Muscle Cholinergic Dilators in the Sinus Baroreceptor Response in Cats

By Toru Takeuchi and John W. Manning

ABSTRACT

Experiments were done to evaluate the effect of the cholinergic vasodilator system on the flow-pressure relation in the isolated, perfused gastrocnemius and soleus muscles of the cat. Reflex changes in the muscle vascular resistance were produced by maintaining the isolated, perfused carotid sinus at different pressure levels. Flow-pressure curves were obtained from the isolated muscles at high, medium, and low carotid sinus pressures before and after administration of adrenergic or cholinergic blocking drugs or denervation, or both. Following cholinergic receptor blockade, flow-pressure relations at high sinus pressure showed a loss of a dilator component, but curves at low and medium sinus pressures were the same as controls. Following adrenergic receptor blockade, flow-pressure curves at low and medium sinus pressures were superimposed; however high sinus pressure still produced a decrease in vascular resistance. The remaining vasodilation could be abated by either denervation or anticholinergic agents. The difference between the untreated and the cholinergically blocked flow-pressure responses represented 15-30% of the range of change in vascular resistance evoked by the sinus baroreceptor reflex. Thus, effective withdrawal of sympathetic vasoconstrictor tone did not account for total vasodilation. The data indicate that the sympathetic cholinergic vasodilator system participated in the vascular responses of skeletal muscle during baroreceptor stimulation.

KEY WORDS baroreceptor reflex sympathetic cholinergic dilator active vasodilation flow-pressure response cholinergic blockade adrenergic blockade vascular resistance

Since Bülbbring and Burn (1) and Folkow et al. (2, 3) demonstrated that the sympathetic vasodilator nerves in muscle were cholinergic, a number of investigators have verified it. The vasodilator pathway is thought to originate in the frontal cortex, to pass through synaptic relays at the hypothalamic and collicular levels, and to run through the ventral medulla to the spinal cord without an additional synaptic relay (4). Under what functional state this sympathetic cholinergic dilator system is called into play has been in question since it was described and demonstrated. Hilton (5) has suggested that the dilator system is activated through hypothalamic mechanisms in the preparatory response to fight or flight.

Numerous investigators have failed to elicit active cholinergic dilation during vascular reflex changes initiated by the carotid sinus baroreceptors in response to transient hypertension (2, 6, 7). There is little doubt of the contribution made by the α-adrenergic vasoconstrictor nerves in altering peripheral resistance of a muscle bed during the baroreceptor reflex (8-10). Recent reports indicate that active vasodilation in skeletal muscle results from histamine release by sympathetic nerve action brought about by increasing pressure in the sinus area (11-13). However, the usual mode of exciting sinus baroreceptors is the application of transient high pressure rather than a prolonged state of hypertension to the sinus region. The purpose of our study was to
investigate the possible role of the cholinergic vasodilator system in determining the flow-pressure relation obtained in isolated, perfused muscle at different levels of maintained sinus pressure.

