Renal afferents responsive to chemical and mechanical pelvic stimuli in the rabbit

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1. Afferent nerve fibres sensitive to changes in the renal chemical environment have been found in the rat. To verify the existence of these fibres in the rabbit and their response pattern, afferent renal nerve activity was recorded during pelvic perfusions with NaCl solutions at different concentrations.

2. The experiments were carried out in 13 anaesthetized rabbits. Arterial pressure from a femoral catheter and afferent renal nerve activity from the distal stump of a cut renal nerve bundle were recorded. Three catheters were inserted into the renal pelvis to measure pelvic pressure, to allow pelvic perfusions at constant rates and to drain pelvic fluids.

3. After a control period, the pelvis was perfused with physiological saline (0.14 mol/l for 2 min), followed by one of a series of solutions containing increasing concentrations of NaCl (0.5, 0.75, 1.0 and 1.5 mol/l for 2 min). Pelvic perfusion was performed both at a low (0.2 ml/min) and a high (0.8 ml/min) flow rate for each solution tested.

4. In all animals arterial pressure was not modified during pelvic perfusions. Physiological saline did not change afferent renal nerve activity at the low perfusion rate, but it significantly increased afferent renal nerve activity and pelvic pressure at the high rate. Hypertonic NaCl solutions caused progressive increases in afferent renal nerve activity at both perfusion rates, and these effects were larger at the high perfusion rate.

5. These data demonstrate, in the rabbit, the existence of renal chemosensitive fibres in any species other than the rat, in which the backflow into the renal pelvis of non-diuretic urine or hypertonic NaCl solutions has been shown to increase afferent renal nerve activity [11]. Katholi et al. [17] suggested that the renal nerves of the dog might contain chemosensitive fibres, because afferent renal nerve activity was enhanced by infusing adenosine into the renal artery or the renal pelvis. However, adenosine can activate many types of fibres and cannot be considered a specific activator of chemosensitive fibres [18].

The demonstration of renal chemoreceptors in other animal species would further support their physiological role. It is also important to define the operating system of these sensory receptors by showing that discrete levels of chemical stimulation would induce different and proportional levels of arterial pressure [1–8], renal venous pressure [1–4, 7, 9] or ureteropelvic pressure [4, 7, 9]; and chemoreceptors sensitive to renal ischaemia [10] or to the composition of pelvic fluid [11].

The existence of mechano-receptors has been shown in dogs [4, 9], cats [2], rats [1, 4], rabbits [5, 6] and monkeys [3], but their physiological role has not been completely defined [12, 13]. Nijima [6] studied the mechano-receptors in the rabbit and suggested that they may play a role resembling that of sinoaortic arterial baroreceptors.

In the rat, Kopp et al. [4] observed that afferent renal nerve activity can also be increased by elevating renal pelvic pressure, within the physiological range. As the increased afferent renal nerve activity was associated with contralateral diuresis and natriuresis, a role of renal mecano-receptors in the control of body water and sodium balance has been suggested [4, 14, 15]. In previous experiments, performed in anaesthetised cats [16], we have demonstrated that diuretic manoeuvres activate renal mecano-sensitive fibres by increasing pelvic pressure, thus suggesting a role of renal mechano-receptors in monitoring urine volume in the cat [16].

At present, there is no conclusive evidence of renal chemosensitive fibres in any species other than the rat, in which the backflow into the renal pelvis of non-diuretic urine or hypertonic NaCl solutions has been shown to increase afferent renal nerve activity [11]. Katholi et al. [17] suggested that the renal nerves of the dog might contain chemosensitive fibres, because afferent renal nerve activity was enhanced by infusing adenosine into the renal artery or the renal pelvis. However, adenosine can activate many types of fibres and cannot be considered a specific activator of chemosensitive fibres [18].

The demonstration of renal chemoreceptors in other animal species would further support their physiological role. It is also important to define the operating system of these sensory receptors by showing that discrete levels of chemical stimulation would induce different and proportional levels of
activation of the renal afferent chemosensitive fibres. Moreover, it should be established whether or not interactions between renal chemoreceptors and renal mechanoreceptors do exist and are capable of modifying the operating system of renal sensory receptors.

Thus, this study was performed: (i) to verify the presence of chemosensitive fibres in the rabbit kidney; (ii) to examine the degree of responsiveness of these chemoreceptors to pelvic perfusion with solutions containing increasing concentrations of NaCl; and (iii) to investigate whether the afferent discharge from renal chemoreceptors can be influenced by the level of activation of renal mechanoreceptors.

