Reduced Electrogenic Sodium-Potassium Pump in Arterioles During Renovascular Hypertension

JULIAN H. LOMBARD, WILLIAM L. JOYNER, AND WILLIAM J. STEKIEL

SUMMARY The goal of this study was to assess the role of the electrogenic Na\(^+\)-K\(^+\) pump in controlling active tone in cremasteric arterioles of normotensive hamsters and hamsters with bilateral (two-kidney, two figure-8) Grollman hypertension. Arterioles of both groups exhibited a large transient dilation when the Na\(^+\)-K\(^+\) pump was stimulated by superfusing the cremaster muscle with physiological salt solution containing 15 mM K\(^+\) after 20 minutes of 0 mM K\(^+\) superfusion. Arteriolar dilation in response to 15 mM K\(^+\) was significantly smaller in the hypertensive animals than in sham-operated controls. Ouabain (10\(^{-5}\) M and 10\(^{-3}\) M) inhibited arteriolar dilation in response to 15 mM K\(^+\) in both groups of animals. Resting diameters, total active tone (assessed by application of 10\(^{-4}\) M adenosine), and arteriolar responses to inhibition of the Na\(^+\)-K\(^+\) pump by superfusion with 0 mM K\(^+\) or ouabain were not significantly different in normotensive and hypertensive animals. These data indicate that an electrogenic Na\(^+\)-K\(^+\) pump can regulate active tone in cremasteric arterioles, and that the maximum response of this pump to stimulation with 15 mM K\(^+\) is reduced in arterioles of hamsters with two-kidney Grollman hypertension. (Hypertension 9 [Suppl III]: III-86–III-90, 1987)

KEY WORDS • electrogenic sodium-potassium pump • microcirculation • two-kidney Grollman hypertension • vascular smooth muscle • renal wrap hypertension • ouabain
Methods

Male golden hamsters (Mesocricetus auratus), obtained from Charles River Breeding Laboratories (Wilmington, MA, USA), were anesthetized with sodium pentobarbital (60 mg/kg i.p.). Hypertension was induced in 23 animals by tying figure-8 ligatures around both kidneys, as described by Click et al.1 Animals in the control group (n = 22) were subjected to identical surgical procedures, except for placement of ligatures around the kidneys. After the animals recovered from anesthesia, they were housed in the Animal Resource Center and allowed normal food and water ad libitum for 3 to 4 weeks. Hamsters with this form of Grollman hypertension exhibit a significant increase in blood volume (Stacy and Joyner, personal communication, 1986), and an enhanced arteriolar reactivity to vasoactive agonists5 relative to their normotensive controls.

On the day of the experiment, hamsters were anesthetized with pentobarbital (60 mg/kg i.p.). A jugular vein was cannulated for the administration of supplemental anesthesia as necessary, the trachea was cannulated to ensure a patent airway, and a carotid artery was cannulated for continuous monitoring of arterial pressure with a Statham transducer (Gould Medical Products Division, Oxnard, CA, USA) and Grass polygraph (Grass Instruments, Quincy, MA, USA). After the initial surgery, the cremaster muscle was prepared for observation via television microscopy and internal diameters of third and fourth order arterioles were measured with a movable raster line system operated through a Model 321 Colorado Video Analyzer (Colorado Video, Boulder, CO, USA). During the experiment, the tissue was continuously superfused at 35°C with bicarbonate-buffered physiological salt solution (PSS) equilibrated with 0% O2, 5% CO2, 95% N2 in a pharmacological organ bath, which served as a delivery reservoir. The normal PSS used in these experiments had the following ionic composition (mM): NaCl, 131.9; KCl, 4.7; CaCl2, 2.0; MgSO4, 1.17; and NaHCO3, 20.0. After the initial equilibration in normal PSS (30–60 minutes), active tone in the arterioles was assessed by measuring the dilation that occurred in response to a brief application of 10-4 M adenosine. After recovery from adenosine, neurogenic tone was assessed by changing the tissue with a test solution delivered from an adjacent reservoir maintained at the same temperature and equilibrated with the same gas mixture. Abrupt changes in [K+]o were achieved by using a stopcock system to change between the two delivery reservoirs. Changes in PSS K+ concentration were balanced by an opposite change in Na+ concentration in order to maintain osmotic balance. Phentolamine (10-6 M) and propranolol (10-6 M) were also added to the PSS before changing K+ concentration in order to block any effects of neurotransmitter release during exposure to K+-free solution or ouabain. Temperature changes were avoided by draining off any superfusate that had cooled in the delivery lines prior to changing solutions.

