Neurogenic regulation of renal tubular sodium reabsorption

DIBONA, GERALD F. Neurogenic regulation of renal tubular sodium reabsorption. Am. J. Physiol. 233(2): F73–F81, 1977 or Am. J. Physiol.: Renal Fluid Electrolyte Physiol. 2(2): F73–F81, 1977.—The evidence supporting a role for direct neurogenic control of renal tubular sodium reabsorption is reviewed. Electron microscopic and fluorescence histochemical studies have demonstrated adrenergic nerve terminals in direct contact with basement membranes of mammalian (rat, dog, and monkey) renal tubular epithelial cells. Low-level direct or baroreceptor reflex stimulation of renal sympathetic nerves produces an increase in renal tubular sodium reabsorption without alterations in glomerular filtration rate, renal blood flow, or intrarenal distribution of blood flow. Antinatriuresis was prevented by prior treatment of the kidney with guanethidine or phenoxybenzamine. Rat kidney micropuncture studies have localized a site of enhanced tubular sodium reabsorption to the proximal tubule. Possible indirect mediation of the antinatriuresis by other humoral agents known to be released from the kidney on renal nerve stimulation (angiotensin II, prostaglandin) was excluded by experiments with appropriate blocking agents. The possible effects of anesthesia and uncertainties about the completeness of surgical renal denervation and other tubular segmental sites of action are critically analyzed. The clinical implications of this mechanism in pathologic conditions of sodium and water retention are discussed and a prospectus for future work is presented.

renal nerves; electron microscopic studies; antinatriuresis; angiotensin II; prostaglandin

THE INFLUENCE OF THE RENAL NERVES on the regulation of renal tubular sodium and water reabsorption has been a subject of controversy since Claude Bernard (8) observed an increased urine flow following section of the splanchnic nerve in the anesthetized animal. Smith (47) criticized this and subsequent similar experiments noting that "the phenomenon of 'denervation diuresis' is observable only under the abnormal conditions of his experiment (i.e., anesthesia), which are such as to excite vasoconstriction in the kidney." He summarized: "Renal denervation in unanesthetized normal dogs and men does not produce renal hyperemia, the vasoconstrictor pathways being quiescent under basal conditions; nor does renal denervation have any significant effect upon the normal urine flow, upon water diuresis, or pituitary antidiuresis." It should be noted that the only intervention under review was one designed to decrease renal nerve impulse frequency. It was generally performed by division of all visible nerves entering the renal hilus or by cutting the splanchnic nerves, despite the contention (41) that complete renal denervation could be secured only by section and resection of the renal artery, vein, and ureter. In addition, functional evidence, such as failure of renal blood flow to decrease following bilateral carotid artery occlusion or supramaximal splanchnic or renal nerve stimulation, verifying the completeness of surgical renal denervation was not presented.

Recent experiments employing interventions designed to increase as well as decrease renal nerve impulse frequency, to denervate the kidney by nonsurgical pharmacological means, and to provide verification of the completeness of renal denervation have contributed new information on this subject.

State of Current Information

Prior to the work of Barajas and Muller (1, 37), it was thought that the renal tubules lacked significant innervation. With use of fluorescence histochemical (19) and electron microscopic techniques, these investigators demonstrated adrenergic nerve terminals in direct contact with basement membranes of proximal and distal tubules in the monkey (37) and rat (1) kidney. Employing methods similar to those of Falck et al. (19) and Muller and Barajas (37), we have demonstrated similar tubular adrenergic innervation in the dog kidney (Figs. 1 and 2).
Previous investigators who studied the effect of increases in renal nerve activity on sodium excretion utilized electrical stimulation parameters or other interventions that produced pronounced changes in intrarenal hemodynamics. These approaches were summarized in 1974 by Schrier (43) who stated: "In fact, in virtually all in vivo experiments demonstrating an effect of alterations in adrenergic tone on sodium excretion, the results do not differentiate between a direct effect on active sodium transport and an indirect effect mediated by some alteration in intrarenal hemodynamics."

