Spontaneous changes in arterial blood pressure and renal interstitial hydrostatic pressure in conscious rats

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1. Previous work has demonstrated a positive relationship between experimentally induced changes in arterial pressure (AP) and renal interstitial hydrostatic pressure (RIHP). The purpose of the present study was to test the hypothesis that RIHP is positively correlated with the normal changes in AP that occur spontaneously in conscious rats.

2. Rats were chronically instrumented for the recording of AP (via an aortic catheter) and RIHP. RIHP was measured by implanting a Millar microtransducer, whose tip had been encapsulated in a 35 μm pore polyethylene matrix (5 mm long, 2 mm o.d.), approximately 5 mm below the renal cortical surface.

3. A total of 56 h of simultaneous analog recording of AP and RIHP was obtained from ten rats. Each 1 h segment was digitized and evaluated at frequencies of 1, 0·1, 0·02 and 0·01 Hz.

4. In forty-nine out of fifty-six of these 1 h recordings taken at 1 Hz, there were significant positive linear correlations between AP and RIHP (mean $r = 0{\cdot}32$) with a mean slope of 0·11 mmHg RIHP/1 mmHg AP. Low-pass filtering to 0·01 Hz significantly increased the r value to 0·48.

5. These results demonstrate that spontaneous changes in AP and RIHP are positively correlated. The spontaneous coupling of AP and RIHP may be of importance in the regulation of salt and water excretion by the pressure diuresis mechanism.

Both theory and experimental evidence indicate that a close correspondence between arterial pressure (AP) and urinary excretion of salt and water (pressure diuresis) is essential for the long-term control of AP (Selkurt, Hall & Spencer, 1949; Thompson & Pitts, 1951; Tobian, Coffee, Ferreira & Meuli, 1964; Guyton, Coleman, Cowley, Scheel, Manning & Norman, 1972; Guyton, Coleman & Granger, 1972; Hall, Mizelle, Woods & Montani, 1988; Roman, 1988; Roman, Cowley, Garcia-Estan & Lombard, 1988; Romero & Knox, 1988; Guyton, 1990; Guyton, Hall, Coleman & Manning, 1990). Although the mechanism of pressure diuresis is not known, experiments with anaesthetized animals demonstrate that transmission of AP to the renal interstitium is critical for the expression of pressure diuresis (Hartupee, Burnett, Mertz & Knox, 1982; Granger, Haas, Pawlowska & Knox, 1988; Granger & Scott, 1988; Roman *et al.* 1988; Khraibi & Knox, 1988; Garcia-Estan & Roman, 1989; Khraibi & Knox, 1989*a*; Khraibi & Knox, 1989*b*; Wehberg, Hassassian, Scott & Granger, 1990). In response to mechanically induced changes in AP, urine flow (UF) and renal interstitial hydrostatic pressure (RIHP) change in parallel (Ott, Navar & Guyton, 1971; Granger & Scott, 1988; Khraibi & Knox, 1988; Roman *et al.* 1988; Garcia-Estan & Roman, 1989; Khraibi, Haas & Knox, 1989; Khraibi & Knox, 1989*b*; Roman & Lianos, 1990). Similarly, a close correspondence between changes in UF and RIHP have been observed during renal venous pressure elevation (Burnett & Knox, 1980), acetylcholine-induced renal vasodilatation (Hartupee *et al.* 1982; Granger & Scott, 1988; Wehberg *et al.* 1990), renal lymphatic outflow blockade (Wilcox, Sterzel, Dunckel, Mohrmann & Perfetto, 1984), and direct or indirect renal interstitial volume expansion (Burnett & Knox, 1980; Granger *et al.* 1988; Roman *et al.* 1988; Garcia-Estan & Roman, 1989; Khraibi & Knox, 1989*a*; Khraibi, 1991).

A role for RIHP in pressure diuresis is further supported by the impaired pressure diuresis observed when the correspondence between AP and RIHP is altered by removal of the renal capsule (Hartupee *et al.* 1982; Garcia-Estan & Roman, 1989; Khraibi & Knox, 1989*a*; Khraibi & Knox, 1989*b*; Wehberg *et al.* 1990). Based on the correlation between induced changes in AP and RIHP (Haas, Granger & Knox, 1986), it has been proposed that

even small changes in RIHP may be essential and sufficient for long-term control of AP (Ott *et al.* 1971; Haas *et al.* 1986; Granger, 1992). Thus, from studies in anaesthetized animals, there is evidence that AP, RIHP and UF are all positively correlated, supporting the hypothesis that changes in RIHP are an essential intermediate step in pressure diuresis.

No previous studies, however, have examined the normal, spontaneous relationship between AP and RIHP in the conscious animal. The importance of evaluating this relationship in conscious animals during spontaneous changes in AP derives primarily from three observations. First, the rapidity of the pressure diuresis response (Steele, Brand, Metting & Britton, 1993) suggests that if changes in RIHP are a critical intermediate step in pressure diuresis, then spontaneous, moment-to-moment changes in AP must be reflected in similar moment-to-moment spontaneous changes in RIHP. Second, the normal, spontaneous influence of AP upon RIHP may be vastly different to that found previously during mechanically controlled changes in AP. For example, the AP–RIHP coupling may be even more pronounced during spontaneous changes in AP because we now know that the predominant renal pressure–flow relationship in conscious dogs and rats is not autoregulation, but a baroreflex-like pattern (Skarlatos *et al.* 1993; Skarlatos, Metting & Britton, 1993). Such a pattern, in which the renal vessels dilate during an increase in AP, would tend to amplify the influence of AP upon RIHP. Third, the effects of anaesthesia and surgery in acute experiments probably influence renal haemodynamics (Walker, Buscemi-Bergin & Gellai, 1983) and thus may affect the relationship between AP and RIHP.

