANP (Burnett, Opgenorth & Granger, 1986; Sosa, Volpe, Marion, Atlas, Laragh, Vaughan & Maack, 1986; Criscione, Burdet, Hänni, Kamber, Truog & Hofbauer, 1987; Davis & Briggs, 1987*a*; Paul, Kirk & Navar, 1987; Takezawa, Cowley, Skelton & Roman, 1987; Firth, Raine & Ledingham, 1988).

Several years ago a method was developed in our laboratory enabling continuous monitoring of the total electrolyte concentration in the renal medullary interstitium based on measurements of tissue electrical admittance (reciprocal impedance, Sadowski & Portalska, 1983; Sadowski, 1985). In order to investigate the possibility that wash-out of renal medullary solutes, possibly resulting from an ANP-induced increase in vasa recta blood flow, may be an important determinant of natriuresis (see e.g. Sosa *et al.* 1986), we measured tissue admittance in all experiments, concurrently with standard parameters of renal haemodynamics and excretion. These measurements provided new information on ANP-dependent fluctuations of the electrolyte component of the renal cortico-papillary solute gradient.

## METHODS

Male Wistar rats weighing 306 ± (S.E.M.) 6 g maintained on a standard rodent diet were used for experiments. The animals had free access to food and water until the day of study. They were anaesthetized with intraperitoneal sodium pentobarbitone, 50 mg/(kg body weight), followed by small intravenous supplements as required. Throughout the surgical preparation and experimental procedures body temperature was maintained at about 37 °C by means of a heated pad and lamps. A polyethylene tube was placed in the trachea to ensure free airways. Aortic blood pressure was recorded by a catheter introduced via the femoral artery and connected with a Statham pressure transducer; the same catheter was also used for sampling aortic blood. In order to compensate for fluid losses, isotonic Ringer solution was infused to match urine flow rate (about 2 ml/h). A cannula was placed in the jugular vein to be later connected with the renal vein–jugular vein shunt tubing (see below).

The left kidney was exposed through a subcostal flank incision, gently freed and placed in a plastic cup similar to that used for micropuncture. For timed urine collections a catheter was introduced into the ureter and passed to the pelvis. The renal artery and vein were dissected free from the adjacent tissue. Then the renal vein was cannulated in order to direct the whole renal vein outflow along an extracorporeal shunt to the jugular vein, to enable measurement of total renal blood flow. This methodology was described in detail in a recent publication (Sadowski *et al.* 1988). Briefly, in heparinized animals the renal vein effluent entered a perpendicular side-branch of the renal vein–jugular vein shunt system provided with a roller pump supporting blood flow along shunt tubing. Raised blood level in the side-branch obscured a phototransistor which activated the pump to return a portion of blood to the animal. The pump stopped when blood level in the side branch had fallen; this cycle was continuously repeated. The renal blood flow was derived from a record in time of turns performed by the pump and a calibration factor equivalent to the output of the pump per one turn.

After completing all the preparatory procedures and starting RBF measurement, electrodes for measurement of tissue electrical admittance were inserted into the kidney.

*Tissue admittance measurements*

Electrical admittance of the renal tissue was measured to obtain an index of total electrolyte concentration in the renal medullary interstitium. The principle and detailed description of this technique were given previously (Sadowski & Portalska, 1983; Sadowski, 1985). It was established that at a relatively low frequency of the measuring alternating current (e.g. 3·5 kHz used here) the current bypasses cells and measurement is restricted to the extracellular compartment.

In essence, the measuring technique was as follows. Three needle electrodes were prepared from 75% platinum – 25% iridium wire, 0·2 mm in diameter. The whole length, except for a 1 mm fragment of a sharpened tip (active surface), was coated with an insulating resin. The tip-to-base length of the electrodes was 6·3, 4·3 and 2·6 mm. Viewed at the base in the horizontal plane, the electrodes were located at the points of an equilateral triangle with 1 mm sides. It was checked that for this measuring set and kidney size usual for rats weighing about 300 g, tissue area between the longest and intermediate electrode (channel 1) corresponded to most of the inner medulla (with exclusion of the tip of the papilla), and that between the intermediate and shortest electrode (channel 2) to the outer medulla. In addition, measurements were made for the area between the longest and shortest electrode, indicating average admittance for the whole renal medulla (channel 3). The electrodes were connected with a laboratory conductometer (N-572, Mera-Elwro, Poland) for direct reading of admittance in microsiemens. A programming device switched the three channels on and off in sequence, each for 10 s, followed by a 40 s resting period; the whole cycle was automatically repeated and recorded throughout the experiment.

Previous extensive *in vitro* studies of the rabbit kidney have shown that admittance is a linear function of tissue sodium concentration (Sadowski & Kulczykowska, 1988). More recently this was also confirmed *in vivo* for the rat kidney: admittance and tissue $Na^+$ (mmoles/(kg wet tissue)) were fairly closely correlated, with $r = 0{\cdot}78$ ($P < 0{\cdot}001$) and narrow confidence limits (Bądzyńska, Sadowski & Kompanowska-Jezierska, 1990).

*Experimental procedures*

Fifteen minutes after the start of RBF recording, the electrode set was inserted into the kidney and a priming dose of 6 $\mu$Ci of [*methoxy*-$^3$H]inulin was injected I.V. followed by an infusion which delivered 6 $\mu$Ci inulin in 1·2 ml/h of 2% saline. After a 30 min equilibration period, timed urine collections were started. Arterial blood was collected in the mid-point of clearance periods whereas renal vein blood samples were withdrawn continuously from the shunt, using a pump, over the entire urine collection period. Blood losses dependent on sampling and oozing from the flank wound (heparinization) were replaced by transfusion from a donor rat of the same strain. The effectiveness of the blood replacement regime was confirmed by repeated determination of arterial haematocrit which remained stable in all groups of rats.

Studies were performed with a control group and four experimental groups receiving ANP. The substance used here was the rat variant (isoleucine in position 17) of the twenty-six amino acid (8–33) atrial natriuretic peptide (Merck). Standard renal excretion and clearance studies together with direct RBF and admittance measurements were performed in each group. However, all variables were not always determined in each animal, hence occasional differences in individual $n$ values in Tables 1–4.