In 1973, La Grange and colleagues (32) identified a level of direct electrical renal nerve stimulation that increased renal venous renin concentration without affecting glomerular filtration rate (GFR) or renal blood flow (RBF). They also observed a 13-26% decrease in urinary sodium excretion (U\textsubscript{Na}V) while the filtered sodium load remained constant. Although these observations were consistent with a direct effect of renal nerves on tubular sodium reabsorption, the authors deemed this possibility unlikely since, at that time, definitive evidence of tubular innervation was lacking. Additionally, as a methodological limitation, it should be noted that GFR was determined by a urineless technique based on the Fick principle; data correlating this technique with the urine collection technique were not presented.

Our own studies as well as those of others (summarized in Refs. 45 and 46) convinced us that increases in renal sympathetic nerve activity had a direct effect on tubular sodium reabsorption in low-cardiac-output sodium-retaining states. We undertook a series of experiments to characterize the role for the renal sympathetic nerves in directly influencing tubular sodium transport in the absence of changes in GFR, RBF, or intrarenal distribution of blood flow. The left renal nerves of saline-loaded anesthetized dogs were stimulated at a level just below the threshold level that caused a reduction in RBF. These stimulation parameters were 10 V, 0.5 ms, 1.25 mA, and 0.5-2.0 Hz. GFR was determined by inulin clearance (C\textsubscript{IN}). In the right kidney, effective renal plasma flow was estimated by the clearance of para-aminohippurate (PAH). In the left kidney, RBF was determined by clearance and extraction of PAH and by electromagnetic flowmeter; agreement between these latter two methods was within 7%. As shown in Fig. 3, low-level direct electrical stimulation of the left renal nerves produced an ipsilateral decrease in U\textsubscript{Na}V in each of 17 individual studies. The mean decrease was 36.0 ± 6.1 μeq/min or 17.3%. This decrease occurred without significant change in left kidney GFR or RBF. Following cessation of stimulation, the U\textsubscript{Na}V returned to control period levels. Right kidney U\textsubscript{Na}V, GFR, and C\textsubscript{IN} were unchanged throughout. As assessed by the radioactive microsphere technique, low-level direct left renal nerve stimulation did not alter intrarenal distribution of blood flow (45). The adrenergic specificity of the antinatriuretic response to low-level direct electrical renal nerve stimulation was verified by studies that employed pharmacological agents known to interrupt renal neurotransmission. In paired studies both phenoxycobenzamine (56), an alpha-adrenergic receptor antagonist, and guanethidine (45), an adrenergic blocking agent, abolished a previously demonstrated antinatriuretic response to low-level direct renal nerve stimulation. These studies demonstrated that there existed a level of direct electrical stimulation of the renal nerves which resulted in an increase in tubular sodium reabsorption in the absence of changes in intrarenal hemodynamics. The adrenergic blocking studies indicated that this response is mediated by direct adrenergic innervation of the renal tubules. These views receive strong support from the anatomic studies of Barajas (1), Muller (37), and ourselves (45, 46), referred to earlier.

Confirmation of these results has come from renal micropuncture and clearance studies in the anesthetized saline-loaded rat by Bello-Reuss and associates (7). Stimulation of the greater splanchnic nerve at 3–16 V, 0.5 ms, and 1–2 Hz produced a 25% decrease in urinary flow rate and U\textsubscript{Na}V, while GFR and renal plasma flow (RPF) were unchanged (Fig. 4). Increases in late proximal tubule fractional (from 27 to 47%) and absolute (from 31 to 64%) water reabsorption occurred without change in the single nephron glomerular filtration rate (SNGFR). Slowly conducting (0.7–1.0 m/s), unmyelinated C fibers appeared to be responsible for the effect. This study confirms the finding that low-frequency electrical stimulation of the renal nerves leads to an antidiuretic and antinatriuretic response in the absence of changes in whole kidney GFR or RPF. The micropuncture data demonstrate that this effect results in part from an increase in sodium and water reabsorption in the proximal tubule, which occurs without significant alterations of either SNGFR or filtered sodium load.