Therefore this study was designed to evaluate the relationship between AP and RIHP in the intact conscious rat during spontaneous operation of all the mechanisms that may influence AP and renal function. Our results demonstrate that spontaneous changes in AP and RIHP are positively correlated, indicating that moment-to-moment changes in AP are transmitted to the renal interstitium to mediate pressure diuresis.

METHODS

Animal preparation

Experiments were performed on ten conscious male Sprague–Dawley rats (Harlan Sprague–Dawley Inc., Indianapolis, IN, USA), weighing 365 ± 35 g. Food (Purina rat chow) and water were provided *ad libitum* throughout the study. At least two days prior to the start of data collection, aseptic surgery was performed to instrument the animals for measurement of AP and RIHP. The rats were anaesthetized with ketamine–xylazine I.P. (Ketaset®, 8 mg (100 g body wt)$^{-1}$; Aveco, Inc., Fort Dodge, IA, USA; Rompun®, 1·2 mg (100 g body wt)$^{-1}$; Miles, Inc., Shawnee Mission, KA, USA) and placed on a thermostatically controlled warming table to maintain body temperature close to 37 °C. The pressure-sensitive region of a microtransducer (model SPR-249A, Millar Instruments Inc., Houston, TX, USA) was secured with Silastic® glue (Dow Corning) inside the narrow, bored-out cavity (< 1 mm i.d.) of a small polyethylene capsule (5 mm long, 2 mm o.d., 35 μm pore matrix). The left kidney was exposed through a flank incision and the capsule containing the pressure microtransducer was implanted within the renal parenchyma with its tip approximately 5 mm below the cortical surface at the dorsolateral aspect of the kidney. This positioned the pressure-sensing part of the microtransducers approximately 2·5 mm below the surface of the kidney. The polyethylene capsule was secured in position by means of a Dacron felt patch (3 mm radius) that surrounded the transducer wire where it exited the kidney and was glued to the surface of the kidney by two to three drops of cyanoacrylate ester gel. The insulated wire of the Millar microtransducer was anchored to the abdominal wall by 5/0 silk sutures to maintain its longitudinal orientation at an approximate right angle to the kidney surface. Through a second incision, the left femoral artery and vein were exposed. A catheter made from Teflon tubing (0·015 in i.d.) was advanced approximately 3·5 cm in a retrograde direction into the lower abdominal aorta for measurement of AP. The AP catheter was connected to PE-50 tubing (Clay Adams, Parsippany, NJ, USA), filled with heparinized saline (100 units ml^{-1}), and closed with a stainless-steel plug. A second catheter made of PE-50 was advanced from the femoral vein into the abdominal vena cava for infusion of saline and drugs. The wire from the microtransducer, and the two catheters, were tunnelled subcutaneously to exit through the skin of the dorsum between the scapulae. The exteriorized catheters and the wire were carried outside and above the animal's cage through a customized spring-tethering system. The lower end of the tethering system was sutured to the skin on the dorsum of the animal in such a fashion that mobility, food seeking, stretching, grooming, and sleeping posture were minimally impaired. The animals were killed at the completion of the studies by administering sodium pentobarbitone I.V. at a dose of 100 mg (kg body wt)$^{-1}$. On postmortem evaluation, the tips of the arterial catheters were found approximately 0·5–1·0 cm caudal to the origin of the left renal artery. The kidney's anatomical integrity was inspected under × 20 magnification. Stereoscopic examination of the capsule was also performed in order to exclude experiments where tissue growth into the capsule may have occurred. We did not observe blockade of the capsule by tissue growth in any of our experiments.

Protocol and measurements

Data were collected starting at approximately 07.00 h, in a quiet, lighted room that was maintained at a temperature of 22–24 °C. The animals were studied while 'resting' in their home cage. Each daily protocol consisted of recording both AP and RIHP for 1–3 h in undisturbed, conscious rats. AP was measured with a Statham strain gauge pressure transducer (Model P23Db, Gould-Statham, Oxnard, CA, USA), with zero pressure reference taken at heart level. The signals from the RIHP microtransducer and the aorta were transduced and amplified with a Sensormedics R-611 polygraph recorder (Anaheim, CA, USA). Both amplified unfiltered signals were sampled at 250 Hz by a PO-NE-MAH digital acquisition and archiving system (Storrs, CT, USA) and then resampled and stored on disk at a frequency of 1 Hz (about 6 cardiac cycles per second).

Table 1. Slopes derived from calibrations of the Millar microtransducer for each rat

Animal No.	Slopes of calibration (mmHg V^{-1}) Before implantation	Inside kidney	After retrieval
1	9·9	—	9·9
2	8·9	8·1	9·8
3	9·8	—	9·8
4	9·8	10·8	10·4
5	9·7	9·3	9·8
6	9·8	9·0	10·5
7	9·5	10·7	9·9
8	9·6	9·9	9·8
9	9·8	9·9	9·8
10	9·7	13·0	9·9
Mean ± S.D.	9·7 ± 0·3	10·1 ± 1·5	10·0 ± 0·3

Calibrations were performed: (1) before implantation; (2) with the transducer still inside the excised kidney; and (3) after retrieval of the transducer from the kidney.

