The Conduction of Dilation along an Arteriole Is Diminished in the Cremaster Muscle of Hypertensive Hamsters

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Abstract

Arteriolar vasomotor responses can include a component that conducts along the vessel through gap junction channels. This study examined conducted vasomotor responses in arterioles of the hypertensive hamster. The cremaster muscle of normotensive (CHF-148) and spontaneously hypertensive (CHF-H4) hamsters was exteriorized. Micropipettes containing phenylephrine (0.1 M) or acetylcholine (ACh; 1.0 M) were positioned along second-order arterioles and diameter responses were recorded locally for every 0.4 mm upstream to 1.6 mm. Substantative local constrictions to phenylephrine(PE) were poorly conducted to the 0.4-mm site in normotensive and hypertensive hamsters. Local dilation to ACh decayed by 3 ± 1 μm/mm as it conducted along arterioles of the normotensive hamster. In contrast, conducted dilation decayed by 7 ± 1 μm/mm ($p < 0.05$) in the hypertensive hamster. This hypertension-induced increase in decay was reversed by α-adrenergic receptor blockade (phentolamine: 1 μM). However, arteriolar constriction to global α_1- (PE) and α_2- (clonidine) adrenergic agonists was unaffected by hypertension. Rather, sympathetic nervous activity was elevated in the hypertensive hamster as indicated by a greater reduction in arterial pressure upon sympathetic ablation (hexamethonium infusion: 30 mg/kg). This study provides the first evidence that vascular cell-cell communication is altered by the elevated sympathetic nervous activity observed in the hypertensive hamster.

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Introduction

Cell-cell communication involves the diffusion of ions and small molecules between cells through gap junction channels composed of six connexin (Cx) proteins in neighboring cells. This ability to communicate is important to normal cellular functions that include growth, apoptosis, coordination of electrical activity, and metabolic stability [1]. Traditionally, in vitro techniques are employed to study intercellular communication (e.g., dye coupling and patch clamp). The relevance of communica-

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tion measured in vitro to cellular function in vivo remains to be determined. However, cell-cell communication can be assessed indirectly by following vasomotor responses that rely on cell-cell communication: arteriolar conducted responses. Vasomotor responses to agonists like acetylcholine (ACh) include a component that conducts rapidly along the arteriolar wall [2, 3]. Once bound to muscarinic receptors on endothelial cells, ACh hyperpolarizes the cell causing current to move from cell to cell through gap junction channels. The movement of current several millimeters along the arteriole can be observed by following arteriolar diameter: the hyperpolarizing current within the endothelium passes from endothelial to smooth muscle cell through myoendothelial gap junctions [4] to dilate the arteriole. In contrast, agonists such as phenylephrine (PE) mediate conducted constrictions by initiating a depolarization that appears to travel along the smooth muscle cell layer [5]. The magnitude of these conducted responses is observed to decrease with distance from the site of stimulation. This reduction in the magnitude of the diameter response suggests that current is reduced as it moves along the arteriole by the electrical resistance of the pathway (endothelial or smooth muscle cell). Thus, the electrical resistance of the pathway the current travels will determine the extent of decay in the diameter response along an arteriole.

The conduction of constriction along the smooth muscle cell layer is compromised by ischemia [6] and sepsis [7] suggesting that electrical resistance of the smooth muscle cell layer is increased under these conditions. However, those studies did not address conducted dilation, and thereby resistance of the endothelium. We were interested in the endothelium because endothelial cell function of arterioles is altered in several models of hypertension [8]. In these models, large artery expression of Cx43 (a vascular gap junction protein) is usually decreased [9–11] which suggests that electrical resistance of vessels (endothelium and/or smooth muscle cells) may be increased by the elevated blood pressure. Further, knocking out Cx43 elevated the blood pressure of mice [12]. Based upon these studies, we hypothesize that hypertension will increase the decay of the conducted dilatory response. This study investigated whether conducted vasomotor responses are altered in cremaster muscle arterioles of the spontaneously hypertensive hamster and the mechanisms responsible for those changes.