To place these observations in a more physiological perspective, it was important to define the role of this
FIG. 2. Transmission electron photomicrograph of dog kidney, perfusion-fixed with aldehyde-osmium tetroxide and stained with uranyl acetate and lead citrate. A nerve ending in contact with basement membrane of proximal tubule is seen to contain granular and agranular vesicles. Magnification x64,000.

regulatory mechanism in response to reflex activation of the renal sympathetic nerves. With the renal perfusion pressure held constant, baroreceptor reflex renal sympathetic nerve stimulation was produced by carotid sinus perfusion (57). As shown in Fig. 5 significant decreases in \( U_{\text{Na}} \) occurred in each of 16 tests in nine dogs. The mean decrease was \( 68 \pm 30 \mu \text{eq/min}, \) or 37\%. This decrease occurred without significant change in GFR or RBF and was completely reversible. In companion studies, the left kidney was infused with either phenoxybenzamine or guanethidine while the right kidney served as the control. The usual antinatriuretic response was observed in the untreated control right kidney while the response was completely abolished in the left kidney that had been treated with the sympathetic blocking agent. These studies indicate that physiological reflex activation of the renal sympathetic nerves results in an increase in tubular sodium reabsorption which is mediated by adrenergic innervation of the renal tubules. That this physiologic reflex can quantitatively and qualitatively produce the same response in the kidney as seen after direct stimulation of the renal nerves suggests that this reflex mechanism could participate in the overall physiologic regulation of renal sodium handling in the maintenance of external sodium balance.

Although the studies with sympathetic blocking agents support the interpretation that the neurogenic effect is direct (an effect of the neurotransmitter or electrical events at the nerve endings on the tubular epithelial cell sodium transport process), an indirect effect involving a humoral mechanism was not excluded. It is known that renal nerve stimulation causes the release of renin (32) and prostaglandins (18) from the kidney. Both angiotensin II (36) and prostaglandin \( \text{E}_1 \) (\( \text{PGE}_1 \)) (26, 35) increase sodium transport by the frog skin and toad bladder. Because of these observations it was considered that the antinatriuresis of low-level direct renal nerve stimulation might be mediated by the enhanced renal release and intrarenal action of angiotensin II or \( \text{PGE}_1 \). Experiments performed under conditions of renal blockade to angiotensin II (55) or of prostaglandin synthesis inhibition with indomethacin (17) have demonstrated that the antinatriuretic response to low-level direct renal nerve stimulation is unaffected. The response, therefore, does not appear to be mediated by the intrarenal action of circulating angiotensin II or prostaglandin.

Another source of controversy concerning the influence of the renal nerves on urinary sodium excretion is the many studies on the effects of renal denervation. Renal denervation results in a natriuresis in many mammalian species. This response has been attributed to a direct effect of the renal nerves on tubular function or to an increase in filtered sodium load derived from increases in GFR (summarized in Ref. 5). However, recent renal clearance and micropuncture studies in anesthetized non-diuretic rats have provided new insight...
FIG. 3. Effect of direct low-level left renal nerve stimulation in dog kidney. \( U_{\text{NaV}} \), urinary sodium excretion, \( \mu \text{eq/min} \); \( C_{\text{IN}} \), inulin clearance, ml/min; TRBF, total renal blood flow, ml/min; \( C_{\text{PAH}} \), para-aminobenzamide (PAH) clearance, ml/min; C, control period; S, left renal nerve stimulation period; R, recovery period. TRBF was measured in left kidney with an electromagnetic flowmeter; \( C_{\text{PAH}} \) was measured in right kidney. Data are means \( \pm \) SE; \( n \), 17.

