Renal Receptors in the Rat Sensitive to Chemical Alterations of Their Environment

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SUMMARY **Two major groups of renal chemosensory neural elements have been identified in the rat: one specifically activated by renal ischemia, the previously described "R" chemoreceptors, and the other by backflow of nondiuretic urine into the renal pelvis. The latter group is the object of the present investigation. In anesthetized, male Sprague-Dawley rats, single-unit recordings were obtained by dissection of the centrally cut nerves of the right kidney. The responses of single units to backflow into the renal pelvis of nondiuretic urine, diuretic urine, and solutions containing urea, mannitol, or inorganic ions were compared. The excitatory effect of the backflow of nondiuretic urine was due to its chemical composition rather than to changes in pelvic pressure and pelvic distension. The same units were activated markedly by renal ischemia. The resting discharge rate of the units was very high in nondiuretic conditions, and it declined progressively when diuresis was induced by expansion of the extracellular fluid volume. It is concluded that this group of sensory elements responds to the chemical environment in the renal interstitium as modified by ions crossing the pelvic epithelium, by leakage of ions out of ischemic cells, and by alterations in the excretory function of the kidney and renal blood flow. This group of renal sensory nerve endings has been termed "R2" chemoceptive receptors, to distinguish them from the previously described group of renal "R" chemoreceptors.** ***Circ Res 46: 395-405, 1980***

LIKE OTHER visceral organs, the kidneys have a profuse sensory innervation. From recordings of impulses conducted along afferent fibers in the renal nerves, several functional classes of renal sensory receptors have been identified so far. In cats, there are receptors sensitive to alterations in ureteral pressure and to venous or arterial perfusion pressure (Beacham and Kunze, 1969; Åström and Crafoord, 1968); in rabbits, receptors affected by changes in ureteral and arterial perfusion pressure, but not changes in venous pressure (Niijima, 1971); in dogs, units responsive to changes in venous, ureteral, or arterial perfusion pressure (Uchida et al., 1971); in rats, receptors sensitive to increase in venous pressure (Åström and Crafoord, 1967). In the renal nerves of the rat at least two other populations exist. The fibers of one group are silent under control conditions and are activated only by renal ischemia; those of the other population exhibit a resting discharge and respond markedly to backflow of urine into the renal pelvis (Recordati et al., 1978). We previously have studied the functional characteristics of the first group of receptors which, because of their sensitivity to ischemia and unresponsiveness to mechanical stimuli, were termed renal, "R," chemoreceptors. The second group, on the other hand, was analyzed only superficially. They were considered to be a population of renal mechanoreceptors because previous investigators (Beacham and Kunze, 1969; Åström and Crafoord, 1968; Niijima, 1971) had thought that changes in pelvic pressure and pelvic distension were the activating stimuli.

The experiments described here were done to investigate the functional characteristics of the latter group. We found that the activation during backflow of urine or other solutions does not depend on mechanical stimuli but rather on the chemical composition of fluids to which they are exposed and that the level of resting discharge is dependent on the excretory function of the kidney and renal blood flow.

It appears now that the rat kidney contains at least two distinct populations of nerve endings whose activity is dependent on alterations in their chemical environment. For the sake of simplicity, these two groups have been provisionally termed renal R1 and R2 chemoceptive receptors, R1 being those described previously and R2 being the receptors described in the present paper.

Methods

Sixty male Sprague-Dawley rats (200–300 g; Charles Rivers Breeding Laboratories) were anesthetized by intraperitoneal injection of sodium pen-

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tobarbital (5 mg/100 g) and maintained at 37°C on a heated operating table. Polyethylene catheters were inserted into (1) the left external jugular vein, for continuous infusion of 0.85% NaCl solution at 20 μl/min, (2) the left carotid artery, to monitor arterial blood pressure, and (3) the right ureter, close to the renal pelvis. Arterial and ureteral pressures were measured with Statham P23Db strain gauges and recorded on a Hewlett-Packard 7414A polygraph recorder.

Each rat was placed on its left side; then the right kidney and its nerve supply were exposed retroperitoneally through a paravertebral incision. A short section of the right renal artery was carefully cleared of connective tissue to permit total occlusion with forceps. In 42 experiments, the right ureter was cannulated at the ureteropelvic junction with a polyethylene catheter (PE 50; Fig. 1A), which allowed urine to flow freely through branches 5 (5 cm long) and 4 (50 cm long), while pelvic pressure was continuously monitored through branch 1. Backflow of urine into the pelvis was accomplished by elevating branch 4 above the level of the kidney while branches 2 and 3 were closed off. For backflow of test solutions into the pelvis, branches 4 and 5 were clamped and the solution introduced through access 2, filling branch 3, whose free end then was elevated above the kidney level. Inevitably, a small amount of urine from branch 5 preceded the test solution into the pelvis. To avoid this, in 12 experiments the ureter was cannulated with a double lumen catheter (Fig. 1B), the first part of which (branch 4) consisted of PE 50 tubing through which the tapered end of a PE 10 cannula was passed into the renal pelvis (branch 2). While test solutions were entering through branch 2, branches 4 and 3 were left open; the resulting free flow of urine and test solutions minimized changes in pelvic pressure. Backflow of urine was produced as described above.

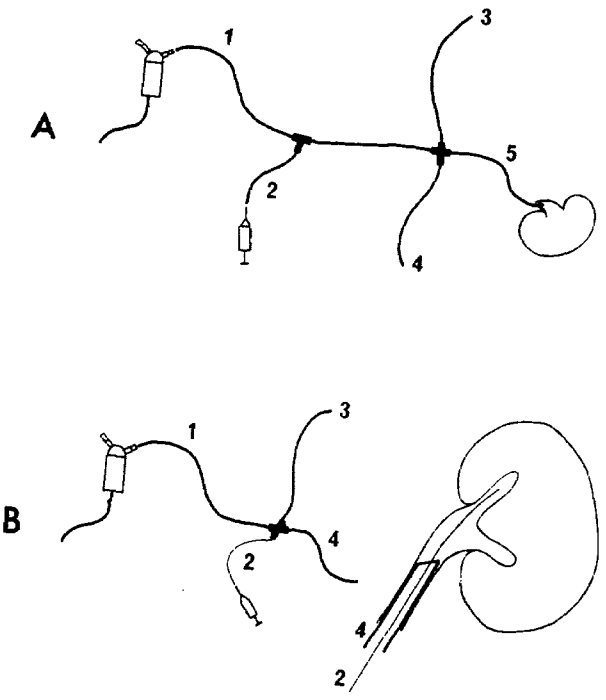

FIGURE 1 *Schematic drawings of the two catheters used to cannulate the ureter. Numbers indicate sections. Description in the text.*

A renal nerve dissected from the tissues along the renal artery was cut centrally and positioned on bipolar hook electrodes for extracellular recordings. The techniques of dissection of a nerve bundle into small filaments and for recording from single units have been described elsewhere (Bessou and Perl, 1969). Nerve activity, arterial blood pressure, and intrapelvic pressure were recorded for storage on magnetic tape (Ampex PR 2200). Nerve activity was analyzed subsequently with the aid of a computer program capable of discriminating between the individual nerve activity of up to four simultaneously recorded units (Capowski, 1976; Recordati et al., 1978). For quantitative descriptions, instantaneous frequency of discharge (1/DT = 1/interval between successive impulses, measured in impulses per second) was plotted, and histograms of the number of impulses occurring every 1 or 2 seconds were made. The analog recordings of the nerve activity were sometimes photographed directly from an oscilloscope screen (Tektronix Dll) with a Grass camera (oscilloscope camera C4).