Methods

Preparation

All procedures employed in the present study were approved by the Ohio University Institutional Animal Care and Use Committee. The spontaneously hypertensive hamster (CHF-H4 [13]) was obtained from Canadian Hybrid Farms along with the normotensive strain (CHF-148). Each hamster was anesthetized with thiopental sodium (pentothal: 100 mg/kg, intraperitoneally) and supplemented as needed (10 mg/kg, intraperitoneally). The carotid artery was cannulated (PE-50 tubing) to measure arterial pressure and replace lost fluids (0.9% saline at 0.4 ml/h). For sympathetic nervous system ablation experiments, the femoral vein was also cannulated for infusion of hexamethonium. In all other experiments, the right cremaster muscle was prepared as described previously [3] while being superfused with physiological saline solution (35°C) of the following composition (in mM): 131.9 NaCl, 4.7 KCl, 1.2 MgSO$_4$, 2 CaCl$_2$ and 18 NaHCO$_3$ (pH was adjusted to 7.4 with 5% CO$_2$). During each experiment, body temperature was maintained at 38°C. Once the surgery was completed, arterioles were viewed with a video monitor (Sony) receiving images from a video camera (Pulnix) coupled to an intravital microscope (Zeiss). Internal diameter was measured at the monitor with video calipers (Microcirculation Research Institute: resolution 1 μm). Mean arterial pressure was calculated as the average of ten pressure pulses. Once an experimental protocol was completed (~4 h), hamsters were euthanized with pentothal (200 mg/kg: intra-arterial). To deliver agonists to a small portion of the arteriole, glass pipettes were pulled (Sutter Instruments) to a 2-μm tip (outer diameter) and backfilled with 1.0 M ACh chloride or 0.1 M PE hydrochloride. Pipettes were positioned with a micromanipulator (Narishige) attached to the microscope stage.

Protocol

Following equilibration (45 min), second-order (2A: range resting diameter 26–100 μm) arterioles with tone (defined by a brisk dilation to topical application of 2–3 drops of 0.1 mM adenosine to the superfusate and termed maximal dilation) were studied (~2 arterioles/muscle). The ACh pipette was positioned adjacent to a 2A arteriole (in the same focal plane) 1.6 mm downstream from its 1A parent (origin: fig. 1 inset) and ACh was applied by iontophoresis (1 μA; World Precision Instruments) for a duration (either 0.1 or 0.5 s depending upon the arteriole depth in the muscle) eliciting a ~15 μm dilation at the first conducted site (0.4 mm). Pipette position was maintained as diameter responses were recorded (0.0, 0.4, 0.8, 1.2 and 1.6 mm), which required multiple ACh applications (the potential for tachyphylaxis was minimized by allowing 2 min between ACh applications). The order in which the upstream sites were evaluated was randomized. The importance of gap junctions in conducted responses was addressed by following ACh dilation (as above) in the absence and presence of 18 β-glycyrrhetinic acid (50 μM: [14]). For analysis, diameter was measured before ACh application and at the peak of the response to ACh. To address the potential contribution of hypertension-induced changes in arteriolar responsiveness to ACh, 2A arteriole responses to 0.1, 1 and 10 μM ACh in the superfusate were quantified in normotensive and hypertensive hamsters.

Since arteriolar responses to norepinephrine (NE) released by sympathetic nerves can be augmented by hypertension [15] and conducted dilatory responses are diminished by elevated sympathetic nervous activity [3], the contribution of sympathetic nervous activity was determined by following conducted dilatory responses in the

presence of 1 μM phentolamine (nonspecific α-adrenergic receptor antagonist) in normotensive and hypertensive hamsters. The ACh pipette was positioned 1.2 mm downstream from the arteriole origin (differences in decay could be resolved at 1.2 mm: fig. 1). Upon equilibration, the muscle was exposed to 1 μM phentolamine for 30 min before conducted dilatory responses were determined as described above. In a separate experiment, ACh pipettes were positioned as outlined above and vasomotor responses to ACh determined in the same arteriole before and after 30 min exposure to 1 μM phentolamine. The efficacy of α-adrenergic receptor blockade was addressed by constricting arterioles with NE (0.1 μM) in the absence and presence of 1 μM phentolamine. The effect of hypertension on α-adrenergic responses was determined by following arteriolar diameter responses to PE (0.1, 1 and 10 mM; $α_1$-adrenergic receptor agonist) and clonidine (1, 10 and 100 μM; $α_2$-adrenergic receptor agonist) in the superfusate. In those arterioles, maximal constrictor capacity was assessed by application of 0.1 μM NE.

Conducted constrictor responses were determined by positioning the PE pipette 1.2 mm from the origin of a 2A arteriole. Thereafter, PE was applied iontophoretically at an intensity (1 μA) and duration (0.5–3 s) eliciting a ~25 μm constriction at the pipette tip. Diameter responses were recorded locally (0.0 mm) and every 0.4 mm upstream.

