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Independent regulation of periarteriolar and perivenular nitric oxide mechanisms in the *in vivo* hamster cheek pouch microvasculature

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Abstract

Objective—We tested the hypothesis that differential stimulation of nitric oxide (NO) production can be induced in pre- and postcapillary segments of the microcirculation in the hamster cheek pouch.

Methods—We applied acetylcholine (ACh) or platelet-activating factor (PAF) topically and measured perivascular NO concentration ([NO]) with NO-sensitive microelectrodes in arterioles and venules of the hamster cheek pouch. We also measured NO in cultured coronary endothelial cells (CVEC) after ACh or PAF.

Results—ACh increased periarteriolar [NO] significantly in a dose-dependent manner. ACh at 1 μ M increased [NO] from 438.1 ± 43.4 nM at baseline to 647.9 ± 66.3 nM, while 10 μ M ACh increased [NO] from baseline to 1035.0 ± 59.2 nM ($P < 0.05$). Neither 1 μ M nor 10 μ M ACh changed perivenular [NO] in the hamster cheek pouch. PAF, at 100 nM, increased perivenular [NO] from 326.6 ± 50.8 nM to 622.8 ± 41.5 nM. Importantly, 100 nM PAF did not increase periarteriolar [NO]. PAF increased [NO] from 3.6 ± 2.1 to 455.5 ± 19.9 in CVEC, while ACh had no effect.

Conclusions—We conclude that NO production can be stimulated in a differential manner in preand postcapillary segments in the hamster cheek pouch. ACh selectively stimulates the production of NO only in arterioles, while PAF stimulates the production of NO only in venules.

Keywords

acetylcholine; platelet-activating factor; microcirculation; nitric oxide synthase; arterioles; venules; regulation of microvascular function

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INTRODUCTION

Cell signaling determines the action of vasomediators in the control of microvascular permeability and diameter. Endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) have emerged as important pleiotropic signaling mechanisms in the cardiovascular system (14). We advanced the concept of differential regulation of pre- and postcapillary segments by selected agonists (13,28). In support of the concept, we demonstrated that vasodilators such as papaverine, adenosine and acetylcholine (ACh) can induce arteriolar dilation without altering venular permeability in the hamster cheek pouch (13,19). Using VEGF (vascular endothelial growth factor), we showed that AKT (protein kinase B) may serve as a pivotal molecule in differentiating whether VEGF stimulation causes changes in vasodilation or in permeability (1). These findings point to a differential regulation of signaling in arterioles and venules.

A striking functional difference between pre- and postcapillary segments in the hamster cheek pouch is demonstrated also by platelet-activating factor (PAF), a phospholipid pro-inflammatory mediator. In the hamster cheek pouch, PAF is a potent vasoconstrictor and increases microvascular permeability and leukocyte adhesion in a dose-dependent manner (6–9). These differential results can be explained, at least partially, on the basis of the presence of different receptors in arterioles and venules in the hamster cheek pouch microvasculature (28). PAF triggers a complex biochemical signaling cascade, which involves the stimulation of eNOS and production of NO, as judged by pharmacological blockade studies and deletion of the gene encoding for eNOS (5,10,14,15,19,26). We tested the hypothesis that differential stimulation of NO production, as measured by direct microelectrode analysis, may exist in pre- and postcapillary segments of the microcirculation. The results should help to better understand the underlying mechanisms of differential microvascular regulation. Because eNOS-derived NO is a fundamental element in the regulation of microvascular hyperpermeability responses to challenge with PAF (15), we chose PAF as a test agonist to investigate whether NO production is differentially stimulated in the preand postcapillary microvascular segments. We chose ACh as a second test agonist because it stimulates vasodilation but does not induce changes in permeability in the hamster cheek pouch (19).

MATERIALS AND METHODS

The experimental protocols for hamsters were approved by the UMDNJ-New Jersey Medical School's Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guidelines for the Use of Animals.