**MATERIALS AND METHODS**

**Animal preparation**

Experiments were conducted in accordance with the National Institutes of Health Guide for the care and use of laboratory animals. Male New Zealand White rabbits (n = 13), weighing 2.4–3.4 kg, were used in this study. Animals were fasted overnight, but water was allowed *ad libitum*. Anaesthesia was induced with sodium pentobarbital (20–30 mg/kg, intravenous) and maintained with additional doses (5–10 mg intravenous) when necessary. Rabbits were placed on a temperature-regulated table, tracheotomized and allowed to breathe spontaneously. Polyethylene catheters (PE 90) were inserted into a femoral artery to measure arterial pressure (Statham P23De transducer, Statham Medical Institute, Los Angeles, CA, U.S.A.) and into a femoral vein (PE 50) were inserted together into the renal pelvis through the left ureter. The first catheter was used to measure urine flow rate (photoelectric drop counter, Keyence PG 602; Keyence Lead Corporation, Osaka, Japan) and to drain the perfusate. The second catheter was connected to a pressure transducer (Statham P23De) to measure the renal pelvic pressure. The third catheter was connected to an infusion pump to perform renal pelvic perfusions at a constant rate. Through a midline laparotomy the left ureter and pelvis were exposed. Three polyethylene catheters were inserted together into the renal pelvis for fluid and drug administration. Physiological saline was intravenously infused at constant rate (12 ml/h) to compensate for fluid losses (Sage syringe pump, model 341A, Cambridge, MA, U.S.A.).

The nerves of the left kidney were identified and cut at a distance from the kidney hilus. After dissection with fine needles to obtain a small bundle with few nerve fibres, the peripheral stump was placed on a bipolar hook electrode and plunged in mineral oil at 37°C. Afferent renal nerve activity was recorded using standard electrophysiological techniques. In brief, the signals were led by a high-impedance probe (Grass HIP511, Grass Instr., Quincy, MA, U.S.A.) to a band-pass amplifier (Grass P511) with a high-frequency cut-off at 3000 Hz and a low-frequency cut-off at 10 Hz. The signals were amplified 5000 times. All nerve bundles studied showed spontaneous and non-synchronized pulse discharge. The background electrical noise was assessed at the end of the experiment by crushing the nerve bundle distally to the recording electrode. Neural activity was displayed on an oscilloscope (Tektronix, Beaverton, OR, U.S.A.), while arterial pressure, urine flow rate and pelvic pressure were continuously recorded on a polygraph (Grass 7C8).

During the experimental trials, all variables were simultaneously recorded on a tape recorder (Racal V-store, Southampton, U.K.). Stored neural activity was analysed by a digital neural-spike analyser (Ing. Malagodi, Milan, Italy) after the threshold level had been set just above the noise level. The impulses counted by the spike analyser over 5 s were digitally transferred, via a serial line, to a computer (M24 SP, Olivetti S.p.A., Ivrea, Italy) to calculate the mean value of renal nerve activity over 20 s periods.

**Experimental protocol**

After surgery a recovery period of 30 min was allowed. Figure 1 shows the experimental design. After a 2 min control period (control), the renal pelvis was perfused at a constant rate of either 0.2 or 0.8 ml/min for 5 min. During the first 2 min, the pelvis was perfused with physiological saline (saline) in order to verify the degree and reproducibility of mechanoreceptor activation, and then with one of the different hypertonic NaCl solutions for 2 min (test), followed by 1 min perfusion with physiological saline to completely wash-out the pelvic fluids. After stopping the perfusion, a 2 min recovery period was recorded (recovery).

Each hypertonic solution employed (0.5, 0.75, 1.0, and 1.5 mol/l) was separately tested by repeating the whole sequence shown in Fig. 1, when all parameters were returned to control values. The order of the hypertonic solutions and pelvic perfusion rates was randomly assigned. In all animals, to verify the constancy of mechanoreceptor activation, the same sequence was performed with perfusion with physiological saline during the test period.

Data presented in this study refer to the second
minute of each period when stable conditions were reached.

Statistical analysis
All results are expressed as means ± SEM. Absolute values of arterial pressure, pelvic pressure and afferent renal nerve activity, measured during each experimental episode, were treated with one-way analysis of variance for repeated measures, followed by the Bonferroni corrected paired t-test for simultaneous multiple comparisons within treatments. Comparisons of absolute changes in afferent renal nerve activity between the low (0.2 ml/min) and high flow rates (0.8 ml/min) were done on the data obtained in the same animal, and were analysed with the paired t-test. Differences are reported as statistically significant when P values are <0.05.

RESULTS
Pelvic perfusion with physiological saline at low and high flow rates
The effects on pelvic pressure and afferent renal nerve activity caused by perfusing the pelvis with physiological saline at low and high flow rates are shown in Table 1.