For sympathetic ablation experiments, arterial pressure was allowed to stabilize after cannulation of the carotid artery and femoral vein. Thereafter, a bolus (30 mg/kg) of hexamethonium chloride (60 mg/ml) was infused through the femoral vein and arterial pressure measured until it had stabilized. Measurements of mean arterial pressure were taken before and 5 min after hexamethonium infusion.

Statistics

Data are reported as mean ± SEM (significant at $p < 0.05$). Local responses to ACh were compared between the normotensive and hypertensive hamsters with unpaired t tests. A two-way ANOVA compared conducted responses between normotensive and hypertensive hamsters (control and phentolamine-treated) with a linear regression addressing differences in slope (decay). A two-way ANOVA was performed to assess global vasomotor responses to ACh, PE and clonidine in the superfusate at three concentrations. These vasomotor responses are presented as percentage maximal dilation (to 0.1 mM adenosine) for ACh responses or percentage maximal constriction (to 0.1 mM NE) for PE and clonidine responses. Finally, a two-way ANOVA with repeated measures and a linear regression for slope compared conducted responses before and after treatment with phentolamine. Arteriolar responses to NE in the absence and presence of phentolamine were compared between normotensive and hypertensive hamsters with an unpaired t test as were the changes in mean arterial pressure induced by hexamethonium treatment. A paired t test compared mean arterial pressure before and after treatment with hexamethonium.
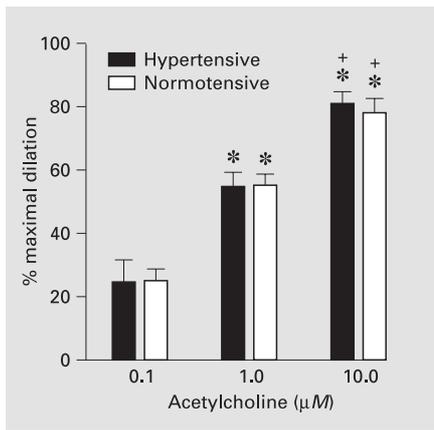
Results

Adult normotensive and hypertensive hamsters were age-matched at 1 year. At that age, the normotensive hamster (n = 22) weighed more (167 ± 7 g: $p < 0.05$) than
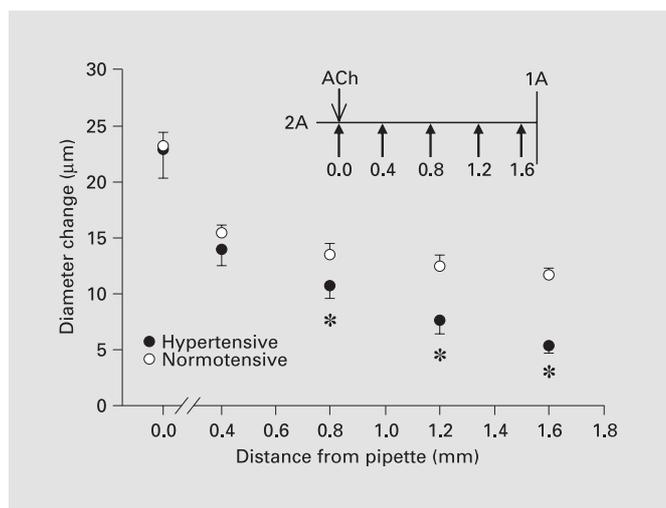
Fig. 1. The effect of hypertension on the decay of conducted dilatory responses to acetylcholine (ACh). Inset diagrams the position of the ACh pipette relative to the first (1A)- and second (2A)-order arterioles and the sites where conducted dilation was measured (0.4, 0.8, 0.2 and 1.6 mm from the pipette). Note the greater decay of the dilation in arterioles of the hypertensive hamster [slope of –7.2 ± 1.2 for the hypertensive (n = 9) vs. –3.0 ± 0.9 μm/mm for the normotensive hamster (n = 9); $p < 0.05$]. * Significant difference ($p < 0.05$) from the normotensive hamster.

their hypertensive counterparts (153 ± 3 g; n = 25). As expected from previous work [13], mean arterial pressure was greater in the hypertensive (182 ± 7 mm Hg; n = 25) than the normotensive (124 ± 6 mm Hg; n = 22, $p < 0.05$) hamster.