The Millar microtransducers were calibrated: (1) before implantation in the kidney; (2) following euthanasia with the transducer still in the excised kidney; and (3) after removing the transducer from the renal tissue. The calibrations were performed by using a saline column and placing the pressure-sensitive portion of the transducer at depths below the free surface of the saline, equivalent to 5, 10, 15 and 20 mmHg pressure. Table 1 shows the slopes (mmHg V^{-1}) of the calibrations obtained during the three conditions. The slopes were similar (about 10 mmHg V^{-1}) under the three experimental conditions and variability was minimal (i.e. coefficients of variability < 0·15 mmHg). In additional tests, we evaluated the drift of the transducer voltage signal at static pressures between 0 and 20 mmHg. The mean drift was always less than 0·5 mmHg h^{-1} and it was directionally random. These calibrations and tests demonstrate that the matrix-enclosed microtransducer has sufficient accuracy and precision to evaluate changes in RIHP. Accurate determination of the absolute value of RIHP was not possible because significant drift does occur in the zero pressure voltage (i.e. baseline) when evaluated over several days and we had no method of applying atmospheric pressure to the transducer while it was implanted in the kidney of a conscious rat. The approximate absolute values of the RIHP were estimated from the transducer voltage in each daily experiment by the following calculation:

$$\mathrm{RIHP} = S(V_E - V_0),$$

where S is the mean slope of the three calibrations shown in Table 1, V_E is the experimental voltage from the amplified transducer output and V_0 is the mean of the three voltages obtained at atmospheric pressure during the calibration trials. Thus, the absolute values of RIHP listed in Table 2 and used in this study represent the true value plus instrument drift. The inability to measure absolute values of RIHP over a time period of several days did not obscure our ability to measure changes in RIHP accurately as they occurred during experiments that lasted 2 h.

Evaluation of the RIHP measurements

The nature of the intrarenal pressure recorded in the present study was evaluated using two manoeuvres. First, we measured the response of AP and RIHP to angiotensin I bolus injections (1 μg kg^{-1}, I.V.) in five conscious animals. Second, we determined the ability of the pressure microtransducer to follow changes in the renal interstitial fluid volume induced by slow intravenous administration of saline (5% body wt given over 2 h). This latter manoeuvre was performed in three of the rats that were anaesthetized after completion of the conscious protocols.

Data analysis

During each experimental session, 1–3 h of recording were obtained and divided into hourly segments. The data for each segment (about 1 h) were stored as individual digital files, each containing, on average, 3500 ± 205 paired values for AP and RIHP, with each value representing one mean value obtained from 250 samples per second (i.e. about 6 cardiac cycles). A total of 56 digital files (about 56 h of observation) were analysed. Prior to analysis, aberrant data, defined as those differing by more than 60% from the immediately preceding and subsequent values, were discarded. This criterion for exclusion of data was based upon our experience that a 60% or greater change in AP or RIHP that occurs within 1 s is always associated with a mechanical or electrical problem and is not of physiological origin. Less than 2% of the data were discarded by this criterion.

The relationship between AP and RIHP was analysed at four different levels of low-pass filtering to gain some insight into the frequency range within which AP and RIHP might be associated. The highest frequency (1 Hz) was equal to the sampling frequency and also approximated the respiration rate of the animals. The data collected at 1 Hz were further filtered at 0·1, 0·02 and 0·01 Hz, using a non-weighted moving-average filter of the appropriate value. For example, each original 1 Hz signal (AP or RIHP) was filtered to 0·1 Hz by sequentially averaging ten consecutive values of the 1 Hz signal.

The relationship between the spontaneous AP and RIHP was evaluated using correlation analysis (Hinkle, Wiersma & Jurs, 1988). One-way analysis of variance (ANOVA) was used to determine differences in the mean correlation coefficients at

Table 2. AP and RIHP in conscious resting rats

Animal No.	n	Mean values AP (mmHg)	RIHP (mmHg)
1	6	103·7 ± 6·2	10·2 ± 1·7
2	7	132·7 ± 7·9	6·4 ± 2·6
3	9	124·4 ± 16·9	9·5 ± 2·7
4	5	114·0 ± 3·9	22·3 ± 2·8
5	5	113·7 ± 2·7	18·0 ± 3·4
6	10	116·9 ± 4·0	3·5 ± 3·0
7	4	118·1 ± 6·9	13·0 ± 0·3
8	4	115·7 ± 6·4	9·7 ± 1·4
9	2	122·5 ± 3·6	14·7 ± 2·6
10	4	104·5 ± 1·4	8·2 ± 0·5
Mean ± S.D.		116·5 ± 8·8	11·5 ± 5·5

Values are means ± S.D.; n, no. of hourly observations.

Table 3. Mean slopes (mmHg RIHP/mmHg AP) at four different levels of low-pass filtering

		Mean slopes (mmHg RIHP/mmHg AP)			
Animal No.	*n*	1 Hz	0·1 Hz	0·02 Hz	0·01 Hz
1	6	0·03 ± 0·21	0·04 ± 0·32	0·04 ± 0·39	0·04 ± 0·42
2	7	−0·03 ± 0·10	−0·08 ± 0·19	−0·11 ± 0·26	−0·12 ± 0·28
3	9	0·11 ± 0·11	0·15 ± 0·17	0·21 ± 0·19	0·27 ± 0·15
4	5	0·34 ± 0·16	0·47 ± 0·22	0·53 ± 0·24	0·55 ± 0·26
5	5	0·40 ± 0·19	0·66 ± 0·28	0·81 ± 0·35	0·85 ± 0·38
6	10	0·10 ± 0·04	0·14 ± 0·06	0·16 ± 0·07	0·16 ± 0·08
7	4	0·01 ± 0·02	0·01 ± 0·03	−0·00 ± 0·16	−0·01 ± 0·05
8	4	0·02 ± 0·01	0·03 ± 0·01	0·03 ± 0·01	0·03 ± 0·02
9	2	0·05 ± 0·02	0·07 ± 0·03	0·08 ± 0·04	0·09 ± 0·05
10	4	0·08 ± 0·11	0·11 ± 0·20	0·13 ± 0·26	0·13 ± 0·29
Mean slope ± S.D.		0·11 ± 0·14	0·16 ± 0·23	0·19 ± 0·28	0·20 ± 0·29

