Altered β-Receptor Control of In Situ Membrane Potential in Hypertensive Rats

William J. Stekiel, Stephen J. Contney, and Nancy J. Rusch

Sympathetic neural activation of vascular smooth muscle β-receptors induces membrane hyperpolarization and arterial relaxation. This response, which likely is mediated by the G, protein-adenyl cyclase—cyclic AMP signaling cascade, is reduced in some hypertensive animal models and in human essential hypertension. Since reduced β-receptor-mediated vasodilation is a potential mechanism for enhanced arterial resistance, this study was designed to identify which step (or steps) in the β-receptor signaling cascade is altered in hypertension. Transmembrane potentials were recorded in situ in small first-order arterioles and venules of cremaster muscle from hypertensive, reduced renal mass rats and normotensive, sham-operated controls. Vascular muscle cells in arterioles and venules of hypertensive rats were 5–7 mV more depolarized than in respective vessels of control rats during superfusion with physiological salt solution. Hyperpolarization and depolarization responses were reduced in hypertensive rats during superfusion with a β-receptor agonist and antagonist, respectively, suggesting attenuated β-receptor responsiveness compared with normotensive rats. Furthermore, direct activation of G, protein by 10 ng/mL cholera toxin did not affect arterial or venous transmembrane potential in hypertensive rats, but hyperpolarized arterial and venous vascular muscle in normotensive controls by 17 mV. However, when the G, protein—adenylate cyclase coupling step of the β-receptor cascade was bypassed by using 10–5 M forskolin to directly activate adenylate cyclase, arterial and venous vascular muscle of hypertensive rats hyperpolarized by 25–27 mV. These data strongly suggest that the β-receptor-mediated cascade regulating arterial and venous vascular muscle hyperpolarization and vasodilation is reduced in this rat model of volume-expanded hypertension and implicate the G, protein—adenylate cyclase coupling step as the likely abnormal cellular event.

KEY WORDS • membrane potentials • vasodilation • cholera toxin • forskolin • G-proteins • hypertension, volume-dependent

An elevated sympathetic efferent control of vascular smooth muscle (VSM) tone has been demonstrated in various subgroups of human essential hypertension1–4 and in numerous rat models of this disease, including the spontaneously hypertensive rat (SHR),1,5,6 genetically salt-sensitive rat (Dahl S rat),7,8 and sodium-dependent renal models of hypertension in the rat (reduced renal mass [RRM]9 and one-kidney, renal wrap models10). However, despite its prevalence in the pathophysiology of hypertension, the abnormal cellular events mediating this enhanced sympathetic efferent regulation of VSM tone are still obscure.3

At the level of the VSM cell, one potential determinant of elevated sympathetically mediated VSM tone in hypertension may be a reduced β-receptor-mediated membrane hyperpolarization and vasodilation. The latter has been reported in VSM of SHR11 and salt-sensitive human hypertensive patients.12,13 This alteration may coexist with an unchanged or elevated α-adrenergic receptor function.3 In view of this, it is possible that a reduced β-receptor-mediated hyperpolarization also may favor an increased sympathetic excitatory control of in situ membrane potential (E, in VSM of RRM rats, as suggested recently by our laboratory.9 However, to define possible cellular sites of impaired β-adrenergic receptor response in small arteries and veins of RRM, one must consider the multiple events involved in the β-receptor-mediated cascade. After β-receptor activation, the guanine nucleotide binding protein G, likely activates adenyl cyclase to elevate cyclic adenosine monophosphate (cAMP) levels and induce VSM hyperpolarization and relaxation. Thus, in the present study of RRM VSM we investigated specific alterations in this intracellular cascade by comparing E, responses of in situ VSM of RRM and sham-operated control rats during pharmacological modulation of different events in the β-receptor-activated signaling cascade. The results suggest that β-receptor–mediated hyperpolarization is compromised in small blood vessels of RRM rats and that the underlying cellular abnormality likely exists at the G, protein level.

