Role of nitric oxide in vasodilation in upstream muscle during intermittent pneumatic compression

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Chen, Long-En, Kang Liu, Wen-Ning Qi, Elizabeth Joneschild, Xiangling Tan, Anthony V. Seaber, Jonathan S. Stamler, and James R. Urbaniax. Role of nitric oxide in vasodilation in upstream muscle during intermittent pneumatic compression. J Appl Physiol 92: 559–566, 2002. First published October 5, 2001; 10.1152/japplphysiol.00365.2001.—This study investigated the dosage effects of nitric oxide synthase (NOS) inhibitor N\textsuperscript{o}-monomethyl-L-arginine (L-NMMA) on intermittent pneumatic compression (IPC)-induced vasodilation in uncompressed upstream muscle and the effects of IPC on endothelial NOS (eNOS) expression in upstream muscle. After L-NMMA infusion, mean arterial pressure increased by 5% from baseline (99.5 ± 18.7 mmHg; \(P < 0.05\)). Heart rate and respiratory rate were not significantly affected. One-hour IPC application on legs induced a 10% dilation from baseline in 10- to 20-μm arterioles and a 10–20% dilation in 21- to 40-μm arterioles and 41- to 70-μm arteries in uncompressed cremaster muscle. IPC-induced vasodilation was dose dependently reduced, abolished, or even reversed by concurrently infused L-NMMA. Moreover, expression of eNOS mRNA in uncompressed cremaster muscle was upregulated to 2 and 2.5 times normal at the end of 1- and 5-h IPC on legs, respectively, and the expression of eNOS protein was upregulated to 1.8 times normal. These increases returned to baseline level after cessation of IPC. The results suggest that eNOS plays an important role in regulating the microcirculation in upstream muscle during IPC.

Deep vein thrombosis (DVT) and pulmonary embolism (PE) have long been recognized as common complications and as preventable causes of morbidity and mortality in patients subjected to prolonged periods of bed rest or limb immobilization (8, 26). The incidence of venography-proven DVT in the lower extremities in immobilized trauma patients exceeds 60% and may range as high as 80% (15, 34). Even patients receiving full prophylaxis have an incidence of DVT as high as 12% (30). This high incidence of thrombotic events makes prophylaxis, screening, and treatment extremely important.

Intermittent pneumatic compression (IPC) has been widely used clinically as an effective means of preventing the development of DVT and PE (44, 62, 63). To compare the efficacy of thromboembolic prophylaxis, a recent meta-analysis reviewed 6,001 total knee arthroplasty patients in 23 studies published between 1980 and 1997. The incidence of DVT was between 29 and 53% in the pharmacological treatment (aspirin, warfarin, and low-molecular-weight heparin) groups but only 17% in an IPC-treated group (58). Considering the side effects and cost of pharmacological prophylaxis (7), particularly when the contraindications to anticoagulation are present, such as in patients suffering from head and spinal cord injuries (26), IPC may provide a strong alternative to pharmacological prophylaxis.

Studies have suggested that IPC effectively reduces the incidence of DVT by reducing stasis in the venous system through an increase in venous velocity and blood flow (6, 25, 28, 39) and by reducing a hypercoagulable state with the enhancement of fibrinolytic activity and tissue-factor pathway inhibitor (22, 27, 51). In addition, lower limb IPC produces upstream hemodynamic alterations (57). For example, IPC application on the foot causes an increase of blood flow in the popliteal artery (41), and IPC on the arms reduces the incidence of DVT in the legs to half of that in control patients (29). Our earlier data have also shown that IPC on the legs significantly increases vessel diameters of both arteries and veins in rat cremaster muscle (36, 37). These findings suggest that IPC has the ability to produce distant effects remote from the compression site. One common explanation for this distant biological response is an increase in the arteriovenous pressure gradient subsequent to compression of venous blood from the limb. However, either increasing or decreasing this pressure gradient can actually produce the opposite effect on flow (20, 48), thus leaving the mechanistic basis of distant IPC protection unknown (8).

