Sympathetic Neural Control of Vascular Muscle in Reduced Renal Mass Hypertension

William J. Stekiel, Stephen J. Contney, and Julian H. Lombard

Vascular smooth muscle (VSM) transmembrane potentials (E_m) were measured in situ in small branch arteries (150–300-μm o.d.), small branch veins (300–400-μm o.d.), arterioles (90–150-μm o.d.), and venules (80–250-μm o.d.) in the mesenteric and gracilis muscle and the arterioles and venules of cremaster muscle vascular beds in anesthetized rats with reduced renal mass hypertension (HT-RRM) and normotensive sham-operated RRM control rats. All rats were given a 4% NaCl diet for 2 weeks with water ad libitum. Relative to sham, HT-RRM mesenteric and gracilis arterial and venous vessels, but not the microvessels of the cremaster muscle bed, were less polarized during superfusion with normal physiological salt solution. Also relative to sham, hyperpolarization responses to local sympathetic neural (SNS) denervation with 6-hydroxydopamine were greater in mesenteric and gracilis small arteries, arterioles, veins, and venules but not in cremaster microvessels. The immediate (less than 5-minute) electrogentic depolarization response to local blockade of VSM Na^+-K^+ pump activity with 10^{-3} M ouabain was similar between each respective HT-RRM and sham vessel pair in each vascular bed. These results suggest that in all three vascular beds: 1) significant SNS control of VSM E_m and active tone exists all the way to the arterial and venous microvasculature (except cremaster venules); 2) in HT-RRM, such SNS control is elevated relative to sham in both arterial resistance and venous capacitance vessels in mesenteric and gracilis vascular beds but not in the cremaster microvessels; and 3) any circulating Na^+-K^+ pump inhibitors in the circulation of this volume-expanded model of hypertension do not appear to affect VSM tone in the vessels studied. (Hypertension 1991;17:1185–1191)

A significant body of experimental evidence supports an elevated sympathetic efferent neural (SNS) regulation of vascular smooth muscle (VSM) tone as one major causal factor in the pathogenesis and maintenance of the early phase of hypertension in both humans and genetic and salt-induced animal models.1-3 A major, long-standing question yet to be clarified concerning the etiology and maintenance of hypertension is the relation between salt intake with concomitant volume expansion and altered SNS regulation of blood pressure. Based on the observed necessity of hypothalamic salt/volume control centers for the normal regulation of blood volume and pressure,4-5 Folkow1 suggested that cardiovascular and pressure changes caused by central neurohormonal adjustments precede the cardiovascular autoregulatory events that are set into motion by salt-induced volume changes.5 In general, however, little direct evidence is available concerning the possibility of an elevated SNS regulation of VSM tone of resistance and capacitance-regulating blood vessels in volume-expanded models of hypertension. Therefore, based on the hypothesis that a poorly defined causal relation exists between salt-induced volume expansion and neural regulation of VSM tone, the major objective of the present study was to determine by measurement of in situ VSM transmembrane potentials (E_m), an index of VSM tone, whether SNS regulation is elevated in small and micro blood vessels of the hypertensive, volume-expanded, reduced renal mass (HT-RRM) rat relative to a normotensive, sham-nephrectomized rat with both on a high salt diet. In addition, in situ E_m measurements were made during initial blockade of the VSM electrogenic Na^+-K^+ pump. It has been postulated that a circulating, endogenous inhibitor of membrane-bound Na^+-K^+-ATPase is released in this volume-expanded model of hypertension and that one of its major actions is to increase VSM tone by the membrane depolarization resulting from blockade of the electrogenic Na^+-K^+ pump.6 The results of our study support a role for an elevated SNS regulation of VSM tone in both the resistance and
capacitance-regulating blood vessels of intestinal and skeletal muscle vascular beds.

**Methods**

Male Sprague-Dawley rats (weight, 250 g) were subjected to a 75% reduction in renal mass via a two-stage operation in which 50% of left kidney (both polar regions) was removed followed by a total right nephrectomy 2 weeks later (HT-RRM). Sham-operated control rats were subjected to similar surgical procedures, except for removal of renal tissue. After a 1-week recovery period, both groups of rats were placed on a 4% NaCl diet for 2 weeks.