Values are means ± S.D.; *n*, no. of hourly observations. There were no significant differences in the mean slopes at the different levels of filtering.

the four levels of low-pass filtering. One-way ANOVA was also used to determine differences in the slopes obtained during the three different calibration conditions, as well as for determining the differences in the slopes (mmHg RIHP/mmHg AP) at the four levels of low-pass filtering. Significant differences ($P < 0·05$) within a given experimental condition were further evaluated using the Student–Neuman–Keuls *post hoc* test.

RESULTS

Data were collected for a total of about 56 h in ten conscious rats. The mean number of paired values for AP and RIHP evaluated from each experiment was 3500 ± 205. Table 2 shows the mean values for AP and the estimated values for RIHP. The mean AP was 116·5 ± 8·8 mmHg for all animals and ranged from 103·7 to 132·7 mmHg. The mean estimated RIHP was 11·5 ± 5·5 mmHg for all animals and ranged from 3·5 to 22·3 mmHg. In 49 of the 56 h of recording (88%) in the ten rats, there was a positive, significant correlation between AP and RIHP.

Typical results from the two manoeuvres used for evaluation of the RIHP measurements are shown in Figs 1 and 2. The intravenous injection of angiotensin I (1 μg kg^{-1}, I.V.) produced a rapid increase in AP to about 160 mmHg and a decrease in RIHP from about 18 to 10 mmHg (Fig. 1). This manoeuvre demonstrates the ability of the microtransducer to measure rapid (moment-to-moment) changes in RIHP. The rapid decline in RIHP

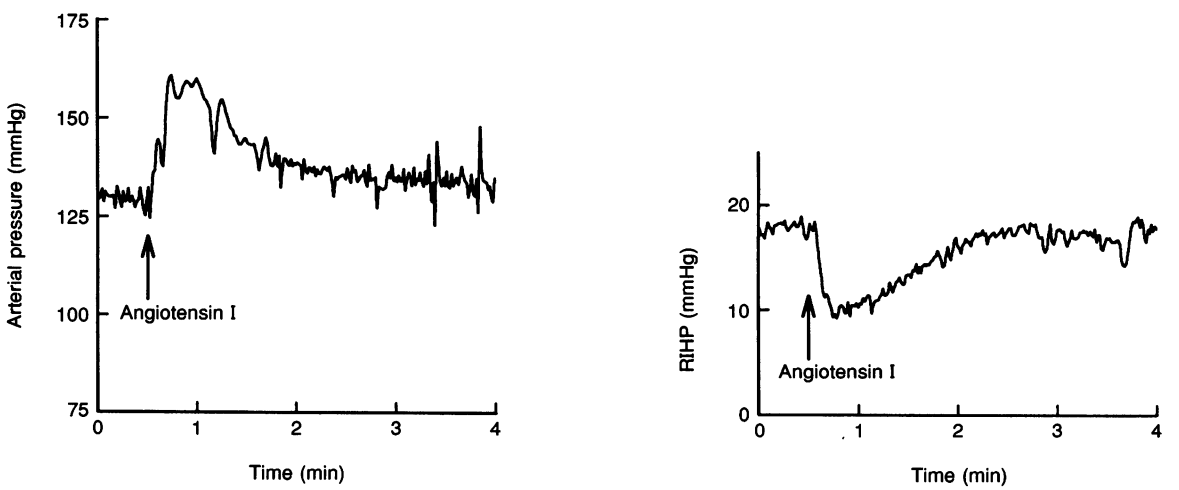

Figure 1. Typical responses of AP (left panel) and RIHP (right panel) to a bolus injection of angiotensin I (1 μg kg^{-1}, I.V.) in a conscious rat
The figure shows that renal haemodynamic changes can produce rapid changes in RIHP.

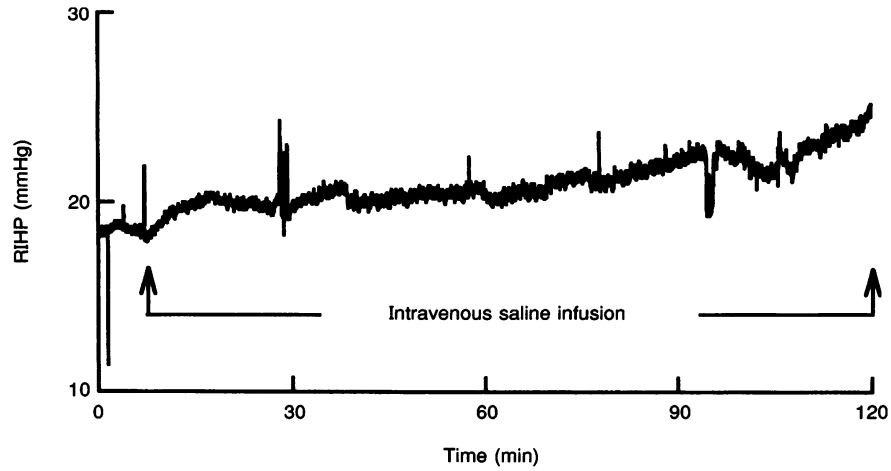

Figure 2. Response of the RIHP to 5% body wt volume expansion in the anaesthetized state
The 7 abrupt changes in RIHP were due to injection of anaesthetic agent or a paroxysmal change in the spontaneous ventilatory excursions.