*Group 1 (low dosage)*. After a 20 min control period, a priming dose of 2 $\mu$g of ANP in 0·2 ml isotonic saline was given I.V. and the first 30 min experimental period was started immediately while the peptide was infused at 0·35 $\mu$g/(kg min) (0·6 ml/30 min). After stopping infusion two further 20 min urine collections were made. To avoid a major decrease in arterial blood pressure, in groups 2 and 3 ANP infusion time was shortened to 10 min.

*Group 2 (medium dosage)*. The protocol differed from that in group 1 in that the prime was 3 $\mu$g/kg and infusion rate was 2 $\mu$g/(kg min), given during the first 10 min of the 15 min experimental clearance period.

*Group 3 (high dosage)*. The protocol was as for group 2 except that the prime was increased to 10 $\mu$g/kg and the infusion rate to 6 $\mu$g/(kg min).

*Group 4 (intra-aortic ANP infusion, constant renal perfusion pressure)*. In seven rats a catheter for ANP infusion and pressure measurement was passed into the aorta via the left carotid artery, its tip was positioned opposite the origin of the left renal artery, and a screw-controlled clamp was

placed on the aorta just above the origin. Then the aorta was ligated below the left renal artery, which raised aortic pressure above the ligature to about 140–145 mmHg. Subsequently, the clamp was tightened to bring the pressure back to about 110 mmHg. In the control period isotonic saline was infused into the aorta at a rate of 0·1 ml/min; this infusion was shown in preliminary observations to have no distinguishable effect on blood pressure. Thereafter, vehicle infusion was replaced by ANP infusion for 10 min, followed by a return to the vehicle until the end of the experiment. From the start of ANP infusion the aortic clamp was loosened gradually to maintain initial pressure. In individual experiments ANP infusion rates were: 1·3, 1·8, 1·9, 2·6, 3·6, 3·6 and 4·4 $\mu$g/(kg min).

Clearance, RBF and admittance measurements were carried out simultaneously as in the other groups.

*Control group.* These experiments were designed to check the stability of our experimental preparation over time. Four 20 min periods were made and all measurements were carried out as in the experimental groups 1–3 but no ANP was given.

*Analytical methods and calculations*

Plasma and urine samples were counted in duplicate in an LKB 1211 liquid scintillation counter. The glomerular filtration rate was calculated as (1) clearance of tritiated inulin ($C_{in}$) given by the urine-to-plasma activity ratio multiplied by urine flow, and as (2) $RPF \times E_{in}$, with $E_{in} = (In_a - In_v)/In_a$, where $In_a$ and $In_v$ denote arterial and renal vein plasma inulin activity, respectively. Urine osmolality ($U_{osm}$) was determined by the freezing-point depression method and sodium by flame photometry.

*Statistical methods*

The standard error of mean (S.E.M.) was calculated. The significance of changes over time was first evaluated by repeat-measurement analysis of variance. When positive and whenever difference between two points in time was evaluated, Student's $t$ test for paired samples was performed and $P = 0{\cdot}05$ was used as significance level. When multiple comparisons (control *vs.* three points in time) were made, the required significance level was raised according to the formula proposed by Christensen *et al.* (1986): $(1-P)^n = 0{\cdot}95$, where $n$ is the number of comparisons. Accordingly, for the three-comparison tests usually performed in this study, the required significance level was $P = 0{\cdot}017$.

## RESULTS

### *Control studies*

Time control experiments (Table 1) carried out exactly as the experimental studies except that ANP was not given, showed stability of almost all parameters characterizing renal haemodynamics and excretory function. The exception was urine osmolality which fell progressively and significantly in four consecutive 10 min urine collection periods. The concurrent increasing tendency observed for urine flow was less consistent and not significant. This impairment of urine concentration is probably inherently connected with our experimental preparation and protocol and should be considered in interpretation of decreases in $U_{osm}$ observed after ANP.

### *Intravenous ANP studies*

The natriuretic response to ANP was quite distinct with the lowest dosage used in this study (group 1, Table 2): sodium excretion ($U_{Na}\dot{V}$) increased 2·8-fold during the 30 min peptide infusion and the peak increase (to the 4·8-fold control value) was delayed to the clearance period that followed cessation of the infusion. Less pronounced changes in $\dot{V}$ and $U_{osm}\dot{V}$ paralleled those in sodium excretion. The increase in renal excretion of sodium, total solutes and water occurred in the absence of significant changes in GFR measured as $C_{in}$ or $RPF \times E_{in}$. RBF increased

TABLE 1. Renal excretion and haemodynamics in control rats

| Time (min) | 0–20 | 20–40 | 40–60 | 60–80 | *n* |
|---|---|---|---|---|---|
| $\dot{V}$ (μl/min) | 11·7±2·2 | 13·5±2·7 | 16·6±3·3 | 16·7±4·0 | 15 |
| $U_{osm}$ (mosmol/kg) | 818±66 | 760±43 | 684±43* | 682±49* | 15 |
| $U_{Na}\dot{V}$ (μmol/min) | 1·2±0·5 | 1·4±0·5 | 1·2±0·3 | 1·2±0·3 | 14 |
| $U_{osm}\dot{V}$ (μosmol/min) | 8·7±1·3 | 9·9±1·7 | 9·1±1·5 | 9·9±1·8 | 15 |
| BP (mmHg) | 116±3 | 118±3 | 117±3 | 116±3 | 15 |
| RBF (ml/min) | 6·4±0·4 | 6·7±0·5 | 6·9±0·5 | 6·9±0·5 | 12 |
| $C_{in}$ (ml/min) | 0·95±0·09 | 0·96±0·10 | 0·95±0·10 | 0·88±0·11 | 13 |
| RPF × $E_{in}$ (ml/min) | 0·96±0·18 | 0·85±0·19 | 0·93±0·20 | 0·86±0·21 | 5 |

Mean values ± S.E.M. $\dot{V}$, $U_{osm}$, $U_{Na}\dot{V}$, BP, RBF, $C_{in}$ and RPF × $E_{in}$ denote urine flow, urine osmolality, sodium excretion, total solute excretion, aortic blood pressure, renal blood flow, inulin clearance and renal plasma flow times renal inulin extraction ratio, respectively. *Significantly different from control at $P < 0.017$, the level required with three comparisons by Student's *t* test (see Methods).