into this controversy (5). Left renal denervation was accomplished by stripping the left renal artery of its adventitia by coating it with a solution of 10% phenol in absolute alcohol. Sham left renal denervation was accomplished by exposing the left renal artery while leaving its adventitia intact and coating it with a 0.9% solution of NaCl. Completeness of denervation was evaluated by determination of renal norepinephrine content at 10 min, 3 h, and 3–6 days after renal denervation. In the left renal denervation studies, no difference between left and right kidney norepinephrine content was observed at 10 min or 3 h, but left kidney norepinephrine content was undetectable at 3–6 days. In the left renal sham denervation studies, no difference between left and right kidney norepinephrine content was observed at 3–6 days. Left renal denervation increased ipsilateral urinary flow rate and \( U_{\text{NaV}} \), but GFR and RPF were unchanged in both kidneys. The innervated kidney showed no change in urinary flow rate and \( U_{\text{NaV}} \). Proximal tubular fractional and absolute sodium and water reabsorption decreased by 40%, but SNGFR was unchanged. Fractional sodium reabsorption was unchanged in the loop of Henle, but decreased along the distal convolution and collecting duct. Absolute sodium reabsorption, although increased in the loop of Henle, distal convolution, and collecting duct, only partially compensated for the increase in sodium delivery out of the proximal tubule. Change in intrarenal hydrostatic pressures was small. Sham-denervation animals showed no change in GFR, RPF, urinary flow rate and \( U_{\text{NaV}} \), SNGFR, or proximal tubular fractional and absolute sodium and water absorption. This study demonstrated that the diuresis and natriuresis observed after acute renal denervation is mediated by a marked depression of sodium and water reabsorption in the proximal tubule with only partial compensation in the more distal nephron segments. These responses occurred in the absence of systemic or intrarenal (peritubular Starling forces) hemodynamic changes. Similar observations have been made following acute unilateral renal denervation in anesthetized rats with extracellular volume expansion (6). These results clearly demonstrate a direct effect of the renal nerves on proximal tubular function.

To summarize, direct or reflex-induced perturbations in renal nerve impulse frequency result in changes in renal tubular sodium reabsorption independent of GFR, RBF, or intrarenal distribution of blood flow. Experiments employing sympathetic blocking agents indicate that this response is dependent on adrenergic innervation of the renal tubules. Ultrastructural studies demonstrate adrenergic innervation of proximal and distal renal tubules, and micropuncture studies localize a site of action of the neurogenic effect to the proximal tubule.

FIG. 4. Effect of low-level direct splanchnic nerve stimulation in rat kidney. GFR, glomerular filtration rate, ml/min per 100 g; RPF, renal plasma flow, ml/min per 100 g; \( U_{\text{NaV}} \), urinary sodium excretion, \( \mu \text{eq/min} \); SNGFR, single nephron glomerular filtration rate, ml/min; percentages are fractional water reabsorption; C, control period; S1, stimulation period (1 Hz); C2, recovery period. Fractional and absolute water reabsorption data are for accessible surface proximal convoluted tubule. Data are means \( \pm \) SE; \( n \), 6.
Critique of Deficiencies in Current Information

There can be little reservation regarding the existence of adrenergic innervation of mammalian renal tubules. By use of fluorescent histochemical and electron microscopic techniques, catecholamine-containing nerve terminals in direct contact with basement membranes of proximal and distal renal tubules have been identified in three mammalian species (monkey, rat, and dog) by two different laboratories (1, 37; and Figs. 1 and 2). These observations provide a firm anatomic rationale for an evaluation of the possible functional significance of such innervation.

The experiments summarized above were performed in anesthetized animals. Berne (9) compared the function of denervated and intact kidneys in both anesthetized and conscious dogs. In the anesthetized animals GFR and \( U_{\text{Na}}V \) were higher in the denervated kidney, whereas in the conscious animal the GFR and \( U_{\text{Na}}V \) of the denervated and intact kidneys were the same. He concluded that anesthesia resulted in vasoconstriction of the intact kidney which was mediated by the renal nerves; and this resulted in a lower GFR and \( U_{\text{Na}}V \) in the intact kidney of the anesthetized dogs. This anesthesia-dependent effect is also seen with the alpha-adrenergic-receptor blocking agent phenoxybenzamine (POB). Intrarenal arterial POB has no effect on \( U_{\text{Na}}V \) in the conscious unanesthetized dog (2, 3), whereas it is natriuretic in the anesthetized surgically manipulated dog (49). However, observations in man clearly indicate that demonstration of a direct neurogenic effect on renal tubular sodium reabsorption is not a by-product of anesthesia. Gill and Bartter (21) found that in man adrenergic blockade with guanethidine inhibited the usual decrease in \( U_{\text{Na}}V \) that occurs with sodium deprivation. The greater \( U_{\text{Na}}V \) occurred despite increases in fecal sodium, decreases in blood pressure and glomerular filtration rate, and unimpaired aldosterone production. In other studies in man (20), these investigators demonstrated an earlier escape from the sodium retaining effect of mineralocorticoid hormone in the presence of adrenergic blockade with guanethidine. Wagner’s (52) observation that individuals with idiopathic autonomic insufficiency (supine position) excrete a saline load more rapidly than normal subjects is in agreement with Shear’s (44) finding that such individuals are unable to normally diminish their \( U_{\text{Na}}V \) when they stand. Taken together these observations indicate a significant effect of renal sympathetic innervation on tubular sodium reabsorption in the conscious unanesthetized state.