Hamster cheek pouch preparation

Male golden Syrian hamsters (80–120g) were anesthetized with sodium pentobarbital (50 mg/kg ip). The right jugular vein was cannulated for administration of supplemental doses of anesthetic as appropriate. The right carotid was cannulated for monitoring blood pressure. Body temperature was maintained at 37 °C by placing the animal on a heating pad. The left cheek pouch was prepared for direct visual observation and intervention (6,16–18). At the completion of the experiment, the hamster was sacrificed by an overdose of sodium pentobarbital (150 mg/kg iv).

Perivascular NO Measurement

NO concentration was measured with a NO-sensitive microelectrode (2–4,16,17,29). The electrode is based on a single carbon fiber core glued with electrically conductive epoxy cement into a glass micropipette, sharpened to a tip diameter of 10–12 µm such that the

glass envelop forms a barrier around the carbon fiber so that only the “open” tip is NO sensitive. To avoid contamination by organic and inorganic molecules, the carbon fiber was electroplated with Nafion (Sigma Chemical, St. Louis, MO, USA) as a membrane. The microelectrodes were polarized at +0.9 V, the current generated was measured with an electrometer (model 6517A, Keithley, Cleveland, OH) and recorded on a PowerLab Data Acquisition (A D Instruments, Colorado Springs, CO). Calibration, using a gas tonometer at 37 °C, was performed for each experiment by measuring the microelectrode current generated by 0,600, and 1,200 nM NO. Microelectrodes having a linear relationship of electrical current to NO concentration were used. After the microelectrode tip was properly located, the microvessel and microelectrode were allowed to stabilize for 30 min before experimental protocols were implemented.

For perivascular measurements of NO, the microelectrode was placed nearly parallel to the vessel, with the microelectrode tip in close proximity to the vessel wall. Our goal for all measurements was to achieve the highest possible NO concentration for a given vessel; thus, we positioned the microelectrode tip as close as possible to the vessel wall. However, we took care to avoid penetrating the vessel wall as this can irritate or damage the vessel wall and cause falsely large NO generation. We determined the relationship of the concentration of NO as a function of distance from the microvascular wall. As we moved the microelectrode tip straight up and away from the vessel wall through the tissue and then into the bath, NO concentration decreased linearly and approached the bath background NO concentration (Fig. 1), which for practical purposes is 0 nM at ~200 µm above the vessel wall in the superfusion media. This finding was identical to those using intestinal and cerebral microvasculatures in rats (2–4,29). As the cheek pouch microvessels are located quite shallowly in the tissue and the tissue is covered with a flowing suffusion solution, the fall in NO concentration on withdrawal from the microvessel does not follow an exponential decay as might be expected in a stable diffusion environment.

Microscopy

Observations were made with an Olympus BH microscope. The recording system comprised an Optronics TEC-470 TV microscope camera (Optronics, Goleta, CA), a Sony monitor, and a MetaMorph image system (Universal Imaging Corporation, Downingtown, PA). The MetaMorph system allowed video recording directly from the TV microscope camera and image processing.

Cell culture

In order to have a NO reference at the cellular level, we grew bovine endothelial cells derived from coronary postcapillary venules ((24); CVEC; a generous gift from Dr. Cynthia Meininger, Texas A&M University) in Dulbecco's modified Eagle's Medium supplemented with 10% fetal bovine serum; 20 units/ml sodium heparin, 50 µg/ml penicillin, 50 µg/ml streptomycin and 10 µg/ml neomycin. We performed the experiments using passage 3–6 CVEC. We grew the cells on coverslips and allowed them to reach confluence. For the experiments, we placed the coverslips containing confluent CVEC in a perfusion chamber. We superfused the cells with media at a rate of 1 ml/min. We additionally administered PAF or ACh through a side-port in the perfusion tubing to achieve a PAF concentration of 100 nM in the chamber. We recorded NO responses in control and stimulated conditions. We also tested for the presence of muscarinic receptor 3 (mAChR-M3) in CVEC using standard Western blot techniques.