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More recently, clues to the biological basis have emerged. Nitric oxide (NO) was suggested as a possible mediator in IPC-induced blood flow alterations, although the authors had no evidence (8), and we found that the IPC-induced vasodilatory effect in the upstream muscle was blocked completely by a higher dose of \( \text{NG-monomethyl-L-arginine acetate (L-NMMA)} \), an inhibitor of NO synthase (NOS) (36). The purpose of this study was to determine the dose effects of systemically infused L-NMMA on IPC-induced vasodilation in uncompressed cremaster muscle during and after IPC application on the legs and the effects of IPC on the expression of endothelial NOS (eNOS) mRNA and protein in uncompressed cremaster muscle. We hypothesized that NO production from eNOS plays an important role in IPC-induced vasodilation in upstream muscle. If this hypothesis can be proven, we will establish a strong relationship between IPC function, NOS expression, and NO-mediated vasodilation and may offer a biological mechanism for clinical IPC application and improvement.

MATERIALS AND METHODS

Ninety male Sprague-Dawley rats weighing 100–150 g were used in this study. The animals were anesthetized with an intraperitoneal injection of Nembutal (50 mg/kg body wt, Abbott, North Chicago, IL).

Experimental Protocol

Systemic effects of L-NMMA administration. Eighteen rats were divided into three groups of six each and received infusion of 100 nmol·min\(^{-1}\)·100 g L-NMMA\(^{-1}\), 10 \( \mu \)mol·min\(^{-1}\)·100 g L-NMMA\(^{-1}\), or the same volume of PBS via the right external jugular vein for 120 min. The left carotid artery was cannulated, and blood pressure, heart rate (HR), and respiratory rate (RR) were monitored by a patient monitor (MR-1300, Mennen Medical, New York, NY) and recorded at 10-min intervals for 120 min.

Dose effects of L-NMMA infusion on microcirculation of the cremaster muscle during and after IPC on legs. The left cremaster muscle of 60 rats was surgically prepared for in vivo visualization by a technique described previously (5), in which a midline longitudinal incision was created on the ventral aspect of the scrotum and the cremaster muscle was opened along the least vascular area. The muscle was separated from the testis and was spread on the surface of a transparent acrylic microscope stage by five 6-0 sutures with minimal tension. The exposed muscle was kept moist by using buffered Ringer's solution and covered with an O\(_2\) impermeable polymer to prevent the diffusion of gases from the environment to the muscle. Temperature was maintained at 34 ± 0.5°C [the in situ scrotal sac temperature of conscious rats (24)] throughout the experiment.

Microcirculation of the cremaster muscle was observed through a binocular microscope (Carl Zeiss, Germany) connected to a video camera, monitor, and recording system. Internal luminal diameters of arteries were measured from the recorded image by means of a video measuring gauge (For/A IV-560, Japan). Muscle preparation was left undisturbed for 30 min to allow any effects of the muscle isolation procedure to dissipate. The viewed area was selected to contain arteries 10–70 \( \mu \)m in diameter, and 10–15 sites within this area were selected for measurement in each muscle. Sequential measurements were taken at the same sites throughout the experiment. The data obtained from each muscle at each time point were divided into three categories [small arterioles (10–20 \( \mu \)m), large arterioles (21–40 \( \mu \)m), and small arteries (41–70 \( \mu \)m)] depending on the baseline diameters of the measured vessels. The diameter change of each vessel at each time point was expressed as a percentage change compared with baseline values, and the mean percentage change in each cremaster muscle was calculated for each vessel size category.

A specially designed IPC device for the rat leg was used (Aircast, Summit, NJ). It included two cuffs connected to a pump through flexible air tubes. Each cuff consisted of two vinyl sleeves, a lateral sleeve 4 cm in length with a 3-cm width proximally and a 1.5-cm width distally, and a medial sleeve 3 cm in length with a 2.3-cm width proximally and a 1.3-cm width distally. Cuffs were placed on the hind limbs from the ankle to the inguinal level, exclusive of the prepared cremaster, and secured with two rubber bands. The pump provided pressure up to 55 mmHg, achieved within <1 s, with an inflation cycle set at 25 s of inflation followed by 5 s of deflation (Fig. 1). This parameter is identical to that of the VenaFlow device (Aircast) used in clinical applications.