Direct electrical stimulation of the renal nerves is a necessarily artificial way of increasing the frequency of nerve impulses impinging on the renal tubular epithelial cell basement membranes. However, activation of the carotid baroreceptor reflex is known to increase renal nerve activity in a physiologic fashion (31). The similarity of overall renal functional response to direct electrical and reflex stimulation of the renal nerves indicates that these two interventions operate through the same physiological effector mechanism. The identical observations in the rat add further support in this regard. The evidence for the adrenergic specificity of the antinatriuretic response is substantial; it is totally abolished by treatment of the kidney with either phenoxybenzamine or guanethidine. Phenoxybenzamine, as an alpha-adrenergic-receptor blocking agent, interferes with the action of both circulating catecholamines and the catecholamines released from nerve endings. Guanethidine, as a sympathetic blocking agent, inhibits the response to sympathetic adrenergic nerve activity while preserving the response to injected norepinephrine. It seems clear that the renal functional response to direct electrical or reflex stimulation of the renal nerves is mediated by a mechanism dependent on adrenergic innervation of some intrarenal structure(s).

The interpretation that this effector mechanism is adrenergic tubular innervation regulating tubular sodium reabsorption is dependent on the exclusion of other factors known to participate in the regulation of sodium excretion. A major factor is the filtered load of sodium, the product of plasma sodium concentration and GFR; neither of these determinants was changed significantly by activation of the renal nerves. The possibility always exists that small undetectable changes

**Fig. 5.** Summary of data for carotid sinus perfusion studies. \( U_{\text{Na}}V \), urinary sodium excretion, \( \mu \text{eq/min} \); \( C_in \), inulin clearance, ml/min; RBF, renal blood flow, ml/min; AP, arterial pressure, mmHg; MAP, mean arterial pressure; RPP, renal perfusion pressure; CSP, carotid sinus pressure; C, control period; E, experimental period; R, recovery period. Data are means ± SE for left kidney representing 16 tests in 9 dogs.
in GFR occurred that could possibly explain the antinatriuresis. There are several arguments that render this possibility unlikely. The response has been reproducible and reversible in dogs and rats under conditions of direct electrical and/or reflex stimulation of the renal nerves. In addition, the studies with adrenergic blocking agents were conducted in a paired experimental design. In the direct electrical stimulation studies, an antinatriuretic response to renal nerve stimulation was established in each dog prior to blockade. The GFR was not significantly changed by renal nerve stimulation before or after blockade, whereas the antinatriuretic response was eliminated after blockade. This sequence of events suggests that either small and similar undetectable decreases in GFR occurred secondary to renal nerve stimulation both before and after blockade and were unrelated to the antinatriuresis of nerve stimulation, or that blockade prevented the small undetectable decrease in GFR usually seen after renal nerve stimulation. If the latter were the case measurable decreases in RBF before blockade would be anticipated. The RBF was monitored by continuous electromagnetic flowmeter recording, and the level of both direct electrical and reflex stimulation of the renal nerves was set well below threshold for a reduction in RBF. A decrease in RBF was not observed. In the reflex stimulation experiments, one kidney served as the control while the contralateral experimental kidney was treated with adrenergic blocking agents. The increase in renal nerve activity produced by carotid baroreceptor stimulation was identical in both the control and the experimental blocked kidney. Therefore, any resulting small undetectable decreases in GFR would also be identical in the two kidneys. By similar reasoning, one would have expected a decrease in RBF in the control kidney as compared to the experimental blocked kidney during carotid baroreceptor stimulation. Such a decrease in RBF was not observed.