At the time of the experiment, the animals were anesthetized intraperitoneally with 40 mg/kg ketamine-HCl (Ketaset, Aveco Co., Inc., Fort Dodge, Iowa) followed by 20–30 mg/kg sodium pentobarbital (Veterinary Laboratories, Inc., Lenexa, Kan.), and the mesenteric, gracilis muscle, or cremaster muscle vascular bed was prepared for $E_m$ measurements. During the experiment, the tissue was continuously superfused with normal physiological salt solution (PSS) at 37°C, pH 7.4, $PCO_2$ of 35–40 mm Hg, and $PO_2$ of 90–120 mm Hg. Its composition was (mM): NaCl, 119; KCl, 4.7; MgSO$_4$, 1.17; CaCl$_2$, 1.6; NaHCO$_3$, 24.0; NaH$_2$PO$_4$, 1.18; and EDTA, 0.026. Catheters were placed into the left femoral artery and vein, respectively, for pressure recording and supplemental anesthesia administration as needed.

For in situ measurements of $E_m$ in mesenteric vessels, a loop of jejunum and its attached mesentery were externalized through a midline abdominal incision and superfused with PSS. Short lengths of a small, flattened metal rod platform supporting the vessel into the Silastic rubber coating on a row of miniature tungsten wire pins inserted alongside the vessel into the Silastic rubber coating on a small, flattened metal rod platform supporting the vessel. Six to 10 individual artery and vein pairs (one pair per animal) were used for sequential $E_m$ measurements under the three experimental conditions. Approximately six impalements were averaged to obtain each $E_m$ value for a single vessel under a particular experimental condition. These averages were then used to calculate each mean $E_m$ and SEM illustrated in the bar graphs (Figures 1–3). Significance of differences between HT-RRM and sham in mean $E_m$ and their responses to chemical sympathectomy and Na$^+$-K$^+$ pump blockade was determined by analysis of variance with repeated measures using the css: GENERAL MANOVA STATS PLUS software program (Stat Soft, Tulsa, Okla.).

**Results**

**Whole Animal Data**

Mean body weight of the HT-RRM was significantly less than that of sham-operated rat. Heart rate and blood pressures measured in anesthetized animals during the initial PSS superfusion control period were significantly elevated in the HT-RRM (Table 1). A variable (0–20 mm Hg) decrease in arterial pressure occurred in both animal types during the period of time that included the local 20-minute superfusion with 6-OHDA and the 1 hour of washout with PSS. No significant decrease in arterial pressure was noted during the 5-minute superfusion with PSS containing 10$^{-3}$ M ouabain.

**Vessel Diameters in Normal, Control PSS**

An approximate measurement of outside diameters of blood vessels used for $E_m$ measurements was made using a calibrated scale mounted in one of the eye pieces of the dissecting microscope (Stereo Zoom

---

**TABLE 1. Body Weight and Hemodynamic Data for Reduced Renal Mass Rats and Their Sham-Operated Controls**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HT-RRM (g)</th>
<th>Sham-operated (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td>384±4.0</td>
<td>397±4.6</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>377±6.8</td>
<td>341±4.5</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>212±3.0</td>
<td>147±1.8</td>
</tr>
<tr>
<td>Systolic</td>
<td>148±2.1</td>
<td>104±1.7</td>
</tr>
<tr>
<td>Mean</td>
<td>169±2.3</td>
<td>118±1.6</td>
</tr>
</tbody>
</table>

Values are given as mean±SEM in anesthetized animals. All rats had been on 4% NaCl diet for 2 weeks.

