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Articles

Interaction Between Conducted Vasodilation and Sympathetic Nerve Activation in Arterioles of Hamster Striated Muscle

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Abstract

Abstract We tested the hypothesis that sympathetic nerve activity can influence the conduction of vasodilation along the arteriolar wall. Arterioles in the superfused cremaster

References

muscle of anesthetized male hamsters (n=21, 109±4 g) were studied. Microelectrodes were positioned adjacent to the distal end of primary arterioles to stimulate sympathetic nerves throughout arteriolar networks (perivascular nerve stimulation [PNS]). Microiontophoresis micropipettes (tip outer diameter, 1 to 2 μm) filled with acetylcholine (ACh, 1 mol/L) were positioned adjacent to the wall of second-order (2A) or third-order (3A) arterioles ≈1 mm distal to their origin to induce local and conducted vasodilation; diameter responses were recorded at the micropipette tip and at vessel origins, respectively. For 2A and 3A arterioles (resting diameters, 15 to 54 and 9 to 30 μm, respectively), vasoconstriction with PNS was frequency dependent (0.5 to 32 Hz); this was attenuated by 65% ($P<.05$) with α-adrenoceptor blockade (phentolamine, 1 μmol/L). Conducted vasodilation was attenuated by >40% during 16-Hz PNS ($P<.05$); this effect was reversed by phentolamine. In a reciprocal fashion, conducted vasodilation diminished PNS-induced vasoconstriction by ≈50% ($P<.05$). Elevating oxygen (from 0% to 10%) in the superfusion solution induced vasoconstriction similar to that with 16-Hz PNS yet had no effect on conduction. Neural blockade with tetrodotoxin (1 μmol/L) eliminated PNS-induced vasoconstriction and enhanced ($P<.05$) conducted vasodilation. These findings indicate that perivascular nerves in striated muscle can influence cell-to-cell communication along the arteriolar wall both at rest and during enhanced sympathetic activity. The attenuation of sympathetic vasoconstriction by conducted vasodilation suggests a novel explanation for functional sympatholysis.

Key Words: microcirculation • perivascular nerve stimulation • microiontophoresis • acetylcholine • adrenoceptors

Introduction

The control of arteriolar diameter reflects the sum of multiple inputs to vascular smooth muscle cells. In skeletal muscle, activation of sympathetic nerves results in vasoconstriction.[1][2][3] In contrast, increasing the metabolic activity of muscle fibers induces vasodilation.[4][5] Arteriolar diameter also reflects changes in transmural pressure and luminal blood flow.[6][7] The interaction between central (ie, neural) and local mechanisms of blood flow control is a long-standing question in cardiovascular and exercise physiology. Of particular interest in the present study is the apparent "competition" between sympathetic vasoconstriction and the vasodilation that occurs during muscular activity.[5][8][9][10][11] Sympathetic outflow increases with exercise, yet there is a preferential increase in blood flow to active muscle. In effect, functional vasodilation "overrides" sympathetic vasoconstriction, giving rise to the concept of "functional sympatholysis."[5][9] Nevertheless, sympathetic nerve activity can limit muscle blood flow by constricting resistance vessels.[11][12][13]

The mechanisms of interaction between sympathetic vasoconstriction and peripheral vasodilation have been studied in some detail. For example, substances released by muscle fibers[1] and motor nerves[14] can inhibit norepinephrine (NE) release and dilate arterioles. Vasomotor responses can also result from stimuli delivered to sites well removed from a particular branch of the arteriolar network. For example, acetylcholine (ACh) microiontophoresis induces a dilation that is conducted rapidly from cell to cell along the arteriolar wall over distances encompassing several millimeters and multiple branch orders.[15][16][17] However, the influence of sympathetic nerve activity on

cell-to-cell conduction in arteriolar networks has not been determined. In the present study, we tested the hypothesis that sympathetic nerve activity can influence the conduction of vasodilation along the arteriolar wall. Our findings in striated muscle show that stimulation of sympathetic nerves substantially depressed conducted vasodilation via activation of α-adrenoceptors. Furthermore, the conduction of vasodilation significantly attenuated sympathetic vasoconstriction of arterioles, suggesting a novel explanation for functional sympatholysis.[5][9]