Chemicals

Acetylcholine (ACh; Sigma Chemical Co., St. Louis, MO) was dissolved initially in distilled water to a concentration of 10^{-2} M and subsequently diluted to the desired experimental

concentration with bicarbonate buffer solution. Platelet-activating factor (PAF; Sigma Chemical Co., St. Louis, MO) was dissolved initially in dimethyl sulfoxide (DMSO) to a concentration of 10^{-2} M and subsequently diluted to the test concentration of 100 nM with a mixture of 1.5% bovine serum albumin (BSA) and bicarbonate buffer solution (6–8). The concentration of PAF was chosen on the basis of our earlier work that established that 100 nM PAF causes maximal changes in microvascular permeability in the hamster cheek pouch, while concentrations of 1 nM or lower demonstrate the chemottractant properties of this phospholipids autacoid (6,7,8). ACh and PAF were administered topically via a side port into the suffusate bicarbonate buffer line at a concentration necessary to achieve the desired final concentration. ACh was applied for 5 minutes, while PAF was applied for 3 minutes. Suffusion with buffer bicarbonate was resumed after topical application of the agents. Neither the vehicle for ACh nor the vehicle for PAF changed perivascular NO concentration.

Statistical Analysis

All data are expressed as mean $\pm$ SEM. Statistical analysis was performed using a one-way analysis of variance. The Student-Newmann-Keuls test was applied to determine significance. Differences were considered significant for values of $P < 0.05$. All statistical analyses were performed using the InStat package (GraphPad, San Diego, CA).

RESULTS

ACh increased only periarteriolar [NO] in the hamster cheek pouch

Twenty male golden Syrian hamsters were used in these experiments. After stabilization, we scanned the pouch, selected either an arteriole or a venule, and measured the baseline perivascular [NO] to serve as a control. The sequence of measurement of periarteriolar and perivenular [NO] was randomized in every experiment.

Arteriolar dilation was clearly seen after ~2 min of ACh topical application; it attained a maximum at ~5 min and declined towards baseline in the subsequent 5–10 min. Part of the slow vascular response was due to the gradual infusion of the ACh to the desired bath concentration to avoid thermal transients that are very detrimental to NO microelectrode measurements. In agreement with an earlier report (11), we observed an excellent correlation between the time course of ACh-stimulated changes in NO production and arteriolar dilation.

Figure 2 shows the time course of perivascular NO concentration after the application of ACh and of PAF. The mean resting diameter of arterioles and venules used for the ACh study was 23 ± 2 and 23 ± 1 μ m, respectively. The mean resting diameter for arterioles and venules in the PAF study was 25 ± 2 and 21 ± 2 μ m, respectively. ACh stimulated an increase in perivascular NO only in arterioles, while PAF stimulated an increase in perivascular NO only in venules. Topical application of ACh induced a rapid increase in periarteriolar NO concentration, which reached a maximum value after 2–3 minutes and reached baseline values within 10 minutes. After topical application of PAF, perivenular NO concentration rose and reached a peak within 2–3 minutes after its onset and declined back to baseline levels within 10 minutes.

To further evaluate the impact of ACh on perivascular NO, we analyzed statistically the peak or maximal NO concentration induced by ACh in arterioles (based on the time course shown on Figure 2), and in venules. Figure 3 shows that topical application of 1 μ M ACh increased maximal periarteriolar [NO] significantly from a baseline of 438.1 ± 43.4 nM to 647.9 ± 66.3 nM ($p < 0.05$). Application of 10 μ M ACh increased maximal periarteriolar

[NO] to 1035.0 ± 59.2 nM; a value that is significantly different from baseline and significantly higher than that induced by $1 \mu\text{M}$ ACh ($p < 0.05$).

Perivenular baseline [NO] in postcapillary venules was 337.0 ± 34.8 nM. Topical application of $1 \mu\text{M}$ ACh did not alter [NO] significantly (340.9 ± 50.7 nM; $p > 0.05$). Similarly, no significantly different changes in [NO] were induced by $10 \mu\text{M}$ ACh (337.0 ± 34.8 nM at baseline vs. 345.0 ± 12.6 nM after ACh; $p > 0.05$).

