Limb venous distension evokes sympathetic activation via stimulation of the limb afferents in humans

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Cui J, McQuillan PM, Blaha C, Kunselman AR, Sinoway LI. Limb venous distension evokes sympathetic activation via stimulation of the limb afferents in humans. Am J Physiol Heart Circ Physiol 303: H457–H463, 2012. First published June 15, 2012; doi:10.1152/ajpheart.00236.2012.—We have recently shown that a saline infusion in the veins of an arterially occluded human forearm evokes a systemic response with increases in muscle sympathetic nerve activity (MSNA) and blood pressure. In this report, we examined whether this response was a reflex that was due to venous distension. Blood pressure (Finometer), heart rate, and MSNA (microneurography) were assessed in 14 young healthy subjects. In the saline trial (n = 14), 5% forearm volume normal saline was infused in an arterially occluded arm. To block afferents in the limb, 90 mg of lidocaine were added to the same volume of saline in six subjects during a separate visit. To examine whether interstitial perfusion of normal saline alone induced the responses, the same volume of albumin solution (5% concentration) was infused in 11 subjects in separate studies. Lidocaine abolished the MSNA and blood pressure responses seen with saline infusion. Moreover, compared with the saline infusion, an albumin infusion induced a larger (MSNA: Δ14.3 ± 2.7 vs. Δ8.5 ± 1.3 bursts/min, P < 0.01) and more sustained MSNA and blood pressure responses. These data suggest that venous distension activates afferent nerves and evokes a powerful systemic sympathoexcitatory reflex. We posit that the venous distension plays an important role in evoking the autonomic adjustments seen with postural stress in human subjects.

Blood pressure is one of the most highly regulated hemodynamic variables measured in humans. It is clear that, in addition to arterial baroreceptors (44, 46) located in the central arteries and the aorta, many additional autonomic reflex loops can serve to regulate blood pressure. Evidence in animals suggests that afferent nerve endings in peripheral veins may sense regional blood volume changes and in turn contribute to the blood pressure regulation via a reflex response in cats (11, 39), rabbits (2), and dogs (12). Haouzi et al. (17) demonstrated that acute venous distension and the intra-arterial infusion of vasodilator papaverine increased the discharge of group III and IV afferent nerves in cats. Abdominal venous distension in rabbits (2) evokes enhanced sympathetic efferent discharge.

Recently, we reported that the infusion of normal saline in the veins of an occluded forearm evokes a large increase in systemic muscle sympathetic nerve activity (MSNA) and raises arterial blood pressure in humans (10). In additional studies, we reported that the magnitude of the MSNA and blood pressure responses depended on the volume and the rate of the infused saline (9). In the experimental model employed, peripheral venous distension was seen without changes in central blood volume (9). Thus the response was disassociated from arterial and cardiopulmonary baroreceptors. In this paper, we tested two crucial hypotheses. The first was that the blood pressure and MSNA responses seen with the infusion of saline could be blocked with lidocaine. Lidocaine is widely employed as a regional anesthetic agent (4). If lidocaine did in fact block the blood pressure and MSNA response, we would be confident that the observed autonomic responses with saline infusion were reflex in nature. Second, we speculated that infused normal saline evoked the increases in MSNA and blood pressure via venous distension per se and not by increasing interstitial fluid and stimulating interstitial afferents. To examine this question, we again used the prior venous distension model and compared the reflex responses of equal volumes of normal saline and albumin. Albumin is clinically used for acute vascular expander (19), and, in normal physiology, albumin contributes importantly to colloid osmotic pressure. We reasoned that if venous distension were the primary determinant of the autonomic responses seen with saline infusion, then albumin should evoke a larger and more sustained increase in blood pressure and MSNA.

METHODS

Subjects

Fourteen subjects (8 male, 6 female) participated in the study. The average age was 27 ± 1 (SE) yr, and all were of normal height (174 ± 3 cm) and weight (72 ± 4 kg). All subjects were normotensive (supine blood pressures <140/90 mmHg), in good health, and none were taking medications. Subjects refrained from caffeine, alcohol, and exercise for 24 h before the study. The experimental protocol was approved by the Institutional Review Board of the Milton S. Hershey Medical Center and conformed with the Declaration of Helsinki. Each subject had the purposes and risks of the protocol explained to them before written informed consent was obtained.