Materials and Methods

Animal Care and Preliminary Surgery

All procedures were approved by the Institutional Animal Care and Use Committee of the John B. Pierce Laboratory. Male golden hamsters (n=21, 109±4 g, Charles River) were maintained at 24°C on a 14-h/10-h (light/dark) cycle and provided food (Purina rodent chow) and water ad libitum. Hamsters were anesthetized with pentobarbital sodium (60 mg/kg IP) and tracheotomized to maintain a patent airway. The right carotid artery was cannulated for monitoring arterial pressure (CDX III transducer, Cobe Laboratories). Another cannula was secured in the left femoral vein to replace fluids and maintain anesthesia during experiments (10 mg pentobarbital per milliliter isotonic saline, infused at 0.41 mL/h).

Cremaster Preparation

The right hamster cremaster muscle was prepared as recently described in detail.[16] Briefly, by use of a stereo microscope (model DRC, Zeiss), the muscle was exposed and positioned onto a transparent acrylic pedestal. The cremaster muscle was opened from the apex to the inguinal

canal along the ventral surface. The spermatic artery and vein were ligated, and then the testis and epididymis were carefully separated from the muscle and removed. Eight to 12 sutures (6-0 silk, Ethicon) were secured around the edge of the muscle and used to spread the tissue radially.

The cremaster preparation was superfused continuously (5 mL/min) with a bicarbonate-buffered physiological saline solution (PSS) (34±1°C, pH 7.4) of the following composition (mmol/L): NaCl 131.9, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2, $NaHCO_3$ 18 (Sigma Chemical Co). Superfusion solutions were gassed continuously with 5% CO_2/95% N_2 unless noted otherwise; in three experiments, a Clark-type electrode (model PHM 7/MK2, Radiometer Copenhagen) was used to measure PO_2 of the PSS on the surface of the preparation. Esophageal temperature was maintained at 38±1°C by positioning the hamster on a copper coil through which warm water (43°C) was circulated. When all experimental procedures for the day were complete, the hamster was given an overdose of pentobarbital through the venous cannula.

Video Microscopy

The preparation was transferred to the stage of an intravital microscope (model ACM, Zeiss) and equilibrated for 45 minutes. Second-order (2A; resting diameter, 15 to 54 μm) and third-order (3A; resting diameter, 9 to 30 μm) arterioles were selected for study on the basis of optical clarity and resting tone, as demonstrated by a brisk and reversible dilation in response to topical application of adenosine (0.1 mmol/L). Microvessels were observed with bright-field microscopy using Köhler illumination (ACH/APL condenser [numerical aperture, 0.32]; objectives, Zeiss UD40 [numerical aperture, 0.41] or Leitz L25 [numerical aperture, 0.35]). A video camera (CCD model C2400, Hamamatsu) was positioned

on a trinocular imaging tube and coupled to a video monitor (model PVM 1343MD, Sony). Final magnification on the monitor was ≈x1400 when either objective was used.

Internal vessel diameters were recorded from the video monitor by using a video caliper. A stage micrometer (100x0.01=1 mm, Graticules Ltd) was used for calibration; spatial resolution was ≤1 μm. The direct effect of ACh was assessed at the micropipette tip (referred to as "local"), and conducted vasodilation was evaluated at the origin of the stimulated branch, which was typically ≈1 mm upstream from the ACh micropipette[16 17] (Fig 1). Control experiments eliminated the possibility of ACh diffusion to the upstream site or of nonspecific effects of iontophoretic current on arteriolar diameter.[15 16] Data were acquired at 100 Hz by using a MacLab system (AD Instruments) coupled to a Macintosh IIVX computer.