Methods

Successful experiments were performed on 22 cats of both sexes weighing 2.5-4.8 kg. Anesthesia was induced with ether and maintained by intravenous injection of α-chloralose (40-50 mg/kg). The common vagosympathetic trunk and the aortic depressor nerve were sectioned at a cervical level. Following vagotomy, respiration was maintained at 10-20 strokes/min with a tidal volume of 50 ml by use of a Harvard respirator. Body temperature (monitored by a probe in the esophagus at the level of the thorax) arterial oxygen saturation, and arterial pH were kept within normal limits. Systemic arterial pressure, carotid sinus pressure, and perfusion pressure of the isolated muscles were measured with Statham transducers and simultaneously displayed on a Grass polygraph. Heparin was administered (500 units/kg iv), and cannulas were placed for separate perfusion of the carotid sinuses by an external pump. The common carotid arteries were cannulated distally, and the left common carotid artery was cannulated proximally. Blood from the left common carotid artery was received by an Emory roller pump and used to perfuse the carotid sinuses. The circuit required a priming volume of about 10 ml; heparinized 5% dextran solution was used. The perfusion pressure of the sinus region was maintained at approximately the same level as the systemic blood pressure. Systemic arterial pressure was recorded through a cannula placed in the right common carotid artery. An arteriovenous collateral channel was made with tubing by connecting the lingual arteries with the right external jugular vein. A schema of the perfusion circuits is given in Figure 1. Hypertension in the sinus region was produced by clamping the external carotid artery and adjusting the perfusion flow rate. Hypotension was elicited by opening the artificial arteriovenous shunt and adjusting the flow rate of the pump. These procedures allowed the carotid sinus pressure to be maintained at three levels; low (25-50 mm Hg), medium (the same level as systemic blood pressure), and high (250 mm Hg) sinus pressures. The latter value was selected to ensure maximum reflex activation of a vasodilator response.

Except for the caudal femoral artery, the gastrocnemius and soleus muscles of the left leg were carefully and completely isolated from all blood vessels, especially from those at the knee joint.

The vascular isolation was confirmed at the end of an experiment by the injection of India ink into the perfusion circuit. Maximum care was exercised to keep nerves intact when ligating vessels. The isolated muscle preparation was perfused with a Holter roller pump with blood taken from the right femoral artery. The muscles were covered with skin, the perfusing circuit and leg were wrapped in a warming jacket, and surface temperature of the muscle was maintained at 38°C. Caution was exercised to avoid any small obstruction of venous return from the muscle. At each of the sinus pressures mentioned above, muscle blood flow was changed stepwise to five different levels, and the perfusion pressure was measured as shown in Figure 2. Each step change was maintained approximately 30-40 seconds with the exception of zero flow, which was maintained only 15-20 seconds. Flow-pressure data points were taken at near steady-state values. All drugs were given by rapid injection
into the muscle perfusion circuit; the drugs were dissolved in 10–50 µliters of physiological saline which was adjusted to a pH of 7.4 when possible. To minimize the effects of formation of edema on the experimental results, all data were collected less than 1.5 hours after the start of perfusion. In preliminary studies, the isolated, perfused gastrocnemius-soleus preparation was weighed and compared with the weight of the identical muscles in the intact opposite limb. No difference in the weights was observed.

Results

An example of the changes in heart rate, systemic arterial pressure, and perfusion pressure in the isolated muscle bed at high, medium, and low carotid sinus pressures is shown in Figure 2. Blocking agents given by close arterial injection into the isolated muscle had little systemic effect: arterial pressure was the same at each sinus pressure level before and after the drug. Table 1 presents the mean systemic pressure for each experimental group before and after drug treatment for the three sinus pressure levels. At a given sinus pressure, no statistical difference in mean pressure was noted within or between groups. These data attest to the near absence of drug effect on the systemic circulation when administered in small volumes into the perfused muscle bed. Because the vagus and the aortic depressor nerves were sectioned, the changes in heart rate were relatively small compared to those usually seen during the carotid sinus reflex. The difference in heart rate between low and high sinus pressure averaged 31 beats/min for

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>No. animals</th>
<th>Low CSP</th>
<th>Medium CSP</th>
<th>High CSP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13</td>
<td>203 ± 19</td>
<td>131 ± 21</td>
<td>56 ± 13</td>
</tr>
<tr>
<td>After cholinergic receptor blockade</td>
<td>13</td>
<td>192 ± 14</td>
<td>128 ± 17</td>
<td>56 ± 13</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>211 ± 21</td>
<td>121 ± 11</td>
<td>49 ± 16</td>
</tr>
<tr>
<td>After α-receptor blockade</td>
<td>7</td>
<td>209 ± 24</td>
<td>118 ± 10</td>
<td>51 ± 14</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>204 ± 19</td>
<td>37 ± 11</td>
<td>37 ± 11</td>
</tr>
<tr>
<td>After α-receptor and cholinergic blockade</td>
<td>5</td>
<td>180 ± 29</td>
<td>56 ± 14</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.D. CSP = carotid sinus pressure.