In 2A arterioles, where conducted responses to ACh were tested, resting and peak diameters were not different between the normotensive (52 ± 5 and 84 ± 5 μm, respectively; n = 9) and hypertensive (55 ± 12 and 85 ± 11 μm, respectively; n = 9) hamsters. Iontophoresis of ACh [stimulus duration was similar between hypertensive (0.36 ± 0.07 s) and normotensive (0.32 ± 0.07 s) hamsters] initiated a submaximal dilation locally (at the 0.0 mm site) that was not different between the normotensive and hypertensive hamster (fig. 1). This is in stark contrast to the conducted response where dilation at three of the conducted sites (0.8–1.6 mm) was greater in the normotensive hamster (fig. 1). These differences are reflected in a greater slope ($p < 0.05$; decay) for arterioles of the hypertensive hamster (note, slope does not include local dilation). As observed in other laboratories, partial closure of gap junctions with 18 β-glycyrrhetinic acid (50 μM) reduced the distance the conducted responses traveled by increasing the slope of the conducted dilatory

Fig. 2. The effect of hypertension on the dilation of 2A arterioles to three concentrations of acetylcholine (ACh). Application of 0.1, 1 and 10 μM ACh to the superfusate resulted in a significant dilation that was not different between the normotensive (n = 5) and hypertensive (n = 5) hamsters. Resting diameter and maximal dilation were not different between normotensive (49 ± 10 and 86 ± 10 μm, respectively) and hypertensive hamster (48 ± 6 and 89 ± 7 μm, respectively). * Significant difference (p < 0.05) from 0.1 μM ACh dilation within the same strain; + significant difference (p < 0.05) from 1 μM ACh dilation within the same strain.

response to ACh to 10 ± 5 μm/mm (n = 3; data not shown). At the same time, arteriolar responses to three concentrations of ACh in the superfusate (fig. 2) were not affected by hypertension.

The efficacy of 1 μM phentolamine was confirmed by a 41% reduction in arteriolar constriction to 0.1 μM NE (n = 6 arterioles; data not shown). In the presence of phentolamine, no differences were apparent in local or conducted dilatory responses between the normotensive and hypertensive hamster (fig. 3A). When compared with the hypertensive hamsters in figure 1, the presence of phentolamine decreased (p < 0.05) the slope of the conducted dilatory response. To strengthen this finding, experiments were repeated with each arteriole acting as its own control (conducted responses were tested in the same arteriole before and after 1 μM phentolamine). Similar reductions in the slope of the conducted dilatory response were observed in the presence of phentolamine in the hypertensive hamster (fig. 3B), whereas slope was not altered in the normotensive hamster. Interestingly, local dilation to 0.5 s stimulus duration was similar between the normotensive and hypertensive hamster. More importantly, the initial (transient) dilatory response of arterioles [includes data from panel A and B – normotensive (n = 11) and hypertensive (n = 10)] to phentolamine (peak 2.7 ± 1.2 min) was greater in the hypertensive hamster (56 ±

4% of maximal dilation) than the normotensive (41 ± 5%) hamster even though the change in diameter after 30 min of phentolamine, while significant (p < 0.05), was not different (normotensive –3 ± 2 and hypertensive –3 ± 3 μm). In contrast, there was no effect of hypertension on arteriole responses to α_1- (PE) or α_2- (clonidine) adrenergic agonists in the superfusate (fig. 4). However, when sympathetic nervous activity was blocked with hexamethonium (bolus, 30 mg/kg), mean arterial pressure decreased to ~60 mm Hg (at 5 min post-infusion) in the normotensive and hypertensive hamster (fig. 5) and did not change further over 15 min. The decrease in mean arterial pressure in the hypertensive hamster was nearly double that observed in the normotensive (fig. 5).

Iontophoresis of PE resulted in a local constriction (p < 0.05) that was not different between the normotensive and hypertensive hamster (fig. 6). More importantly, no conducted constriction was observed when the stimulus duration was <2 s. When stimulated for 3 s, a small constriction was observed at 0.4 mm site but not at the 0.8 mm site (fig. 6). Thus, constrictor responses to PE appear to be poorly conducted along cremaster muscle arterioles of the normotensive and hypertensive hamster.

Discussion

The present study investigated the effect of hypertension on conducted vasomotor responses in arterioles embedded within skeletal muscle. In the cremaster muscle of the normotensive hamster, constriction to PE did not conduct well along arterioles (fig. 6), whereas dilation to ACh conducted over 1.6 mm with little decay (fig. 1). In the hypertensive hamster, the decay (slope) of the conducted dilatory response was greater (steeper) suggesting an increase in electrical resistance of the endothelium (fig. 1). Interestingly, decay of the conducted response was reversed (decreased) in the presence of phentolamine indicating that α-adrenergic receptor activity is altered in the hypertensive hamster (fig. 3A, B). The source of those alterations was not increased vasomotor responsiveness, as hypertension did not affect arteriolar responses to α_1- or α_2-adrenergic receptor agonists (PE or clonidine, respectively; fig. 4). Rather, ablation experiments indicated that sympathetic nervous activity was elevated in the hypertensive hamster (fig. 5). Thus, the increase in sympathetic nervous activity associated with hypertension appears to affect skeletal muscle arterioles by increasing the electrical resistance of the endothelium, thereby attenuating conducted dilation.