Rats were randomly divided into six groups of 10 animals each. Group A was subjected to IPC for 60 min and simultaneously infused with PBS for 120 min. Groups B to E were treated with 60-min IPC and simultaneous infusion of NOS inhibitor L-NMMA at dosages of 10 nmol/min·1·100 g, 100 nmol/min·1·100 g, 5 \( \mu \)mol/min·1·100 g, and 10 \( \mu \)mol/min·1·100 g, respectively, for 120 min (Fig. 2). Group F was infused with L-NMMA (10 \( \mu \)mol·min·1·100 g) for 120 min without IPC application. The right external jugular vein of each animal was cannulated with polyethylene tubing (PE-10, Clay Adams) for systemic infusion of L-NMMA or PBS. The tubing was connected to a 3-ml syringe fixed to a Sage syringe pump (model M362, Analytical Technology, Boston, MA). Immediately before IPC was applied and L-NMMA or PBS was infused into the rats, the diameter of each selected vessel was recorded as a baseline and recordings were repeated at 10-min intervals for the duration of the experiment. The agent infusion was started at the time IPC was started.

Effects of IPC application on the expression of eNOS mRNA and protein in the cremaster muscle. The remaining 12 rats were divided into three groups and underwent 50 min of IPC application, 5 h of IPC application, and 1 h of IPC application.
followed by 4 h without IPC, respectively. Muscle samples were harvested immediately after cessation of the protocol. eNOS mRNA expression was determined in one-half of each harvested cremaster muscle, and eNOS protein expression was determined in the remaining half of the muscle.

**Quantitative PCR.** Procedures were based on our laboratory’s earlier studies (45). Muscle samples were lysed in lysis/binding buffer (Sigma Chemical, St. Louis, MO), sonicated, centrifuged, and then combined with Dynabeads oligo(dT)25 (Dynal, Lake Success, NY). Poly(A) RNA was then eluted from the Dynabeads in diethyl pyrocarbonate-treated water at 67°C, and its concentration was measured immediately by using DNA Dip Stik (Invitrogen, San Diego, CA) according to the manufacturer’s instructions. Poly(A) RNA was reverse transcribed in a PCR machine (model 2400, Perkin-Elmer, Norwalk, CT). A 20-μl reaction mixture contained 2 ng of mRNA, 2.5 μM oligo(dT)25 as a primer, 5 mM MgCl2, 20 units RNase inhibitor, 1 mM dNTPs, and 50 units Moloney murine leukemia virus reverse transcriptase (Perkin-Elmer, Branchburg, NJ). First-strand cDNA was synthesized at 25°C for 10 min, 42°C for 15 min, and 99°C for 5 min. In a selected tube, the reverse transcriptase was omitted to control for amplification from contaminating cDNA or genomic DNA.

Simultaneous coamplification of both the target cDNA and a reference template (MIMIC) with a single set of primers was performed. MIMIC for eNOS and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was constructed by using a PCR MIMIC construction kit (Clontech Laboratories, Palo Alto, CA). Each PCR MIMIC consists of a heterologous DNA fragment with 5’ and 3’ end sequences that are recognized by known concentrations of MIMIC were added to PCR amplification reactions containing the first-strand cDNA. PCR MIMIC amplification was performed in 100 μl of a solution containing 1.5 mM MgCl2, 0.4 μM of primer, PCR digoxigenin labeling mix (200 μM dNTP), 0.002 μM Taq DNA polymerase, and 0.056 μM TaqStart antibody (Clontech). After an initial denaturation, the cycle condition was 15 s at 95°C and 30 s at 60°C for 45 cycles (Nos) or 35 cycles (GAPDH). Digoxigenin-labeled PCR products were subjected to electrophoresis on agarose gels and transferred to nylon membranes. Membranes were air dried and chemiluminescent detection was carried out by using a Wash and Block Buffer (Boehringer-Mannheim, Indianapolis, IN). Autoradiogram band intensity was quantitated by densitometry by using NIH Image software and calculated from the point of equal density of the sample and MIMIC PCR products. The eNOS mRNA level was normalized with the levels of GAPDH mRNA present in each sample, which served to control for variations in RNA purification and cDNA synthesis. Relative mRNA expression of eNOS in each group was compared with those from respective normals and expressed as a percentage of normal.