In five experiments, diuresis was induced by expanding the extracellular fluid volume. To do so, an amount of isotonic saline equal to 5% of the body weight was infused, iv, over a 1-hour period, followed by a maintenance infusion rate of 100 μl/min. *Before and after expansion, three periods of 5 minutes* each were randomly selected, and the number of nerve impulses per 10 seconds was counted. The average of these three periods was used to express the mean discharge rate before and after expansion. Urine was analyzed as follows: (1) sodium and potassium concentration by flame spectrophotometry (Zeiss PMQII), (2) urea concentration by the method of Wybenga et al. (1971) as described by Di Giorgio (1974), (3) osmotic concentration by freezing point depression (Advanced Digimatic Mk. IV).

In 12 experiments, a sidebranch of the renal artery was cannulated with tapered PE 10 tubing. This enabled us to perfuse the kidney with isotonic saline during the afferent excitation caused by backflow of nondiuretic urine or renal artery occlusion (wash-out experiments).

Results

When a long ureteral catheter, positioned at the *ureteropelvic junction, is raised above the kidney* level, urine flows back into the renal pelvis and pelvic pressure increases. In nondiuretic rats this maneuver always elicited a prompt, marked increase in the activity of the afferent fibers in multifiber preparations. We noted, however, that when

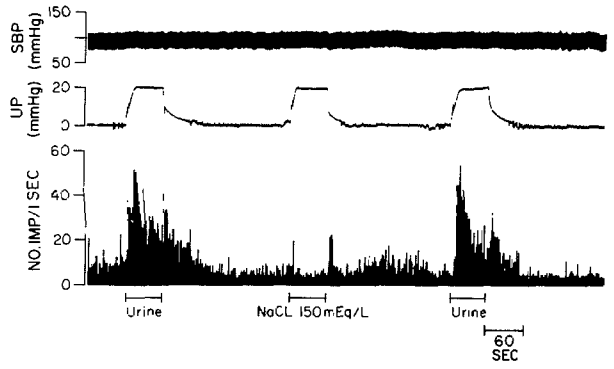

FIGURE 2 *Response of a multifiber preparation to backflow into the pelvis of nondiuretic urine and isotonic saline. Traces from top to bottom are: systemic blood pressure (SBP), ureteral pressure (UP), and number of impulses per second.*

isotonic saline, rather than urine, was used to raise pelvic pressure, the afferent discharge failed to increase. Figure 2 shows such an experiment, in which a branched catheter was used to raise and lower pelvic pressure three times, by backflow of nondiuretic urine, isotonic saline, then urine again. The urine produced marked and prolonged excitations, which slowly declined after the release of the stimulus. In contrast, only brief and small increments in activity appeared at the beginning and the end of the backflow of isotonic saline; the initial response was probably due to the residual urine inside the catheter (branch 5 in Fig. 1A). It appeared that the stimulus responsible for activating some afferent nerve fibers might be the composition of the fluid inside the pelvis, rather than the changes in pelvic pressure, which were similar in all cases. To test this hypothesis, we studied the responses of single-unit preparations during backflow into the renal pelvis of urine and solutions differing in ionic composition and total osmolality.

Effects of Sodium and Potassium Chloride, Urea, and Mannitol

Single units sensitive to the backflow of nondiuretic urine were isolated. They had a low resting discharge rate (average: 2.3 ± 0.5 impulses/second; 35 units), sputtering at times into brief trains of impulses. Backflow of urine markedly raised their instantaneous firing rate (average peak frequency 44.1 ± 6.9 impulses/sec; 22 units), as shown in Figure 3. Figure 4 illustrates a typical single-unit response to backflow of NaCl solutions and molar solutions of urea and mannitol. Nondiuretic urine elicited a high frequency discharge, whereas 1.0 M

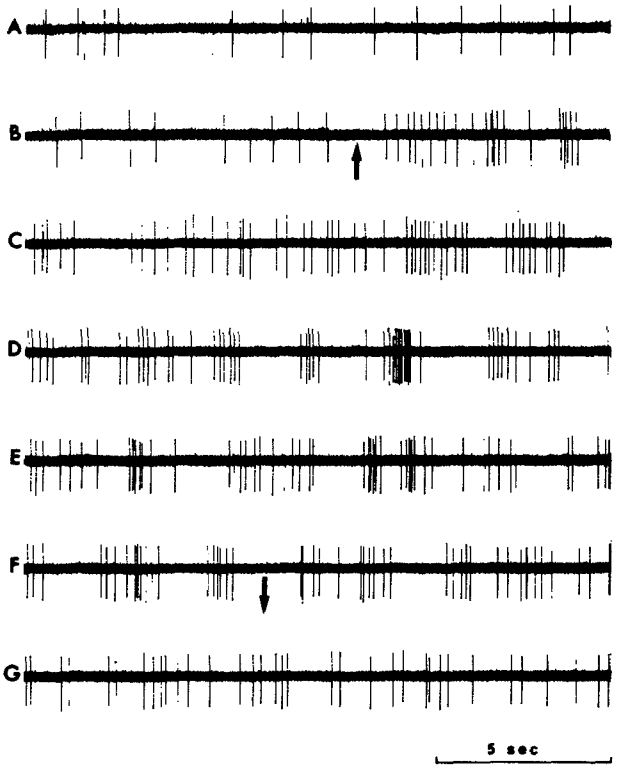

FIGURE 3 *Activity of a single unit with a low resting discharge under control conditions and during backflow into the pelvis of nondiuretic urine (between arrows). The activity subsided towards control levels after the release of the stimulus in F.*

