Oscillatory Contractions in Tail Arteries from Genetically Hypertensive Rats

FRED S. LAMB, J. HURLEY MYERS, MICHAEL N. HAMLIN, AND R. CLINTON WEBB

SUMMARY This study characterizes a cellular mechanism for oscillatory contractions induced by norepinephrine in vascular smooth muscle from spontaneously hypertensive stroke prone rats (SHRSP). Helically cut strips of tail arteries from SHRSP and normotensive Wistar-Kyoto rats (WKY) were mounted in a muscle bath for measurement of isometric force generation. Norepinephrine-induced responses of arteries from SHRSP were characterized by fluctuations in contractile activity, whereas those in arteries from WKY remained constant with time. The magnitude of the oscillatory contractile activity (frequency \times \text{mean amplitude}) varied directly with norepinephrine concentration ($5.9 \times 10^{-8}$ to $1.8 \times 10^{-7}$ M). The oscillatory contractile activity varied inversely with the potassium concentration (0.1-5.0 mM) of the buffer solution and directly with the calcium concentration (0.1-5.0 mM). The oscillatory activity was converted to maintained contraction by barium ($10^{-4}$ M), quinidine ($3 \times 10^{-6}$ M), sparteine ($10^{-3}$ M), D-600 ($10^{-7}$ M), and nifedipine ($10^{-4}$ M). Tetraethylammonium and 3,4-diaminopyridine, inhibitors of voltage-dependent potassium channels, did not alter the oscillatory contractile activity induced by norepinephrine. These observations suggest that oscillatory contractile activity in tail arteries from SHRSP is caused by an abnormal variation in potassium efflux during stimulation with norepinephrine. The altered potassium efflux appears to be related to calcium entry, which is sensitive to inhibition by channel blockers. This altered membrane property may contribute to changes in vascular sensitivity in hypertension.

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KEY WORDS • potassium • calcium • norepinephrine • quinidine • sparteine • calcium channel blockers

A complex series of alterations in both structure and function of blood vessels contributes to the development of arterial hypertension. These alterations include increases in sensitivity to contractile agonists and altered membrane ionic permeabilities. Jones and Miller have proposed that these changes in vascular smooth muscle function in hypertension may parallel the difference between tonic and phasic types of smooth muscle. Indeed, isolated blood vessels from hypertensive animals often exhibit spontaneous rhythmic contractions.

Previous experiments in our laboratory have identified an unusual contractile response in isolated tail arteries from spontaneously hypertensive stroke prone rats (SHRSP). These vessels manifest a contractile response to several agonists (norepinephrine, serotonin, and histamine, but not angiotensin II or vasopressin) that is characterized by large oscillations in force development. These oscillations are not observed in tail artery strips from SHR or normotensive Wistar-Kyoto rats (WKY). The purpose of this study was to identify the cellular mechanism responsible for oscillatory contractions induced by norepinephrine in tail arteries from SHRSP.

Methods

Adult male and female SHRSP and WKY (age matched) were obtained from rat colonies maintained in the Department of Anatomy, University of Michigan (Ann Arbor, MI). Systolic blood pressures were measured by an indirect tail cuff method (SHRSP = 214 ± 7 mm Hg, n = 17; WKY = 125 ± 3 mm Hg, n = 3). Rats were anesthetized with sodium pentobarbital (50 mg/kg), and tail arteries were removed and cut helically into strips (0.7 \times 10 mm). These strips were mounted in an organ chamber containing physiological salt solution (PSS) for measurement of isometric force development, as described elsewhere.
All preparations were allowed to equilibrate for 90 minutes before an experiment was begun. The PSS was maintained at 37°C and was aerated with a mixture of 95% O₂ and 5% CO₂. The composition of the PSS (mmol/L) was as follows: NaCl, 130; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄·7H₂O, 1.17; NaHCO₃, 14.9; CaCl₂·2H₂O, 1.6; dextrose, 5.5; and CaNa₂EDTA, 0.03. The potassium and calcium concentrations of the buffer were altered without compensating for changes in tonicity.