*Significantly different from sham-operated reduced renal mass rats (RRM) (p<0.05).
A. Small Mesenteric Branch Artery

**FIGURE 1.** Bar graphs showing effect of local chemical sympathectomy (6-hydroxydopamine [6-OHDA]) and 10⁻³ M ouabain (ouab.) (during 5-minute exposure) on in situ vascular smooth muscle transmembrane potential (Em) mesenteric vessels in hypertensive, reduced renal mass (RRM) and normotensive sham-operated RRM control rats. For each type of paired artery and vein, sequential Em's are given as mean±SEM calculated using six to 10 average values obtained from each of six to 10 pairs (one per rat). In turn, each average value was obtained from approximately five impalements. Left side of each panel: Absolute value. Right side of each panel: Response to treatment. All rats had been on 4% NaCl diet for 2 weeks. *RRM significantly different from sham (p<0.05). †Significantly different from control physiological salt solution (PSS) (p<0.05).

In Situ Vascular Muscle Eₘ

**Mesenteric vessels.** The left side of each mesenteric vessel panel in Figure 1 illustrates that in control PSS, the VSM of each of the four HT-RRM mesenteric vessel types was significantly less polarized than VSM of respective sham vessels. Local sympathetic denervation produced a hyperpolarization in all HT-RRM and sham vessels. The difference in absolute magnitude of mean Eₘ between HT-RRM and sham was eliminated by the denervation for each respective type except for the first-order arteriole. Also, 10⁻³ M ouabain depolarized each HT-RRM and sham vessel compared with each respective Eₘ magnitude after local sympathetic denervation. During the 5-minute period of ouabain superfusion, the mean Eₘ level of each HT-RRM vessel was not significantly different relative to its respective sham vessel.

The right side of each mesenteric vessel panel in Figure 1 compares mean Eₘ hyperpolarization of HT-RRM relative to sham in response to local sympathetic denervation and mean Eₘ depolarization in response to superfusion with 10⁻³ M ouabain.
It is important to note that relative to sham vessels, the hyperpolarization response to local sympathetic denervation is greater in all HT-RRM vessels except the first-order arteriole. Also, this difference is greater on the venous than on the arterial side of the circulation. The \( E_m \) response of the two sham venous vessels to local denervation is relatively small. No significant differences exist between HT-RRM and sham in the depolarizing responses to \( 10^{-3} \) M ouabain in any of the four mesenteric vessel types studied.

**Gracilis muscle vessels.** For each of the four gracilis muscle vessels studied, the pattern of differences in mean \( E_m \) magnitudes between HT-RRM and sham under each experimental condition was very similar to that for respective mesenteric vessels (Figure 2). The same was true for the differences between HT-RRM and sham in the hyperpolarization response to local sympathetic denervation and in the depolarization response to \( 10^{-3} \) M ouabain. The only exception was in the HT-RRM gracilis first-order arteriole, where the hyperpolarization to local sympathetic denervation was significantly greater relative to sham than in the mesenteric bed.

**Cremaster muscle microvessels.** During the control period of superfusion with normal PSS, the VSM of HT-RRM cremaster muscle vessels was less polarized than VSM of sham vessels in only the first-order arteriole (Figure 3). As with the respective microvessel types in the mesenteric and gracilis muscle vascular beds (except mesenteric first-order arteriole), there was no longer a difference between HT-RRM and sham in absolute magnitudes of \( E_m \) after local sympathetic denervation. Also, such local sympathetic denervation produced a significant hyperpolarization in both first- and second-order cremaster arterioles, but the difference in hyperpolarization between HT-RRM and sham was not significant for each respective vessel size. Of particular note is the very small hyperpolarization that occurred in both HT-RRM and sham venous microvessels in response to local sympathetic denervation. This is in contrast to the relatively large local denervation-induced hyperpolarization in the VSM of venous microvessels in both mesenteric and gracilis muscle vascular beds. For each of the cremaster muscle microvessel pairs, the depolarization response to \( 10^{-3} \) M ouabain was the same for HT-RRM and sham, similar to respec-
A. Cremaster Muscle 1st Order Arteriole

B. Cremaster Muscle 2nd Order Arteriole

C. Cremaster Muscle 1st Order Venule

D. Cremaster Muscle 2nd Order Venule

**FIGURE 3.** Bar graphs showing effect of local chemical sympathectomy (6-hydroxydopamine [6-OHDA]) and $10^{-3}$ M ouabain (ouab.) (during 5-minute exposure) on in situ vascular smooth muscle transmembrane potential ($E_m$) of cremasteric vessels in hypertensive, reduced renal mass (RRM), and normotensive sham-operated RRM control rats. For each type of paired artery and vein, sequential $E_m$s are given as mean±SEM calculated using six to 10 average values obtained from each of six to 10 pairs (one per rat). In turn, each average value was obtained from approximately five impalements. Left side of each panel: Absolute value. Right side of each panel: Response to treatment. All rats had been on 4% NaCl diet for 2 weeks. *RRM significantly different from sham (p<0.05). †Significantly different from control physiological salt solution (PSS) (p<0.05).