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the controls and 29 beats/min after the blocking agents. At each of the three sinus pressures, the muscle flow rate was adjusted to five levels before and after administration of a pharmacological blocking agent. The abrupt change in high flow values produced some degree of autoregulation in the muscle bed. The values for perfusion pressure were taken at the end of each 30- to 40-second step change in flow. Assuming that the change in perfusion pressure in response to a step change in flow follows a nearly exponential function, the rapidity of change recorded from the perfused muscle (Fig. 2) ensured that values taken at the end of 30 seconds represented better than 90% of the maximum change in the system. The altered vascular resistance in the muscle is depicted better by constructing flow-pressure curves obtained under each experimental condition. The data presented in Figures 3-6 are mean values for each group and curves have been drawn through the means.

EFFECTS OF CHOLINERGIC RECEPTOR BLOCKADE

In 13 animals, the vascular cholinergic receptors in the gastrocnemius and soleus muscles were blocked by 300-400 μg of atropine (six cats) or by 0.05-0.1 μg of methscopolamine bromide (Pamine; seven cats). The efficiency of the blocking agents was tested by injection into the perfusion circuit of three dose levels of acetylcholine (0.1, 0.5, 1.0 μg). This test was made immediately before and after muscle perfusion pressure measurements at high, medium, and low sinus pressures. Complete blockade was always observed. Injections of equal volumes of saline had no effect on the muscle vascular response. No difference in the effects on the muscle vascular bed was noted between the two anticholinergic agents.

The plot of average flow-pressure points ± se before and after cholinergic receptor blockade is given in Figure 3. The flow-pressure curves were taken at low, medium, and high sinus pressures. Each animal served as its own control, and the P values represent levels of significant difference between the means at high sinus pressure. The degree of vasodilation in the muscle at high sinus pressure was greater before blockade of cholinergic transmission than after. However, there was no appreciable change before and after the drug in the vascular resistance of the muscle bed during medium or low sinus pressures. The reduction in the dilator response caused by cholinergic receptor blockade represented approximately 20% of the range of change in resistance evoked by the sinus baroreceptor reflex.

EFFECTS OF ALPHA-ADRENERGIC RECEPTOR BLOCKADE

Figure 4 represents the flow-pressure relations in the isolated muscles of seven animals before and after α-adrenergic receptor blockade with phenoxybenzamine (0.45-0.5 mg).
Flow-pressure curves obtained from muscle bed of 7 cats before and after infra-arterial administration of phenoxybenzamine HCl (Dibenzyline), 0.45–0.5 mg, at low, medium, and high sinus pressures. Perfusion pressure expressed as mean ± SE for each flow rate. P values are levels of significant difference from paired t-test of difference in mean perfusion pressure at medium and high sinus pressure after α-adrenergic blockade.

Again, complete blockade was demonstrable, because injection of 0.3, 0.6 and 1.0 μg of norepinephrine into the muscle perfusion circuit produced no vasoconstrictor response. The control curves were similar to those obtained for Figure 3. They represent the range through which the sinus reflex could drive the vascular resistance of the isolated muscle bed. If the decrease in vascular resistance as the sinus pressure was manipulated from low to high values was due entirely to withdrawal of sympathetic adrenergic activity, then there should be no difference following adrenergic receptor blockade between the flow-pressure relations at any of the sinus pressures. However, there was a significant vasodilator response at high sinus pressure not seen at the transition from low to medium sinus pressure. The overlap of flow-pressure curves at medium and low sinus pressures was an index of the functional mitigation of α-adrenergic activity by phenoxybenzamine. The P values are the level of significant differences between the means at medium and high sinus pressure following the drug. At flow values of 7.5 and 10 ml/100g min⁻¹, the mean perfusion pressures during sinus hypertension were statistically different before and after the drug (P<0.01). At flow rates above 2.5 ml/100g min⁻¹, the perfusion pressures in the isolated muscles during high sinus pressure were consistently less than the paired control values. Thus, as represented by the lowest broken line in Figure 4, the sinus reflex could actively decrease vascular resistance in the muscle bed beyond the point of inhibition of adrenergic constrictor tone.