Methods

Experimental Animal Model

Adult male Sprague-Dawley rats (weight 250 g) (Sasco, Inc., Madison, Wis.) were subjected to a 75% reduction in renal mass by a two-stage operation under intraoperative anesthesia with 3 mg/kg acepromazine and 123 mg/kg ketamine-HCl (Aveco, Inc., Fort Dodge, Iowa).
Iowa) to produce a rat with RRM (HT RRM). Nor-motensive control rats were subjected to similar surgical procedures except for removal of renal tissue (NT SHAM). After a 1-week recovery period, both groups of rats were placed on a 4% NaCl diet for 2 weeks. At the end of this time, the animals were anesthetized intra-peritoneally with 40 mg/kg ketamine-HCl followed by 20–30 mg/kg sodium pentobarbital (Veterinary Labs., Inc., Lenexa, Kan.). Catheters were placed into the left femoral artery and vein, respectively, for continuous arterial pressure recording and supplemental anesthesia administration as needed. The cremaster muscle vascular bed was exteriorized, with collateral vessels between muscle and epididymis left intact. 14 Throughout the experimental protocols, the entire tissue preparation was superfused with a normal physiological salt solution (PSS) maintained at 37°C and adjusted to pH of 7.4, Pco2 of 35–40 mm Hg, and Po2 of 100–120 mm Hg. The PSS composition was (in mM): NaCl 119, KCl 4.7, MgSO4 1.17, CaCl2 1.6, NaHCO3 24, NaH2PO4 1.18, and EDTA 0.026.

In Situ Measurement of VSM E\( _m \)

For in situ measurement of VSM E\( _m \), small segments of first-order arterioles (1A) and venules (1V) were carefully dissected from the connective tissue without disturbing luminal blood flow and adventitial innervation. Measurements of E\( _m \) were made with Ag-AgCl wire and capillary glass microelectrodes filled with 3 M KCl and connected to a high impedance biological amplifier using measurement criteria previously described. 39 Tip diameters were ≤0.1 \( \mu \)m with impedances between 60 and 80 MO. Arterial pulsations were reduced by stabilizing the vessel wall with a row of miniature tungsten wire pins, also as previously reported. 9

Experimental Protocols

Three series of E\( _m \) measurements were made in three groups of 6–7 HT RRM and NT SHAM, respectively. In the first series, for each rat, five to six successful in situ impalements of VSM cells per vessel were made in cremaster 1A and 1V to obtain an E\( _m \) value during control PSS superfusion. Over the next 30 minutes, E\( _m \) measurements were repeated in the same 1A and 1V with a \( \beta \)-receptor agonist (10\(^{-6} \) M isoproterenol) or antagonist (10\(^{-4} \) M propranolol) in the PSS superfusate. Then E\( _m \) measurements were repeated during superfusion with normal PSS 1 hour after local chemical sympathectomy with 6-hydroxydopamine. 15 In the second series of experiments, the same protocol was used except that the E\( _m \) response to an \( \alpha \)-receptor blocker (10\(^{-4} \) M phentolamine) alone, and in combination with the \( \beta \)-receptor blocker 10\(^{-4} \) M propranolol, were measured in vessels of each rat model. In the third series of experiments, the same general protocol was used to evaluate the effect of 10 ng/ml chola toxin (a G\( _\text{ protein activator and chola toxin plus 10} ^{-3} \) M forskolin (a direct activator of adenyl cyclase) on in situ E\( _m \) of 1A and 1V of HT RRM and NT SHAM rats. All drugs were purchased from Sigma Chemical Company, St. Louis, Mo. Chola toxin was dissolved in PSS superfusate. Forskolin was dissolved in absolute ethanol as a 10\(^{-2} \) M stock solution and diluted in PSS to obtain a final concentration of 10\(^{-5} \) M in the superfusate. The final concentration of ethanol in the PSS superfusate was 0.1%, which did not affect E\( _m \) in 1A or 1V vessels.