**Discussion**

The $E_m$ results of the present study provide evidence that the SNS contributes significantly to the regulation of vascular resistance and capacitance as manifested by the absolute magnitude of VSM $E_m$ and its responses to local sympathetic denervation. In addition, the greater hyperpolarization response to local sympathetic denervation in HT-RRM small arteries and veins, relative to sham, suggests that SNS regulation of VSM $E_m$ (and tone) is clearly greater in these HT-RRM vessel types. However, the equal hyperpolarization responses to local sympathetic denervation in the cremaster muscle first- and second-order arterioles and venules and the mesenteric bed first-order arterioles do not provide evidence for a greater SNS regulation of VSM $E_m$ and tone in these HT-RRM microvessels. The reason for the presence of a significant difference between HT-RRM and sham responses to local sympathetic denervation in some but not all of the microvessels is not clear; it may be the result of a differential contribution of intraluminal pressure-induced myogenic changes in $E_m$ or of differences in the relative density of sympathetic innervation in these microvessels.12

The elevated SNS control of venous capacitance vessels in HT-RRM confirms the importance of a neural contribution to the reduction in venous compliance that may be an initiating factor in the hemodynamic sequence and autoregulatory mechanisms leading to the elevated total peripheral resistance in volume-expanded hypertension.5-11

The validity of a negative linear correlation between the absolute magnitude of graded $E_m$ changes and graded active contractile force changes (i.e., electromechanical coupling) is assumed in VSM of the small blood vessels used in the present study. Thus, the greater hyperpolarization response to local sympathetic denervation suggests an elevated SNS control of both $E_m$ and coupled active tone in VSM of resistance and capacitance-regulating blood vessels in the HT-RRM. A significant amount of experimental evidence exists in support of the validity of electromechanical coupling, particularly over the normal range of in situ $E_m$ magnitudes exhibited by VSM,8-10 when activated in vitro with physiological concentrations of endogenously occurring humoral...
agents, including oxygen. However, one unanswered question concerns the validity of the assumption that equal magnitudes of $E_m$ in VSM of vessels in hypertensive and normotensive animals represent equal magnitudes of active stress in respective VSM cell types. This question is difficult to answer—not because of problems in measuring in situ $E_m$ in blood vessels of hypertensive and normotensive rats but primarily because of problems in attempting to assess and compare active contractile force generated by single VSM cells in the vessel media of such animals. Some of the more important factors that can affect the physiological significance of such comparative assessments of active contractile force generation, particularly in isolated in vitro vessels either under resting conditions or in response to electrical or neurohumoral stimuli, include vessel length, axial stress, lumen geometry, intraluminal pressure, VSM cell circumferential orientation, passive length, and medial density.

The reasons for an elevated SNS control of resistance and capacitance-regulating blood vessels in this salt/volume-dependent HT-RRM model of established hypertension are not clear. In a study of hemodynamic changes using an HT-RRM model very similar to ours, Ylitalo and Gross also reported elevations of mean blood pressure and heart rate. In addition, they reported a sustained elevation of serum osmolality due to a threefold increase in serum urea retention resulting in a sustained enlargement of both extracellular fluid and intravascular volumes. Hence, they concluded that the hypertension and elevated heart rate resulted from an elevated cardiac output, in turn resulting from a sustained elevation of extravascular and intravascular fluid volumes.