After 40 minutes of adrenergic receptor blockade, [K+]o was reduced from 4.7 to 0 mM to inhibit the electrogenic Na+-K+ pump in the arterioles. After 20 minutes in K+-free solution, [K+]o was abruptly elevated from 0 mM to 15 mM by changing delivery reservoirs. After 10 minutes exposure to 15 mM K+, 4.7 mM K+ superfusion was restored and the arterioles were allowed to recover for 30 to 60 minutes in normal PSS. In most experiments, adrenergic responses were blocked again by adding phentolamine and propranolol to the PSS after the recovery period. Forty minutes later, ouabain was added to the superfusion solution to achieve a final concentration of 10-5 M or 10-3 M. Arteriolar responses to changes in [K+]o were retested after 20 minutes of ouabain superfusion, utilizing the same protocol as before (i.e., 0 mM K+ superfusion for 20 minutes followed by 15 mM K+ for 10 minutes).

Data were summarized as mean ± standard error of the mean. Differences between means were assessed via analysis of variance followed by a Newman-Keuls test.11 A probability level of p<0.05 was considered to be statistically significant.

Results

Baseline Data

At the time of the experiment, mean arterial pressure was significantly elevated (p<0.05) in hamsters with Grollman hypertension (155 ± 4 mm Hg, n = 23) relative to their sham-operated controls (127 ± 4 mm Hg, n = 22). However, heart rates (364 ± 11 beats/min in the hypertensive animals versus 349 ± 8 beats/min in the normotensive animals) and body weights (111 ± 4 g in the hypertensive animals vs 116 ± 2 g in the normotensive animals) were not significantly different in the two groups.

Resting Diameters and Arteriolar Responses to Adenosine and Tetrodotoxin

Resting diameters of third and fourth order arterioles were not significantly different in the hypertensive and normotensive animals. The mean diameter (± SE) of third order vessels was 24 ± 1 μm in both the hypertensive group (n = 24) and the normotensive group (n = 27). Mean diameters of fourth order arterioles were 14 ± 1 μm in the normotensive group (n = 29) and 13 ± 1 μm in the hypertensive group (n = 26). Arteriolar responses to tetrodotoxin and adenosine were also similar in the hypertensive and normotensive animals (Figure 1).

Arteriolar Responses to Changes in K+ Concentration of Superfusion Solution

Figure 2 summarizes arteriolar responses to changes in [K+]o in the hypertensive and normotensive animals. There were no significant differences in arteriolar responses to 0 mM K+ superfusion between the two
Alterations in Na⁺-K⁺ pump activity or vascular responses to K⁺ have been reported in several forms of hypertension. For example, electrogenic Na⁺-K⁺ pump activity appears to be enhanced in VSM of groups. When the K⁺ concentration of the PSS was abruptly increased from 0 mM to 15 mM, both orders of arterioles exhibited a transient dilation, which subsided after 5 to 10 minutes in 15 mM K⁺. The maximum dilation in response to 15 mM K⁺ superfusion was significantly smaller in the hypertensive animals than in their normotensive controls.

**Effect of Ouabain on Arteriolar Responses to Changes in K⁺ Concentration**

Table 1 summarizes the effects of ouabain upon resting diameters in normal PSS and upon arteriolar dilation in response to 15 mM K⁺ superfusion in normotensive and hypertensive hamsters. Ouabain inhibited arteriolar dilation in response to 15 mM K⁺ in both groups of animals. There were no significant differences in the effect of ouabain upon resting diameters in the hypertensive and normotensive animals.

**Discussion**

Although the electrogenic Na⁺-K⁺ pump in VSM may have an important role in controlling vascular resistance and modulating vessel reactivity in vivo, it is difficult to precisely determine its role in the control of the peripheral circulation, since the electrogenic component of VSM Eₚ varies considerably in the blood vessels in which it has been studied. Microcirculatory studies have suggested that the electrogenic Na⁺-K⁺ pump may be an important regulator of arteriolar tone in some tissues (e.g., cat pial circulation, muscular portion of the hamster cheek pouch, and hamster cremaster muscle), but not in others (e.g., the guinea pig ileal submucosa). Although Duling and Kuschinsky et al. proposed that the electrogenic Na⁺-K⁺ pump may mediate arteriolar responses to changes in external K⁺ concentration, they did not specifically test for its role in controlling arteriolar diameter.