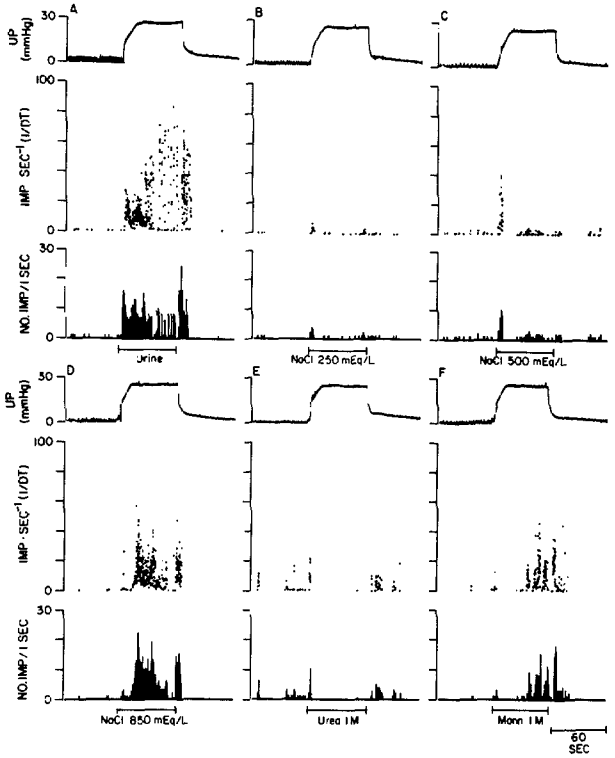

FIGURE 4 *Single-unit response to backflow into the pelvis of nondiuretic urine (A) and solutions of NaCl (B–D), urea (E), and mannitol (F). Traces from top to bottom are: ureteral pressure (UP), instantaneous frequency of discharge, and number of impulses per second.*

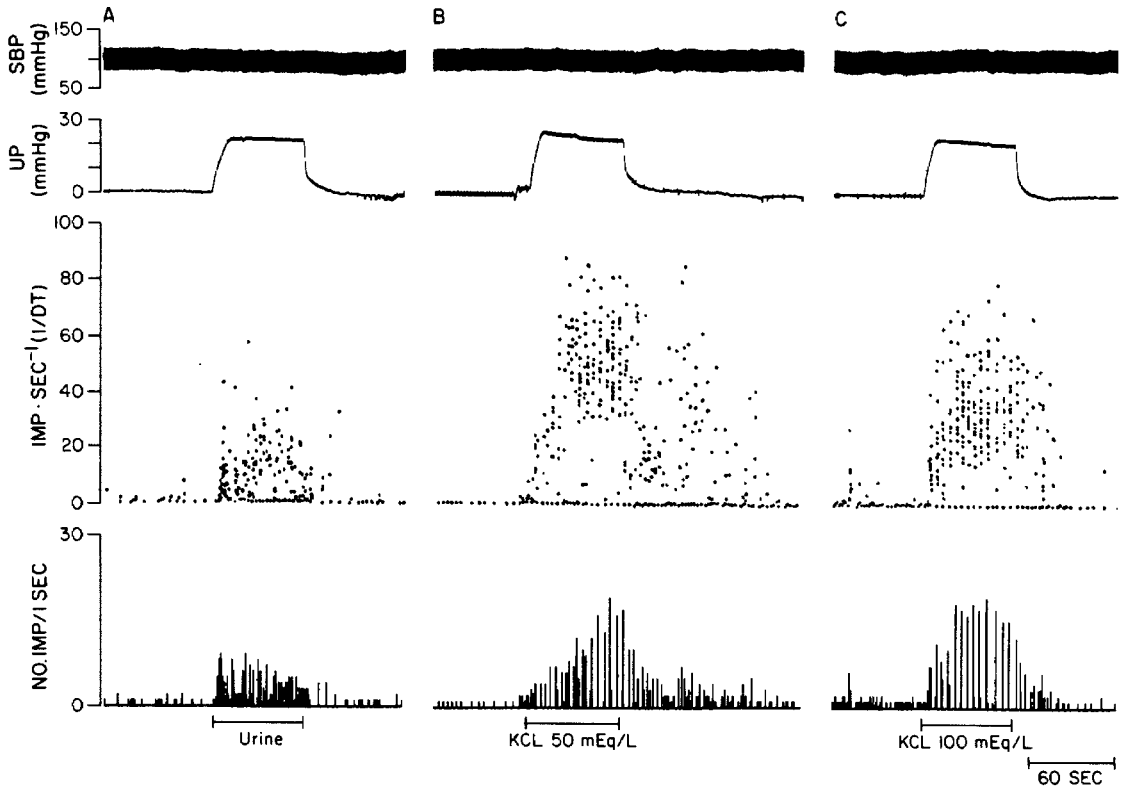

FIGURE 5 *Single-unit response to backflow into the pelvis of urine (A) and KCl solutions (B and C). Traces from top to bottom are: systemic blood pressure (SBP), ureteral pressure (UP), instantaneous discharge rate, and number of impulses per second.*

urea, like NaCl solutions of 150 (not shown in Fig. 4) and 250 mEq/liter, had no consistent effect. NaCl at 500 mEq/liter elicited a slight activation, whereas 850 mEq/liter NaCl and 1.0 M mannitol were clearly excitatory. Initial brief increases in activity always accompanied the rise in ureteral pressure, probably due to the residual urine in the catheter, as noted above. Of the 13 single units tested with these solutions, none responded to 150 and 250 mEq/liter NaCl or to 1.0 M urea; 500 mEq/liter NaCl elicited a slight effect, but all were clearly excited by 850 mEq/liter NaCl and, usually after a short delay, by 1.0 M mannitol (Fig. 4F).

All units tested (15 units, 10 experiments) were excited by backflow of KCl solutions of 50, 100, and 150 mEq/liter. Of these, three units were excited maximally by 50 mEq/liter KCl. In such cases, higher concentrations of KCl, which were invariably excitatory, elicited no greater activity (Fig. 5, B and C). The response to the KCl solutions was characterized by a high frequency of discharge and trains of impulses.

To distinguish further any effects of changes in pelvic pressure from those of fluid composition, and to avoid the initial excitatory effect caused by backflow of urine remaining in the catheter, we employed a second catheter system through which test solutions could be introduced into the pelvis by low pressure perfusion as well as by backflow (Fig. 1B). Even though each method alters pelvic pressure at a different rate and to a different degree, nondiuretic urine introduced both ways evoked similar responses from two multifiber preparations and from three single units; data for one is shown in Figure 6. Likewise, 14 units activated by NaCl, KCl,