TABLE 2. Renal excretion and haemodynamic responses to ANP in group 1

| | Control | ANP | | | |
|---|---|---|---|---|---|
| Time (min) | −20–0 | 0–30 | 30–50 | 50–70 | *n* |
| $\dot{V}$ (μl/min) | 8·9±1·2 | 23·0±3·8* | 32·3±8·7* | 22·4±3·1* | 9 |
| $U_{osm}$ (mosmol/kg) | 899±80 | 749±53 | 642±42* | 628±53* | 9 |
| $U_{Na}\dot{V}$ (μmol/min) | 1·2±0·2 | 3·4±0·6* | 5·7±1·6* | 4·0±0·8* | 9 |
| $U_{osm}\dot{V}$ (μosmol/min) | 7·4±0·6 | 14·1±1·8* | 20·0±4·5* | 14·5±2·0* | 9 |
| BP (mmHg) | 128±6 | 123±5 | 121±5 | 122±5 | 9 |
| RBF (ml/min) | 6·9±0·3 | 7·1±0·3 | 7·4±0·3* | 7·9±0·4* | 11 |
| $C_{in}$ (ml/min) | 1·11±0·09 | 1·17±0·11 | 1·21±0·19 | 0·89±0·10 | 7 |
| RPF × $E_{in}$ (ml/min) | 0·85±0·09 | 0·84±0·07 | 0·94±0·06 | 0·89±0·08 | 11 |

ANP dosage: prime, 2 μg/kg I.V., followed by 0·35 μg (kg min) for 30 min. *Significantly different from control value at $P < 0.017$, as required with three comparisons by Student's *t* test. Abbreviations as in Table 1.

TABLE 3. Renal excretion and haemodynamic responses to ANP in group 2

| | Control | ANP (10 min) | | | |
|---|---|---|---|---|---|
| Time (min) | −20–0 | 0–15 | 15–35 | 35–55 | *n* |
| $\dot{V}$ (μl/min) | 8·5±1·2 | 41·9±8·6** | 29·3±5·6** | 24·5±5·1** | 7 |
| $U_{osm}$ (mosmol/kg)*** | 712±47 | 542±34 | 570±47 | 586±49 | 7 |
| $U_{Na}\dot{V}$ (μmol/min) | 1·0±0·3 | 6·9±1·5** | 5·3±0·7** | 4·2±0·7** | 7 |
| $U_{osm}\dot{V}$ (μosmol/min) | 6·9±0·8 | 22·8±4·0** | 16·6±2·4** | 13·9±2·3 | 7 |
| BP (mmHg) | 126±8 | 107±9** | 116±7** | 117±8 | 7 |
| RBF (ml/min) | 8·3±0·5 | 9·1±0·7 | 9·5±0·7 | 9·6±0·6 | 8 |
| $C_{in}$ (ml/min) | 0·85±0·10 | 1·10±0·16* | 0·82±0·10 | 0·79±0·07 | 5 |
| RPF × $E_{in}$ (ml/min) | 0·99±0·12 | 1·02±0·11 | 0·80±0·11 | 0·94±0·15 | 6 |

ANP dosage: prime, 3 μg/kg I.V., followed by 2 μg/(kg min) for 10 min. *$P < 0.05$, significantly different from control, single comparison; **$P < 0.017$, as required with three comparisons by Student's *t* test; ***overall change during experiment significant when tested by repeat-measurement ANOVA but not significant by *t* test (three comparisons). Abbreviations as in Table 1.

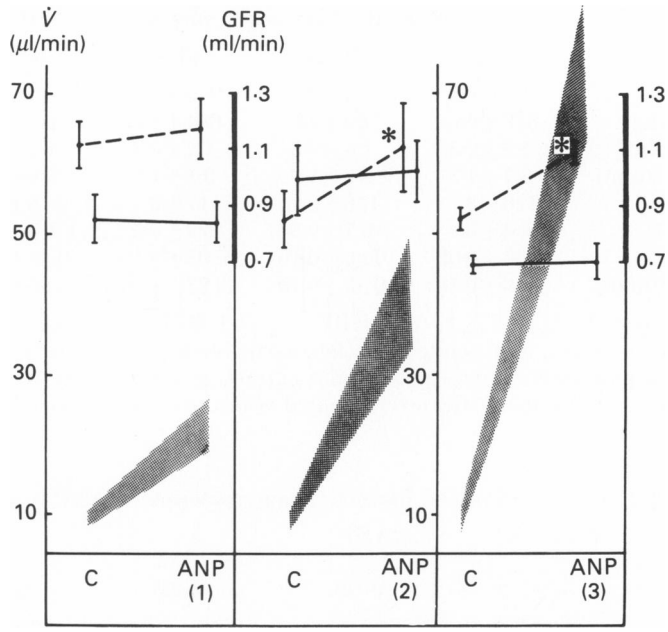


Fig. 1. Acute changes in $C_{in}$ (dashed lines) and RPF × $E_{in}$ (continuous lines) compared with increases in urine flow ($\dot{V}$, shaded areas representing mean ± S.E.M.) in three ANP dosage groups. The dynamics of change from control ($C$) to the infusion period (1, 2 or 3) is shown. * Significantly different from control ($P < 0{\cdot}01$–$0{\cdot}05$).