PAF increases only perivenular [NO] in the hamster cheek pouch

To further evaluate the impact of PAF on perivascular NO, we analyzed statistically the peak NO concentration induced by PAF in venules (based on the time course shown on Figure 2), and in arterioles. Figure 4 shows the results of the statistical analysis. PAF (100 nM), applied topically for 3 minutes, increased maximal perivenular [NO] from a baseline of 326.6 ± 50.8 nM to 622.8 ± 41.5 nM. In contrast, 100 nM PAF did not alter significantly periarteriolar [NO] (430.3 ± 18.9 nM at baseline vs. 374.3 ± 14.5 nM after PAF; $p > 0.05$).

Stimulated production of NO in postcapillary endothelial cells

To further determine whether the differential NO production in response to ACh and PAF is a property of venular endothelial cells, we measured pericellular [NO] in CVEC. Interestingly, ACh, at $1 \mu\text{M}$ and $10 \mu\text{M}$, failed to increase NO production in CVEC (Fig. 5). To determine whether or not CVEC possess eNOS-linked receptors for ACh, we performed Western blots using antibodies against M3, a muscarinic receptor (mAChR-M3). Figure 5 shows that mAChR-M3 are present in CVEC. In contrast to ACh, 100 nM PAF caused a robust increase in pericellular [NO] in CVEC (Fig. 5).

DISCUSSION

Our results demonstrate that NO production can be differentially regulated in the pre- and postcapillary segments of the microcirculation of the hamster cheek pouch. The differential response in NO production was closely associated to the selected agonists and their microvascular function.

We interpret the ACh-induced NO as an index of eNOS activity. This interpretation is reasonable because ACh does not induce relaxation in endothelium-denuded arteries (12,27). In the hamster cheek pouch, eNOS is detected mainly in the endothelial lining of arterioles and venules and as such, neither ACh nor PAF should induce appreciable NO generation by vascular smooth muscle cells (10,25). When applied topically at 100 nM for 3–5 min to the hamster cheek pouch, PAF causes vasoconstriction, hyperpermeability, and minimal leukocyte adhesion (6–9). Pharmacologic blockade of NOS in the hamster cheek pouch prevents PAF-stimulated hyperpermeability, but not arteriolar constriction (19). We reported that 100 nM PAF increased NO release *in vivo* using a chemiluminescence method. However, the chemiluminescence method measured global cheek pouch NO production (10). The current study using the excellent spatial resolution of NO microelectrodes that only measure NO at their open tip conclusively demonstrated that venules - but not arterioles - are the source of NO in response to PAF stimulation of the cheek pouch microvasculature.

Our results provide further *in vivo* biochemical mechanistic support to the concept that there are mechanisms that regulate the microvascular precapillary segment independently and differentially from the microvascular postcapillary segment. Based on a concept derived from experiments reported in the early 1960's (20,21); our laboratory provided proof for independent regulation of microvascular segments upon the advent of intravital microscopy using selected agonists (13,28). It remains to be explored whether the differential NO

production in response to specific agonists is a universal microvascular property or a characteristic of the hamster cheek pouch microcirculation. In our study, we have confirmed the demonstration that both arterioles and venules have the capacity to generate NO in response to stimuli (4). We had anticipated earlier that PAF stimulated NO mainly from venules; a deduction based on the combined observations that PAF-induced arteriolar constriction is completely blocked by inhibition of thromboxane A₂ synthesis (8,9) and that inhibition of NOS with L-NMMA does not diminish PAF-induced constriction, but abrogates PAF-induced hyperpermeability (19). In these series, using NO-sensitive microelectrodes, we located the exact microvascular origin of NO and found that PAF stimulates NO production in venules where it increases permeability. Importantly, we determined that PAF has no significant influence on periarteriolar [NO].