EFFECTS OF ALPHA-ADRENERGIC AND CHOLINERGIC RECEPTOR BLOCKADE

An estimate of the contribution that the cholinergic dilator system made to decreasing...
vascular resistance in a muscle bed in response to sinus hypertension was obtained in five animals by the use of both blocking agents (Fig. 5). Following \( \alpha \)-adrenergic and cholinergic receptor blockade, the flow-pressure curves at high and low sinus pressures were approximately equal. There was no statistical difference between the pairs of mean perfusion pressures of the two curves at each of the five flow rates. This was taken to indicate the elimination of neural vasomotor activity in the isolated muscles during the reflex response. Prior to treatment, muscle vascular resistance attained a much lower value during sinus baroreceptor stimulation. Under these conditions, the mean perfusion pressure with high sinus pressure at flow rates of 2.5 ml/100g min\(^{-1}\) and above were significantly less than after drug blockade. At flow rates above 2.5 ml/100g min\(^{-1}\), 20–30% of the decrease in vascular resistance in the muscle bed was accounted for by activity of the cholinergic system.

**EFFECT OF ALPHA-ADRENERGIC RECEPTOR BLOCKADE AND DENERVATION**

The participation of the cholinergic dilator system was further demonstrated in four animals in which muscle vasoconstrictor activity was first blocked with phenoxybenzamine (0.5 mg) before baroreceptor stimulation. Flow-pressure curves were then repeated at high and low sinus pressures following surgical denervation of the muscles. A comparison of the vascular resistance under these conditions is given in Figure 6. Again, following pharmacological inactivation of the \( \alpha \)-adrenergic component, high sinus pressure evoked a decrease in muscle vascular resistance. This dilator response was abolished by surgical denervation. The negation of muscle adrenergic constrictor participation in the reflex was most marked in these experiments: flow-pressure curves at low sinus pressure were nearly coincident with those after denervation.

**Discussion**

There is some debate concerning the role, if any, of active vasodilation in the baroreceptor reflex effecting a decrease in skeletal muscle vascular resistance compared with the well-established role of passive withdrawal of sympathetic vasoconstrictor tone. The neurogenic release of histamine as part of the reflex dilation has been implied from determinations of radioactive labeled histamines collected in the venous effluent of muscles (12, 14). However, phenoxybenzamine markedly attenuated the amount of \(^{14}\)C-histamine found in the venous blood of isolated, perfused gracilis muscle during sinus nerve stimulation. The results of our present study would support the concept that part of the baroreceptor vasodilation in muscle can be accounted for by activation of the cholinergic dilator system and is not solely due to a purely passive cessation of norepinephrine release from postganglionic sympathetic nerve endings.

The part of the dilator response that is truly cholinergic has been demonstrated in a number of ways. Figures 3 and 5 show the loss of a dilator component in response to sinus...
hypertension following blockade of cholinergic receptors. That this dilator response is in addition to the inhibition of adrenergic constrictor tone is demonstrated in Figures 4 and 6, where neurally activated vasodilation remained after adrenergic receptor blockade. Indeed, phenoxybenzamine proved an efficient agent in abating constrictor activity, for after treatment, low and medium sinus pressures evoked no change in muscle vascular resistance. The remaining dilator component is definitely neurogenic, for sectioning of nerves to the muscles abolished the response (Fig. 6).