TABLE 4. Renal excretion and haemodynamic responses to ANP in group 3

| | Control | ANP (10 min) | | | |
|---|---|---|---|---|---|
| Time (min) | −20–0 | 0–15 | 15–35 | 35–55 | $n$ |
| $\dot{V}$ (μl/min) | 9·8 ± 1·4 | 72·5 ± 10·6* | 56·1 ± 6·5* | 38·4 ± 5·3* | 8 |
| $U_{osm}$(mosmol/kg) | 720 ± 93 | 462 ± 24 | 466 ± 19* | 511 ± 36 | 8 |
| $U_{Na}\dot{V}$ (μmol/min) | 1·0 ± 0·2 | 11·4 ± 1·3* | 8·4 ± 1·0* | 5·7 ± 0·8* | 8 |
| $U_{osm}\dot{V}$ (μosmol/min) | 6·5 ± 1·0 | 32·3 ± 3·0* | 25·5 ± 2·4* | 18·7 ± 2·1* | 8 |
| BP (mmHg) | 120 ± 3 | 101 ± 3* | 110 ± 3* | 117 ± 3 | 8 |
| RBF (ml/min) | 6·1 ± 0·3 | 6·5 ± 0·3 | 6·6 ± 0·3* | 7·1 ± 0·2* | 8 |
| $C_{in}$ (ml/min) | 0·79 ± 0·04 | 1·08 ± 0·04** | 0·85 ± 0·04 | 0·79 ± 0·04 | 8 |
| RPF × $E_{in}$ (ml/min) | 0·69 ± 0·03 | 0·70 ± 0·06 | 0·87 ± 0·05* | 0·88 ± 0·03* | 8 |

ANP dosage: prime, 10 μg/kg I.V., followed by 6 μg/(kg min) for 10 min. *$P < 0{\cdot}017$, as required with three comparisons by Student's $t$ test; **$P < 0{\cdot}01$, significantly different from control, single comparison. Abbreviations as in Table 1.

progressively but these changes were dissociated from those in $U_{Na}\dot{V}$: towards the end of experiments a visible increase in the renal blood flow occurred while sodium excretion was decreasing. In this group aortic blood pressure fell slightly and insignificantly during ANP infusion; however, this minor change combined with a distinct increase in RBF indicated a significant decrease in renal vascular resistance.

When peptide infusion was shortened to 10 min while overall dosage was increased, sodium excretion rose 7-fold in group 2 and more than 11-fold in group 3; the peak increase occurred during infusion (Tables 3 and 4). Less pronounced changes in $\dot{V}$ and $U_{osm}\dot{V}$ paralleled those in $U_{Na}\dot{V}$. The aortic blood pressure decreased after both higher

ANP doses but remained within the RBF autoregulatory range. Since simultaneously the RBF increased during experiments, it was obvious that the renal vascular resistance decreased considerably. The changes in renal excretion were again clearly dissociated from those in renal blood flow.

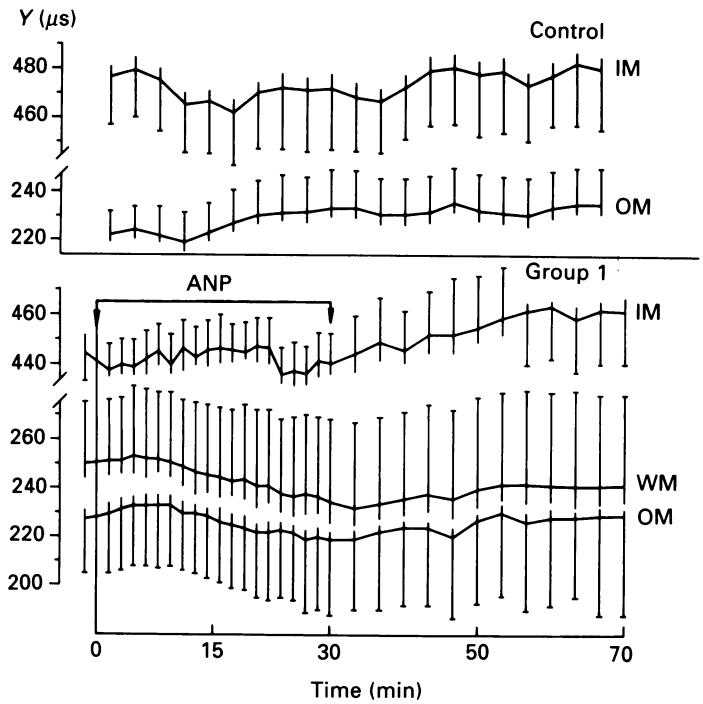


Fig. 2. Tissue admittance ($Y$) in control rats (upper panel, $n = 8$) and in group 1 (low ANP dosage, $n = 6$). Mean values ± S.E.M. Inner, outer and whole medulla denoted by IM, OM and WM, respectively. The large scatter of data for WM and OM in group 1 is due to very low values recorded in one rat. When this animal was excluded, there was still no significant change in WM or OM admittance during ANP infusion.

There were important differences in post-ANP changes in GFR estimated as $C_{in}$ as opposed to RPF × $E_{in}$. Figure 1 puts together the changes in both indices for three experimental groups (1–3) and relates them to concurrent changes in urine flow. It is seen that with moderate gradual increase in diuresis, as in group 1, neither $C_{in}$ nor RPF × $E_{in}$ changed significantly during ANP infusion. On the other hand, with higher ANP dosage and greater increases in urine flow (group 2 and 3), $C_{in}$ showed an immediate significant increase that was not confirmed by any change in RPF × $E_{in}$. However, later on in the experiments RPF × $E_{in}$ (but not $C_{in}$) increased significantly in group 3 (Table 4).

Profiles of tissue electrical admittance ($Y$) reflecting total electrolyte concentration in the medullary interstitium are shown in Figs 2 and 3. A common feature of tracings for both control and experimental groups (1–3) and for all channels (inner medulla, outer medulla and whole medulla) were large S.E.M. values, the result of major interanimal variations. There can be little doubt that the scatter was due to inevitable differences in positioning of electrodes in the kidney. Modest transient

decreases in admittance of the inner medulla were noted in groups 2 and 3. Since analysis of variance and the paired Student's $t$ test separated interanimal from time-dependent variations, despite large scatter of the data these changes proved significant by both methods.