Fig. 3. The effect of α-adrenergic receptor blockade with 1 μ*M* phentolamine on conducted vasodilatory responses of 2A arterioles to acetylcholine (ACh) in normotensive and hypertensive hamsters. **A** Local and conducted dilatory responses in the presence of phentolamine were not different between normotensive (–2.8 ± 2.8 μm/mm) and hypertensive (–3.4 ± 3.0 μm/mm) hamsters. Resting diameter and maximal dilation before 1 μ*M* phentolamine application were not different between normotensive (46 ± 10 and 83 ± 11 μm, respectively; n = 6) and hypertensive (52 ± 11 and 80 ± 9 μm, respectively; n = 6) hamsters. **B** Local and conducted vasomotor responses to ACh in the normotensive before (–4.5 ± 1.4 μm/mm) and after 30 min exposure to 1 μ*M* phentolamine (–4.0 ± 1.7 μm/mm) were not different. However, while local responses were not affected in the hypertensive, the slope of the conducted vasomotor responses was decreased (p < 0.05) by the application of phentolamine (from –7.1 ± 1.8 to –4.1 ± 2.1 μm/mm). Resting diameter and maximal dilation before phentolamine were not different between normotensive (63 ± 6 and 98 ± 4 μm, respectively; n = 5) and hypertensive (57 ± 10 and 93 ± 8 μm, respectively; n = 4). * Significant difference (p < 0.05) from diameter responses in the presence of 1 μ*M* phentolamine.
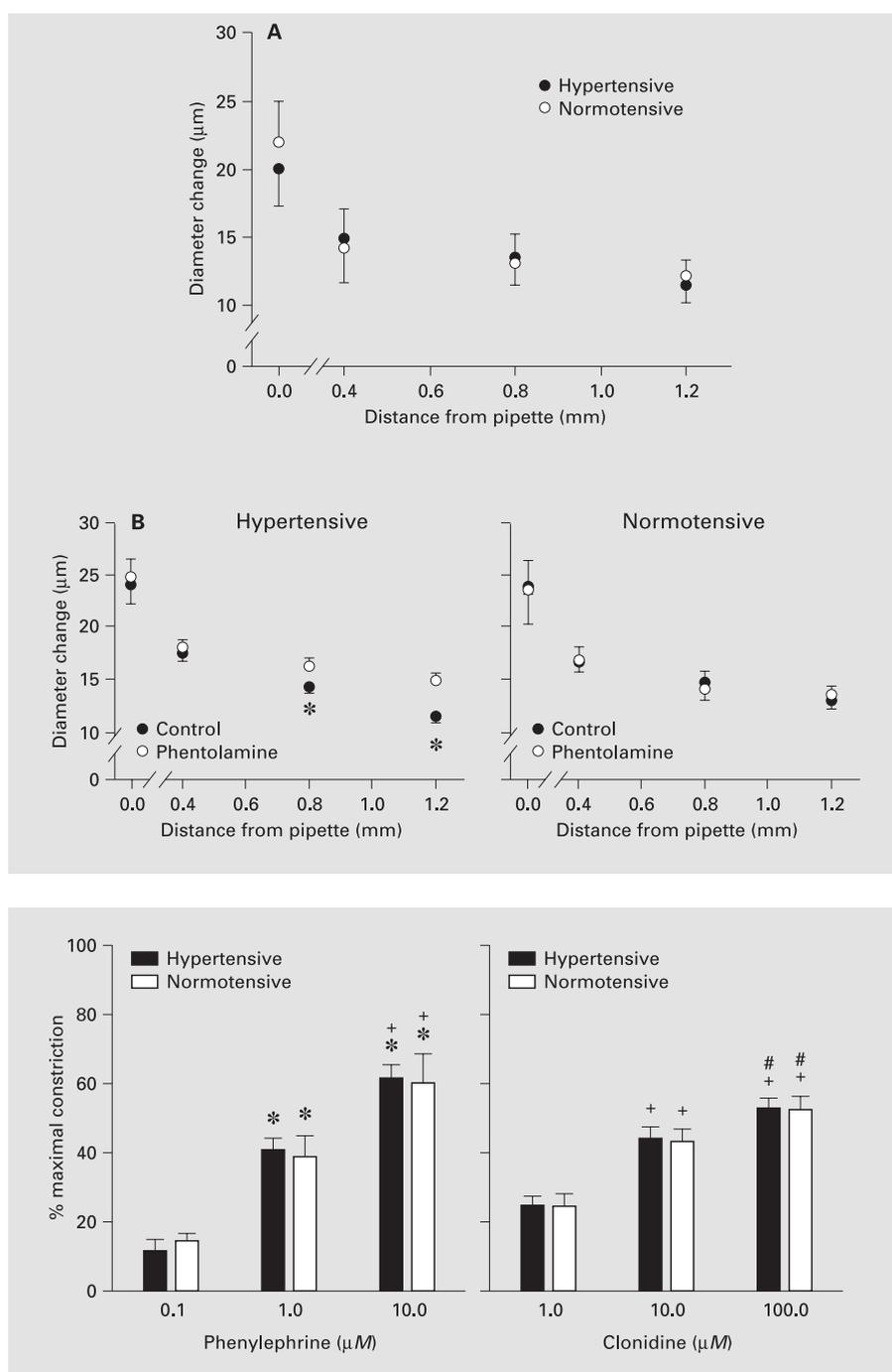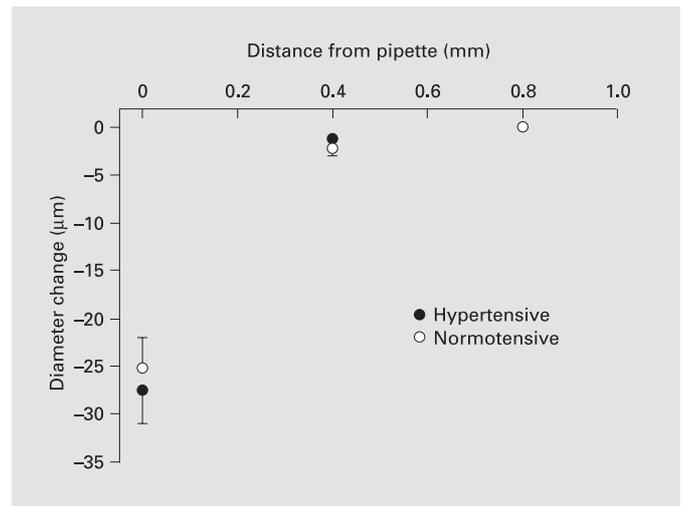
Fig. 4. The effect of hypertension on constriction of 2A arterioles to three concentrations of phenylephrine (PE) and clonidine. Application of 0.1, 1 and 10 μ*M* PE to the superfusate resulted in a significant constriction that was not different between the normotensive and hypertensive hamsters. Resting