Measurements

Forearm volume (i.e., from elbow to wrist) was assessed by the water displacement method (13). The volume of the forearm was obtained by subtracting the hand (to wrist) volume from the limb (to elbow) volume. Beat-by-beat blood pressure was recorded from a finger (Finometer; Finapres Medical Systems, Amsterdam, The Netherlands) with resting values verified by auscultation of the brachial artery (SureSigns VS3, Philips; Philip Medical System) from the nontreated arm. Heart rate was monitored from the electrocardiogram (Cardicap/5, Datex-Ohmeda; GE Healthcare). Respiratory frequency was monitored using piezoelectric pneumography. Multifiber recordings of MSNA were obtained with a tungsten microelectrode inserted in the peroneal nerve of a leg. A reference electrode was placed...
subcutaneously 2–3 cm from the recording electrode. The recording electrode was adjusted until a site was found in which muscle sympathetic bursts were clearly identified using previously established criteria (51). The nerve signal was amplified, band-pass filtered with a bandwidth of 500–5,000 Hz, and integrated with a time constant of 0.1 s (Iowa Bioengineering, Iowa City, IA). The nerve signal was also routed to a loudspeaker and a computer for monitoring throughout the study.

Protocols

Effects of lidocaine. The lidocaine trial and the respective saline trial (control) were performed in six subjects during two separate visits. The two visits were separated by over one month.

All subjects were tested in the supine position. An intravenous catheter was inserted in the antecubital fossa of the nondominant arm. After instrumentation, baseline (6 min) heart rate, blood pressure, MSNA, and respiratory excursion data were collected. The basic procedure was the same as described in a previous report (9). Briefly, the arm was elevated and was fitted with three occlusion cuffs from the wrist to the elbow. A forth cuff was placed on the upper arm. From the wrist to the upper arm, the cuffs were inflated to 250 mmHg in sequence. Next, the three cuffs on the forearm were deflated and removed. When the upper arm cuff remained inflated, the arm was put back to the horizontal position. We term this procedure “W-E occlusion.” This procedure allows the infusate (e.g., lidocaine solution) to quickly distribute in the vascular system of the limb. After 4 min of preinfusion, ischemia data were collected, and normal saline (0.9% concentration) equal to 5% arm volume was infused in the occluded forearm via the intravenous catheter in the antecubital fossa (saline trial). A syringe pump was employed to maintain a constant infusion rate of 30 ml/min. The volume and rate of infusion were based on the previous study (9). After 5 min from the end of infusion, the upper arm cuff was deflated.

On a separate day, the lidocaine trial was performed. This trial was identical to the trial above, except that 90 mg of lidocaine were added to the saline infusate. The dose of lidocaine was based on pilot studies, and the dose used was less than that employed during a regional anesthetic procedure (4).

Effects of albumin. The albumin trial and the respective saline trial were performed in 11 subjects during two separate visits performed in random sequence. The two visits were separated by approximately one month. In the albumin trial, an albumin solution (5% concentration) equal to 5% arm volume was infused using the above procedure. In the saline trial, the same volume of saline was infused. Only three subjects were studied in all three trials (lidocaine, albumin, and saline) on three separate visits.

Data Analysis

Data were sampled at 200 Hz via a data acquisition system (MacLab; AD Instruments, Castle Hill, Australia). MSNA bursts were...
first identified in real time by visual inspection of the data, coupled with the burst sound from the audio amplifier. These bursts were further evaluated by a computer program that identified bursts based upon fixed criteria, including an appropriate latency following the R wave of the electrocardiogram (8). Integrated MSNA was normalized by assigning a value of 100 to the mean amplitude of the top 10% largest bursts during the 6-min baseline period. Normalization of the MSNA signal was performed to reduce variability between subjects attributed to factors including needle placement and signal amplification. Total MSNA was identified from burst area of the integrated neurogram with the computer program (8). MSNA was expressed as burst frequency and as total activity. Mean arterial blood pressure (MAP) was calculated from the Finometer waveform during each trial, whereas the baseline MAP was verified by an automated sphygmomanometer from an upper arm.

The mean values for MSNA, MAP, and heart rate were analyzed over the 6-min freely perfused baseline, W-E occlusion, the last 3 min of the preinfusion, the last 30 s during the infusion, the first and the second 30 s of postinfusion, and the 2nd and the 3rd min of postinfusion. Because the peak MSNA and MAP responses occurred toward the end of the infusion period, the mean values obtained during the last 30 s of infusion and the first 30 s of the postinfusion period (i.e., the entire 60 s) were defined as the maximal responses. The changes (Δ) from the preinfusion to the Max response were also used for comparisons.