Furthermore, because measurements of GFR and RBF were constant throughout these experiments, the filtration fraction, a major determinant of peritubular capillary plasma colloid osmotic pressure, was also unchanged. In the rat micropuncture studies, direct measurement of peritubular Starling forces showed them to be unchanged. Therefore, a separate and independent role for peritubular Starling forces to mediate the increase in tubular sodium reabsorption is unlikely. The fall in renal blood flow and the rise in filtration fraction (i.e., unchanged GFR) could explain the antinatriuresis observed by Gill and Casper (23) in experiments in which the renal nerves of a hemorrhaged animal were left intact but the blood perfusing the animal's kidneys was derived from another normal animal.

Exogenous antidiuretic hormone and mineralocorticoid were provided to ensure that endogenous variations in these substances were not mediating the increase in tubular sodium reabsorption. The possibility of other hormonal involvement, i.e., prostaglandin and angiotensin II, was also investigated. Although renal prostaglandin secretion rates were not determined in the indomethacin experiments discussed above, our previous studies (28) indicated that a 2 mg/kg intravenous dose of indomethacin reduced the renal arteriovenous prostaglandin secretion rate to 0 within 20 min of administration, and both measurements were maintained at 0 for the 140-min observation period. Therefore, the assessment of the renal response to direct electrical stimulation of the renal nerves was routinely made within 140 min of indomethacin administration, i.e., during the time when renal prostaglandin synthesis and secretion were completely inhibited. The experiments employing an angiotensin II antagonist demonstrated that circulating angiotensin II does not mediate the antinatriuretic response to direct electrical stimulation of the renal nerves. However, the possibility remains that infusion of the angiotensin II antagonist into the renal artery a) might not provide access to the angiotensin II receptors, presumably extravascular, which could mediate the antinatriuretic response, or b) might not antagonize the action of angiotensin II formed intrarenally. Even though low-level direct electrical stimulation of the renal nerves substantially increases renal renin secretion rate, this problem might be profitably examined in animals with verified renal renin depletion.

The nature of the interventions employed to study the role of the renal nerves is deserving of comment. Direct electrical stimulation of the renal nerves is known to produce graded renal vasoconstriction in a dose-related fashion. The recorded compound action potentials suggest that the slowly conducting, unmyelinated C fibers are responsible for the antinatriuretic effect of renal nerve stimulation in the rat. Carotid baroreceptor reflex activation elicits a measurable increase in frequency of the recorded renal nerve impulse frequency. A quantitative definition of renal denervation is more difficult. Pharmacological denervation with phenoxybenzamine or guanethidine was assessed quantitatively. Phenoxybenzamine blockade was deemed complete when a renal arterial dose of phenylephrine, which prior to blockade reduced RBF by 90%, no longer decreased RBF. Guanethidine blockade was deemed complete when bilateral carotid artery occlusion or a level of direct electrical renal nerve stimulation, which prior to blockade reduced RBF by 90%, no longer decreased RBF. Surgical renal denervation by stripping the renal pedicle of all visible nerves and the local application of ethanol/procaine/phenol results in a marked diminution in kidney norepinephrine content, which has been offered as evidence of complete renal denervation (10, 54). However, seldom has functional evidence, such as failure of RBF to decrease following bilateral carotid artery occlusion or supramaximal splanchnic or renal nerve stimulation (45), been offered to verify the completeness of surgical renal denervation. In the rat studies described above, a greater increment in urinary flow rate was noted after the renal artery was stripped of its adventitia by coating it with phenol than after the left splanchnic nerve was crushed. The authors concluded that the magnitude of the denervation was greater with the former method. Additionally, surgical renal denervation in its simplest form as described above or in more complex forms involving auto-transplantation (11) or section and resuture of the renal...
artery, vein, and ureter (41) often adversely affects overall renal function (30). It is possible that the previously reported (summarized in Ref. 5) variable responses to surgical renal denervation are related to a combination of uncertainty as to the completeness of the denervation in the absence of a functional evaluation thereof and a deleterious effect on renal function of the procedure itself. The similarity of responses with phenoxybenzamine and guanethidine under conditions of both direct electrical and reflex stimulation of the renal nerves suggests that pharmacological renal denervation is reliable, reproducible, and essentially complete.