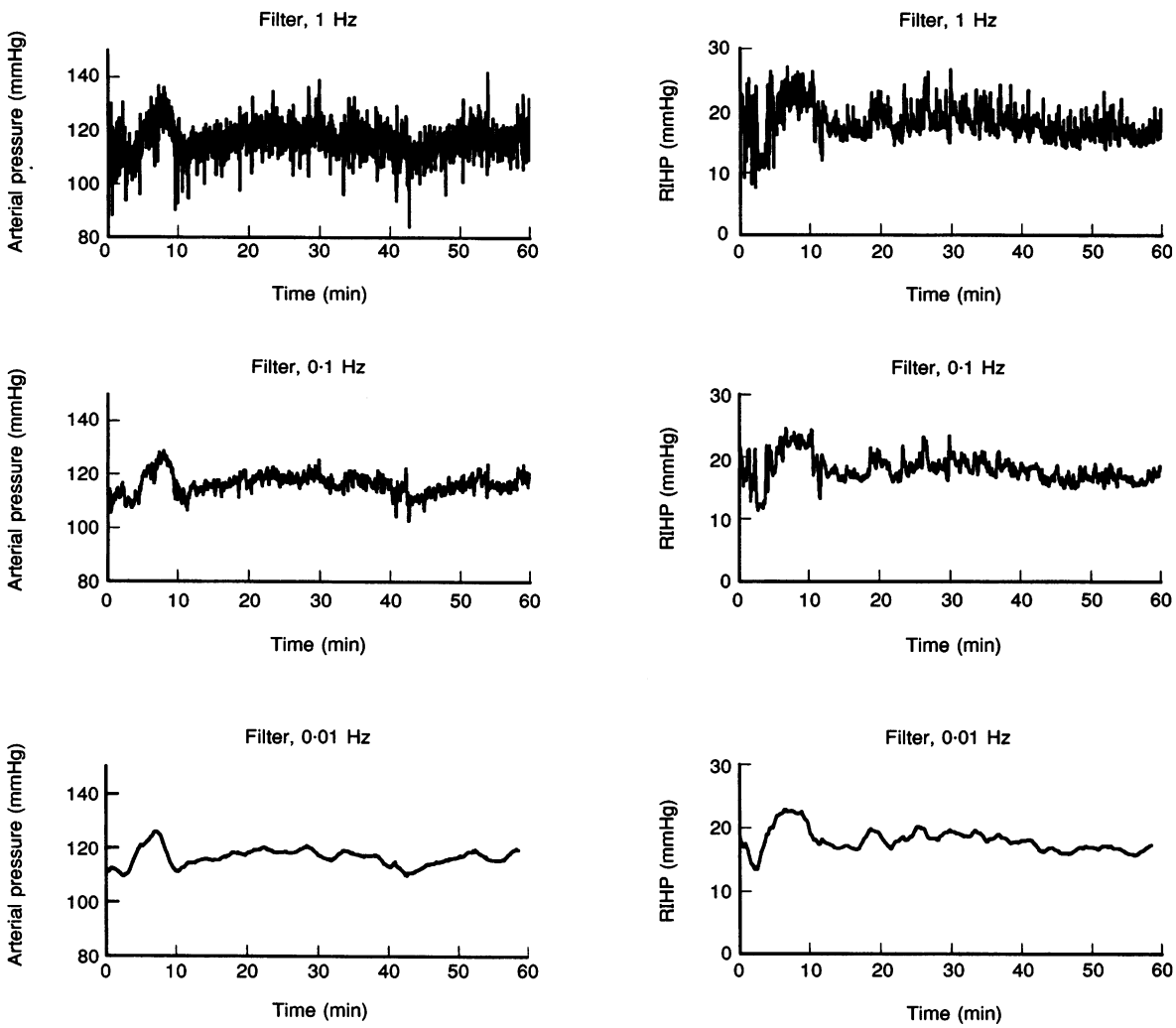

Figure 3. Typical spontaneous AP and RIHP recordings from a conscious rat
The responses are from rat No. 4 at 3 different levels of low-pass filtering; 1 (upper panel), 0·1 (middle panel) and 0·01 Hz (bottom panel).

Table 4. Correlation coefficient for the relationship between AP and RIHP at four different levels of low-pass filtering

		Mean correlation coefficient (r)			
Animal No.	n	1 Hz	0·1 Hz	0·02 Hz	0·01 Hz
1	6	0·37 ± 0·13	0·44 ± 0·14	0·49 ± 0·13	0·53 ± 0·12
2	7	0·24 ± 0·07	0·39 ± 0·04	0·51 ± 0·07	0·54 ± 0·08
3	9	0·22 ± 0·10	0·26 ± 0·10	0·35 ± 0·10	0·39 ± 0·11
4	5	0·47 ± 0·10	0·56 ± 0·11	0·62 ± 0·11	0·64 ± 0·12
5	5	0·42 ± 0·07	0·52 ± 0·10	0·58 ± 0·12	0·60 ± 0·12
6	10	0·31 ± 0·11	0·41 ± 0·13	0·46 ± 0·16	0·48 ± 0·16
7	4	0·22 ± 0·16	0·27 ± 0·17	0·33 ± 0·16	0·37 ± 0·13
8	4	0·22 ± 0·06	0·25 ± 0·08	0·23 ± 0·08	0·21 ± 0·09
9	2	0·35 ± 0·07	0·41 ± 0·10	0·49 ± 0·17	0·52 ± 0·21
10	4	0·34 ± 0·12	0·43 ± 0·15	0·49 ± 0·17	0·52 ± 0·17
Mean r ± S.D.		0·32 ± 0·09	0·40 ± 0·11	0·46 ± 0·12*	0·48 ± 0·13*