**Western Blot.** The muscle samples were homogenized in lysis buffer and centrifuged at 100,000 g at 4°C for 1 h. Protein concentration in the supernatant was measured with a bicinchoninic acid kit (Pierce, Rockford, IL) and 50 μg of total protein was loaded onto an 8% SDS-PAGE, then transferred onto nitrocellulose membrane (Micron Separations, Westborough, MA). The membrane was blocked with 5% nonfat dry milk in Tris-buffered saline (TBS)-T at room temperature for 1 h, followed by incubation with monoclonal primary antibody for eNOS (1:1,000) (Transduction Laboratories, Lexington, KY) at 4°C overnight. After a 10-min washing in TBS-T, the blot was incubated with 1:4,000 horseradish peroxidase-labeled goat anti-mouse IgG (Calbiochem, La Jolla, CA) for 1 h at room temperature and detected with an enhanced chemiluminescence detection kit (Amersham). Kodak film was used to detect chemiluminescent signals.

**Statistics**

The effects of L-NMMA and PBS on mean arterial blood pressure (MAP), HR, RR, and vessel diameter changes were analyzed by a repeated-measures two-way ANOVA. Post hoc analysis of specific values at each time point was performed by repeated-measures one-way ANOVA. Post hoc analysis was performed by Tukey’s test. The significance level was set at P < 0.05. Data for the expressions of eNOS mRNA and protein were analyzed by a paired t-test. Values of MAP, HR, RR, and the expressions of eNOS mRNA and protein were reported as means ± SD whereas values of vessel diameter changes were reported as means ± SE.

**RESULTS**

**Systemic Effects of L-NMMA Administration on Normal Rats**

Baseline values of MAP, HR, and RR are summarized in Table 1. MAP decreased slightly and remained between 92 ± 5% and 99 ± 4% of baseline in the PBS group throughout a duration of 120 min (n = 6). MAP was maintained at the initial level in the 10 μmol·min⁻¹·100 g L-NMMA−¹ group and increased slightly in the 100 μmol·min⁻¹·100 g−¹ group, with a range from 99 ± 4% of baseline at 10 min to 105 ± 6% at 110 min. Although a statistically significant overall difference in MAP was present between groups, there was no significant difference at each time point throughout the experiment (Fig. 3A). HR and RR in the L-NMMA groups remained stable, and no statistical differences were observed between groups.

<table>
<thead>
<tr>
<th>PBS group</th>
<th>MAP, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Respiratory Rate, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 μmol/min group</td>
<td>104.1 ± 9.2</td>
<td>82.3 ± 8.2</td>
<td>78.5 ± 7.8</td>
</tr>
<tr>
<td>10 μmol/min group</td>
<td>101.4 ± 13.4</td>
<td>204.8 ± 19.4</td>
<td>89.8 ± 5.9</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6 animals/group). MAP, mean arterial blood pressure. *P < 0.05 when compared with the value in the 10 μmol/min group.

**Table 1. Baseline values of MAP, heart rate, and respiratory rate in each experimental group**

<table>
<thead>
<tr>
<th>Group</th>
<th>MAP, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>Respiratory Rate, breaths/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>101.4 ± 13.4</td>
<td>204.8 ± 19.4</td>
<td>89.8 ± 5.9</td>
</tr>
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Values are means ± SD (n = 6 animals/group). MAP, mean arterial blood pressure. *P < 0.05 when compared with the value in the 10 μmol/min group.
groups B–F received increasing doses of l-NMMA (n = 10 for each group; Fig. 4). In small arterioles (10–20 μm), vessel diameter in groups A and B increased moderately during IPC application, with a maximum increase of 10% from baseline, and gradually returned to the baseline level after cessation of compression. Groups A and B did not differ significantly at any time point during the 120-min observation period. In group C, vessel diameter remained at the baseline level throughout the experiment. In groups D–F, diameter decreased up to 8% from baseline throughout the experiment. Statistical analysis showed a significant decrease (P < 0.01 to <0.001) in vessel diameter in

difference existed at any time point throughout 120 min of duration (Fig. 3B).