The rat renal micropuncture studies localized the site of the neurogenic effect on tubular sodium reabsorption to the proximal tubule. In the renal nerve stimulation experiments, $U_{Na,V}$ decreased whereas proximal tubule absolute sodium reabsorption increased. Assuming a homogeneous nephron population of 35,500 per kidney, absolute sodium reabsorption beyond the proximal tubule decreased. In the acute renal denervation experiments, $U_{Na,V}$ increased whereas proximal tubule absolute sodium reabsorption decreased. Absolute sodium reabsorption increased in the loop of Henle, distal convolution, and collecting duct. The changes in $U_{Na,V}$, therefore, were mainly attributable to the effect on proximal tubule sodium reabsorption. No specific contribution of altered sodium reabsorption in more distal nephron segments could be identified. In fact, absolute sodium reabsorption in the more distal nephron segments changed in a direction opposite to the proximal tubule. Recent experiments (48) have indicated that the collecting duct is a critical final regulator of urinary sodium excretion in states of both extracellular volume expansion and depletion. Currently available data do not indicate such a role for the collecting duct in alterations in $U_{Na,V}$ produced by changes in renal sympathetic nerve activity. Furthermore, although adrenergic innervation of the distal tubules has been demonstrated, evidence supporting adrenergic innervation of the collecting ducts is lacking.

**Clinical Implications**

Several studies indicate a significant role for the renal sympathetic nerves in mediating the sodium retention of cardiac failure in human subjects and experimental animals. The intravenous administration of dibenamine to human subjects with congestive heart failure produced an increase in RPF and $U_{Na,V}$ (12). Intrarenal arterial administration of phenoxybenzamine to unanesthetized conscious dogs produced an ipsilateral increase in urinary sodium excretion without a change in GFR or $U_{Na,V}$ (12). Interruption of renal neurotransmitter transmission by surgical or pharmacological (phenoxybenzamine, guanethidine, or pentolinium) renal denervation reversed the antinatriuresis and decreased proximal tubule fractional reabsorption in dogs with acute or chronic thoracic inferior cava constriction in the absence of changes in GFR, RBF, or intrarenal distribution of blood flow (22, 46). These observations demonstrate that a portion of the enhanced renal tubular sodium reabsorption characteristic of cardiac failure is mediated by a direct tubular effect of renal sympathetic innervation independent of systemic and intrarenal hemodynamic changes. These findings in cardiac failure were based on indirect experiments, i.e., a natriuresis was observed after renal sympathetic blockade without change in GFR and RBF. The results of the direct experiments involving direct and reflex renal nerve stimulation before and after renal sympathetic blockade provide firm support for the observations made in cardiac failure. The studies involving interruption of neuroadrenergic transmission in experimental sodium-retaining conditions and the direct or reflex renal nerve stimulation and renal denervation experiments all indicate the proximal tubule as a major locus of action for the direct neurogenic control of tubular sodium reabsorption. However, recent micropuncture studies in chronic experimental sodium-retaining conditions (33, 48) have demonstrated that SNGFR and proximal tubule fractional and absolute sodium reabsorption rates are normal during hydropenia and respond normally to saline volume expansion. These studies indicated that the loop of Henle is the major nephron segment responsible for the excessive sodium and water retention; this defect was more evident during saline volume expansion than during hydropenia. Although the loops of Henle may have adrenergic innervation, currently available studies do not indicate an effect of alteration in renal sympathetic nerve traffic on sodium and water reabsorption in this segment. Further studies, perhaps employing in vivo continuous microperfusion of the loop of Henle, are required to evaluate the effect of alterations in renal sympathetic nerve activity on sodium and water absorption in the loop of Henle.