Data Analysis

Approximately six impalements were averaged to obtain a single E\( _m \) value for each vessel type under a specific experimental condition. Each E\( _m \) mean±SEM illustrated in Tables 1–4 was calculated from single E\( _m \) values obtained from 6 or 7 HT RRM or NT SHAM rats. Significance of differences of control E\( _m \) and matched drug-induced responses between HT RRM and NT SHAM rats (p≤0.05) was determined by analyses of variance with repeated measures using the CSS/GENERAL MANOVA STATS PLUS software program (Stat Soft, Tulsa, Okla.) and Duncan’s Multiple Range Test.

Results

Whole Animal Data

After 2 weeks on a 4% NaCl diet, body weight was 419±5 g in NT SHAM (n=24) and 405±3 g in HT RRM (n=25) rats, showing similar values. Mean arterial pressure under anesthesia at this time was significantly higher in the HT RRM, averaging 179±2 mm Hg compared with 116±1 mm Hg in the NT SHAM rats. Heart rate also was elevated at 404±3 beats per minute compared with 362±4 beats per minute in HT RRM and NT SHAM rats, respectively. A variable (0–20 mm Hg) but sustained decrease in mean arterial pressure occurred in both HT RRM and NT SHAM rats after local chemical sympathectomy, which presumably was due to variable absorption of 6-OHDA.

Response of In Situ Vascular Smooth Muscle Membrane Potential to \( \beta \)-Adrenergic Receptor Agonist and Antagonist

In general, respective VSM E\( _m \) magnitudes of 1A and 1V were less in HT RRM compared with NT SHAM rats in control PSS (Tables 1–4). This difference did not reach statistical significance for 1A vessels shown in Table 2. Table 1 illustrates that stimulation of \( \beta \)-receptors by 10\(^{-6} \) M isoproterenol produced less hyperpolarization of both 1A and 1V from HT RRM compared with NT SHAM, consistent with a compromised \( \beta \)-receptor response in hypertension. In the same vessels, local sympathetic denervation by 6-OHDA did not affect E\( _m \) responses to isoproterenol, suggesting that reduced \( \beta \)-receptor modulation of HT RRM E\( _m \) is a function of the VSM cell and is independent of adrenergic innervation. Table 2 shows that 10\(^{-4} \) M propranolol depolarized VSM cells from NT SHAM, consistent with a loss of \( \beta \)-receptor–mediated hyperpolarization. However, \( \beta \)-receptor block did not change VSM E\( _m \) of HT RRM, due presumably to the already attenuated \( \beta \)-adrenergic response in this model. Elimination of neurosympathetic influences by 6-OHDA hyperpolarized VSM cells from both HT RRM and NT SHAM rats similarly, implying that sympathetic neural influences other than those mediated by \( \beta \)-receptors have a net depolarizing effect that is comparable between VSM of NT SHAM and HT RRM rats.
Response of In Situ Vascular Smooth Muscle Membrane Potential to α-Adrenergic Antagonist

Table 3 illustrates the isolated role of α-receptors in establishing \( E_m \) of VSM cells in situ. α-Adrenergic receptor blockade by 10^{-6} M phentolamine hyperpolarized 1A and 1V of both rat models, compatible with loss of α-receptor-mediated depolarization. Further addition of 10^{-4} M propranolol did not change \( E_m \) of HT RRM VSM, again suggesting an absence of β-receptor influence. In contrast, propranolol induced a prominent depolarization in both 1A and 1V of NT SHAM rats, indicating a large β-receptor-mediated contribution to \( E_m \) amplitude in NT SHAM vessels.