Dose Effects of l-NMMA Infusion on Microcirculation of the Cremaster Muscle During and After IPC on Legs

The baseline diameter of each vessel category in each experimental group is summarized in Table 2. Group A rats were treated with IPC alone, whereas rats in

Table 2. Baseline values of vessel diameters in three vessel categories in each experimental group

<table>
<thead>
<tr>
<th>Group</th>
<th>10–20 μm Arterioles</th>
<th>21–40 μm Arterioles</th>
<th>41–70 μm Arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (PBS)</td>
<td>17.5 ± 1.8</td>
<td>28.5 ± 1.9</td>
<td>44.0 ± 2.9</td>
</tr>
<tr>
<td>Group B (IPC + 10 nmol l-NMMA/min)</td>
<td>16.2 ± 1.8</td>
<td>29.9 ± 5.2</td>
<td>52.7 ± 9.4</td>
</tr>
<tr>
<td>Group C (IPC + 100 nmol l-NMMA/min)</td>
<td>16.1 ± 1.6</td>
<td>30.3 ± 3.3</td>
<td>51.9 ± 8.7</td>
</tr>
<tr>
<td>Group D (IPC + 5 μmol l-NMMA/min)</td>
<td>16.4 ± 1.2</td>
<td>29.6 ± 3.6</td>
<td>51.9 ± 6.3</td>
</tr>
<tr>
<td>Group E (IPC + 10 μmol l-NMMA/min)</td>
<td>17.4 ± 1.8</td>
<td>29.8 ± 2.8</td>
<td>51.0 ± 7.6</td>
</tr>
<tr>
<td>Group F (10 μmol l-NMMA/min)</td>
<td>16.8 ± 1.6</td>
<td>30.1 ± 2.5</td>
<td>52.2 ± 7.1</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 10 animals/group). IPC, intermittent pneumatic compression; l-NMMA, N6-monomethyl-l-arginine.
groups C–F at each time point during IPC compared with groups A and B (Fig. 4A).

In larger arterioles (21–40 μm), obvious vasodilatation (10–20%) was evident in group A during the 60 min of IPC application. The diameter returned to baseline soon after stopping IPC and further reduced slightly at the end of the experiment. Similar but less dramatic changes were seen in group B during IPC application. Compared with group A, significantly less (P < 0.05 to <0.001) vasodilatation was present in group B from 20 to 60 min of IPC. In group C, vessel diameter remained very near baseline level throughout the experiment. In groups D and E, the diameter decreased 15% from baseline at 10 min and decreased further to 20% from baseline at 60 min. After stopping IPC, no further vasoconstriction was found in these two groups. Compared with group C, significant (P < 0.001) vessel constriction existed in these two groups at each time point of observation. Group F showed a slightly greater decrease in vessel diameter than groups D and E but without statistical significance among these three groups (Fig. 4B).

For the small arteries (41–70 μm), the general tendency of diameter changes in each group paralleled that for the larger arterioles (21–40 μm). There was a significant diameter difference between groups A and B from 20 to 60 min (P < 0.01 to <0.001) and between group C and each of groups D–F at each time point during the 120-min observation period (P < 0.001; Fig. 4C).

Effects of IPC Application on the Expression of eNOS mRNA and Protein of the Cremaster Muscle

IPC application upregulated eNOS mRNA expression in the muscle. After 1 h of IPC application, eNOS mRNA in the cremaster muscle increased to 2 times normal and further increased to 2.5-times normal after 5 h of IPC (n = 4). There was a significant increase in eNOS mRNA expression at each time point compared with normal. The expression of eNOS mRNA returned to a normal level after cessation of IPC application (in the 1-h IPC plus 4-h lapse of the time group; Fig. 5).