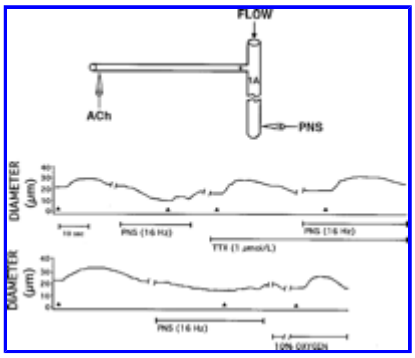

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Figure 1. Top, Diagram of arteriolar networks studied. PNS indicates perivascular nerve stimulation; ACh, acetylcholine. The break in the first-order (1A) arteriole accounts for the distance between the PNS microelectrode and the sites where PNS and ACh responses were assessed (see "Materials and Methods" for details). The ACh micropipette was positioned ≈1 mm downstream from the vessel origin (*), where conducted responses were measured. Bottom, Two representative tracings of conducted responses at the origin of second-order arterioles to ACh microiontophoresis (1 mA, 500 ms; given at arrowheads), 16-Hz PNS, and 10% O_2 or tetrodotoxin (TTX , 1 μmol/L) in the superfusion solution. Solid bars below each tracing

indicate the duration of corresponding manipulation (for TTX, application was begun 30 minutes before); the 10 sec calibration bar pertains to both tracings. In the upper tracing, note vasoconstriction and suppression of conducted vasodilation during 16-Hz PNS; TTX augmented conducted vasodilation above the control level and eliminated both vasoconstriction and suppression of conduction induced by PNS. In the lower tracing, whereas 16-Hz PNS suppressed conducted vasodilation, equivalent vasoconstriction with 10% O_2 had no effect on the magnitude of conduction.

Micropipettes and Microelectrodes

Borosilicate glass capillary tubes (Corning No. 7740; outer diameter, 1.2 mm; inner diameter, 0.68 mm; Warner Instrument Corp) were pulled (model P-87, Sutter Instruments) to produce micropipettes with tips (outer diameter) of 1 to 2 μm (for microiontophoresis) or 2 to 3 μm (for perivascular nerve stimulation [PNS]); tip dimensions were measured at x630 optical magnification with bright-field microscopy. Micropipettes were backfilled with 1 mol/L ACh (Sigma) or 0.9% NaCl after filtering to remove particles >0.2 μm (Acrodisc, Gelman Sciences).

Microiontophoresis

Micropipettes containing ACh were secured in a holder and connected to an iontophoresis programmer (model 160, World Precision Instruments) via a Ag/AgCl wire; the programmer was gated externally by the MacLab system. A second Ag/AgCl wire secured at the edge of the preparation served as the reference electrode. Micropipettes were positioned ≈1 mm distal to the vessel origin (Fig 1) with a micromanipulator (model M, Leitz); a large movable stage enabled the

entire preparation and micromanipulators to be moved as a unit without disturbing the spatial relation between arterioles, micropipettes, and microelectrodes.[16] The retaining current (0.1 to 0.2 mA) was adjusted to just prevent leakage (indicated by vasodilation) from the micropipette tip. Based on dose-response curves to ACh microiontophoresis (data not shown), the amplitude of ejection current was held at 1 mA; stimulus durations were selected to elicit maximal and half-maximal conducted responses (500 and 200 ms, respectively). To quantify the effect of ACh on arteriolar diameter, the magnitudes of local and conducted responses to microiontophoresis were calculated as follows: peak response diameter (in micrometers) minus preceding baseline diameter (in micrometers).

Perivascular Nerve Stimulation

A distal segment of the first-order (1A) arteriole was exposed by microdissection of adjacent striated muscle fibers. A stimulating microelectrode was prepared by using a micropipette filled with 0.9% NaCl; this was secured in a Leitz micromanipulator and positioned adjacent to the exposed 1A segment (Fig 1). The microelectrode was connected via a Ag/AgCl wire to the negative terminal of a stimulator (model S48, Grass Instruments Co); the positive terminal of the stimulator was connected to the Ag/AgCl reference wire. With constant pulse duration (1 ms) and stimulation frequency (8 Hz), voltage for PNS was adjusted (average, 120 V) until maximal constriction was observed in an arteriolar branch located ≈5 mm proximal to the microelectrode; observations at such remote sites ensured that vasoconstriction was not due to direct depolarization of smooth muscle cells at the microelectrode tip.