PAF-induced NO production was characterized by a rapid onset and NO levels returned to baseline after removal of PAF. This is an important point because the short term use of PAF did not induce either injury or loss of regulation of eNOS as might be expected of a proinflammatory agent. The extent and time course of perivenular [NO] in response to PAF is comparable to the changes in periarteriolar [NO] stimulated by topical application of ACh, even though their vascular functions are markedly different. It is worth noting that PAF causes a shorter NO response in CVEC, which increases almost immediately upon topical application, reaches a peak at about 2–3 minutes and subsides by 5 minutes after the onset of PAF application (22).

In designing this study, we were somewhat concerned that global application of ACh and PAF could generate sufficient NO so that the NO responses in arterioles would influence the venular NO either by diffusion through tissue or by NO entering plasma and being swept downstream. For example, the time delay of plasma flowing from small arterioles to small venules would be of the order of 2–3 seconds, well within the life span of NO in the relatively low oxygen concentration of small microvessels. As the results in Figures 3 and 4 clearly demonstrate, arteriolar NO responses to ACh had no effect on venules and PAF effects on venules did not functionally influence [NO] near arterioles. Given the large increases in NO by small arterioles exposed to ACh (Figs. 2, 3), our observations would argue that arteriolar contributions of NO to downstream venules are of minimal significance.

We also report the novel finding that cultured coronary venular endothelial cells produce NO in response to 100 nM PAF, but do not respond with NO production to ACh at either 1 or 10 μ M (Figure 5). It is remarkable that bovine endothelial cells of postcapillary venular origin exhibit *in vitro* the same functional properties as *in vivo* postcapillary venules of the hamster cheek pouch in regards to NO production. This observation supports the concepts that a) venular phenotype is conserved in CVEC at least up to passage 6, and b) differential translocation of eNOS from plasma membrane to subcellular compartments (possibly via caveolar endocytosis) may determine the functional outcome of eNOS activation (22,23).

It is plausible that differential distribution of ACh receptors may exist in pre- and postcapillary segments of the hamster cheek microvasculature. Because a histochemical study of the distribution of the five reported different ACh muscarinic receptors in the microvasculature would be excessively time consuming, we chose to examine whether or not ACh M3 receptors are present in CVEC. This approach takes advantage of a) the established observation that ACh activation of ACh M3 receptors increases Ca⁺⁺ and causes increased synthesis of NO by eNOS in endothelial cells; and b) the novel observation that ACh does not stimulate NO production in CVEC. The data in Figure 5 –showing ACh M3 receptors in postcapillary venular endothelial cells that do not produce NO in response to ACh - indicate that the differential *in vivo* response is also likely to involve elements of the eNOS-associated signaling cascade (1). Consequently, and in accordance with our previous

in vitro results, functional regulation of NO generation by venular endothelium may depend upon their particular receptor expression and upon processes associated with agonist stimulated-eNOS signaling cascades (22,23).

The details of the mechanisms by which eNOS and perhaps other NOS isoforms differentially regulate segmental microvascular function remain to be further elucidated. Our results demonstrate *in vivo* (hamster cheek pouch) that functional differential activation of eNOS leading to NO production exists in arteriolar and venular segments after challenge with ACh and PAF. This finding is supported *in vitro* (CVEC). We propose that the mechanisms involved in differential activation of eNOS are determinants of NO-related microvascular functions (22,23).