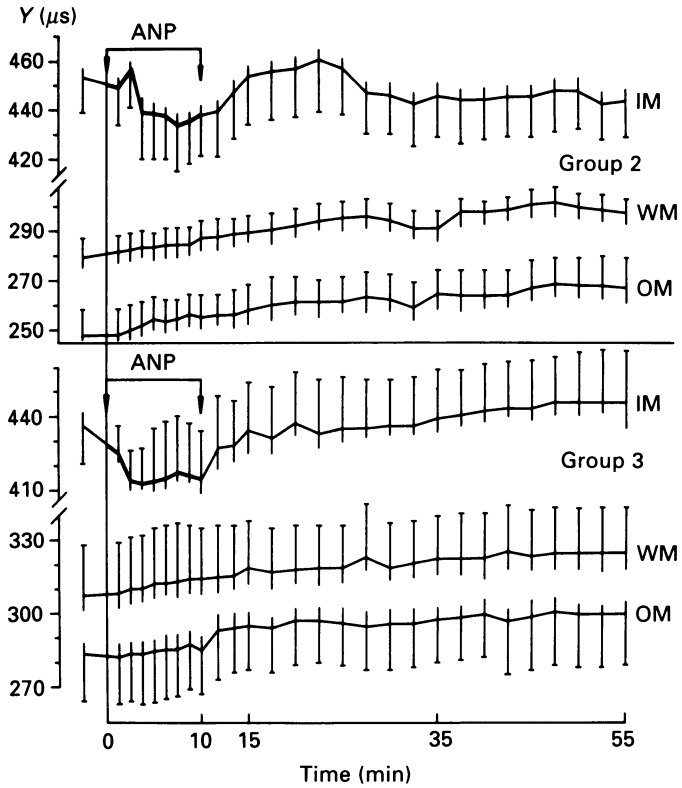


Fig. 3. Tissue admittance ($Y$) changes after ANP in group 2 (medium dosage, $n = 8$, upper panel) and in group 3 (high dosage, $n = 7$, lower panel). Thickened segments of two curves indicate significant decreases in $Y$ within the inner medulla (IM) when tested by repeat-measurement ANOVA and Student's $t$ test. Mean values ± S.E.M. Whole or outer medulla: WM or OM, respectively.

*Intra-aortic ANP with perfusion pressure control*

The main variables describing renal function in this group are shown in Fig. 4 which demonstrates also that the control of renal perfusion pressure was efficient and the required pressure stability was achieved. Individual curves rather than averaged data have been given in this series since ANP infusion rate was varied, ranging from 1·3 to 4·4 μg/kg min. Moreover, the actual amount of peptide entering the left as compared to the right kidney could not be defined, depending critically upon the exact position of the infusion catheter in the aortic pouch.

The largest increases in $U_{Na}\dot{V}$ occurred in the three experiments with the highest ANP infusion rates (3·6–4·4 μg/(kg min)). The renal blood flow and the glomerular filtration rate measured as $C_{in}$ or RPF × $E_{in}$ did not show significant changes though

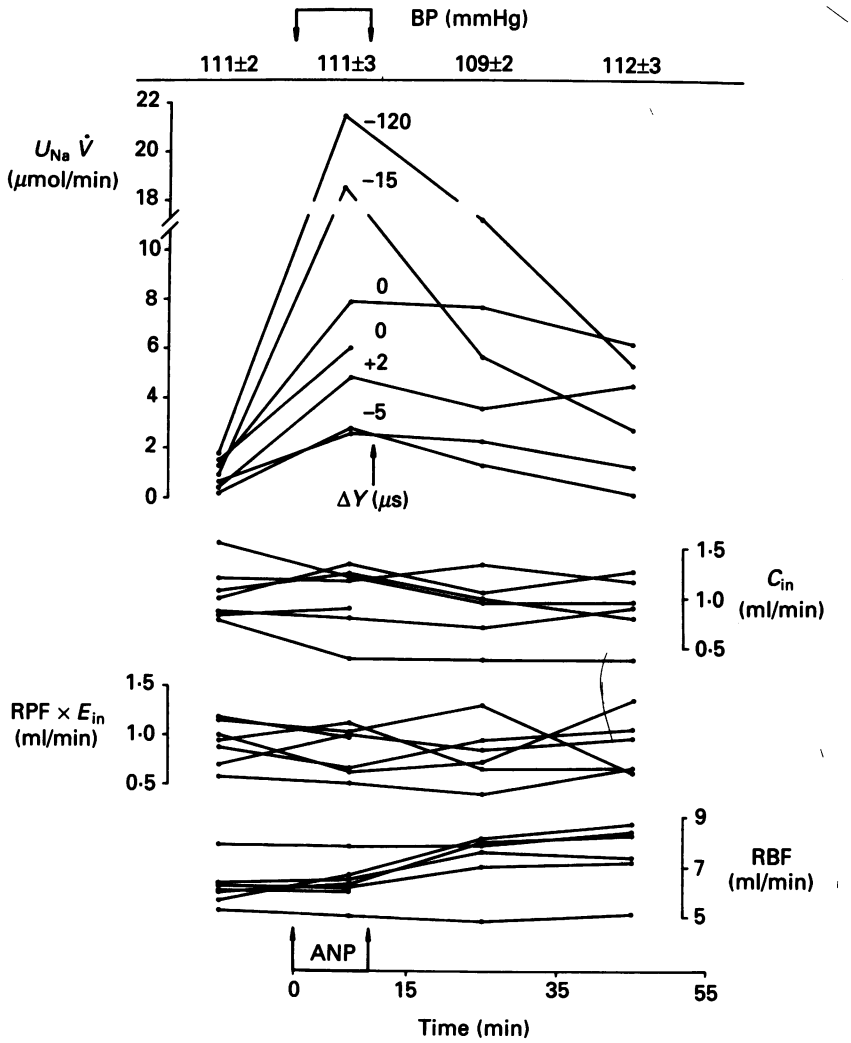


Fig. 4. Main experimental data for seven ANP-treated rats in which renal perfusion pressure (BP) was maintained constant. Admittance changes ($Y$) are indicated on the corresponding $U_{Na}\dot{V}$ curves. ANP dosage varied between 1·3 and 4·4 μg/(kg min).

an increasing trend in RBF was visible. The only major decrease in inner medullary tissue admittance (120 μS) occurred in the study with the highest ANP infusion rate (4·4 μg/(kg min)) and highest post-ANP sodium excretion.