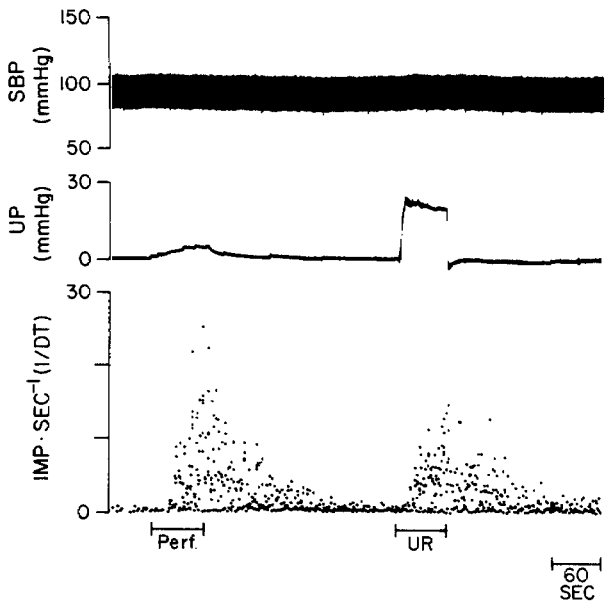

FIGURE 6 *Single-unit response to perfusion of the pelvis (PERF) and backflow into the pelvis (UR) of nondiuretic urine. Traces from top to bottom are: systemic blood pressure (SBP), ureteral pressure (UP), and instantaneous discharge rate.*

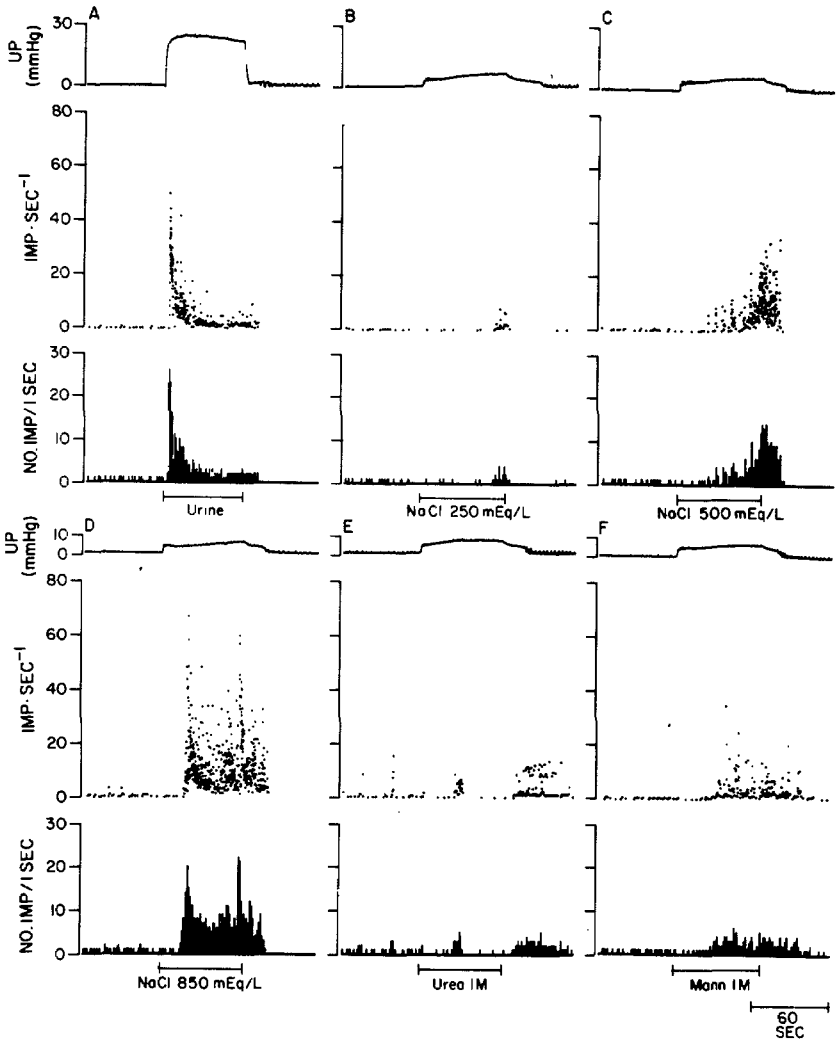

FIGURE 7 *Single-unit response to backflow of urine into the pelvis (A) and to perfusion of the pelvis with solutions of NaCl (B–D), urea (E), and mannitol (F). Traces as in Figure 4.*

urea, and mannitol responded the same to perfusion as to backflow. As Figure 7 illustrates, the perfusion technique eliminated the initial brief activation. Moreover, it demonstrated that some responses arose swiftly (Fig. 7D) when changes in pelvic pressure were slow and slight.

Renal Ischemia

Figure 8 shows that clamping the renal artery during antidiuresis promptly activated multifiber preparations that also responded to backflow of urine into the renal pelvis. To see whether the response to renal ischemia came from the same units responsive to urine composition, single fibers were isolated. All 29 single units sensitive to backflow of urine proved to be activated by renal ischemia lasting 1 minute. Immediately after release of the occlusion, the background activity usually was briefly depressed. The average peak firing rate attained during renal artery occlusion was 48.3 ± 5.1 impulses/sec. Unit B of Figure 9 was typical of this group: it had a resting discharge rate under control conditions, it responded to backflow of urine into the pelvis, and it fired irregularly during clamping of the renal artery. Another unit activated by such clamping (unit A, Fig. 9), which was recorded simultaneously with unit B, had no resting discharge and was not activated by backflow of urine into the renal pelvis. Such a response is character-

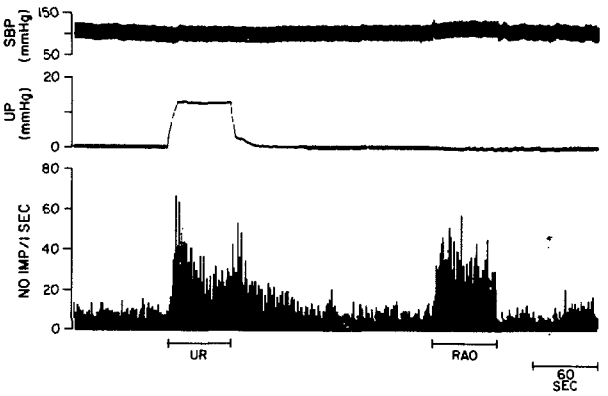

FIGURE 8 *Response of a multifiber preparation to backflow of nondiuretic urine (UR) and occlusion of the renal artery (RAO). Traces as in Figure 2.*

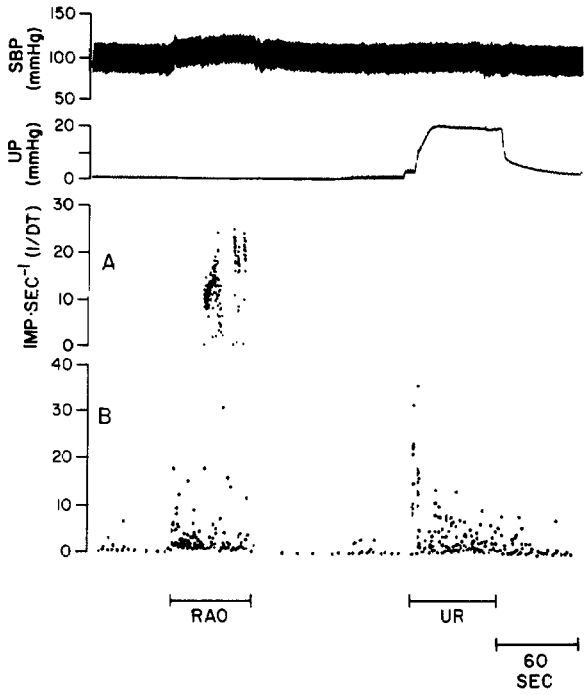

FIGURE 9 *Two single units (A and B) recorded simultaneously from a nerve bundle during clamping of the renal artery (RAO) and backflow of urine into the pelvis (UR). Traces as in Figure 6. The responses of units A and B are characteristic of R1 and R2 chemoreceptors, respectively.*

istic of the "R1" chemoreceptors (Recordati et al., 1978).