At a low flow rate, in spite of a small and progressive increase in pelvic pressure, afferent renal nerve activity did not significantly change during the whole period of perfusion, indicating that these increases in pelvic pressure were sub-threshold for mechano-receptor activation, and that physiological saline did not stimulate chemosensitive fibres.

At the high flow rate, a consistent increase in pelvic pressure was observed. Afferent renal nerve activity significantly increased and then persisted unmodified throughout the whole period of pelvic perfusion, in spite of the further small increase in pelvic pressure. During the recovery, all variables returned to control values.

Arterial pressure did not change during pelvic perfusion both at low and high pelvic-perfusion rates with physiological saline.

Pelvic perfusion with hypertonic NaCl solutions at low pelvic perfusion rate
The effects on pelvic pressure and afferent renal nerve activity caused by perfusing the pelvis at a low flow rate with different test solutions containing increasing concentrations of NaCl are shown in Table 2.

During the perfusion, pelvic pressure increased moderately. In all trials, the increases in pelvic pressure were equal to those observed using physiological saline at a low perfusion rate (Table 1). Confirming that these small changes are sub-threshold, afferent renal nerve activity did not change when the pelvis was perfused with physiological saline for each group of trials (Table 2, saline).

Later on, afferent renal nerve activity significantly increased when the pelvis was perfused with hypertonic NaCl solutions (Table 2, test). The increases in afferent renal nerve discharge were progressively larger when solutions containing increasingly higher concentrations of NaCl were employed, thus indicating a selective renal chemoreceptor activation. The maximal effect was observed with 1.5 mol/l NaCl solution (Table 2). Perfusion of the pelvis with 2.0 mol/l NaCl (n = 5) caused an increase in the afferent neural discharge (+390% compared with saline) similar to that caused by 1.5 mol/l NaCl (+401%).

During the recovery, afferent renal nerve activity returned to control values except after pelvic perfusion with 1.5 mol/l NaCl solution, which required a longer recovery time (5–10 min).

Arterial pressure did not change during pelvic perfusion at a low flow rate with different hypertonic NaCl solutions.

Pelvic perfusion with hypertonic NaCl solutions at high pelvic perfusion rate
The effects on pelvic pressure and afferent renal nerve activity caused by perfusing the pelvis at a