diameter, maximal dilation and maximal constriction of arterioles treated with PE were not different between the normotensive (51 ± 11, 75 ± 10 and 10 ± 1 μm, respectively; n = 6) and hypertensive (48 ± 6, 78 ± 4 and 9 ± 1 μm, respectively; n = 5) hamster. In addition, responses to clonidine (1, 10, and 100 μ*M*) were similar between normotensive and hypertensive hamster. Resting diameter, maximal dilation and maximal constriction of arterioles treated with clonidine were not different between normotensive (62 ± 8, 88 ± 7 and 10 ± 1 μm, respectively; n = 6) or hypertensive hamsters (64 ± 7, 88 ± 6 and 11 ± 2 μm, respectively; n = 6). * Significant difference (p < 0.05) from 0.1 μ*M* PE constriction within the same strain; ⁺ significant difference (p < 0.05) from 1 μ*M* PE or clonidine constriction within the same strain; # significant difference (p < 0.05) from 10 μ*M* clonidine constriction within the same strain.
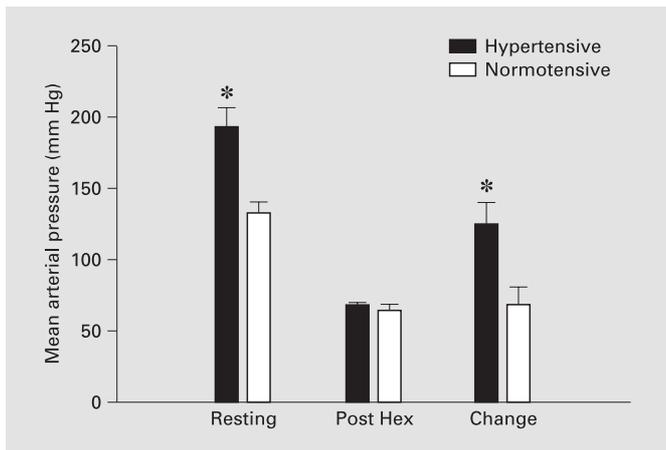
Fig. 5. The effect of sympathetic nerve ablation with hexamethonium on blood pressure in the hypertensive hamster. Mean arterial pressure was ~60 mm Hg greater (p < 0.05) in the hypertensive (n = 3) than the normotensive (n = 3) hamster before hexamethonium treatment (resting). However, 5 min after hexamethonium infusion (post-hex), mean arterial pressure was not different between the two strains. While the decrease in mean arterial pressure was significant for both strains (p < 0.05), the decrease in mean arterial pressure (change) was greater (p < 0.05) in the hypertensive hamsters suggesting that sympathetic nervous activity was increased by hypertension. * Significant difference (p < 0.05) from the normotensive hamster.