Statistics

The absolute values of cardiovascular variables and MSNA were used to examine the effects of the interventions in the protocol (e.g., infusion, etc.) and the drug (lidocaine vs. saline) via repeated-measures two-way ANOVA. When appropriate, Tukey post hoc analyses were employed. In a similar manner, the cardiovascular variables and MSNA in the study of effects of albumin were compared. To further examine the different effects of the albumin infusion from the saline infusion, the differences among the changes (Δ) from the preinfusion to the Max response were evaluated via paired t-test (36, 37). All values are reported as means ± SE. P values of <0.05 were considered statistically significant.

RESULTS

Effects of Lidocaine

The effects of lidocaine on the reflex to saline infusion are shown in Fig. 1. Infusion of saline induced significant increases in MSNA and MAP. Lidocaine abolished the MSNA and MAP responses. Heart rate did not change in either trial.

Effects of Albumin

The absolute MSNA and the cardiovascular variable values for the albumin and the respective saline trials are shown in
Fig. 2. MSNA and MAP increased significantly toward the end of the infusions in both trials. During the last 30 s of the albumin infusion, MSNA was significantly greater than it was during the saline trial. Albumin induced greater maximal MSNA (burst rate $14.3 \pm 2.7$ vs. $8.5 \pm 1.3$ bursts/min, $P < 0.01$, total activity $467 \pm 98$ vs. $275 \pm 52$ U/min, $P < 0.01$) and MAP increases (i.e., the change from the preinfusion to the Max period) than the infusion of saline (Fig. 3). Moreover, the MSNA and MAP responses in the albumin trial lasted longer than during the saline trial. For example, MSNA during the 30–60 s of postinfusion in the albumin trial was significantly greater than in the saline trial (burst rate $32.2 \pm 2.7$ vs. $25.3 \pm 3.6$ bursts/min, $P < 0.05$, total activity $761 \pm 89$ vs. $546 \pm 82$ U/min, $P < 0.005$, Fig. 2). During the postinfusion (0–3 min), the absolute MAP in the albumin trial was significantly greater than in the saline trial (e.g., 30–60 s, $97.9 \pm 3.2$ vs. $91.1 \pm 1.4$ mmHg, $P < 0.005$). In Fig. 4, we present the representative recordings of integrated MSNA and blood pressure during albumin, saline, and lidocaine trials in one subject.

**DISCUSSION**

The main findings of this study are that: 1) lidocaine eliminates the increases in MSNA and blood pressure seen during the venous distension with saline infusion; and 2) albumin evokes a greater and more sustained increase in MSNA and blood pressure than does saline. The results suggest that the observed MSNA and blood pressure increases seen during the saline infusion are due to the reflex stimulation of forearm afferents. The albumin studies suggest that venous distension and not an increase in interstitial volume is a crucial factor in evoking the observed autonomic response.

Our recent reports have shown that saline infusion in an occluded arm induces an increase in MSNA and blood pressure (9, 10). Prior studies suggest that peripheral group I and II fiber activations do not evoke autonomic reflex (34, 47). Other studies have clearly established that muscle contraction activates thin fibers that are both myelinated (group III) and unmyelinated (group IV) afferent fibers. When engaged, they evoke a pressor response (25, 27, 35). Both chemical and mechanical stimuli can activate group III and IV afferent fibers (25–28, 35, 38). It is interesting to note that many of these free nerve endings are located within the adventitia of the small venous vessels (48, 52, 53). Moreover, it has been shown that distension of the femoral-saphenous vein in cats (11, 39) and large abdominal veins in rabbits (2) increases the discharge of group III and group IV afferents. Venous distension is noted to increase sympathetic efferent activity in rabbits (2) and dogs (12), and group III and group IV afferents with free nerve endings in the triceps surae muscle increase their discharge in response to both the infusion of vasodilatory agents and in response to venous occlusion in cats (16, 17). We suggest that the present data and our previous observations (9, 10) in humans are consistent with the concept that saline infusions in an arterially occluded forearm stimulates group III and IV afferents and evokes an autonomic pressor reflex.

Both rat (14) and human (3) studies showed that venous afferents are polymodal and may have nociceptive function. Direct stretch of hand veins in humans has been shown to evoke the sensation of pain (3). In our experiments, pain was not noted during the infusions of saline, albumin, or lidocaine. Subjects reported a pressure sensation in the forearm during the infusion. Whether the afferents responsible for this sensation are the same as the ones evoking the MSNA and blood pressure responses is not clear.