Contractile responses to norepinephrine were measured in all experiments. Norepinephrine (1.8 × 10⁻⁹ to 1.8 × 10⁻⁵ M) was added to the organ bath, and the strips were allowed to contract for 20 minutes. The drug was rinsed from the bath between responses, and the vessels were allowed to recover to baseline. Force generation was measured as the average of minimum points of oscillatory contractions over the final 10 minutes of each response (see Figure 1). Oscillatory activity was defined as the product of mean contractile amplitude and the frequency over this 10-minute period. The effects of pharmacological interventions and alterations in extracellular ionic concentrations on oscillatory activity induced by norepinephrine were determined. All responses are normalized as percent change from control value at a similar magnitude of contractile force.

A variety of compounds were used in this study: sparteine (Aldrich Chemical Co., Milwaukee, WI); tetraethylammonium chloride (TEA, Sigma Chemical Co., St. Louis, MO); BaCl₂ (Aldrich Chemical Co.); quinidine gluconate (Eli Lilly Co., Indianapolis, IN); 3,4-diaminopyridine (Aldrich Chemical Co.); nitroprusside sodium (Hoffman La-Roche, Inc., Nutley, NJ); nifedipine (DelBay Pharmaceuticals, Inc., Bloomfield, NJ); and D-600 (Knoll Pharmaceutical Co., Whippany, NJ).

Data are displayed as means ± SEM. Statistical comparisons were performed by paired t test. A p value < 0.05 was considered significant.

Results

The tracings in the left panel of Figure 1 illustrate the procedure used to evaluate oscillatory contractile activity in tail arteries from SHRSP. In response to norepinephrine (5.9 × 10⁻⁹ to 1.8 × 10⁻⁵ M), these vessels displayed an initial fast component of contraction followed by a maintained component consisting of oscillating contractions and relaxations beginning 3 to 6 minutes into the response. Contractile responses to norepinephrine in tail arteries from WKY did not show this oscillatory contractile activity.

The right panel of Figure 1 shows the relationship between norepinephrine concentration and oscillatory activity. Force development is defined as the average magnitude of contraction from baseline to the bottom (trough) of the oscillating contraction during the final 10-minute interval. Both oscillatory activity and force development were dependent on the concentration of norepinephrine added to the muscle bath. Values are the mean ± SEM for eight SHRSP.

![Figure 1](https://hyper.ahajournals.org/cover.png)
contractile activity and force generation in tail arteries from SHRSP. Both force development and oscillatory activity were dependent on the concentration of norepinephrine. Maximal oscillatory activity occurred at approximately $10^{-7}$ M norepinephrine. In all subsequent figures, the oscillatory contractile activity is expressed as a percent change from a control response (i.e., idealized control values were predicted from these curves) of similar force generation to account for changes in contractile magnitude induced by the experimental intervention.

Oscillatory contractile activity was increased when the concentration of extracellular potassium was decreased from 5.9 to 3.0 mM (Figure 2). Elevating extracellular potassium levels inhibited oscillatory activity induced by norepinephrine.

Because alterations in potassium concentration influenced the oscillatory activity, several experiments were performed to test the effects of drugs that inhibit membrane potassium channels (each drug was added 5 minutes before the response was measured). Barium, sparteine, and quinidine inhibited oscillatory contractile activity (Figure 3), whereas 3,4-diaminopyridine and tetraethylammonium did not significantly alter the oscillatory contractile activity induced by norepinephrine. In addition to inhibiting the oscillatory contractile responses, quinidine inhibited contractile responses to norepinephrine. As the magnitude of force generation may influence oscillatory activity (see Figure 1), a nonspecific vasodilator, nitroprusside, was used to alter contractile force generation. Nitroprusside (4 x $10^{-4}$ to 4 x $10^{-6}$ M) inhibited contractile force generation but did not significantly affect oscillatory activity normalized for the reduced force developed.

The magnitude of oscillatory activity was inhibited by low concentrations of calcium ion (0.1–0.5 mM) and enhanced when the concentration of calcium was increased from 1.6 to 5.0 mM (Figure 4). The calcium channel blockers D-600 and nifedipine inhibited the oscillatory contractile activity induced by norepinephrine (Figure 5).