Response of In Situ Vascular Smooth Muscle Membrane Potential to Cholera Toxin and Forskolin

Table 4 illustrates that 10 ng/ml cholera toxin, an activator of the guanine nucleotide binding protein \( G_i \), caused large hyperpolarizations (17 mV) in 1A and 1V of NT SHAM but had minimal effect on \( E_m \) of HT RRM vessels. In contrast, addition of 10^{-7} M forskolin (a direct activator of adenylyl cyclase) to the PSS in the presence of cholera toxin had no further effect on VSM \( E_m \) of NT SHAM vessels but hyperpolarized the HT RRM 1A and 1V (26 mV) to a VSM \( E_m \) level equal to that measured previously in the NT SHAM vessels.

**Discussion**

It is important initially to acknowledge that the conclusions of the present study are based on two assumptions. First, the design of this study is based on the premise that alteration of α- and β-receptor activa-

**Table 1. In Situ Transmembrane Potential Responses of Cremaster Muscle First-Order Arterioles and Venules to β-Receptor Antagonist and Local Chemical Sympathectomy in Reduced Renal Mass Hypertensive and Sham-Operated Control Rats**

<table>
<thead>
<tr>
<th>Vessel and condition</th>
<th>Mean ( E_m ) (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT SHAM</td>
<td>HT RRM</td>
</tr>
<tr>
<td><strong>1A arteriole</strong></td>
<td></td>
</tr>
<tr>
<td>Control PSS (1)</td>
<td>-44±1.0</td>
</tr>
<tr>
<td>Isoproterenol (2)</td>
<td>-62±1.2†</td>
</tr>
<tr>
<td>( \Delta E_m ) (1)</td>
<td>+18±1.0</td>
</tr>
<tr>
<td>Isoproterenol+denervation (3)</td>
<td>-62±0.7†</td>
</tr>
<tr>
<td>( \Delta E_m ) (2)</td>
<td>-0.3±1.1</td>
</tr>
<tr>
<td><strong>1V venule</strong></td>
<td></td>
</tr>
<tr>
<td>Control PSS (1)</td>
<td>-51±0.9</td>
</tr>
<tr>
<td>Isoproterenol (2)</td>
<td>-68±1.8†</td>
</tr>
<tr>
<td>( \Delta E_m ) (1)</td>
<td>+17±1.1</td>
</tr>
<tr>
<td>Isoproterenol+denervation (3)</td>
<td>-68±0.6†</td>
</tr>
<tr>
<td>( \Delta E_m ) (2)</td>
<td>-0.5±1.8</td>
</tr>
</tbody>
</table>

\( E_m \), transmembrane potential; NT SHAM, normotensive sham-operated control rats; HT RRM, hypertensive 75% reduced renal mass rats; 1A, first-order arterioles; PSS, physiological salt solution; 1V, first-order venules; positive \( \Delta E_m \), hyperpolarization; negative \( \Delta E_m \), depolarization; isoproterenol, 10^{-6} M isoproterenol; denervation, local chemical sympathectomy with 6-hydroxydopamine. Both animal groups were fed 5% NaCl diet for 2 weeks. Values are mean±SEM of \( E_m \) (or \( \Delta E_m \)).

\(*p<0.05\) different from NT SHAM.

\(†p<0.05\) different from control PSS.

\(‡p<0.05\) different from zero.

**Table 2. In Situ Transmembrane Potential Responses of Cremaster Muscle First-Order Arterioles and Venules to β-Receptor Antagonist and Local Chemical Sympathectomy in Reduced Renal Mass Hypertensive and Sham-Operated Control Rats**