Values are means ± S.D.; n, no. of hourly observations. * $P < 0·05$ *vs.* 1 Hz.

is presumably the result of renal vasoconstriction that resulted in a decline in glomerular capillary pressure. Other investigators have also observed opposite changes in AP and RIHP in rats in response to exogenous agents that can produce constriction of the afferent renal arterioles (Miles, Ventom & de Wardener, 1954). The data presented in Fig. 2 show the influence of an intravenous infusion of saline (5% body wt) given evenly over 120 min in an anaesthetized rat. The saline infusion increased the RIHP from a starting value of about 18 mmHg to a final value of 25 mmHg. Other studies have found similar changes in RIHP in response to volume expansion (Burnett & Knox, 1980; Garcia-Estan & Roman, 1989).

Figure 3 shows 60 min of representative AP and RIHP data (animal No. 4) at three different levels of filtering (1, 0·1 and 0·01 Hz). This animal consistently demonstrated a positive relationship between AP and RIHP in five different 1 h recording sessions (Tables 2 and 3). Figure 4 shows these same data in the form of x–y scatter plots at the three different levels of filtering.

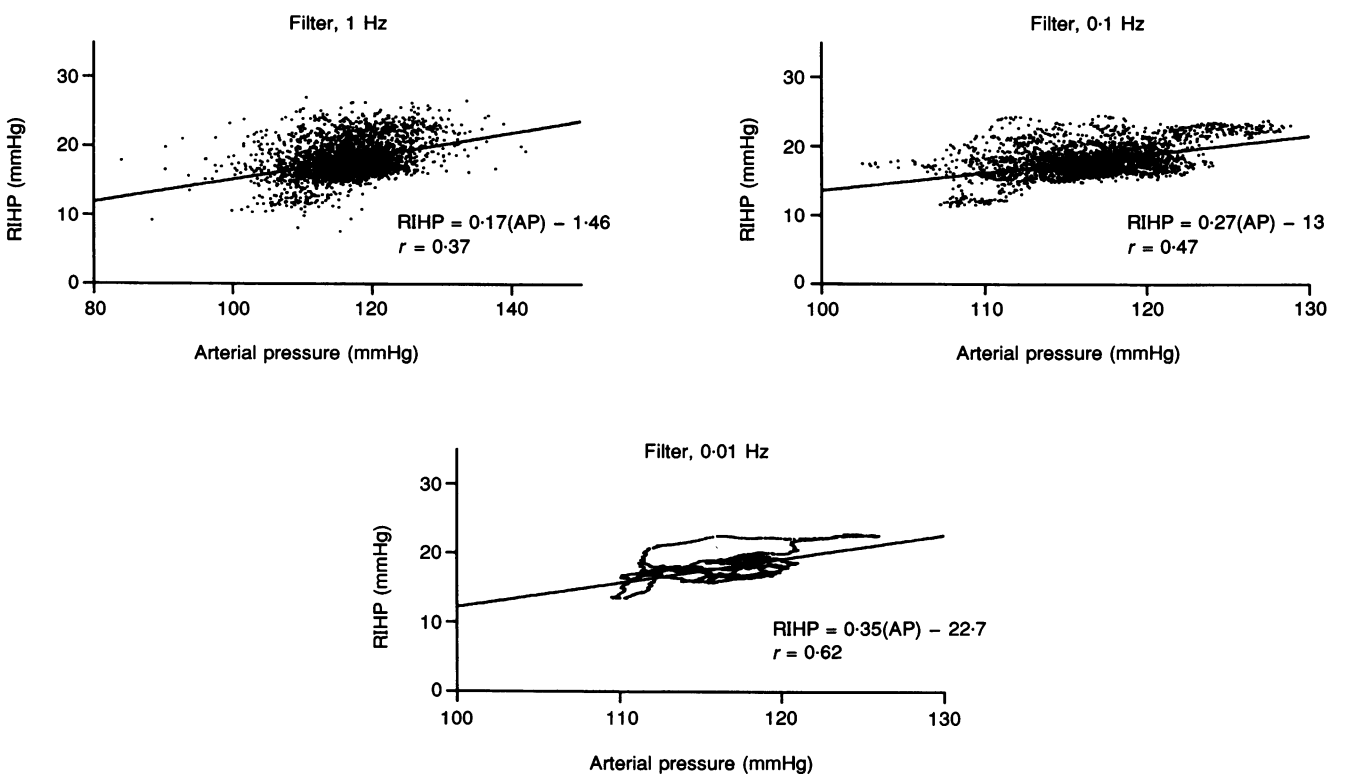

Figure 4. x–y scatter plots of AP *versus* RIHP at three different levels of low-pass filtering
The filtering was at 1 (upper left panel), 0·1 (upper right panel) and 0·01 Hz (lower panel). Data are the same as shown in Fig. 3 (from rat No. 4).