## DISCUSSION

An almost standard protocol applied in the early studies of the effects of ANP on renal excretion and haemodynamics involved a sequence of control, ANP infusion (or bulk injection), and recovery measurements. In most studies performed in the rat, the renal blood flow was not measured at all or estimated from the clearance of *p*-

aminohippurate ($C_{PAH}$) not corrected for renal extraction of PAH (Hirata, Ishii, Sugimoto, Matsuoka, Kangawa & Matsuo, 1985; Sosa *et al.* 1986; Kiberd, Larson, Robertson & Jamison, 1987; Harris, Skinner & Zuho, 1988; Mendez, Dunn, Troy & Brenner, 1988), obviously due to difficulties of sampling renal vein blood or applying non-cannulating renal artery flow probes in this animal species. The glomerular filtration rate was always measured on the basis of inulin clearance. Such data should be interpreted with caution since, because of the pelvic dead space effect, the $C_{PAH}$ and $C_{in}$ values can be artificially elevated, especially in the acute phase of the experiment, i.e. at a rapid transition from the low to high urine flow. In addition, the stability of $E_{PAH}$ in the presence of high plasma ANP levels has never been verified.

In a few rat studies employing the basic protocol described above the total renal blood flow was measured directly by means of electromagnetic (Pollock & Banks, 1983; Pollock & Arendshorst, 1986; Criscione *et al.* 1987) or Doppler (Lappe, Smits, Todt, Debets & Wendt, 1985; Sonnenburg, Muir, Criscione, Kraetz & Hofbauer, 1985) flow probes on the renal artery. The responses to ANP were highly variable: no change or decrease in blood flow with no change or increase in renal vascular resistance were usually reported. The actual result visibly depended on ANP dosage, mode of administration (bolus injection *vs.* infusion), the functional status of animals (conscious *vs.* anaesthetized) and, perhaps most important, on the extent of peptide-induced fall in arterial blood pressure. In contrast, we found that with renal perfusion pressure within the autoregulation range, directly measured total blood flow of the rat kidney increased after ANP and renal vascular resistance fell. The reason for this discrepancy is not clear, but our data accord with the reported vasorelaxant action of the peptide on the isolated rat arcuate artery (Aalkjaer, Mulvany & Nyborg, 1985) as well as on the pre-glomerular vessels of the *in vitro* blood-perfused juxtamedullary nephron (Veldkamp, Carmen, Inscho & Navar, 1988) and of the split hydronephrotic kidney (Marin-Grez, Fleming & Steinhausen, 1986).

In the present study RBF increased 14–16% over about 1 h; the change appeared unrelated to the dose. The early increase in flow, parallel to peak natriuresis and diuresis, did not exceed a modest 10% and towards the end of experiments sodium and water excretion decreased while RBF was still increasing. On the whole, the natriuretic and RBF responses to ANP were dissociated and there was no indication of a causal relationship between them.

A common finding in many studies with exogenous ANP was a rapid onset of copious diuresis associated with an elevation of the glomerular filtration rate measured as $C_{in}$. This acute increase in GFR was, understandably, regarded as suspicious. Aware of the dead space effect, Hansell & Ulfendahl (1987) simply concluded that the transient increase in GFR was an artifact. Mendez *et al.* (1988) discarded measurements from the transient phase and started proper clearance collections when urine flow had stabilized. Davis & Briggs (1987*a*) attempted to quantify the magnitude of the dead space error using a rather complex procedure and formula but still had to discard the GFR data from the first minutes of ANP action.

In the present study we circumvented difficulties with measuring GFR during the development of ANP diuresis by using $RPF \times E_{in}$ as an index of filtration, an approach that does not require steady-state conditions with respect to urine flow and is not subject to the dead space error. Application of the $RPF \times E_{in}$ formula was quite

practical and easy in our experiments for RBF was measured directly and accurately and renal vein blood could be continuously sampled from the renal vein–jugular vein shunt. For comparison $C_{in}$ was determined simultaneously.

We found that at two ANP dosage levels associated with rapid increases in urine flow (groups 2 and 3, Fig. 1), $C_{in}$ increased significantly while $RPF \times E_{in}$ remained constant. This disparity indicates, not unexpectedly, that $C_{in}$ was not a reliable measure of GFR at the onset of ANP action. A similar disparity between the clearance and extraction method was recently reported from ANP studies in the dog performed by Banks (1988) who used creatinine as a marker for filtration measurement. Even with the fairly high pharmacological doses applied in our experiments, the genuine rate of glomerular filtration ($RPF \times E_{in}$) did not change. Consequently, an increase in GFR appeared not to be a mechanism contributing to the major natriuresis and diuresis. A late increase in GFR observed with the highest dosage (group 3) occurred while excretion rates were already decreasing.

In a study that employed small doses of ANP in the anaesthetized rat Soejima, Grekin, Briggs & Schnermann (1988) showed that an infusion rate as low as 20 ng/(kg min) (which doubled plasma ANP levels) was clearly natriuretic. It will be noticed that a 100 times higher rate as applied in our group 2 still did not produce a genuine, early or delayed, increase in the filtration rate, even though plasma ANP levels attained in these experiments must have largely exceeded those that occur under extreme physiological or pathological conditions. Taken together, these data suggest that GFR changes do not have a part in the postulated regulation of body fluid balance by ANP. At the same time, there can be little doubt that with appropriately high pharmacological dosage the peptide can increase GFR and the resulting natriuresis would possibly be enhanced.

We have shown previously that tissue electrical admittance as measured in the present study is an index of total electrolyte (mostly NaCl) concentration in the medullary interstitium (Sadowski & Portalska, 1983; Portalska & Sadowski, 1984; Sadowski, 1985; Sadowski & Kulczykowska, 1988). Considering the widespread speculation on the role of solute wash-out in the mechanism of ANP natriuresis, we used this methodology to examine the peptide's effect on the electrolyte component of the cortico-medullary solute gradient. The observed decrease in admittance (i.e. in tissue electrolyte concentration) occurred only at higher peptide infusion rates (groups 2 and 3), was limited to the inner medullary zone and quantitatively modest. This was in striking contrast to a major response to frusemide. With ANP an increase in $U_{Na}\dot{V}$ by a factor of 12 (group 3) was required to result in only one-fourth of the decrease in admittance observed with a frusemide dose which increased $U_{Na}\dot{V}$ by a factor of 3·5 (Bądzyńska *et al.* 1990).