To determine the frequency-response characteristics of arterioles to PNS, diameter responses were characterized at seven stimulation frequencies

(0.5, 1, 2, 4, 8, 16, and 32 Hz)[18] in five hamsters. The train durations for stimulation were selected to provide stable diameter responses and varied inversely with stimulation frequency: 30 s for 0.5 and 1 Hz; 20 s for 2, 4, and 8 Hz; 16 s for 16 Hz; and 8 s for 32 Hz. At 16 Hz, for example, constriction began ≈3 s after initiating PNS and peaked within 16 s. To test for activation of sympathetic nerves, the above procedures were repeated after 30 minutes of exposure to phentolamine (1 μmol/L, Research Biochemicals Inc), an α-adrenoceptor antagonist, added to the superfusion solution.

For summary frequency-response curves, changes in arteriolar diameter (peak diameter response [in micrometers] minus resting diameter [in micrometers]) at a given frequency of PNS were divided by the maximal diameter change and expressed as percentage of maximum for each arteriole. These summary data were used to ascertain the stimulus frequencies required to elicit 50% and 100% of the maximal PNS responses (4 and 16 Hz, respectively), which were used in subsequent experiments. Although 32 Hz was often the stimulus giving maximum response, preliminary experiments revealed sympathetic "escape" (ie, dilation after constriction during nerve stimulation) in 3A arterioles during stimulation at this frequency.[1] Thus, responses to 16-Hz PNS were taken as maximal without escape.

Interaction Between PNS and ACh

For these experiments, eight hamsters were used to test whether PNS (ie, sympathetic vasoconstriction) would alter conducted vasodilation in arterioles. After positioning the ACh micropipette as shown in Fig 1, ACh was applied (1 mA, 500 and 200 ms) under control conditions; each ACh stimulus was then tested during 4- and 16-Hz PNS. Because PNS results in arteriolar constriction, control experiments were necessary to account for the change in diameter per se. Therefore, responses to

identical ACh stimuli were also evaluated during vasoconstriction induced by elevating superfusate O_2 concentration from 0% to 10%. For all experiments, diameter values were quantified at resting baseline and when responses to experimental manipulations (eg, PNS or 10% O_2) had stabilized. Local and conducted responses to ACh microiontophoresis were recorded at the peak of the diameter response at rest and during PNS or 10% O_2. Representative diameter tracings for individual 2A arterioles are presented in Fig 1 to illustrate experimental protocols.

Conduction and PNS With Sympathetic Blockade

These experiments tested whether inhibition of α-adrenoceptors with phentolamine (1 μmol/L) or blockade of nerve action potentials with tetrodotoxin (TTX, 1 μmol/L; Sigma) would alter the influence of PNS on conducted vasodilation. On the basis of the similarity of results between 2A and 3A branches in the above experiments (see "Results"), only 2A arterioles were studied with these protocols. Micropipettes containing ACh were positioned as in Fig 1. Responses to PNS (16 Hz) and ACh (1 mA, 500 ms) were studied before and after 30 minutes of exposure to either phentolamine or TTX in the superfusion solution (Fig 1). Eight hamsters were studied with these protocols (phentolamine, n=3; TTX, n=5). Arteriolar diameters at rest and in response to PNS were measured under control conditions and in the presence of phentolamine or TTX (Table). Responses to ACh microiontophoresis at rest and during PNS were recorded locally and at upstream (conducted) sites in the absence and presence of phentolamine or TTX as described above.

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Table 1. Influence of Tetrodotoxin and Phentolamine on Baseline Diameter and Response to 16-Hz Perivascular Nerve

Stimulation in Second-Order Arterioles of Hamster Cremaster Muscle

Statistics

One to three arterioles were studied in each preparation; each vessel was treated as a separate experiment.[16] [17] Experimental treatments affecting the entire preparation (eg, PNS, 10% O_2, TTX, and phentolamine) are referred to as "global." Unpaired *t* tests were performed to determine whether responses to PNS or to ACh varied between 2A and 3A arterioles. Repeated-measures ANOVA was used to compare the effect of global treatments on resting diameter and on vasomotor responses to ACh. Post hoc comparisons of cell means were performed by multiple linear comparisons with the family-wide error rate adjusted to $P \leq .05$. Thus, critical *P* values for individual comparisons were determined by dividing .05 by the number of comparisons of interest as determined a priori for each analysis. All statistical comparisons were performed with SUPERANOVA (Abacus Concepts Inc). Summary data are presented as mean±SEM.