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REFERENCES

1. Aramoto H, Breslin JW, Pappas PJ, Hobson RW 2nd, Durán WN. Vascular endothelial growth factor stimulates differential signaling pathways in *in vivo* microcirculation. *American journal of physiology*. 2004; 287:H1590–1598. [PubMed: 15155260]
2. Bauser-Heaton HD, Bohlen HG. Cerebral microvascular dilation during hypotension and decreased oxygen tension: a role for nNOS. *American journal of physiology*. 2007; 293:H2193–2201. [PubMed: 17630350]
3. Bauser-Heaton HD, Song J, Bohlen HG. Cerebral microvascular nNOS responds to lowered oxygen tension through a bumetanide-sensitive cotransporter and sodium-calcium exchanger. *American journal of physiology*. 2008; 294:H2166–2173. [PubMed: 18326806]
4. Bohlen HG. Mechanism of increased vessel wall nitric oxide concentrations during intestinal absorption. *The American journal of physiology*. 1998; 275:H542–550. [PubMed: 9683443]
5. Chao W, Olson MS. Platelet-activating factor: receptors and signal transduction. *The Biochemical journal*. 1993; 292(Pt 3):617–629. [PubMed: 8391253]
6. Dillon PK, Durán WN. Effect of platelet-activating factor on microvascular permselectivity: dose-response relations and pathways of action in the hamster cheek pouch microcirculation. *Circ Res*. 1988; 62:732–740. [PubMed: 2450695]
7. Dillon PK, Fitzpatrick MF, Ritter AB, Durán WN. Effect of platelet-activating factor on leukocyte adhesion to microvascular endothelium. Time course and dose-response relationships. *Inflammation*. 1988; 12:563–573. [PubMed: 3220518]
8. Dillon PK, Ritter AB, Durán WN. Vasoconstrictor effects of platelet-activating factor in the hamster cheek pouch microcirculation: dose-related relations and pathways of action. *Circ Res*. 1988; 62:722–731. [PubMed: 2832096]
9. Durán WN, Dillon PK. Acute microcirculatory effects of platelet-activating factor. *Journal of lipid mediators*. 1990; 2(Suppl):S215–227. [PubMed: 2133284]
10. Durán WN, Seyama A, Yoshimura K, Gonzalez DR, Jara PI, Figueroa XF, Boric MP. Stimulation of NO production and of eNOS phosphorylation in the microcirculation *in vivo*. *Microvasc Res*. 2000; 60:104–111. [PubMed: 10964584]
11. Figueroa XF, Gonzalez DR, Martinez AD, Durán WN, Boric MP. ACh-induced endothelial NO synthase translocation, NO release and vasodilatation in the hamster microcirculation *in vivo*. *The Journal of physiology*. 2002; 544:883–896. [PubMed: 12411531]
12. Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature*. 1980; 288:373–376. [PubMed: 6253831]
13. Gawlowski DM, Durán WN. Dose-related effects of adenosine and bradykinin on microvascular permselectivity to macromolecules in the hamster cheek pouch. *Circ Res*. 1986; 58:348–355. [PubMed: 3087654]