In seven experiments (seven units) the effects of a prolonged occlusion (5–10 minutes) of the renal artery were studied. Within the first minute of renal ischemia, the frequency of discharge increased, and the activation persisted throughout the period of occlusion. The pattern of discharge involved trains of impulses like those seen during backflow of KCl into the renal pelvis (cf. Fig. 10, A and B).

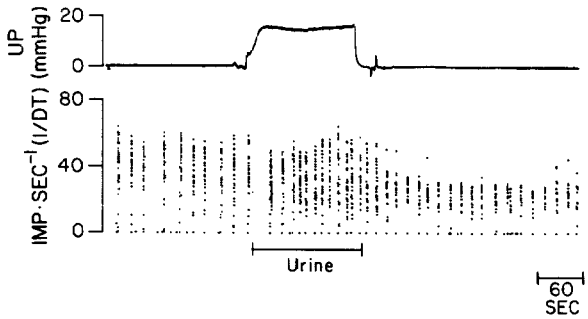

FIGURE 11 *Single-unit response to backflow into the pelvis of nondiuretic urine, recorded up to 15 minutes after the death of the rat. Upper trace, ureteral pressure (UP). Lower trace, instantaneous firing rate.*

Single units responsive to both backflow of urine and renal artery occlusion continued to yield trains of impulses for 20–30 minutes after the death of the rat. As shown in Figure 11, there was a strong resistance to anoxia; after 10–15 minutes of ischemia, the units still responded to backflow of urine into the pelvis.

Wash-out Effects

We have reported previously that the activation of R1 chemoreceptors elicited by clamping of the renal artery is stopped by perfusing isotonic saline

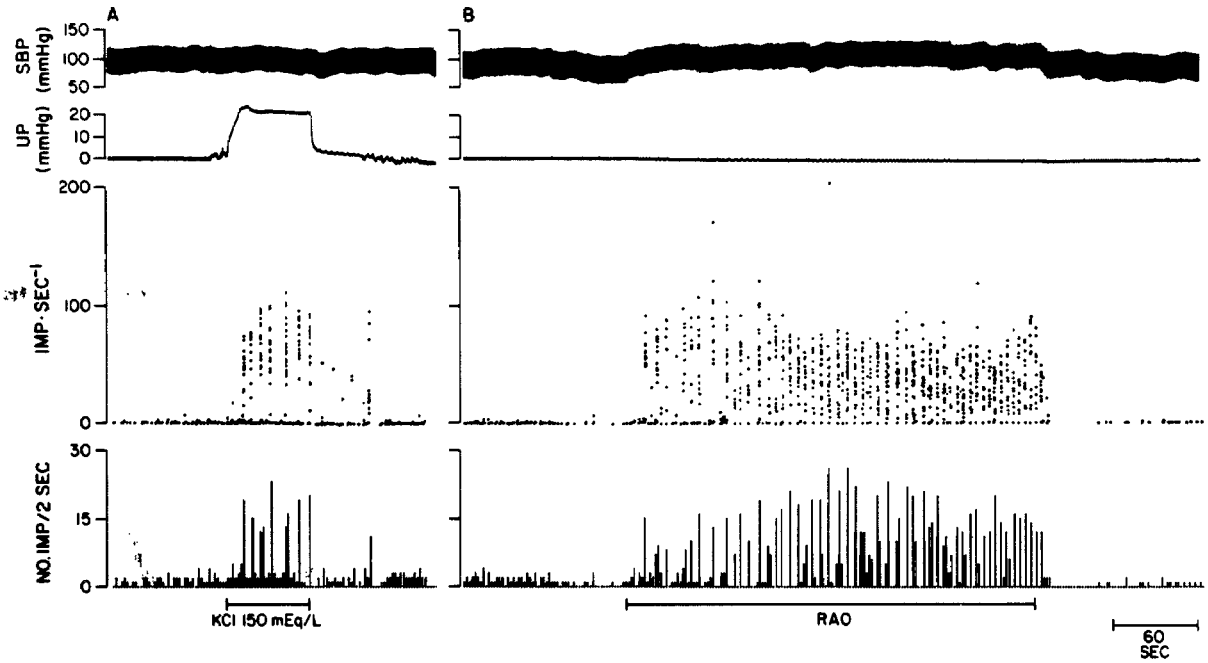

FIGURE 10 *Single-unit response to backflow into the pelvis of KCl (A) and to prolonged (5-minute) occlusion of the renal artery (RAO). Traces as in Figure 5.*