As expected from previous work [13], blood pressure was ~60 mm Hg greater in the hypertensive (CHF-H4) than in the normotensive strain (CHF-148). The mechanisms responsible for this elevated blood pressure were not resolved by earlier studies although renin was increased in the hypertensive hamster [13]. The release of renin is regulated, in part, by sympathetic nervous activity. More importantly, an elevation in sympathetic nervous activity (increases in NE release or arteriolar responses to NE) could account for the greater decay in the conducted dilatory response [3]. The greater reduction in blood pressure in the hypertensive hamster upon ablation of sympathetic nervous system activity (hexamethonium, fig. 5) is consistent with a hypertension-induced increase in sympathetic nervous activity. Further, this greater sympathetic activity was directed, in part, to the arterioles of the cremaster muscle as evidenced by the greater transient dilation to phentolamine in the hypertensive hamster. Transient dilation was a better indicator of sympathetic nervous activity than the resting diameter after 30 min of equilibration to phentolamine because the autoregulatory mechanisms active in skeletal muscle arterioles during the equilibration period were able to compensate for the loss of sympathetic constriction and return diameter to values

Fig. 6. The effect of hypertension on the decay of conducted constrictor responses to phenylephrine (PE). Local constriction to PE elicited a small constriction (p < 0.05) at the 0.4 mm conducted site (only when stimulated for 3 s) in the normotensive (n = 8) and hypertensive (n = 10) hamster that did not reach 0.8 mm.

similar to control (fig. 3A, B). One concern when studying responses altered by sympathetic nervous activity is the effect of the anesthetic (pentothal) itself on sympathetic nervous activity. While similar doses of pentothal were required to anesthetize the two strains, we could not rule out the possibility that pentothal affects sympathetic activity. However, blood pressure under pentothal anesthesia was greater than previously observed in the normotensive or hypertensive hamsters [13]. This elevated blood pressure could reflect differences in the response of hamsters to the anesthetic employed (sodium pentobarbitol [13] vs. pentothal) or an age-related increase (8 months [13] vs. 1 year).

An endothelial dysfunction is observed in other models of hypertension and is characterized by a reduction in endothelial-dependent dilatory responses [8]. A similar endothelial dysfunction was not observed in the hypertensive hamster as evidenced by the similarity in ACh dilation between the normotensive and hypertensive hamster (fig. 2 and 3B). However, this finding conflicts with an earlier study showing that ACh responses of cheek pouch arterioles were reduced in the hypertensive hamster [16]. These differences in arteriolar responses to ACh could result from: (1) location of the vasculature (cheek pouch vs. cremaster muscle), (2) greater blood pressure in the normotensive hamster (the 20 mm Hg elevation in blood pressure may have affected vasomotor responses to ACh

in the normotensive hamster) or (3) age-related changes in vascular responses [17] (6 months [16] vs. 1 year). The current experiments were unable to resolve between these possibilities although studies are in progress to address this discrepancy.