Table 3 displays the mean slope (mmHg RIHP/mmHg AP) for each of the ten animals studied as calculated at four different levels of low-pass filtering (1, 0·1, 0·02 and 0·01 Hz). Thirty-four of the forty slopes displayed in Table 3 were positive and six were negative. Removal of high-frequency components by digitally filtering to 0·01 Hz increased the mean slope from 0·14 to 0·20 mmHg RIHP/1 mmHg AP. While the slopes at each level of filtering were significantly different from zero, one-way ANOVA revealed no significant differences in slope between the four different levels of filtering ($P < 0·05$). The lack of a significant effect of filtering on the slope is probably related to the occasional negative slopes (7 of the total 56 h of recording had a negative slope) which appear to be a small but consistent component of the variability in the relationship between AP and RIHP.

Table 4 shows the mean correlation coefficients obtained from the same ten rats for AP *versus* RIHP at four levels of digital filtering. At the 1 Hz level, the correlation coefficients ranged from 0·22 to 0·47 with a mean of 0·32. In nine of the ten rats (i.e. all rats except No. 8), each sequential level of low-pass filtering ($1 \rightarrow 0·1 \rightarrow 0·02 \rightarrow 0·01$ Hz) increased the correlation coefficients. Significantly higher ($P < 0·05$) correlations were observed only at the lower frequencies of 0·02 and 0·01 Hz. At 0·01 Hz the correlation coefficients ranged from 0·2 to 0·64 (mean r of 0·48).

DISCUSSION

This study is the first to examine the relationship between AP and RIHP during spontaneous changes in AP in intact, conscious animals. Our results demonstrate that there is a positive correlation between spontaneous changes in AP and RIHP with a mean correlation coefficient of 0·48. We also observed that changes in renal haemodynamics produced by vasoconstrictors may be rapidly communicated to the renal interstitium in the resting conscious state (Fig. 1).

Previous experiments using anaesthetized animals have demonstrated a positive correlation between induced, steady-state changes in AP and RIHP (Ott *et al.* 1971). Nonetheless, it is necessary to evaluate the dynamic relationship between AP and RIHP in conscious animals because it is widely believed that changes in AP must induce changes in RIHP in order for pressure diuresis to occur (Burnett & Knox, 1980; Granger *et al.* 1988; Garcia-Estan & Roman, 1989; Khraibi *et al.* 1989; Khraibi & Knox, 1989*a*; Granger, 1992), and because our laboratory has recently observed that the delay time between changes in AP and UF averages 6 s (Steele *et al.* 1993). Given the short delay time between changes in AP and UF, if changes in RIHP are an intermediate step in the pressure diuresis mechanism, then there must be an ongoing, moment-to-moment correlation between spontaneous changes in AP and RIHP.

Furthermore, although the value of the correlation coefficient between AP and RIHP that we observed in conscious animals ($r = 0·48$, $P < 0·05$ after applying a low-pass filter at 0·01 Hz) is similar to the value obtained with induced changes in AP in anaesthetized animals (Ott *et al.* 1971), one could not predict with confidence, from responses to induced pressure changes, what the effect of AP will be on RIHP in the intact, conscious state. Major differences would be expected in the response of the kidney in the anaesthetized animal to induced changes in AP, compared to the effects of spontaneous changes in the conscious state.

For example, the nature of the predominant renal pressure–flow relationship probably differs in conscious *versus* anaesthetized animals, and these differences are likely to influence the relationship between AP and RIHP. In conscious dogs and rats during spontaneous changes in AP, the predominant renal pressure–flow relationship is baroreflex-like and dependent on intact autonomic activity (Skarlatos *et al.* 1993*a*, *b*). That is, when AP increases, renal blood flow increases proportionately more than AP, and vice versa. These results suggest that the renal circulation, in the conscious, undisturbed animal, participates in baroreflex regulation of systemic AP. However, in previous studies of the relationship between AP and RIHP, AP has been fixed at different steady-state levels, and the effect on RIHP measured (Ott *et al.* 1971). With a mechanically fixed renal perfusion pressure, autoregulation predominates (Ott *et al.* 1971). The expected effects on renal vascular resistance of a baroreflex response and autoregulation are opposite in direction. For example, if the baroreflex predominates, an increase in AP will result in renal vasodilatation, probably resulting in augmented transmission of AP to the renal interstitium. In contrast, the autoregulatory response to an increase in AP will be vasoconstriction, which will dampen the transmission of AP to the interstitium. Thus the different pressure–flow relationships occurring spontaneously in conscious animals compared to responses to induced changes in AP in anaesthetized animals may exert opposite effects on the transmission of AP to the renal interstitium.

The effect on the AP–RIHP relationship of anaesthesia and surgery in acute experiments must also be considered. Anaesthesia and surgery decrease renal blood flow and glomerular filtration rate (Grady & Bullivant, 1992; Khraibi, 1992) and elevate AP and renal vascular resistance (Walker *et al.* 1983). The increase in renal vascular resistance is likely to limit the extent to which changes in AP are transmitted to the renal interstitium in anaesthetized animals. In contrast, in the conscious animal, normal activity influences neural control of renal blood flow (Grady & Bullivant, 1992) and the interaction of the pressure diuresis mechanism with the autonomic nervous system (Brand, Coyne, Kostrzewski, Shier, Metting & Britton, 1991). Additionally, in conscious

animals, AP fluctuates considerably even in the resting state (Trapani, Barron & Brody, 1986; Alper, Jacob & Brody, 1987; DeBoer, Karemaker & Strackee, 1987). These spontaneous changes in AP might produce corresponding changes in RIHP, and thus salt and water excretion, to a greater extent than would occur in the anaesthetized animal with increased renal vascular resistance.