Results

Preparations were stable throughout each day's experiments (duration, 3 to 5 hours) as assessed by the maintenance of vasomotor tone (2A and 3A arterioles typically increased diameter 50% to 100% with adenosine) and the stability of mean arterial pressure from the beginning (96±3 mm Hg, n=20) to the end (98±4 mm Hg) of the experiments. Mean arterial pressure during PNS (95±1 mm Hg) was not different from control pressure. Responses to 200-ms ACh and 4-Hz PNS were similar in direction yet reduced in magnitude when compared with 500-ms ACh

and 16-Hz PNS; therefore, only the latter data are presented for clarity.

Perivascular Nerve Stimulation

The magnitude of vasoconstriction increased with the frequency of stimulation in both 2A and 3A arterioles (Fig 2); there were no differences between responses (percentage of maximal response) of 2A or 3A arterioles at either 4 or 16 Hz. The greatest vasoconstrictions occurred at 16 or 32 Hz, irrespective of vessel order.

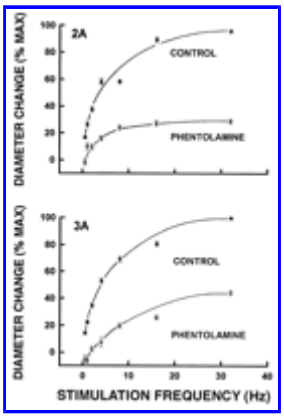

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Figure 2. Frequency-response curves of arteriolar diameter to perivascular nerve stimulation (PNS). Responses to PNS were evaluated in second-order (2A, n=8, top panel) and third-order (3A, n=9, bottom panel) arterioles under control conditions and in the presence of 1 μmol/L phentolamine. Diameter change (% MAX) is defined in "Materials and Methods." Resting diameters of these arterioles were 40.6±5.5 μm (2A) and 20.4±2.9 μm (3A); corresponding diameters with topical adenosine (0.1 mmol/L) were 61.2±5.8 and 30.7±4.4 μm, respectively.

Responses to Global Stimuli

The PO_2 of control PSS (gassed with 5% CO_2/95% N_2) averaged ≈30 mm Hg on the surface of the preparation and increased to ≈100 mm Hg when gassed with 10% O_2/5% CO_2/85% N_2. The vasoconstriction induced by 10% O_2 was not different from that elicited with 16-Hz PNS in either 2A or 3A branches (Fig 3). Whereas phentolamine did not affect baseline diameter, arterioles stabilized at smaller ($P<.05$) diameters in the presence of TTX (Table). Arteriolar constriction to PNS was reduced by 65% in the presence of phentolamine (Fig 2) and eliminated completely during TTX exposure ($P<.05$). Vasoconstrictor

responses to PNS returned after washout (45 minutes) of phentolamine or TTX with control PSS (data not shown).

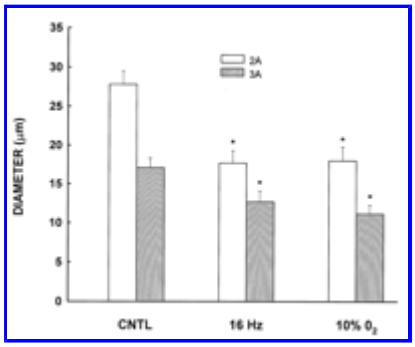

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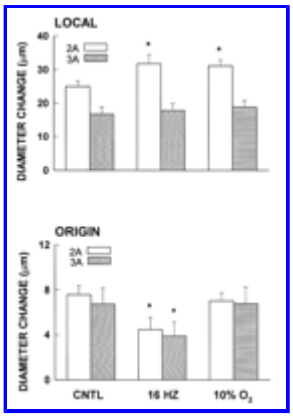
Figure 3. Bar graph showing the diameter of second-order (2A, open bars, n=12) and third-order (3A, hatched bars, n=10) arterioles adjacent to acetylcholine-filled micropipette tip (local) under control conditions (CNTL), during 16-Hz perivascular nerve stimulation, and during 10% O_2 in the superfusion solution. These data were recorded 1028±45 and 998±37 μm distal to the origin of 2A and 3A arterioles, respectively; corresponding responses at the vessel origins were not different. Maximal diameters during dilation with adenosine were 50.2±2.9 and 33.0±2.5 μm for 2A and 3A branches, respectively, and did not differ from local responses to acetylcholine (1 mA, 500 ms). *Significantly different from CNTL ($P<.05$).