However, this proposed mechanism for elevated blood pressure and heart rate does not preclude a contribution from an elevated SNS regulation in our HT-RRM. Such an elevation has recently been suggested to occur in rats made hypertensive by a 5/6 nephrectomy, even in the absence of a high salt diet. This suggestion is based on a measured elevation of serum creatinine, urea nitrogen, and plasma catecholamines despite an elevation of urinary excretion of catecholamines. Such a relation between uremia in rats with subtotal renal ablation and elevated SNS activity is further supported by an observed elevated release of norepinephrine into plasma of uremic patients despite an elevation of its metabolic clearance.

In situ $E_m$ measurements made in the present study do not provide a method of discerning loci involved in the increased SNS regulation in salt/volume-induced hypertension. However, much new evidence is available to implicate a salt-induced elevation in the central neural control of peripheral resistance in human essential, genetic, renal, and salt-sensitive, volume-expanded models of hypertension. Particularly in the latter, aberrations in the function of sodium receptors and the thirst center located in the anterior hypothalamus may play essential roles in the generation of salt/volume-induced hypertension. Besides exerting an effect at a central locus, it is possible that a high salt diet may lead to a greater enhancement of quanta1 release of neurotransmitter per nerve impulse at the vascular neuroeffector junction in the HT-RRM relative to sham. Such a relatively greater salt-induced enhancement has been shown to occur in vitro in isolated mesenteric artery branch vessels of spontaneously hypertensive rats with established hypertension relative to Wistar-Kyoto control rats. A third possible locus for increased SNS regulation control of $E_m$ and tone in our HT-RRM is the VSM membrane itself. This would imply an elevated VSM sensitivity to the adrenergic neurotransmitter (i.e., norepinephrine). As described above, comparative evaluation of the true active stress response of a single VSM cell in an isolated vessel maintained under its physiological conditions is difficult and remains to be done in the HT-RRM.

The lack of differences between HT-RRM and sham in the absolute magnitude of $E_m$ after local sympathetic denervation and in the depolarization in response to $10^{-3}$ M ouabain were unexpected results. Substantial evidence exists in support of a circulating, digitalislike factor in low renin, volume-expanded models of hypertension. This factor has been hypothesized to increase VSM tone by enhancing the concentration of Ca$^{2+}$ in the adrenergic synaptic cleft and in the VSM cell by reducing presynaptic uptake of norepinephrine and the electrogenic component of the VSM membrane $E_m$, respectively. Possible explanations for the lack of differences between HT-RRM and sham in ouabain-induced depolarizations in the present study include 1) absence of the digitalislike factor in our HT-RRM model despite a volume expansion, 2) absence of a depolarizing effect of the factor on the VSM membranes of vessels studied, and 3) competitive displacement of the factor by the relatively high concentration of ouabain used in the present study (neglecting the equality of mean $E_m$ after local sympathetic denervation). It should be emphasized that relative to the second possible explanation, the $E_m$ results of the present study do not eliminate the possibility that a circulating digitalislike factor, if present in our volume-expanded HT-RRM rat, could act directly to elevate VSM tone (e.g., by reducing the inwardly directed Na$^+$ gradient and consequently the rate of Na$^+$-Ca$^{2+}$ exchange). However, based on the significant contribution of an electrogenic Na$^+$-K$^+$ membrane pump to the $E_m$ of VSM, it is not clear how such an electromechanical coupling could occur.

In summary, the results of the present study suggest that the SNS contributes significantly to the elevated VSM tone of both resistance and capacitance-regulating blood vessels in HT-RRM used in the study. These vessels include the first-order arterioles and venules of the mesenteric and gracilis muscle, but not cremaster muscle, vascular beds. Evidence is lacking in the vessels used in the present study for a depolarizing action of
any circulating digitalis-like VSM Na⁺-K⁺ membrane pump inhibitor that may be present in this volume-expanded model of hypertension.

References


KEY WORDS • membrane potentials • microcirculation • renovascular hypertension • ouabain • vascular resistance
Sympathetic neural control of vascular muscle in reduced renal mass hypertension.
W J Stekiel, S J Contney and J H Lombard

*Hypertension*. 1991;17:1185-1191
doi: 10.1161/01.HYP.17.6.1185

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/17/6_Pt_2/1185

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Hypertension* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Hypertension* is online at:
http://hyper.ahajournals.org//subscriptions/