One of the goals of the present study was to test the hypothesis that an electrogenic Na⁺-K⁺ pump can regulate active tone in skeletal muscle arterioles of normotensive and hypertensive hamsters. This was accomplished by measuring arteriolar diameters during changes in superfusion solution K⁺ concentration in the presence and absence of ouabain. This is a classic test for the presence of electrogenic pump mechanisms in blood vessels, since changes in active VSM tone in response to changes in [K⁺], parallel the effects of many known variables upon Na⁺,K⁺-ATPase (e.g., internal Na⁺ concentration, ouabain, and temperature reduction). In our experiments, cremasteric arterioles exhibited a large transient dilation when [K⁺] was abruptly elevated from 0 mM to 15 mM. The dilation of the vessels in response to the change from 0 mM to 15 mM K⁺ was inhibited by ouabain, demonstrating that cremasteric arterioles possess an electrogenic Na⁺-K⁺ pump that can regulate their active tone.

![Figure 1](image1.png)  
**Figure 1.** Response of third and fourth order cremasteric arterioles to neural blockade with 10⁻⁷ g/ml tetrodotoxin (TTX) or maximal dilation with 10⁻⁴ M adenosine (ADO) in 24 to 26 hamsters with two-kidney, two-figure-8 Grollman hypertension (shaded bars) and 27-29 sham-operated controls (open bars). Data are expressed as mean change ± SE from diameter at the end of 0 mM K⁺ superfusion. Parentheses indicate number of animals, and asterisks denote a significant difference (p<0.05) in arteriolar response to 15 mM K⁺ in normotensive and hypertensive animals. See text for details.

![Figure 2](image2.png)  
**Figure 2.** Response of third and fourth order arterioles of normotensive hamsters (open bars) and hypertensive hamsters (shaded bars) to 20-minute superfusion of cremaster muscle with physiological salt solution (PSS) containing 0 mM K⁺ (left panel) followed by abrupt change to 15 mM K⁺ superfusion (right panel). Data for 0 mM K⁺ superfusion are expressed as mean change ± SE from diameter in normal (4.7 mM K⁺) PSS. Data for 15 mM K⁺ superfusion are expressed as maximum change (mean ± SE) from diameter at the end of 0 mM K⁺ superfusion. Parentheses indicate number of animals, and asterisks denote a significant difference (p<0.05) in arteriolar response to 15 mM K⁺ in normotensive and hypertensive animals. See text for details.
deoxycorticosterone acetate (DOCA)–salt hypertensive pigs and spontaneously hypertensive rats (SHR), while dilator responses to intra-arterial K+ infusion are reduced in human essential hypertensives, two-kidney, one clip renal hypertensive rats, and perinephritic hypertensive dogs. There is also evidence that a circulating humoral factor may inhibit the Na+-K+ pump in many forms of volume-expanded hypertension.

In our experiments, the transient dilation of arterioles in response to 15 mM K+ superfusion was significantly smaller in the hypertensive animals than in their normotensive controls. Since resting diameters and arteriolar responses to adenosine were virtually identical in hypertensive and normotensive animals, the smaller response to 15 mM K+ in the hypertensive animals does not reflect a difference in the reserve capacity for arteriolar dilation in the two groups, but must be due to an actual reduction in the response of the electrogenic Na+-K+ pump to stimulation with an elevated [K+]o. This reduced electrogenic pump response may be due to a number of factors, including a reduction in the number and/or affinity of Na+-K+ pump sites in the VSM cells, changes in key regulatory variables such as intracellular Na+, or inhibition of existing Na+-K+ pump sites by a circulating humoral factor.

The similarity of resting diameters and arteriolar responses to adenosine in the hypertensive and normotensive animals might suggest that a reduced electrogenic Na+-K+ pump activity in VSM cells does not contribute to arteriolar constriction in this form of hypertension. However, caution must be exercised when extrapolating experimental findings from an individual vascular bed to the entire circulation. Although the exact contribution of reduced electrogenic Na+-K+ pump activity to arteriolar constriction in renovascular hypertension remains unclear, an early ionic or electrogenic alteration may be essential for the initiation of an enhanced vascular reactivity and a sustained elevation of blood pressure in hamsters with bilateral Grollman hypertension. Further studies of the primary and secondary changes in ionic permeabilities and electrogenic pump activity in VSM cells of hypertensive animals should provide valuable insight into the mechanisms that control vascular reactivity and total peripheral resistance in hypertension.

Acknowledgments
The authors thank Joann Schmidt and Glenda Sharpe for their excellent technical assistance.

References
Reduced electrogenic sodium-potassium pump in arterioles during renovascular hypertension.

J H Lombard, W L Joyner and W J Stekiel

Hypertension. 1987;9:III86
doi: 10.1161/01.HYP.9.6_Pt_2.III86

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/9/6_Pt_2/III86

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/