Local Responses to Vasomotor Stimuli

At the tip of the ACh micropipette, diameter increased significantly in response to ACh in both 2A and 3A arterioles (Fig 4). During vasoconstriction with 16-Hz PNS or elevated O_2, ACh elicited greater dilation of 2A arterioles than was elicited during control conditions.

Figure 4. Bar graphs showing local and conducted vasodilation to acetylcholine microiontophoresis (1 mA, 500 ms) in second-order (2A, open bars) and third-order (3A, hatched bars) arterioles. Corresponding diameters and n values are reported in Fig 3.



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Top, Local diameter changes under control conditions (CNTL), during 16-Hz perivascular nerve stimulation, and during 10% O_2 in the superfusion solution. Each diameter increase was statistically significant ($P<.05$). *Significantly different from CNTL ($P<.05$). Bottom, Magnitude of conducted vasodilation (ie, diameter change) at the origin of arterioles. Each diameter increase was significant ($P<.05$). *Significantly different from CNTL and from 10% O_2 ($P<.05$).

Conducted Responses

Representative tracings of conducted vasodilation in 2A arterioles are presented in Fig 1. As shown previously,[15,16] a 2- to 3-s delay preceded dilation, which peaked ≈10 s thereafter. Conduction increased arteriolar diameter at the vessel origin under all conditions ($P<.05$); the amplitude of conducted responses did not differ between 2A and 3A branches (Fig 4). Conducted vasodilation was significantly less during 16-Hz PNS than during control conditions or equivalent vasoconstriction with 10% O_2 (Fig 4); this effect of PNS on conduction was not different between branch orders. In four arterioles, vasoconstriction was not observed during PNS; nevertheless, PNS attenuated conducted vasodilation in each case.

Phentolamine had no affect on conducted vasodilation under control conditions (Fig 5), yet it eliminated the PNS-induced depression of conducted responses. In the presence of TTX, conducted vasodilation was enhanced by ≈25% at rest and was more than twofold greater during PNS (Fig 5).

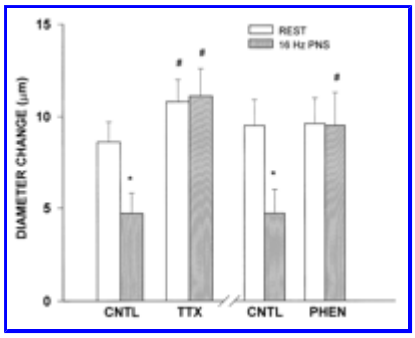

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Figure 5. Bar graph showing the magnitude of conducted vasodilation at the origin of second-order (2A) arterioles under control conditions (CNTL) and in the presence and absence of tetrodotoxin (TTX, 1 μmol/L) or phentolamine (PHEN, 1 μmol/L); local responses to acetylcholine were not affected by these treatments (data not shown). Acetylcholine-filled micropipettes were positioned 956±38 and 1024±47 μm from the vessel origin for PHEN and TTX experiments, respectively. Corresponding n values and diameters at rest (baseline) and during perivascular nerve stimulation (PNS) are shown in the Table. *Significantly different between rest and PNS ($P<.05$). #Significantly different between TTX or PHEN and corresponding CNTL response ($P<.05$).

Discussion

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We have investigated the interaction between perivascular nerve activity and conducted vasodilation in arterioles of striated muscle. In response to PNS, frequency-dependent vasoconstriction occurred in 2A and 3A arterioles, which was suppressed by phentolamine and abolished with TTX. Whereas local vasodilation to ACh (ie, at the site of direct action) increased during PNS, the corresponding conducted responses were diminished by nearly half (Fig 4). The suppression of conduction by PNS was eliminated with TTX or phentolamine; TTX also enhanced conduction at rest. The present data are the first to demonstrate an interaction between sympathetic nerve activity and the conduction of vasomotor responses along arterioles. Our findings indicate the presence

of neural modulation of cell-to-cell communication in arterioles of striated muscle both at rest and during elevated sympathetic outflow.