Table 1. Afferent renal nerve activity (ARNA) responses to low (0.2 ml/min) and high (0.8 ml/min) pelvic perfusion rates with physiological saline. Values are means ± SEM during the second minute of each period. *P < 0.05 compared with the control period; †P < 0.05 between the two subsequent saline periods.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Saline</th>
<th>Saline</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic perfusion rate 0.2 ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvic pressure (mmHg)</td>
<td>6.0 ± 1.0</td>
<td>8.1 ± 0.9*</td>
<td>8.5 ± 0.9**</td>
<td>6.5 ± 0.9</td>
</tr>
<tr>
<td>ARNA (impulses/s)</td>
<td>4.7 ± 1.4</td>
<td>5.2 ± 1.4</td>
<td>5.2 ± 1.4</td>
<td>3.9 ± 1.1</td>
</tr>
<tr>
<td>Pelvic perfusion rate 0.8 ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pelvic pressure (mmHg)</td>
<td>6.3 ± 1.1</td>
<td>15.7 ± 1.4*</td>
<td>17.4 ± 1.3**</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>ARNA (impulses/s)</td>
<td>6.0 ± 1.9</td>
<td>11.5 ± 3.6*</td>
<td>11.3 ± 3.6*</td>
<td>5.6 ± 1.9</td>
</tr>
</tbody>
</table>
Table 2. Afferent renal nerve activity (ARNA) responses to low (0.2 ml/min) pelvic perfusion rate with solutions at different NaCl concentrations. Values are means ± SEM during the second minute of each period. The concentration of NaCl is given for each group of trials; n, number of trials. *P < 0.05 compared with control period; †P < 0.05 compared with the preceding pelvic perfusion period with physiological saline.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Saline</th>
<th>Test</th>
<th>Recovery</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mol/l NaCl</td>
<td>6.4 ± 1.0</td>
<td>8.2 ± 0.9*</td>
<td>8.4 ± 1.0†</td>
<td>6.2 ± 0.9</td>
<td>11</td>
</tr>
<tr>
<td>Pelvic pressure (mmHg)</td>
<td>4.1 ± 1.3</td>
<td>4.6 ± 1.4</td>
<td>6.0 ± 1.8†</td>
<td>3.6 ± 1.0</td>
<td>11</td>
</tr>
<tr>
<td>ARNA (impulses/s)</td>
<td>5.5 ± 0.9</td>
<td>7.0 ± 0.9*</td>
<td>8.0 ± 0.9†</td>
<td>5.9 ± 0.8</td>
<td>11</td>
</tr>
<tr>
<td>0.75 mol/l NaCl</td>
<td>3.4 ± 0.9</td>
<td>5.1 ± 1.0</td>
<td>6.4 ± 2.1†</td>
<td>3.0 ± 0.8</td>
<td>11</td>
</tr>
<tr>
<td>1.0 mol/l NaCl</td>
<td>5.4 ± 0.6</td>
<td>7.1 ± 0.8*</td>
<td>7.7 ± 0.9*†</td>
<td>5.7 ± 0.7</td>
<td>11</td>
</tr>
<tr>
<td>Pelvic pressure (mmHg)</td>
<td>2.4 ± 0.7</td>
<td>2.6 ± 0.7</td>
<td>10.3 ± 1.1†</td>
<td>2.7 ± 0.8</td>
<td>11</td>
</tr>
<tr>
<td>ARNA (impulses/s)</td>
<td>5.2 ± 0.7</td>
<td>5.4 ± 1.7</td>
<td>18.6 ± 2.1*†</td>
<td>12.1 ± 6.7</td>
<td>10</td>
</tr>
<tr>
<td>1.5 mol/l NaCl</td>
<td>6.6 ± 1.4</td>
<td>8.4 ± 1.4*</td>
<td>9.4 ± 1.3*†</td>
<td>7.0 ± 1.3</td>
<td>12</td>
</tr>
<tr>
<td>Pelvic pressure (mmHg)</td>
<td>4.8 ± 1.6</td>
<td>5.4 ± 1.7</td>
<td>18.6 ± 5.4*†</td>
<td>12.1 ± 6.7</td>
<td>10</td>
</tr>
<tr>
<td>ARNA (impulses/s)</td>
<td>5.4 ± 1.2</td>
<td>6.8 ± 1.3*</td>
<td>19.0 ± 1.3*†</td>
<td>7.6 ± 0.9</td>
<td>12</td>
</tr>
<tr>
<td>Pelvic pressure (mmHg)</td>
<td>4.8 ± 1.8</td>
<td>9.2 ± 2.5*</td>
<td>25.4 ± 6.5*†</td>
<td>9.2 ± 2.7*</td>
<td>12</td>
</tr>
<tr>
<td>ARNA (impulses/s)</td>
<td>6.5 ± 1.4</td>
<td>16.0 ± 1.3*</td>
<td>19.3 ± 1.3*†</td>
<td>7.9 ± 1.1</td>
<td>11</td>
</tr>
<tr>
<td>Pelvic pressure (mmHg)</td>
<td>6.7 ± 2.4</td>
<td>10.3 ± 3.1*</td>
<td>37.0 ± 9.3*†</td>
<td>11.1 ± 4.1*</td>
<td>11</td>
</tr>
</tbody>
</table>

During the perfusion, pelvic pressure markedly and progressively increased. In all trials, the increases in pelvic pressure were equal to those observed using physiological saline at a high perfusion rate (Table 1). Afferent renal nerve activity significantly increased in each group of trials during the first 2 min of pelvic perfusion with physiological saline (Table 3, saline).

Later, afferent renal nerve activity further increased when the pelvis was perfused with hypertonic NaCl solutions (Table 3, test). Under these conditions, the increases in afferent renal nerve discharge were progressively larger when increasingly more concentrated NaCl solutions were employed.
Again the maximal effect was observed with 1.5 mol/l NaCl (Table 3). No further increase in afferent neural discharge was observed when perfusing the pelvis with 2.0 mol/l NaCl (n = 5) as compared with the increase caused by 1.5 mol/l NaCl (+780% and +990% respectively).

During the recovery, afferent renal nerve activity returned to control values, except after perfusion with the more concentrated NaCl solutions (1.0 and 1.5 mol/l), which required a longer recovery time (5–10 min).

Arterial pressure did not change during pelvic perfusion at a high flow rate with hypertonic NaCl solutions.

Comparisons between the increments in afferent renal nerve activity during low and high pelvic-perfusion rate with physiological saline and hypertonic NaCl solutions

Increments in afferent renal nerve activity caused by physiological saline at the high perfusion rate were constant during the whole perfusion period (Table 1) and highly reproducible from episode to episode (Table 3, saline periods). Thus both at low and high perfusion rates, additional increases in afferent renal nerve activity observed during the different test periods are due to the chemical composition of the solutions. These additional increments (differences between the test and corresponding saline values) in afferent renal nerve activity caused by the hypertonic NaCl are shown in Fig. 2. At both high and low flow rates, afferent renal nerve activity increments were greater when more concentrated solutions were used. In addition, the changes in the neural response at the high flow rate were larger than those observed during the low flow rate, reaching statistical significance at 1.0 and 1.5 mol/l. Thus, the effects of hypertonic NaCl solutions on renal chemoreceptors were enhanced by the concomitant pelvic pressure increases.