Other systemically mediated dilator agents have been proposed. Boerth, et al. (12) presented data to support the idea that an active component of the baroreceptor-reflex vasodilation was mediated by release of histamines from sympathetic nerves. However, this histamine release was blocked by agents which interfere with adrenergic neurotransmission, e.g., phenoxybenzamine. Histamine must not play a role in the dilator response we described, for phenoxybenzamine in doses which block adrenergically mediated vasoconstriction did not prevent the vasodilation. Further, the active vasodilation seen during high sinus pressure was eliminated by anticholinergic drugs.

It has been reported that stimulation of the lumbar sympathetic chain after atropine results in a muscle vasodilation which is prevented by β-adrenergic receptor-blocking agents (15). Although this activity was elicited by sympathetic nerve stimulation, the β-vasodilation was shown not to be a component of the pressor-receptor reflex resistance change in skeletal muscle of dog. Beck et al. (16) divided active vasodilator responses in the isolated hind limb of the dog into two phases, a transient and a sustained component. About 56% of the transient vasodilation that developed in the first 30 seconds of sympathetic stimulation was blocked by atropine, whereas only 37% of the sustained component that remained after 2 minutes of continued stimulation was affected by the drug. The remainder of the dilator activity was not accounted for by cholinergic, β-adrenergic, or histaminic transmitters. In our studies of the isolated gastrocnemius and soleus muscles of the cat, active vasodilation was maintained with high carotid sinus pressure for 5-minute periods during which time a response was effectively and consistently blocked by anticholinergic agents (Fig. 1). From flow-pressure measurements before and after blocking agents, active dilator responses in muscles effected by the sinus reflex appeared to be cholinergic in nature. This does not deny the possible participation of other neural mediators as dilator agents that could be released by direct electrical stimulation of sympathetic nerves supplying muscle as suggested by other investigators mentioned above.

A number of reflex maneuvers have been reported to yield active dilator responses. Jones and Berne (17) described the induction of muscle vasodilation, in addition to passive withdrawal of adrenergic tone in response to elevated aortic pressure. Stimulation of the carotid and aortic chemoreceptors activated sympathetic dilator fibers supplying prevenous resistance vessels in the paw of the dog (18). This dilation was not mediated by release of acetylcholine, histamine or bradykinin, by β-receptors, or by withdrawal of sympathetic constrictor tone. Given the variety of active dilator mechanisms described, it should not be a surprise to find a cholinergic dilator component participating in changes in muscle vascular resistance during baroreceptor stimulation. Perhaps our ability to demonstrate such a response resulted from the techniques employed to produce sustained levels of carotid sinus pressures. This mode of reflex activation of the cholinergic system was not plagued by the transient response observed with electrical stimulation of the sinus nerve or of the lumbar sympathetic chain. Indeed, a pronounced decrease in vascular resistance could be maintained for prolonged periods by perfusing the sinus at high pressure. During this time 15-30% of the increased flow in the muscle bed could be accounted for by active cholinergic dilation. No attempt was made to
equalize the pulse pressures at the three levels chosen to activate the baroreceptors. It was fortuitous that an elevated pulse pressure existed at high sinus pressure levels, because this is a more effective stimulus for driving baroreceptors to maximum firing range. An additional aid was the ability to compare altered vascular resistance obtained from multiple flow-pressure points with those of paired controlled values.

As sinus pressure was changed from low to medium to high levels, the baroreceptor reflex provoked a nearly complete passive withdrawal of adrenergic tone before it activated additional dilation through cholinergic mechanisms. Such sequencing would suggest a higher threshold to carotid sinus input for activation of the cholinergic system than for inhibition of sympathetic adrenergic tone. Considering the low level of impulse traffic in the sympathetic adrenergic nerves necessary to maintain physiological activity (19), a chain-link system of constrictor inhibition followed by dilator activation would give muscle vasculature a far wider range of central nervous system control.

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References

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