Perivascular Nerve Stimulation

Previous studies in the rat have activated sympathetic nerves by stimulating paravertebral ganglia.[2] [18] In the hamster, we have found these ganglia to be extremely difficult to isolate. Therefore, an alternative approach was taken. Because sympathetic nerves run as a plexus around the arteriolar network of striated muscle[2] [3] (S.S. Segal and B.D. Walker, unpublished data, 1994), we reasoned that action potentials triggered distally in the network should propagate in a retrograde direction. Thus, a segment of the primary arteriole was exposed near the distal edge of the tissue and the stimulating microelectrode positioned adjacent to the exposed vessel. In response to PNS, vasoconstriction propagated into arterioles located in the central region of the muscle, which confirmed our reasoning.

Arterioles constricted in response to PNS over the range of 0.5 to 32 Hz, which is consistent with previous functional studies[2] [18] and recordings of sympathetic activity from peripheral nerves.[19] The attenuation of PNS-induced vasoconstriction by phentolamine confirmed the activation of sympathetic nerves. The slight dilation observed at the lowest PNS frequencies in the presence of phentolamine (Fig 2) may have reflected activation of ß-adrenoceptors.[14] Alternatively, this vasodilation may have resulted from other substances released by perivascular nerves.[20] [21] Nevertheless, the effects of PNS observed in the present study primarily involved the release of NE (Figs 2 and 5).

The frequency-response characteristics of rat cremaster arterioles to sympathetic stimulation were found to vary with network location: 2A branches were less sensitive to low-frequency stimulation (0.2 to 4 Hz)

than 3A branches.[18] These differences have been explained by corresponding variation in the distribution of α-adrenoceptors within the arteriolar network (1A and 2A, α_1 and α_2; 3A and 4A, α_2)[18 22] and provided a rationale for our studying both 2A and 3A arterioles in the hamster cremaster muscle. Although the direct effect of ACh on 2A arterioles was enhanced by vasoconstriction (Fig 4), we found no difference between branch orders in sensitivity to PNS (Fig 2) or to the interaction between conduction and PNS (Fig 4). Hamster arterioles may have more uniform α-adrenoceptor distribution than observed in the rat[18 22]; however, this remains to be ascertained.

Responses to ACh

Local responses to ACh were either maintained or increased by global treatments (eg, PNS and elevated O_2); in no case was the direct effect of ACh attenuated. In contrast, PNS attenuated conducted vasodilation (Figs 4 and 5). This reduction was not dependent on vasoconstriction per se because equivalent constriction with 10% O_2 did not affect conduction. Because phentolamine eliminated the attenuation of conduction during PNS, we conclude that the effect of PNS on conducted vasodilation is mediated via NE activation of α-adrenoceptors.

In a reciprocal fashion, ACh-induced vasodilation overcame the PNS-induced vasoconstriction both directly and at sites of conduction. In addition to its direct action as a vasodilator, ACh could attenuate sympathetic vasoconstriction via presynaptic inhibition of NE release.[14] However, this effect would occur only at the site of ACh release. Because NE is released throughout the perivascular nerve plexus,[2 3 23] conducted vasodilation must interact with sympathetic vasoconstriction by a mechanism other than presynaptic inhibition.

The conduction of vasodilation occurs via coupling between endothelial

cells and smooth muscle cells along the arteriolar wall; a key component appears to involve the spread of hyperpolarization triggered locally by ACh.[17] [24] In the hamster cheek pouch, micropipette application of depolarizing KCl solution (137 mmol/L) or the microiontophoresis of NE onto arterioles was found to induce vasoconstriction that conducted along arterioles and attenuated conducted vasodilation.[15] [17] Nevertheless, the cheek pouch microcirculation is devoid of sympathetic nerves,[25] and the influence of sympathetic nerve activity on conduction in arterioles has not previously been investigated. Arteriolar smooth muscle cells depolarize in response to sympathetic nerve stimulation or exposure to NE.[23] [26] Therefore, the present findings lead us to hypothesize that depolarization of arteriolar smooth muscle cells induced by NE release during PNS may underlie the attenuation of hyperpolarization and conducted vasodilation triggered by ACh.