The specific and highly reproducible decreases in renal medullary tissue admittance demonstrated in our laboratory for the rabbit, dog and rat observed after frusemide as well as after ethacrynic acid, as opposed to ineffectiveness of acetazolamide and chlorothiazide (Sadowski & Portalska, 1983; Portalska & Sadowski, 1984; Sadowski, 1985; Sadowski & Kulczykowska, 1985) suggest that inhibition of reabsorption of salt without water from the ascending limb of Henle's loop is a crucial factor in decreasing NaCl concentration in the medullary interstitium. In this context the small effectiveness of ANP speaks against a major action in this tubule segment, in

agreement with the data from studies of isolated *in vitro* perfused rabbit loop segments (Kondo, Imai, Kangawa & Matsuo, 1986). A similar conclusion has come from the comparison of ANP and frusemide action on the isolated perfused rat kidney (Firth, Raine & Ledingham, 1988) and from experiments in the dog (Sosa *et al.* 1986). In their analytical studies of tissue slices Davis & Briggs (1987*b*) demonstrated that ANP decreased the $Na^+$ concentration of the inner medulla, at least when the data were expressed per solute-free dry tissue weight. There was no clear difference between the ANP group and three frusemide experiments. However, although our admittance values present at best a semi-quantitative index, the resolution they provide in estimating the cortico-papillary electrolyte (mostly NaCl) gradient is probably higher than with conventional analytical studies because the data reflect changes that occur almost exclusively in the interstitial fluid rather than in a sum of all tissue compartments.

In studies other than that by Davis & Briggs (1987*b*) and the present work, the dissipation of medullary tissue solutes after ANP was assumed on the basis of a decrease in $U_{osm}$, a conclusion that may not be warranted since at very high urine flows, as often seen after infusion of the peptide, osmotic equilibration in the terminal collecting duct would probably be incomplete (Lote & Snape, 1977). According to Davis & Briggs (1987*b*) and our present results the decrease in medullary tissue $Na^+$ was small or modest, in contrast to a virtual abolition of urea gradient (Davis & Briggs, 1987*b*). However, a dissipation of papillary and medullary tissue urea is known to be a consequence, rather than a cause, of any major diuresis. Therefore, these data do not necessarily support the postulated role of a wash-out of medullary solutes in ANP natriuresis. Growing evidence against this hypothesis includes a demonstration of the natriuresis in normal rats without any decrease in $U_{osm}$ and in diabetes insipidus (Brattleboro) rats with initially flat cortico-papillary solute gradient (Awazu, Granger & Knox, 1988), and in rats with necrotic papilla (Hildebrandt & Banks, 1987). Also the reports on a dissociation in time of the ANP-induced increase in papillary blood flow and peak natriuresis speak against a causative role of the solute wash-out (Kiberd *et al.* 1987; Takezawa *et al.* 1987).

A decrease in arterial blood pressure observed in our experimental groups 1–3 complicates interpretation both of the ANP-dependent renal haemodynamic changes and of the relationship between haemodynamics and natriuresis. For instance, one could speculate that the observed increase in RBF, an uncommon finding in the rat (see above), could occur only as a compounded effect of a direct vasorelaxant action of ANP plus an autoregulatory response of renal resistance vessels to a decrease in renal perfusion pressure (RPP). A profound effect of RPP on ANP natriuresis has been amply documented (Burnett *et al.* 1986; Davis & Briggs, 1987*a*; Sosa *et al.* 1986; Firth *et al.* 1988); specifically, the peptide was shown to enhance (i.e. increase the slope of) the pressure–natriuresis relationship (Paul *et al.* 1987; Takezawa *et al.* 1987).

In the experiments of our group 4, the suprarenal aortic clamp was used in a way to maintain renal perfusion pressure during ANP dependent renal and systemic vasodilatation. In this setting, changes in renal haemodynamics and excretion did not qualitatively differ from those seen in groups 1–3: a clear increasing trend in RBF was visible, dissociated from the natriuresis, and GFR did not change. These data reinforce our conclusions based on data of groups 1–3 an indicate that the

modest decreases in blood pressure observed in our studies did not significantly modify the renal response pattern to ANP.

This work was supported by the CPBP 06–02 grant. Synthetic ANP (8–33) was a gift from Dr R. J. Winquist of the Merck, Sharp and Dohme Research Laboratories, West Point, PA, USA.

## REFERENCES

AALKJAER, C., MULVANY, M. J. & NYBORG, N. C. B. (1985). Atrial natriuretic factor causes specific relaxation of rat renal arcuate arteries. *British Journal of Pharmacology* **86**, 447–453.

AWAZU, M., GRANGER, J. P. & KNOX, F. G. (1988). Effect of atrial natriuretic peptide in diabetes insipidus rats. *Proceedings of the Society for Experimental Biology and Medicine* **187**, 165–168.

BĄDZYŃSKA, B., SADOWSKI, J. & KOMPANOWSKA-JEZIERSKA, E. (1990). Tissue electrical admittance (electrolyte concentration) in rat renal medulla – effects of furosemide and acetazolamide. *Archives internationales de physiologie et de biochimie* (in the Press).

BANKS, R. O. (1988). Effects of physiological dose of ANP on renal function in dogs. *American Journal of Physiology* **255**, F907–910.

BURNETT, J. C. JR, OPGENORTH, T. J. & GRANGER, J. P. (1986). The renal action of atrial natriuretic peptide during control of glomerular filtration. *Kidney International* **30**, 16–19.

CHRISTENSEN, S., STEINESS, E. & CHRISTENSEN, H. (1986). Tubular sites of furosemide natriuresis in volume-replaced and volume-depleted conscious rats. *Journal of Pharmacology and Experimental Therapeutics* **239**, 211–218.

CRISCIONE, L., BURDET, R., HÄNNI, H., KAMBER, B., TRUOG, A. & HOFBAUER, K. G. (1987). Systemic and regional hemodynamic effects of atriopeptin II in anaesthetized rats. *Journal of Cardiovascular Pharmacology* **9**, 135–141.

DAVIS C. L. & BRIGGS, J. P. (1987*a*). Effect of reduction in renal artery pressure on atrial natriuretic peptide-induced natriuresis. *American Journal of Physiology* **252**, F146–153.

DAVIS, C. L. & BRIGGS, J. P. (1987*b*). Effect of atrial natriuretic peptides on renal medullary solute gradients. *American Journal of Physiology* **253**, F679–684.