Because D-600 and nifedipine also inhibited contractile force development, one further experiment was performed to evaluate the ability of these drugs to inhibit oscillatory contractile activity (Figure 6). In these experiments the strips were made to contract in response to norepinephrine (3 x $10^{-7}$ M). Fifteen minutes into this contraction interval, D-600, nifedipine (not shown), or nitroprusside was added to the muscle bath. All three drugs inhibited force development, but only D-600 and nifedipine inhibited the oscillatory contractile activity. Fifteen minutes after addition of the inhibitor, the concentration of norepinephrine in the muscle bath was increased to 1.8 x $10^{-5}$ M. The strips contracted in response to this concentration of norepinephrine, but those treated with the calcium channel blockers did not show oscillatory contractile activity at this level of increased force development. Oscillatory contractile activity was not inhibited by nitroprusside under these conditions. As quinidine also reduced force development (see above), this same protocol was used to determine if its inhibitory effect on oscillatory contractile activity was related to decreased force generation. Increasing the magnitude of force development by elevating the norepinephrine concentration did not induce oscillatory contractile activity in strips treated with quinidine (see Figure 6).

**Discussion**

The SHRSP are a substrain of SHR that acquire elevated arterial pressures and display a high incidence of cerebral lesions when fed a diet low in certain amino acids. Little information is available about vascular function in this experimental model of hypertension, but it seems likely that the model shares several pathophysiological characteristics with SHR.
In SHR, multiple alterations in cell membrane function have been described that may relate to the cellular mechanism for oscillatory contractile activity in blood vessels from SHRSP. Vascular smooth muscle cells from SHR show an increased membrane permeability to sodium, potassium, and chloride and a less negative membrane potential when active electrogenic transport is inhibited. Activation of aortic smooth muscle of SHR with norepinephrine causes a greater increase in cellular potassium efflux than that from WKY. Alterations in membrane transport of calcium ions also have been reported for vascular tissue from SHR.  

The current studies provide evidence that oscillatory contractile activity in tail arteries from SHRSP is related to alterations in the membrane handling of calcium.
Oscillatory contractions in SHRSP arteries/Lamb et al.

D-600, in M (N=6)  |  Nifedipine, in M (N=4)

![Graph showing effects of calcium channel blockers.](image)

*Effects of calcium channel blockers. Oscillatory activity induced by norepinephrine (3 x 10^-7 M) was inhibited by D-600 and nifedipine. Values are the mean ± SEM for four (nifedipine) and six (D-600) SHRSP. Asterisks indicate a statistically significant difference from control values (p < 0.05, paired t test).*

and potassium (Figure 7). The oscillatory activity varied inversely with the potassium concentration of the buffer and directly with the calcium concentration. The oscillations induced by norepinephrine were inhibited by the potassium channel blockers barium,11 sparteine,12 and quinidine.13 Potassium channel blockers that act on voltage-sensitive potassium channels (tetraethylammonium and 3,4-diaminopyridine) did not alter the oscillatory activity.14,15 The oscillatory activity also was inhibited by calcium channel blockers.

These characteristics are consistent with the hypothesis that the oscillatory activity results from the activation of a calcium-dependent potassium current. Following receptor activation with norepinephrine, the intracellular concentration of calcium ion would increase to cause contraction. This elevated calcium concentration also would activate potassium efflux channels,16 which would hyperpolarize the cell membrane, decrease excitability, and produce relaxation by reduced calcium entry (the falling phase of the oscillatory activity). As the intracellular calcium concentration fell, the potassium channels would close, which would permit a new cycle of contraction to begin because norepinephrine would still be available for membrane activation. The contractile event would then be followed by an enhanced potassium efflux and relaxation. A similar mechanism has been described for bursting pacemaker activity in neurons.17,18

![Graph showing effects of quinidine, D-600, and nitroprusside on oscillatory activity.](image)

*Effects of quinidine, D-600, and nitroprusside on oscillatory activity. Tail arteries from SHRSP were made to contract with norepinephrine (3 x 10^-7 M). Treatment with quinidine (3 x 10^-6 M) and D-600 (10^-7 M) reduced force development and inhibited the oscillatory activity, whereas nitroprusside (4 x 10^-6 M) only reduced the force development. Increasing the concentration of norepinephrine to 1.8 x 10^-5 M caused an enhancement of force generation, but the oscillatory activity was still absent in strips treated with quinidine and D-600.*

![Graph showing calcium entry associated with potassium channel activation.](image)

*In tail arteries from SHRSP, calcium entry is associated with activation of a potassium channel that decreases membrane excitability and causes relaxation. In the presence of continued stimulation with norepinephrine, the smooth muscle exhibits oscillatory activity (see text for details).*
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F S Lamb, J H Myers, M N Hamlin and R C Webb

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