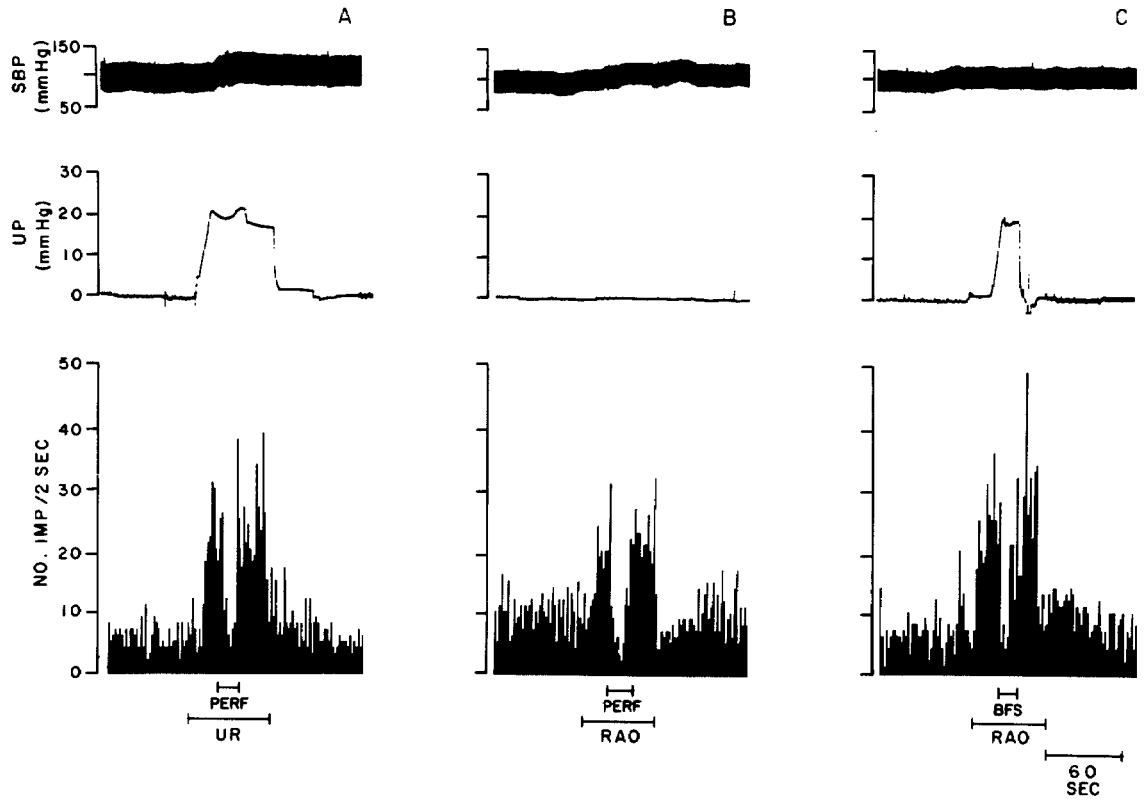

FIGURE 12 *Multifiber response to three successive washout procedures. A: Backflow of nondiuretic urine (UR) and perfusion of isotonic saline into the renal artery (PERF); B: occlusion of the renal artery (RAO) and perfusion of the kidney with isotonic saline (PERF); C: occlusion of the renal artery (RAO) and backflow of isotonic saline into the renal pelvis (BFS). Traces as in Figure 2 except impulses are per 2 seconds.*

into the ischemic kidney. We called this a "washout" effect (Recordati et al., 1978). To verify whether R2 chemoreceptors behave similarly, in 12 experiments the kidney was perfused through the renal artery with isotonic saline during backflow of urine into the pelvis and clamping of the renal artery. The excitatory effects of both these stimuli were inhibited during saline perfusion (Fig. 12, A and B). In addition, the activation elicited by renal ischemia also could be "washed-out" by backflow of isotonic saline into the pelvis (Fig. 12C).

Diuretic vs. Nondiuretic State

The background afferent activity of multifiber preparations in nondiuretic rats was always quite high (Fig. 13, A and B). To determine whether the level of resting discharge was dependent on the excretory function of the kidney, in five experiments afferent nerve activity was recorded before, during, and after 5% expansion of the extracellular fluid volume with isotonic saline. The resting discharge rate progressively declined during the infusion, and in the expanded steady state it averaged 55.7 ± 1.9% less than in the nondiuretic state (Table 1; Fig. 13, C and D). During expansion, urinary flow rate and concentration of sodium increased, while the potassium and urea concentrations and osmolality declined (Table 1). As Figure 14 illustrates, the responses of multifiber preparations to clamping of the renal artery and backflow of urine were much less in the diuretic (Fig. 14B) than in the nondiuretic state (Fig. 14A). Nonetheless, backflow of previously collected nondiuretic urine into a diuretic

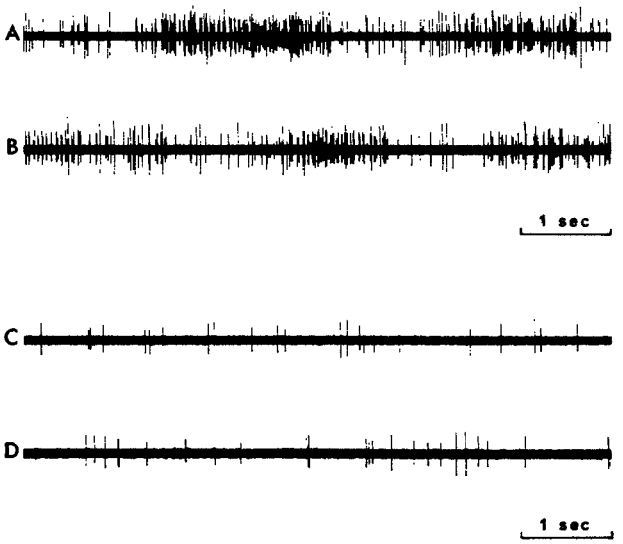

FIGURE 13 *Analog recordings of the resting discharge of a multifiber preparation in nondiuretic conditions (A and B) and after diuresis was induced by expansion of the extracellular fluid volume with isotonic saline (C and D).*

TABLE 1 *Effects of Extracellular Volume Expansion on Renal Afferent Nerve Activity and Urine Composition*

	Afferent nerve activity (imp/10 sec)			$\dot{V}$ (μl/min)		U_{Na} (mEq/liter)		U_K (mEq/liter)		U_{urea} (mEq/liter)		U_{Osm} (mOsm/kg)	
Rat	C	E	% decrease	C	E	C	E	C	E	C	E	C	E
1	462 ±5	244 ±3	47.1	5.6	60.0	91.8	191.0	287.9	142.0	553	52	1347	515
2	279 ±4	117 ±2	57.8	12.0	23.0	102.2	265.0	237.5	196.6	597	198	1357	835
3	145 ±3	61 ±3	57.7	5.1	78.0	25.0	239.2	374.4	161.5	872	280	2128	695
4	295 ±7	137 ±3	53.6	13.8	78.2	39.5	165.4	194.2	119.1	612	276	1412	503
5	284 ±5	118 ±2	58.3	4.3	54.0	52.5	49.0	312.8	130.0	811	345	1669	592

$\dot{V}$ = urine flow rate, C = control, E = after extracellular volume expansion, and U_x = urinary concentration of x.

kidney produced a response like that elicited in the nondiuretic state. Figure 15 contrasts the effect of backflow of diuretic and nondiuretic urine on a single unit in a volume-expanded rat. Backflow of the rat's own diuretic urine provoked a minimal response, but backflow of previously collected nondiuretic urine produced an intense discharge from this unit. The differences in responsiveness demonstrated in nondiuretic and diuretic states have not been quantified.

Discussion

These results indicate that the renal receptors which responded to backflow of urine into the renal pelvis were not sensitive to changes in pelvic pressure or pelvic distension but rather to the chemical composition of urine and of their environment. These receptors are provisionally termed R2 chemoceptive receptors to distinguish them from the previously described group of renal R1 chemoreceptors (Recordati et al., 1978).