Alternatively, NE may alter cell-to-cell coupling in the arteriolar wall and thereby reduce the amplitude of conduction. In support of this argument are the findings that NE increases intracellular Ca^{2+} in smooth muscle cells through binding to α-adrenoceptors,[27] which may reduce gap junctional conductance.[28] Whereas the present results are the first to indicate that PNS depresses conducted vasodilation via α-adrenoceptor activation in vivo, further experiments are required to identify the subsequent event(s) that influence conduction.

Tetrodotoxin

TTX blocks the fast voltage-sensitive Na^{+} channels and thereby inhibits the propagation of action potentials. In previous studies, administration of TTX to cheek pouch arterioles had no affect on conduction.[16] [17] This finding argued against a role for nerves in conduction and contributed to the conclusion that cell-to-cell coupling was the basis of conduction in arterioles.[17] [24] In contrast to the cheek pouch,[25] arterioles of the hamster

cremaster muscle are richly invested with perivascular nerve fibers[18 21] (S.S. Segal and B.D. Walker, unpublished data, 1994); therefore, TTX should inhibit neurotransmitter release. In the presence of TTX, the elimination of PNS-induced vasoconstriction and augmented conducted responses (Fig 5) are consistent with this interpretation.

Arterioles developed sustained constriction during exposure to TTX. In fact, the magnitude of constriction to TTX was not different from that obtained with PNS (Table). Although the cause of this response is unclear, vasoconstriction by itself (eg, with 10% O_2) does not influence conduction (Fig 4). In addition, TTX does not directly affect the membrane potential of vascular smooth muscle cells.[29] Therefore, whereas enhanced conduction during TTX exposure may be explained by elimination of perivascular nerve activity, the inability of phentolamine to alter conduction at rest suggests than neuromodulators in addition to NE could influence cell-to-cell coupling in arterioles.

Significance

Functional hyperemia occurs in the cremaster muscle in response to electrical stimulation[30] and during physical exercise[31]; cremaster preparations have proven highly useful in studies of blood flow control in the microcirculation of striated muscle.[2 7 16 18 21 22 30] Muscular exercise also increases the activity of the sympathetic nervous system.[5 11] The mechanism by which active muscle overrides this vasoconstrictor stimulus is unclear in spite of the volume of research on this topic[1 5 8 9 10 12 13]; the products of muscle metabolism cannot completely account for this phenomenon. ACh release at neuromuscular junctions increases greatly during exercise. As shown in the present study and in previous studies,[15 16] this molecule is highly effective in triggering conducted vasodilation. Recent work suggests that neuromuscular junctions in

striated muscle could provide a vasomotor stimulus to arterioles.[32] Therefore, we speculate that conducted vasodilation triggered at neuromuscular junctions may contribute to functional sympatholysis. Although the present findings demonstrate that the direct effects of ACh are not impaired in the presence of increased sympathetic activity, conduction is clearly suppressed. Nevertheless, the persistence of conducted vasodilation during PNS indicates that it may still contribute to the rapid increase in capillary surface area that occurs with the onset of muscular exercise.[9,16]

Summary and Conclusion

Perivascular nerve stimulation at the distal end of primary arterioles activated sympathetic nerves throughout arteriolar networks in hamster striated muscle. During PNS, ACh microiontophoresis reversed vasoconstriction in 2A and 3A arterioles locally and by triggering conducted vasodilation. The magnitude of conducted vasodilation was diminished similarly by PNS in both vessel orders, and this effect was reversed with phentolamine. In the presence of TTX, responses to PNS were eliminated, and the magnitude of conducted vasodilation was increased. These findings indicate that sympathetic nerves can influence cell-to-cell communication along the arteriolar wall, both at rest and during enhanced sympathetic activity. The attenuation of sympathetic vasoconstriction by conducted vasodilation suggests a novel explanation for functional sympatholysis.

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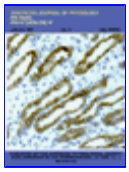

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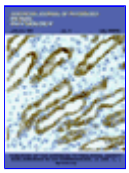

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