For these reasons it was important to examine the relationship between AP and RIHP during spontaneous changes in AP in conscious animals, even though this relationship had previously been examined under steady-state conditions in anaesthetized animals. Despite the differences in renal haemodynamics and autonomic activity between conscious and anaesthetized animals, it is interesting to note the similarity in correlation coefficient of the AP–RIHP relationship measured by us compared to previous observations in anaesthetized animals (Granger *et al.* 1988; Garcia-Estan & Roman, 1989; Khraibi & Knox, 1989*a*; Granger, 1992). Similarly, the slope of the AP–RIHP relationship associated with spontaneous changes in AP is in close agreement with previous reports on the magnitude of changes in RIHP following experimentally induced changes in renal perfusion pressure. We observed a slope between 0·11 and 0·20 mmHg RIHP/1 mmHg AP (Table 3), compared to a value of about 0·14 reported by Khraibi & Knox (1989*b*). One may speculate that these similarities in correlation and slope indicate that the AP–RIHP relationship is an expression of a fundamental mechanism that is minimally affected by differences in renal haemodynamics and autonomic activity between the conscious, spontaneous condition and the anaesthetized state. On the other hand, the similarities may be coincidental and of little significance. Our data do not allow us to distinguish between these two possibilities.

Our observations on the spontaneous relationship between AP and RIHP may also be relevant to the mechanism underlying the long-term character of pressure diuresis. Pressure diuresis is usually thought of as a long-term regulatory mechanism that acts via changes in RIHP to set the level of arterial blood pressure (Guyton *et al.* 1972*a*; Guyton *et al.* 1972*b*; Garcia-Estan & Roman, 1989; Khraibi & Knox, 1989*a*; Guyton, 1990; Guyton *et al.* 1990). Although Guyton (1990) postulated in 1966 that pressure diuresis is a long-term mechanism, there is as yet no explicit definition in the literature of the nature of a long-term mechanism, nor has there been any experimental investigation of the mechanism responsible for the long-term aspect of pressure diuresis. Our group has recently proposed that the long-term aspect of the pressure diuresis mechanism may be a consequence of the cumulative effect of moment-to-moment changes in AP on UF (Steele *et al.* 1993). This hypothesis was based on our observation that experimentally induced changes in AP result in changes in UF within 6 s (Steele *et al.* 1993). If this hypothesis is correct, and changes in RIHP are an intermediate step in the pressure diuresis mechanism, then it follows that spontaneous changes in AP and RIHP will be correlated. Our observation that there is a significant positive correlation between spontaneous changes in AP and RIHP supports the hypothesis that the long-term nature of the pressure diuresis mechanism may be a consequence of the cumulative effect of moment-to-moment changes in AP on RIHP and thus UF.

The hypothesis that changes in RIHP can occur rapidly in response to changes in renal haemodynamics is supported by the rapid response of RIHP to exogenously administered angiotensin I (Fig. 1). The decline in RIHP produced by angiotensin I indicates a predominant renal afferent arteriolar vasoconstriction that caused a decline in glomerular capillary pressure, and thus RIHP, despite the increase in arterial pressure. This indicates that only changes in pressure that become transmitted to the glomerulus can influence RIHP. Indeed, others have also observed that mechanically induced changes in AP produce directionally similar changes in RIHP within a few seconds (Miles & de Wardener, 1954; Roman *et al.* 1988; Garcia-Estan & Roman, 1989). Our present finding that 88% (49 of 56) of the 1 h recordings demonstrated a significant positive relationship between AP and RIHP is consonant with the view that renal haemodynamics are not consistently protected from normal, spontaneous changes in AP. Our previous observations that the predominant, spontaneous pressure–flow relationship in the renal circulation of conscious rats follows a baroreflex-like pattern is also consistent with changes in AP being transmitted to the renal interstitial fluid (Skarlatos *et al.* 1993*a*).

Combining these observations, one may speculate that the spontaneous changes in AP that occur at a frequency of about six per minute (DeBoer *et al.* 1987) in the conscious state could rapidly induce parallel changes in RIHP and UF. A frequency of six per minute is equivalent to 0·10 Hz, a value that is well within the frequency range examined in this study. The magnitude of these changes in AP and UF taken as individual events may be small, but their cumulative effect would act to modulate blood volume and thus regulate AP over the long term.

The hypothesis that moment-to-moment changes in AP, RIHP and UF may participate in the long-term regulation of pressure diuresis is also supported by previous observations that changes in RIHP as small as 1 mmHg have been shown to approximately double the UF (Selen & Persson, 1983). Numerous other reports confirm that relatively small changes in RIHP are associated with significantly increased diuresis (Khraibi & Knox, 1989*b*; Khraibi, 1991). Following an artificially induced 20% increase in renal perfusion pressure, significant increases in sodium excretion were observed, with a concurrent 67% increase in RIHP (Khraibi & Knox, 1989*b*). These data suggest that small changes in RIHP that might occur following spontaneous changes in AP can be effective in changing UF and Na^+ excretion.

In summary, the present study demonstrates a close association between the spontaneously occurring changes in AP and RIHP in resting, conscious rats. These results support our hypothesis (Steele *et al.* 1993) that pressure diuresis acts dynamically, such that spontaneous changes in AP are rapidly transmitted to the renal interstitium, inducing moment-to-moment changes in RIHP and thus in UF. The cumulative effect of these moment-to-moment changes in UF may account for the long-term character of pressure diuresis.

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