FIRTH, J. D., RAINE, A. E. G. & LEDINGHAM, J. G. G. (1988). Low concentrations of ANP cause pressure-dependent natriuresis in the isolated kidney. *American Journal of Physiology* **255**, F391–396.

HANSELL P. & ULFENDAHL, H. R. (1987). The influence of atriopeptins I and II on the relationship between glomerular filtration and natriuresis. *Acta physiologica scandinavica* **129**, 361–369.

HARRIS, P. J., SKINNER, S. L. & ZHUO, J. (1988). The effects of atrial natriuretic peptide and glucagon on proximal glomerulo-tubular balance in anaesthetized rats. *Journal of Physiology* **402**, 29–42.

HILDEBRANDT, D. A. & BANKS, R. O. (1987). Effect of atrial natriuretic factor on renal function in rats with papillary necrosis. *American Journal of Physiology* **252**, F977–980.

HIRATA, Y., ISHII, M., SUGIMOTO, T., MATSUOKA, H., KANGAWA, K. & MATSUO, H. (1985). The effects of human atrial 28-amino acid peptide on systemic and renal hemodynamics in anesthetized rats. *Circulation Research* **57**, 634–639.

KIBERD, B. A., LARSON, T. S., ROBERTSON, C. R. & JAMISON, R. L. (1987). Effect of atrial natriuretic peptide on vasa recta blood flow in the rat. *American Journal of Physiology* **252**, F1112–1117.

KONDO, Y., IMAI, M., KANGAWA, K. & MATSUO, H. (1986). Lack of direct action of $\alpha$-human atrial natriuretic polypeptide on the *in vitro* perfused segments of Henle's loop isolated from rabbit kidney. *Pflügers Archiv* **406**, 273–278.

LAPPE, R. W., SMITS, J. F. M., TODT, A., DEBETS, J. J. & WENDT, R. L. (1985). Failure of atriopeptin II to cause arterial vasodilatation in the conscious rats. *Circulation Research* **56**, 606–612.

LOTE, C. J. & SNAPE, B. M. (1977). Collecting duct flow rate as a determinant of equilibration between urine and renal papilla in the rat in the presence of a maximal antidiuretic hormone concentration. *Journal of Physiology* **270**, 533–544.

MARIN-GREZ, M., FLEMING, J. T. & STEINHAUSEN, M. (1986). Atrial natriuretic peptide causes preglomerular vasodilatation and post-glomerular vasoconstriction in rat kidney. *Nature* **324**, 473–476.

MENDEZ, R. E., DUNN, B. R., TROY, J. L. & BRENNER, B. M. (1988). Atrial natriuretic peptide and furosemide effects on hydraulic pressure in the renal papilla. *Kidney International* **34**, 36–42.

PAUL, R. V., KIRK, K. A. & NAVAR, L. G. (1987). Renal autoregulation and pressure natriuresis during ANF-induced diuresis. *American Journal of Physiology* **253**, F424–431.

POLLOCK, D. M. & ARENDSHORST, W. J. (1986). Effect of atrial natriuretic factor on renal hemodynamics in the rat. *American Journal of Physiology* **251**, F795–801.

POLLOCK, D. M. & BANKS, R. O. (1983). Effect of atrial extract on renal function in the rat. *Clinical Science* **65**, 47–55.

PORTALSKA, E. & SADOWSKI, J. (1984). Renal medullary electrolytes: effects of furosemide assessed by studies *in vivo* of electrical admittance. *Archives internationales de physiolgie et de biochimie* **92**, 345–354.

RAINE, A. E. G., FIRTH, J. G. & LEDINGHAM, J. G. G. (1989). Renal actions of atrial natriuretic factor. *Clinical Science* **76**, 1–8.

SADOWSKI, J. (1985). Estimation of changes in renal tissue electrolytes from measurements of electrical admittance: application in the rat. *Acta physiologica polonica* **36**, 339–344.

SADOWSKI, J. & KULCZYKOWSKA, E. (1985). Renal medullary electrolyte responses to three diuretics assessed by measurement of electrical admittance of the *in situ* rat kidney. *Proceedings of the 5th European Colloquium on Renal Physiology, Frankfurt*, p. 54.

SADOWSKI, J. & KULCZYKOWSKA, E. (1988). Studies of tissue electrical admittance: relation to tissue sodium for different zones of rabbit kidney. *Physiologia bohemosloaca* **37**, 185–187.

SADOWSKI, J., KULCZYKOWSKA, E., KULCZYKOWSKA, M. & BĄDZYŃSKA, B. (1988). Renal vein outflow recording in rats and rabbits: alternative method of RBF measurement. *Acta physiologica hungarica* **72**, 335–342.

SADOWSKI, J. & PORTALSKA, E. (1983). Dynamic evaluation of renal electrolyte gradient by *in situ* tissue impedance studies. *Kidney International* **24**, 800–803.

SOEJIMA, H., GREKIN, R. J., BRIGGS, J. P. & SCHNERMANN, J. (1988). Renal responses of anesthetized rats to low-dose infusion of atrial natriuretic peptide. *American Journal of Physiology* **255**, R449–455.

SONNENBURG, C., MUIR, A. C., CRISCIONE, L., KRAETZ, J. & HOFBAUER, K. G. (1985). Is the natriuretic effect of ANP in conscious rats mediated by renal vasodilatation? *Journal of Hypertension* **3**, 303–305.

SOSA, R. E., VOLPE, M., MARION, D. N., ATLAS, S. A., LARAGH, J. H., VAUGHAN, E. D. JR & MAACK, T. (1986). Relationship between renal hemodynamic and natriuretic effects of atrial natriuretic factor. *American Journal of Physiology* **250**, F520–524.

TAKEZAWA, K., COWLEY, A. W. JR, SKELTON, M. & ROMAN, R. J. (1987). Atriopeptin III alters renal medullary hemodynamics and the pressure–diuresis response in rats. *American Journal of Physiology* **252**, F992–1002.

VELDKAMP, P. J., CARMEN, P. K., INSCHO, E. W. & NAVAR, L. G. (1988). Direct evaluation of the microvascular actions of ANP in juxtamedullary nephrons. *American Journal of Physiology* **254**, F440–444.