Effects of Urine and Test Solutions

According to anatomical studies, the renal papilla and the surface of the pelvic epithelium lack nerve endings. A well-developed nervous plexus exists, however, in the submucosal layers of the pelvic wall and upper third of the ureter, forming a network underlying the basement membrane of the epithelium. This plexus is thought to be sensory in function (Notley, 1968; Gosling, 1970; Dixon and Gosling, 1971; Barajas and Wang, 1978). Another group of nerve fibers, in the outer medullary region, arises from nerves that penetrate the kidney along the distribution of the arterial tree (Gosling, 1969; Dixon and Gosling, 1971). Hence, it would appear

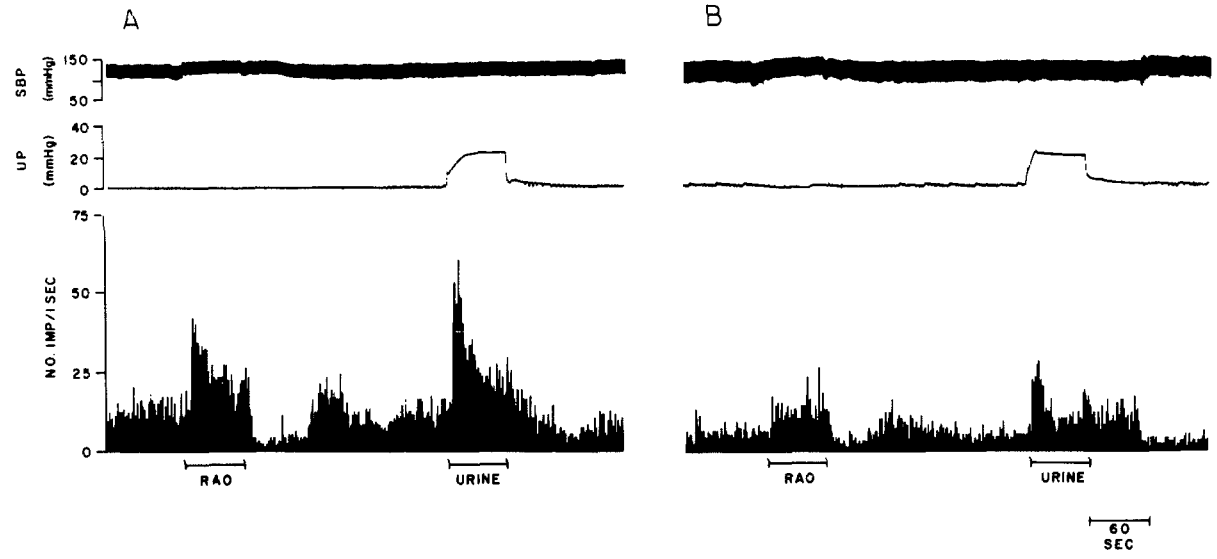

FIGURE 14 *Response of a multifiber preparation to clamping of the renal artery (RAO) and backflow of urine into the pelvis (Urine) in nondiuretic (A) and diuretic conditions (B). Traces as in Figure 2.*

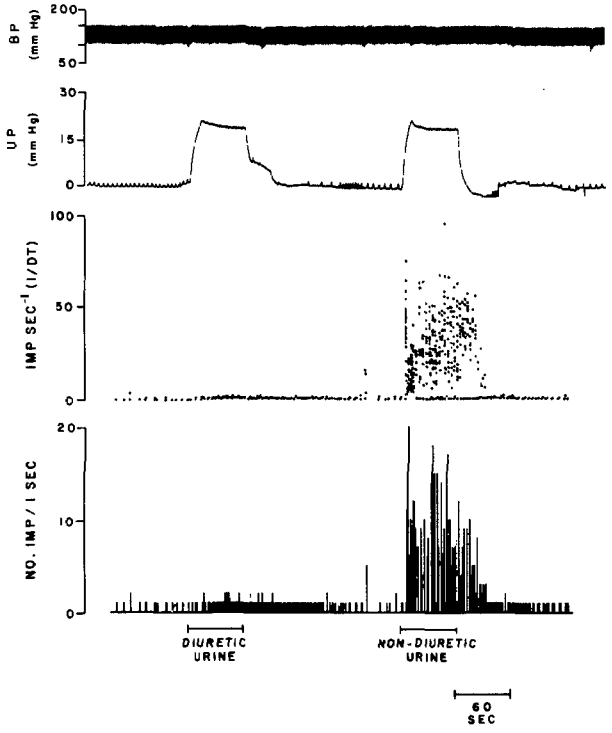

FIGURE 15 *Single-unit response to backflow of diuretic urine and previously collected nondiuretic urine in a diuretic rat. Traces as in Figure 5.*

that urinary solutes exert their effects on nerve endings across an epithelium. Half the pelvic wall is lined by a transitional epithelium, which is impermeable to water and solutes; the remainder is lined by a very thin epithelium, which, in the region of the fornices, separates pelvic urine from the outer medullary tissue. This portion is thought to be permeable to urea and water and may contribute to the urine-concentrating mechanism (Schmidt-Nielsen, 1977; Khorshid and Moffat, 1974). Solutes and water might gain access to this region when pelvic distention opens the fornices, aided by the pressure gradient generated during backflow of urine or perfusion of the pelvis (Schmidt-Nielsen, 1977).

On the basis of these data it appears that urinary solutes and test solutions exert their effects on nerve endings within the renal interstitium. Ions such as K^+, Na^+, and others that we have not tested in these experiments (hydrogen, ammonium, calcium, and anions) might cross the pelvic epithelium and act directly on sensory nerve endings. Mannitol, on the other hand, which presumably does not cross the pelvic epithelium and which is not a charged ion, might exert its effect indirectly by producing an osmotic gradient along which water moves from the renal interstitium into the pelvic cavity. As a consequence, the concentration of solutes in the renal interstitium would rise, leading to an activation of sensory endings. In both circumstances, alterations in the ionic concentration of the renal interstitium would be the event initiating sensory discharges. R2 receptors might behave, therefore, like receptors in other tissues and organs which are known to be activated by increments in the local concentration of ions such as H^+, K^+, Na^+, and NH_4^+ (Khayutin et al., 1976).

Alternatively, it could be argued that R2 receptors respond to total solute concentration and function as osmoreceptors in the manner originally described by Verney (1947). Although the lack of response to urea, which readily crosses cellular membranes, and the excitatory effect of mannitol are highly suggestive of this mechanism of function, it would be difficult to explain the excitatory effect of hypotonic KCl which contrasts with the effects of NaCl, which is excitatory only in hypertonic concentrations.

To discriminate between total osmolality and the effect of individual solutes, the effects of urine would have to be compared with those of mixed solutions whose osmolality was held constant while the concentrations of individual solutes were varied. Mixed solutions would be worth investigating, since the absence of sodium or potassium could have a direct effect on the transmembrane potentials of the nerve endings (Eyzaguirre and Nishi, 1976).