Similar results were found in eNOS protein expression. eNOS protein in the IPC only groups was significantly upregulated after both 1 h and 5 h of IPC application, with a 1.8-fold increase at the 1-h time point. In the 1+4 h group, eNOS protein returned to 88 ± 12% of the normal level (Fig. 6).

**DISCUSSION**

The major finding of this study was that IPC on the legs elicited vasodilation and upregulated the expression of eNOS mRNA and protein in the uncompressed cremaster muscle. Moreover, this IPC-induced vasodilation in the uncompressed cremaster muscle was significantly and dose dependently reduced, abolished, or reversed by systemic infusion of NOS inhibitor L-NMMA. To the best of our knowledge, this is the first study to connect IPC function and eNOS expression in skeletal muscle.

Our findings appear to support that eNOS plays a role in IPC-induced vasodilation in upstream muscle. NO from eNOS is produced by endothelial cells (ECs) and skeletal muscle cells, and its basal release regulates resistance vessel tone and blood pressure (31, 54). Vascular ECs function as a biosensor of fluid dynamic shear force reducing arterial diameter when blood flow rate decreases and increasing the diameter when the flow rate increases (14). NO from eNOS can create vasodilation, reduce leukocyte adhesion, and inhibit platelet activation and aggregation, thereby increasing blood flow and enhancing fibrinolysis and antithrombotic activity. Our findings suggest that the increased release of NO or related compounds from eNOS is a major pathway for vasodilation induced by IPC. Our data thus establish a strong relationship between IPC function, NOS expression, and NO-mediated vasodilation.

We speculate that there are two sources of NO generation by IPC application. The external compression of the legs by IPC causes elevated shear stress in the walls of the underlying vasculature through increasing flow velocity in the deep veins of the extremity (40). This stimulates ECs to release NO, which subsequently modulates blood flow from the legs to the cremaster muscle. Modulation of the response to shear...
stress occurs by stimulating NOS mRNA expression (18, 38, 56) and/or augmenting NOS protein production and the ability of ECs to release NO (17, 21, 42, 43). Although blood flow was not measured in this study, we indeed observed increased movement of blood cells in the cremaster muscle during IPC application on legs. In addition, it is possible that large amounts of NO are produced by the compressed skeletal muscles themselves during IPC because eNOS is present in skeletal muscle fibers (31). Significant increases in NO production by contracting skeletal muscle subsequently incubated in vitro have been reported (3). Muscle contraction results in intramuscular pressure development (1), creating extravascular shear forces and augmenting the shear-induced NO production by ECs (2). A similar phenomenon may occur in skeletal muscle during IPC compression. Our findings from the anterior tibialis muscle in which IPC application induced an upregulation of eNOS mRNA and protein similar to that in the cremaster muscle support this hypothesis (unpublished data).

The findings that mechanical compression on the legs induces increased blood flow in uncompressed tissues appear to be explained by an ascending vasodilation theory in which the locus of blood flow control moves upstream from the microvessels to larger feed arteries over distances of several centimeters (16, 35, 61). Ascending vasodilation may be the additive interaction of myogenic and flow-mediated responses (35). Dilation in small vessels results in decreased pressure in upstream intermediate sizes of arterioles, which in turn may elicit myogenic dilation of these vessels. The dilation decreases arteriolar resistance and hence increases flow that further recruits larger upstream vessels to dilate because of EC-dependent flow-induced responses. ECs and smooth muscle cells may also be involved in propagating vasodilator signals along arterioles to parent and daughter vessels (9). Caution, however, should be paid to the myogenic factor because others (55) demonstrated in the human forearm that 1-min rhythmic mechanical compression (100 mmHg) produced an upregulation of eNOS mRNA and protein similar to that in the cremaster muscle support this hypothesis (unpublished data).