The blood pressure response to the albumin infusion was slightly but significantly greater than the saline infusion (see Figs. 2 and 3). A study in dogs showed that the plasma volume retention rate for an infusion of Tyrode’s solution (i.e., isotonic solution) was approximately half of the retention rate for dextran (i.e., a volume expander) when the infusion rate was 15
release of some substance(s) from the vascular walls and/or the pressure gradient across the vessel may directly stimulate the mechanically sensitive afferent endings. How-

ever, we cannot exclude that venous distension leads to the movement of the fluid in the interstitium was the key distinction between the "venoarteriolar reflex" and the "Bainbridge reflex." It should be noted that its role in human physiology has been difficult to categorically document (7, 33). To our knowledge, there are no studies documenting that distension of the central great veins leads to sympathoexcitation and an increase in peripheral vascular resistance (15). In fact, human studies employ approaches that lower central venous pressure as a way to increase MSNA and vascular resistance (i.e., baroreflex) (43).

On the other hand, distension of abdominal veins in rabbits (2) and femoral-saphenous vein in dogs (12) has been shown to evoke sympathetic activity. Studies in cats demonstrate that afferents from femoral-saphenous (49, 50, 54) and triceps surae muscle veins (17) sense the limb venous distension (11, 17, 39). Our report demonstrates that this reflex is indeed present and quite active in humans. Current physiological teaching does not emphasize such a sensory role for limb veins in humans (1, 32, 33). We believe that a greater understanding of this system may afford opportunities to better understand blood pressure regulation in health and disease.

Fig. 4. Representative tracing of MSNA and blood pressure during infusion of albumin solution (top), saline (middle), or lidocaine (90 mg) solution (bottom) in the venous circulation of an arterially occluded arm in one subject. The volume of the infusion in the 3 trials was equal to 5% forearm volume. Notice the clear MSNA activation and blood pressure increases during the infusion of saline or albumin solution while the responses were not evoked during the infusion of lidocaine solution.

ml/min for both trials (30). Thus, when normal saline (i.e., isotonic) is infused in a peripheral vein, a sizeable fluid volume will diffuse out of the vessel and in the interstitium. Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41). Thus saline infusion will cause venous distension (9) and an increase in interstitial volume. It should be noted that albumin is the largest protein constituent of plasma, and it plays an important role in maintaining osmotic pressure. In fact, intravenous albumin is often used for rapid plasma volume expansion (19, 31, 41).

Perspective on the Importance of the Limb Venous Distension Reflex

Prior animal studies examining the role cardiovascular structure can play as sensor organs have focused on a more central portion of this system. In particular, work has focused on the role of central venous structures, overall venous return, afferents in pulmonary veins, and afferents within the cardiac muscle (5, 15, 23, 24, 29). For example, it has been proposed that afferents located at the junction of the right atrium and caval veins or at the junctions of the pulmonary veins and the left atrium when stimulated evoked a reflex increase in heart rate in dogs (24, 29). This response has been termed the "Bainbridge reflex." It should be noted that its role in human physiology has been difficult to categorically document (7, 33). To our knowledge, there are no studies documenting that distension of the central great veins leads to sympathoexcitation and an increase in peripheral vascular resistance (15). In fact, human studies employ approaches that lower central venous pressure as a way to increase MSNA and vascular resistance (i.e., baroreflex) (43).

When venous pressures are raised above 25 mmHg via venous stasis, subcutaneous and cutaneous vascular resistance increases within the region in question (6, 18, 20–22, 42). This response has been termed the venoarteriolar reflex. Previous observations suggest that local mechanisms [e.g., the sympathetic axon reflex (21, 40) and myogenic autoregulatory mechanisms (6, 42)] may be responsible for this local response. It should be noted that prior work on the venoarteriolar reflex has focused on blood flow within the region in question. The venoarteriolar reflex does not increase blood pressure (42). Thus, a key distinction between the "venoarteriolar reflex" and any number of different sensory inputs from veins, cutaneous vessels, or the skin may stimulate the cutaneous sympathetic nerve endings.
the venous distension reflex is that the venous distension reflex causes a rise in systemic blood pressure and sympathetic nerve traffic, whereas venaarteriolar reflex describes a local flow regulatory system.

Study Limitations
Lidocaine was infused through a large vein in the occluded arm. We suggest that the afferent endings responsible for the reflex are in close proximity to veins. However, the present data do not allow us to make any statements as to the size of the veins in question. Further studies are necessary to examine this issue.

In conclusion, the present study shows that volume infusion in an occluded arm activates afferents, evokes an autonomic reflex, and raises sympathetic activities and systemic blood pressure. Distension of veins and/or the increase in pressure gradient across the vascular wall plays a key role in evoking this autonomic reflex. Thus, venous distension in limbs “directly” contributes to cardiovascular adjustment in humans.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

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