Effects of Renal Ischemia

The units that responded to backflow of urine into the pelvis also were activated promptly during occlusion of the renal artery. Their activation was characterized by an initial, irregular discharge followed by trains of impulses that reached a high frequency. Cessation of renal blood flow might activate sensory endings in several ways: by a direct effect of hypoxia and hypercapnia, by release of substances such as renin and prostaglandins, or by changes in cellular volume and interstitial accumulation of ions. Our data do not allow a clear distinction among these possibilities. One could postulate, however, that the excitatory stimulus is similar to that caused by backflow of urine into the renal pelvis, i.e., an accumulation of ions at the receptor site. This possibility is supported by the observations that the activation due to renal ischemia is "washed-out" by backflow of isotonic saline into the renal pelvis and that during a prolonged ischemia the receptors discharge with trains of impulses quite similar to those elicited during backflow of KCl into the renal pelvis. Hypoxia causes a leakage of K^+ out of cells, which could be the activating stimulus (Macknight and Leaf, 1977). Afferent activity persists throughout prolonged ischemia and after the death of the animal; a response to backflow of urine still can be obtained 15–20 minutes after death. The resistance of these sensory units and axons to anoxia is reminiscent of that reported for the chemoreceptors of the carotid body (Mills and Jöbsis, 1972).

Wash-out Effects

The activation of R2 chemoreceptors elicited by clamping the renal artery was stopped either by perfusing the ischemic kidney with isotonic saline through the renal artery or by backflow of isotonic saline into the renal pelvis. Moreover, the excitatory effect of backflow of nondiuretic urine into the renal pelvis could be washed out by perfusing isotonic saline into the renal artery. The first two wash-out effects might be explained by assuming that substances released by the ischemic kidney or which accumulated at the receptor site were washed out during the perfusion, as we suggested for the R1 chemoreceptors (Recordati et al., 1978). The third effect, however, might support a different interpretation. Isotonic saline under hydrostatic and osmotic forces might have moved from the renal vasculature or renal pelvis into the interstitium and thus diluted any chemical stimuli acting on the receptors. Although we do not know the precise mechanism involved, the wash-out effects support the view that the units involved are chemoreceptive in function (Eyzaguirre and Koyano, 1965) and that their receptive field is easily accessible through either the pelvic epithelium or the renal vasculature.

Effects of Diuresis

When the extracellular fluid volume was expanded by an amount of isotonic saline equivalent to 5% of the body weight, marked diuresis ensued, characterized by a 10-fold increase in the urine flow rate and a decrease in urine osmolality. Backflow of diuretic urine was less excitatory than nondiuretic urine in both nondiuretic and diuretic rats, suggesting that the decreased responsiveness reflects the composition of the urine, rather than any alterations in the receptors' ability to react to the stimulus. This observation is consistent with the demonstration that ionic and non-ionic solutions introduced into the renal pelvis have different and selective effects.

The transition from the nondiuretic to the diuretic state is accompanied by a clear and progressive decline in the rate of resting discharge. It is known that many changes occur within the kidney during diuresis, including a reduced osmotic gradient between cortex and medulla, more water in the renal interstitium, and increased renal blood flow (Atherton et al., 1968a, 1968b). All these changes in renal circulation and function, as suggested earlier, might contribute to a decrease in the ion concentration at the receptor site, thus decreasing the afferent discharge rate. The decreased responsiveness to renal artery occlusion in diuretic conditions could reflect this.

Renal Chemoceptive Receptors

In summary, we conclude that R2 receptors respond to changes in ion concentration in the renal interstitium, caused by urinary solutes crossing the pelvic epithelium, by alterations in renal blood flow, or by demands on renal function. R2 receptors differ from R1 receptors in at least three respects: their resting discharge, their response to backflow of urine, and their maintained discharge during prolonged occlusion of the renal artery. These apparently different functional attributes might be traceable to differences in the location of receptors inside the renal parenchyma. For example, if R2 chemoreceptors were located beneath the pelvic epithelium in the outer medullary region, they would be accessible to urinary solutes, whereas R1 receptors might be deeper within the kidney, perhaps within the cortex. Since it was possible to record from single units of both types simultaneously, their clearly distinct response characteristics argue strongly that these are two different groups of sensory units. (Fig. 9).

Physiological Role

Although sudden distension of the pelvis provokes pain (DeWolf and Fraley, 1975), and chemoreceptors of tissue subserve painful sensation caused by tissue damage, it is unlikely that the unique role of the R2 receptors is nociception. On the contrary, R2 receptors are activated during perfusion of the pelvis at low pressures, are unaffected by distension of the pelvis with isotonic saline, and in nondiuretic conditions have a high resting discharge rate which can not be assumed to give rise to a painful sensation.

A recent hypothesis (Khayutin et al., 1976) addressing the pressor reflexes elicited by stimulation of intestinal chemoreceptors suggests that mild, "natural" stimulation may produce a moderate reflexive rise in blood pressure, whereas maximal stimulation of the afferent nerves causes a much greater increase in systemic blood pressure and elicits simultaneous pseudaffective reactions. The painful sensation might therefore depend on the degree to which the afferent nerves are stimulated. Renal nerves might behave similarly. In the cat, for example, electrical stimulation of afferent renal nerves can raise blood pressure and produce tachycardia without concomitant pseudaffective reactions (Calaresu et al., 1976).

Although R2 receptors do not seem to be sensitive to urine osmolality as a whole, their sensitivity to ion concentration in the renal interstitium might enable them to act as peripheral osmoreceptors, sensitive to extracellular dehydration. The high resting discharge in antidiuresis and its decline during expansion of the extracellular fluid volume are highly suggestive in this sense. Interestingly, moreover, a number of authors link the pelvic cavity to the process of urine concentration. Opening the pelvic cavity decreases the osmolality of urine (Gottschalk et al., 1963), and the osmolality of the solution bathing the pelvis appears to influence the

osmolality of the fluid leaving the ducts of Bellini (Schmidt-Nielsen, 1977; Schütz and Schnermann, 1972). Spontaneous pelvic and ureteral contractions might increase the area of contact between urine and the pelvic epithelium by forcing urine into the fornices (Schmidt-Nielsen, 1977). If R2 receptors lie close to the pelvic epithelium, they would be ideally situated to monitor the ion concentration in the renal interstitium in a region where the influence of urinary solutes is most pronounced.

The kidney may thus be the source of nerve impulses which, through spinal and supraspinal nervous reflexes (Pearce and Sonnenberg, 1965; Gill and Casper, 1969; Calaresu et al., 1978; Brody et al., 1979), may participate in the homeostatic regulation of body fluids (Gottschalk, 1979).

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