The endothelium provides the primary pathway by which conducted dilatory responses travel along skeletal muscle arterioles [18] and feed arteries [19, 20]. This is in contrast to cheek pouch arterioles, where conducted dilation can also travel along the smooth muscle cell layer [5, 21]. Interestingly, while constriction to PE conducts along cheek pouch arterioles [5, 21], no conducted responses to PE are observed in skeletal muscle arterioles or feed arteries [22] suggesting that current travels poorly along the smooth muscle cell layer in these vessels. Since vasomotor responses to ACh were unaffected by hypertension (fig. 2) and PE did not initiate a conducted constriction (fig. 6), the greater decay of the conducted dilatory response likely reflects an increase in the electrical resistance of the endothelium. In order for sympathetic nervous activity to affect the electrical resistance of endothelial cells, α-adrenergic receptors must be present on the endothelium. Several studies have provided functional evidence for the presence of α_1- [23] and α_2- [24] adrenergic receptors on the endothelium. The activation of α-adrenergic receptors would lead to an increase in intracellular second messengers (i.e., protein kinase C and mitogen-activated protein kinase [25]) that reduce gap junction communication and whose activity is elevated by hypertension [26, 27]. With gap junctions providing the pathway whereby current moves along the endothelium, a decrease in cell-cell communication could account for the increased electrical resistance of the endothelium. A case for the involvement of second messengers is strengthened by the time course of the changes in electrical resistance which was reversed after a 30-minute exposure to phentolamine (fig. 3B). However, this does not preclude the possibility that changes in Cx expression might also be involved. Relative expression of the four vascular Cx proteins affects the Cx composition of heteromeric connexons (hemichannel formed by 6 Cx proteins that are not of the same isotype). These heteromeric gap junction channels demonstrate unique gating properties [28, 29], which could include differences in sensitivity to second messengers. In fact, the absence of an effect of phentolamine on the decay of conducted dilation in the normotensive hamster argues that sensitivity of gap junctions to second messengers may be altered by hypertension. Alternatively, the electrical resistance of the endothelium could also be affected by hypertension-induced changes in ion channel activity within

endothelial cells. Resolving between ion channels and gap junctions is complicated by the fact that many of the signals affecting gap junctions (e. g. protein kinase C) also alter ion channel activity [30]. Thus, further studies are required to address whether the hypertension-induced changes in electrical resistance of the endothelium are caused by gap junctions or ion channels.

Conducted constriction is mediated by the depolarization of smooth muscle cells, which has been proposed to travel along that same layer [5]. Interestingly, conducted constrictor responses to PE are not observed with the same regularity as conducted dilatory responses to ACh. Cheek pouch arterioles conduct constrictor responses to PE [2], whereas similar responses were not reported in arterioles of the retractor [22] or cremaster muscle of the hamster (fig. 6; the small constriction at 3 s of stimulation is likely caused by diffusion of PE to the 0.4 mm site). Differences in cell-cell communication do not appear to be involved since gap junction protein expression was similar between cheek pouch and cremaster muscle arterioles [4]. Previous work [22] suggested that the absence of sympathetic innervation to cheek pouch arterioles might be responsible for the lack of conducted constrictor responses in those vessels. Our results are consistent with those findings since cremaster muscle arterioles, like those of the retractor muscle, are innervated by sympathetic nerves [3] and do not appear capable of initiating a conducted constrictor response. Under chronic sympathetic stimulation, cell-cell communication between smooth muscle cells could be reduced by differences in the expression of gap junctions or the chronic activation of second messengers. Thus, while the source of the differences in conducted constrictor responses must still be resolved, no differences were apparent in conducted constrictor responses between the normotensive and hypertensive hamsters.

At the beginning of this study, we hypothesized that conducted responses would be altered by hypertension. Results indicated that conducted dilation was reduced in the hypertensive hamster as a consequence of an elevated sympathetic nervous activity. The increase in decay of the conducted dilatory response in skeletal muscle arterioles suggests that electrical resistance of the endothelium is increased by hypertension, which can be explained by a decrease in cell-cell communication between endothelial cells. One of the interesting aspects of this study is the in vivo evidence for the regulation of cell-cell communication through α-adrenergic receptor-mediated activation of second messengers. While considerable in vitro evidence has accumulated on the regulation of gap junction com-

munication by second messengers, this is the first study to show that communication can be altered by hypertension. Further, since cell-cell communication is involved in many other cellular functions, the ultimate consequence of a reduction in communication may be broader than just the changes in conduction of vasomotor responses along arterioles that are reported here. For example, cell-cell communication is altered by apoptosis. In other models of hypertension, apoptosis is responsible for decreasing arteriolar density, which contributes to the increase in peripheral resistance. Whether cell-cell communication affects apoptosis or vice versa remains to be determined. Thus, the precise role these reductions in cell-cell communication might play in apoptosis, or any of the other cellu-

lar functions affected by cell-cell communication, warrants further study. In conclusion, hypertension decreases the ability of electrical signals to travel along the endothelium, thereby reducing the extent to which dilation is conducted along that layer. The source of this reduction in communication appeared to involve an increase in sympathetic activation in the hypertensive hamster.

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