DISCUSSION

The results of our experiments in anaesthetized rabbits, showing that afferent renal nerve fibres can be specifically activated by chemical stimuli, demonstrate the existence of renal chemoreceptors in an animal species other than the rat. Our results also indicate that the neural discharge of chemosensitive fibres is proportionally related to the degree of the chemical stimulus. Indeed, pelvic perfusions with solutions containing increasing concentrations of NaCl caused progressive increases in afferent nerve activity. Under our experimental conditions, the whole range of chemical activation of renal afferents was explored, since the neural response was saturated by the highest concentrations.

We are aware that 1.5 mol/l or more concentrated solutions are beyond the concentration capacity of the rabbit kidney. Nevertheless 0.5, 0.75 and 1.0 mol/l solutions (employed in our experiments) are within the physiological range of urinary osmolarity of the rabbit [19]. Furthermore, it is likely that the actual electrolyte concentrations achieved at the receptor level is lower than concentrations of pelvic solutions because, (i) a complete equilibrium between interstitial and pelvic fluid concentrations is unlikely to be reached within a few minutes, and (ii) the lower concentration of intratubular or interstitial fluids should have diluted the solutions employed to perfuse the pelvis. Therefore, our data, by showing discrete neural responses to discrete changes in chemical composition of the pelvic solutions, and by defining the range of the chemical activation, suggest a physiological role for renal chemoreceptors.

It is known that the anatomical structure of the pelvis of rodents allows a large area of contact between the pelvic urine and the inner and outer medulla [20]. Superfusion of the renal papilla with NaCl solutions enhances the osmolarity of the superperfused papilla and of urine [21]. Although our experiments do not clarify the site of renal chemoreceptors, it is likely that our manoeuvres have modified the chemical composition of the renal medulla and its osmolarity.

Pelvic pressure increments caused by physiological saline are currently used to activate renal mecano-receptors [1, 4, 16]. Our data also show the presence of fibres sensitive to mechanical stimuli. The changes in pelvic pressure we have induced by pelvic perfusion are likely in the physiological range. Similar changes in pelvic pressure were observed during changes in urine flow rate caused by diuretic manoeuvres [16, 22, 23], during spontaneous contractions of the pelvis [24], and at different degrees of fullness of the urinary bladder [25]. In the rabbit, renal receptors capable of responding to changes in renal perfusion pressure or renal vein pressure, have
mechanoreceptors are also sensitive to changes in pelvic pressure of 30 mmHg were enhanced by pelvic nerve activity caused by chemical stimuli at the high perfusion rate were larger than those obtained during the low perfusion rate. Therefore, our data indicate that the mechanical stimuli may influence the neural discharge elicited by chemical stimuli. As fluid leakage from the pelvis to the medulla has been described in rodent kidneys [20], it is likely that increased pelvic pressure can favour the contact between pelvic fluids and medullary segments. This influence was particularly evident when more concentrated NaCl solutions were employed (1.0 and 1.5 mol/L), whereas it was less clear when using NaCl solutions at lower concentrations (0.5 and 0.75 mol/L).

In the anaesthetized rat [14] it has recently been shown that neural responses to increasing renal pelvic pressure of 30 mmHg were enhanced by pelvic peristalsis with 0.9 mol/L NaCl solutions. These and our present findings suggest that mechanical and chemical stimuli can potentiate each other at high levels of pelvic pressure and high NaCl concentration in pelvic fluid.

Although the precise role of these sensing intrarenal mechanisms remains unknown, combined afferent signals from pelvic mechano- and chemo-receptors might monitor the amount of urinary electrolyte output under physiological conditions. Under extreme conditions (high pelvic pressure and high NaCl concentration), the reciprocal strengthening might represent aspecific sensory information such as pain. Alternatively it might convey information on abnormal natriuretic states, such as those observed in salt-wasting conditions or following ureteral obstructions [26].

In conclusion, our study demonstrates the existence of renal chemoreceptors in the rabbit that are able to monitor discrete changes in urinary ionic or osmotic composition, and shows that rabbit renal mechanoreceptors can be activated by pelvic pressure changes. In addition, our results indicate that interactions between mechanical and chemical stimuli can exist.

REFERENCES