In addition, countercurrent exchange of NO may contribute to the ascending vasodilation in upstream muscle. In this study, the maximal IPC pressure of 55 mmHg would be expected to mechanically distort venous vessels to a greater degree than arterial vessels. Under this experimental condition, shear stress-induced NO release from eNOS could predominantly be generated by venular ECs. Although we did not measure venous NO release, studies by others (12, 13) have shown that topical application of acetylcholine or bradykinin to rat venules produces significant vasodilation in EC-denuded arterioles, which is completely abolished by l-NMMA. Therefore, communication from venules to nearby arterioles through NO release may be of functional significance. Another study in hamster cremaster muscle supports this concept. Denudation of venular ECs attenuated the vasodilation of adjacent arterioles in response to electrical stimulus-induced muscle contraction, suggesting that venular ECs release a relaxing factor partially responsible for arteriolar dilation in response to muscle contraction (49). If these findings are verified, countercurrent exchange of NO might play a functional role in modulating upstream muscle flow. It is speculated that IPC application stimulates venular ECs to release NO that then diffuses toward the arterioles and elicits upstream vasodilation. However, exactly how the NO signal is delivered from venules to the uncompressed upstream muscle remains a subject for further study.

Evidence suggests that NO is released at basal levels and in response to increased shear stress within the arterial system (32). NO does appear to affect vascular tone at rest and during recovery from exercise (16, 46). However, its role in modulating the hyperemic response at the onset of and during skeletal muscle exercise is rather controversial (4, 16, 46), with evidence both for (2, 10, 19) and against (11, 23, 47, 52, 53) a role for it. Results from the present study, in conjunction with that from others (4), indicate that NO is involved in elevated blood flow during mechanical compression. This effect may require venous distension such that the muscle pump effect is activation (55).

Our earlier data have shown that application of IPC with the same device and parameters used in the present study does not induce significant systemic effects compared with controls (36), excluding the possibility of passive vasodilation via increasing cardiac output or perfusion pressure. Clinical data have also shown no statistically significant influence of IPC application on MAP and HR (59, 60). In the present study, infusion of l-NMMA at different dosages resulted in an increase in systemic MAP but had no effect on HR and RR compared with a PBS-treated group (Fig. 2A), a finding similar to those reported by others (33, 47). Compared with the huge difference (−30–45%) in cremaster arterial diameters between group A (IPC + PBS) and group F (10 μmol/min−1·100 g l-NMMA infusion−1) (Fig. 4), this mild increase (−10–20%) in systemic MAP is unlikely to be a major factor in the l-NMMA-induced local effects. Therefore, the dose-dependent counter effect of l-NMMA on IPC-induced vasodilation is not related to systemic effects of l-NMMA but rather to the local changes in vascular tone of the cremaster muscle.

Methodology Considerations

The IPC device was designed with two characteristics. First, IPC pressure reached its maximum of 55 mmHg within <1 s, which causes a marked and rapid venous collapse in the lower extremities, thereby creating strong shear stress on the venous wall. Earlier data have demonstrated that an increase in arterial blood flow emerges only when relatively rapidly increasing compression is applied (37, 39). Second, the device applied asymmetric compression to the lateral and medial sides of the limb. A recent study has shown that asymmetric compression produces greater venous collapse and larger shear stress (peak value > 20 Pa)
than circumferentially symmetric compression (peak value < 5 Pa) (8). Taken together, the data offer a solid reference for future improvements in IPC in prophylaxis and treatment of DVT clinically.

Only eNOS mRNA and protein expression were measured in this study. Upregulation of eNOS transcriptional and translational expression does not always mean an increase of eNOS activity and NO generation because of the influence of mRNA degradation control and posttranslational control. Direct measurement of authentic NO production from living tissues is important but is difficult because of NO’s short half-life and its inactivation by hemoglobin (16).

In conclusion, vasodilation in rat cremaster muscle produced by IPC application on the legs was accompanied by upregulation of eNOS mRNA and protein expression and was suppressed by inhibition of NOS in a dose-dependent fashion. This suggests that NO, particularly eNOS, plays an important role in regulation of microcirculation during IPC used in prophylaxis against DVT. This IPC-induced NO pathway also suggests that there may be a potential for pharmacological manipulation of systemic NO for DVT prophylaxis in postoperative and trauma situations. Further study in this model using NO donors is necessary to better establish this possibility.

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