14. Harrison DG, Cai H. Endothelial control of vasomotion and nitric oxide production. *Cardiology clinics*. 2003; 21:289–302. [PubMed: 14621446]
15. Hatakeyama T, Pappas PJ, Hobson RW 2nd, Boric MP, Sessa WC, Durán WN. Endothelial nitric oxide synthase regulates microvascular hyperpermeability *in vivo*. *The Journal of physiology*. 2006; 574:275–281. [PubMed: 16675496]
16. Kim DD, Pica AM, Durán RG, Durán WN. Acupuncture reduces experimental renovascular hypertension through mechanisms involving nitric oxide synthases. *Microcirculation*. 2006; 13:577–585. [PubMed: 16990216]
17. Kim DD, Sánchez FA, Durán RG, Kanetaka T, Durán WN. Endothelial nitric oxide synthase is a molecular vascular target for the Chinese herb Danshen in hypertension. *American journal of physiology*. 2007; 292:H2131–2137. [PubMed: 17172272]
18. Mayhan WG, Joyner WL. The effect of altering the external calcium concentration and a calcium channel blocker, verapamil, on microvascular leaky sites and dextran clearance in the hamster cheek pouch. *Microvasc Res*. 1984; 28:159–179. [PubMed: 6209532]
19. Ramirez MM, Quardt SM, Kim D, Oshiro H, Minnicozzi M, Durán WN. Platelet activating factor modulates microvascular permeability through nitric oxide synthesis. *Microvasc Res*. 1995; 50:223–234. [PubMed: 8538502]
20. Renkin EM, Rosell S. Effects of different types of vasodilator mechanisms on vascular tonus and on transcapillary exchange of diffusible material in skeletal muscle. *Acta physiologica Scandinavica*. 1962; 54:241–251. [PubMed: 14491475]
21. Renkin EM, Rosell S. Independent sympathetic vasoconstrictor innervation of arterioles and precapillary sphincters. *Acta physiologica Scandinavica*. 1962; 54:381–384. [PubMed: 14491476]
22. Sánchez FA, Kim DD, Durán RG, Meininger CJ, Durán WN. Internalization of eNOS via caveolae regulates PAF-induced inflammatory hyperpermeability to macromolecules. *American journal of physiology*. 2008; 295:H1642–H1648. [PubMed: 18708444]
23. Sánchez FA, Savalia NB, Durán RG, Lal BK, Boric MP, Durán WN. Functional significance of differential eNOS translocation. *American journal of physiology*. 2006; 291:H1058–1064. [PubMed: 16679407]
24. Schelling ME, Meininger CJ, Hawker JR Jr, Granger HJ. Venular endothelial cells from bovine heart. *The American journal of physiology*. 1988; 254:H1211–1217. [PubMed: 2454587]
25. Segal SS, Brett SE, Sessa WC. Codistribution of NOS and caveolin throughout peripheral vasculature and skeletal muscle of hamsters. *The American journal of physiology*. 1999; 277:H1167–1177. [PubMed: 10484439]
26. Shukla SD. Platelet-activating factor receptor and signal transduction mechanisms. *Faseb J*. 1992; 6:2296–2301. [PubMed: 1312046]
27. Simonsen U, Wadsworth RM, Buus NH, Mulvany MJ. *In vitro* simultaneous measurements of relaxation and nitric oxide concentration in rat superior mesenteric artery. *The Journal of physiology*. 1999; 516(Pt 1):271–282. [PubMed: 10066940]
28. Tomeo AC, Durán WN. Resistance and exchange microvessels are modulated by different PAF receptors. *The American journal of physiology*. 1991; 261:H1648–1652. [PubMed: 1659229]
29. Zani BG, Bohlen HG. Transport of extracellular L-arginine via cationic amino acid transporter is required during *in vivo* endothelial nitric oxide production. *American journal of physiology*. 2005; 289:H1381–1390. [PubMed: 15849232]

Figure 1. Concentration of NO as a function of distance from the microvascular wall. NO concentration decreases linearly as the electrode is moved away from the microvascular wall. Data from three animals.

Figure 2.

Time course of stimulated NO production in hamster cheek pouch microvessels. LEFT PANEL: ACh was applied topically at two concentrations. The time course of periarteriolar NO concentration is displayed. ACh did not stimulate venular NO production. RIGHT PANEL: PAF was applied topically at 100 nM. The time course of perivenular NO concentration is displayed. PAF did not stimulate arteriolar NO production. ACh and PAF stimulated a rapid increase in perivascular NO concentration, which reached a peak within 2–4 minutes and returned to baseline by 10–12 minutes after topical application.

Figure 3.

ACh increases perivascular NO concentration in hamster cheek pouch. The bar graphs show the maximal increments in response to ACh. The data represent the mean $\pm$ SEM. The numbers in parentheses show the number of animals included in each group. *P<0.05 compared with corresponding control. #P<0.05 compared with ACh 1 μ M.

Figure 4.

PAF increases perivascular NO concentration in hamster cheek pouch. The bar graphs show the maximal increments in response to PAF. The data represent the mean $\pm$ SEM. The numbers in parentheses show the number of animals included in each group. *P<0.05 compared with corresponding control.

Figure 5.

PAF increases NO concentration in bovine coronary postcapillary endothelial cells (CVEC). Administration of 100 nM PAF to cultured coronary endothelial cells (passage 3–6) caused a robust increase in NO production. Administration of 1 μM and 10 μM ACh failed to cause changes in NO production. The bottom panel shows the presence of muscarinic ACh receptor M3 (mAChr-M3) in CVEC as detected by western blotting.