It is known that hypertension, in man or in experimental animals, is characterized by an exaggerated natriuresis which is present during hydropenic conditions but which becomes more marked after saline volume expansion (39). By utilization of the spontaneous hypertensive rat, an animal model of essential hypertension, the nephron segment responsible for the exaggerated natriuresis has been identified as the loop of Henle (16). Volume expansion is known to decrease renal nerve impulse frequency, probably via activation of cardiopulmonary baroreceptors (42). Therefore, it is possible that the exaggerated natriuresis of the hypertensive state is due to cardiopulmonary baroreceptor reflex diminution in renal sympathetic nerve activity, with a resultant decrease in sodium and water reabsorption in the loop of Henle.

The magnitude of the effect of alteration in renal sympathetic nerve activity on renal tubular reabsorption is modest. However, the observed changes in fractional sodium excretion of 1–2% in the rat and dog studies would translate into increases or decreases in urinary sodium excretion in man of 518 meq/day (GFR = 120 ml/min, plasma water sodium concentration = 150 meq/liter). Therefore, it is clear that the portion of renal tubular sodium reabsorption regulated by renal sympathetic nerve traffic may be a substantial contribution to the overall physiologic regulation of renal sodium handling in the maintenance of external sodium balance. The fact that adrenergic blockade with guan-
ethidine impaired the ability of human subjects to normally conserve sodium in response to sodium deprivation further underscores the physiologic importance of this neurogenic control mechanism.

**Prospectus for Future Work**

The studies to date indicate that alterations in renal sympathetic nerve traffic can directly influence renal tubular sodium reabsorption. More information is required to give proper perspective to this control mechanism in the daily regulation of external sodium and water balance in both normal and pathophysiological states. Concurrent measurements of renal nerve activity and renal function are required in conscious unanesthetized animals subjected to maneuvers known to increase or decrease renal sympathetic nerve traffic. The importance of the quantitative contributions of this regulatory system may be more manifest under the extreme conditions of marked dietary sodium deprivation or excess. While interventions, either direct or reflex, designed to increase renal sympathetic nerve activity are relatively well characterized, acute surgical renal denervation to decrease renal sympathetic nerve traffic is not as precisely defined. However, there are several other maneuvers which are known to reflexly decrease renal sympathetic nerve impulse frequency (14, 29, 33, 38, 40, 42, 51, 53). Assessment of their effects on renal tubular sodium reabsorption and hemodynamics would significantly add to the acute surgical renal denervation experiments in the understanding of this control mechanism.

With the current evidence indicating a significant direct tubular effect of alterations in renal sympathetic nerve impulse frequency, one might consider whether the chronically denervated kidney would be deficient in its ability to respond to various stimuli. Is the renal secretory response to variations in sodium balance or renal perfusion pressure different? Does the renal hemodynamic and functional response to increased ureteral pressure differ in the innervated and chronically denervated kidney? Does chronic renal denervation impair the compensatory response of the kidney following deleterious interventions on the contralateral kidney (acute and chronic reduction in mass, renal artery occlusion, and ureteral occlusion)? The answers to these and similar questions should help to further define the physiologic role of the renal nerves.

Although existing ultrastructural data place the adrenergic nerve terminal with its content of catecholamine-containing neurosecretory granules in contact with the renal tubular epithelial cell basement membrane, little information is available on the nature of the ensuing events which result in enhanced renal tubular sodium reabsorption. Norepinephrine increases short-circuit current in the toad bladder (27) and frog skin (4), suggesting an increase in active sodium transport. Other information suggests that the effect of adrenergic stimulation on tubular sodium reabsorption may involve a cyclic nucleotide-mediated mechanism (24, 25). However, more information is needed on the cellular events that follow catecholamine release from the adrenergic nerve terminal: its translocation across tubular epithelial cell basement membrane, the nature and site of its interaction with the transporting cell, and the intracellular mechanism(s) responsible for the final effect of increased net sodium transport.

In 1973 a major review (15) of the intrarenal control of sodium excretion stated: "Even the possibility that renal nerve impulses have a more direct effect on renal tubular sodium reabsorption cannot be excluded with certainty." While it seems clear that renal sympathetic nerve impulses can, indeed, directly alter renal tubular sodium reabsorption, more information is required to understand the quantitative significance of this control mechanism in the daily overall maintenance of external sodium balance and to understand its cellular mechanism of action.

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