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</tr>
<tr>
<td><strong>1A arteriole</strong></td>
<td></td>
</tr>
<tr>
<td>Control PSS (1)</td>
<td>-41±1.2</td>
</tr>
<tr>
<td>Propranolol (2)</td>
<td>-38±0.9†</td>
</tr>
<tr>
<td>( \Delta E_m ) (1)</td>
<td>-4±1.5‡</td>
</tr>
<tr>
<td>Propranolol+denervation (3)</td>
<td>-52±1.3†</td>
</tr>
<tr>
<td>( \Delta E_m ) (2)</td>
<td>+14±1.4*</td>
</tr>
<tr>
<td><strong>1V venule</strong></td>
<td></td>
</tr>
<tr>
<td>Control PSS (1)</td>
<td>-53±0.9</td>
</tr>
<tr>
<td>Propranolol (2)</td>
<td>-47±1.9†</td>
</tr>
<tr>
<td>( \Delta E_m ) (1)</td>
<td>-6±1.7†</td>
</tr>
<tr>
<td>Propranolol+denervation (3)</td>
<td>-55±2.0</td>
</tr>
<tr>
<td>( \Delta E_m ) (2)</td>
<td>+9±2.9†</td>
</tr>
</tbody>
</table>

\( E_m \), transmembrane potential; NT SHAM, normotensive sham-operated control rats; HT RRM, hypertensive 75% reduced renal mass rats; 1A, first-order arterioles; PSS, physiological salt solution; 1V, first-order venules; positive \( \Delta E_m \), hyperpolarization; negative \( \Delta E_m \), depolarization; propranolol, 10^{-6} M propranolol; denervation, local chemical sympathectomy with 6-hydroxydopamine. Both animal groups were fed 5% NaCl diet for 2 weeks. Values are mean±SEM of \( E_m \) (or \( \Delta E_m \)).

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Experimental justification for this idea has come from many laboratories and indicates that adrenergic receptors regulate vascular excitability by modulation of VSM transmembrane potential. Second, to correlate \( E_m \) changes to vascular contraction, we have assumed that...
Another principal finding of the present study is that the abnormal cellular component underlying the reduced β-receptor-induced hyperpolarization of HT RRM vessels is likely the GTP-binding protein \( G \). This conclusion is supported by 1) the minimal hyperpolarizing response observed during direct activation of \( G \) by cholera toxin, which suggests an inability of \( G \) to activate adenyl cyclase in small arteries and veins from HT RRM rats, and in contrast, 2) the large and presumably normal hyperpolarization of VSM \( E_m \) in HT RRM rats observed during direct activation of adenyl cyclase by forskolin. Conceptually combined, the latter findings suggest that events in the VSM β-receptor cascade distal to, and including, the adenyl cyclase enzyme are not altered in HT RRM vessels and imply a reduced activation of adenyl cyclase by \( G \) as the primary aberration in this signaling cascade.

These findings closely parallel the results of vascular reactivity studies by Asano et al.\(^{11}\), who reported attenuated relaxation responses of SHR femoral and mesenteric arterial strips to cholera toxin but not to forskolin. Using a radioligand binding technique, the same authors also have demonstrated that the decreased responsiveness of the β-receptor vasodilatory cascade in SHR arteries is not associated with changes in β-receptor function or membrane density.\(^{11}\) Thus, they hypothesize that a localized alteration of \( G \) protein is responsible for the attenuated relaxation of SHR arteries. Our measurements in HT RRM vessels indicate reduced VSM \( E_m \) response to cholera toxin but normal \( E_m \) response to forskolin and concur with their hypothesis.\(^{11}\) Our findings further show that reduced β-receptor responsiveness in VSM of HT RRM rats is characterized by a loss of β-receptor-mediated membrane hyperpolarization. The cellular basis for this hyperpolarization in normal VSM is not defined by experiments in this study, but studies by others have linked the β-receptor signaling cascade to activation of membrane K⁺ channels in cultured aortic VSM.\(^{20}\) This implies that reduced β-receptor-mediated vasodilation in some hypertensive models may be related to an inability to activate this membrane ionic pathway for hyperpolarization. In this situation, the enhanced sympathetic neural control demonstrated in the HT RRM would favor α-receptor-mediated depolarization and contraction, contributing to elevated VSM tone in hypertension.

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