Role of Muscular Contraction in the Reflex Vascular Responses to Stimulation of Muscle Afferents in the Dog

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ABSTRACT

The effect of stimulation of somatic afferents from muscle was investigated in 24 anesthetized dogs. The carotid sinuses were isolated and kept at constant pressure, the vagi were cut, and the left iliac artery was isolated and perfused at constant flow. Muscle afferents were stimulated by either inserting electrodes into the muscles of the right thigh or stimulating the central end of the right femoral nerve. Muscle stimulation at 5 Hz decreased perfusion pressure in the iliac artery $76 \pm 6$ mm Hg (mean $\pm SE$); the decrease was not prevented by paralysis of the stimulated muscles, administration of atropine or antihistamines, or $\beta$-receptor blockade. Muscle stimulation at 40 Hz increased perfusion pressure in the iliac artery $28 \pm 3$ mm Hg. This increase was prevented or changed to a depressor response by muscle paralysis. Similar effects were obtained with femoral nerve stimulation. Both depressor and pressor responses persisted after the leg had been skinned and the muscles had been separated from their tendons, but they were abolished by left sympathectomy. Thus, skeletal muscle contraction is necessary for activation of the muscle afferents which cause reflex vasoconstriction but not for activation of those which cause reflex vasodilatation.

KEY WORDS muscle receptors muscular exercise sympathectomy peripheral mechanoreceptors somatic afferents muscle contraction limb circulation $\beta$-receptor blockade

Since the turn of the century, investigators have observed that muscle activity is accompanied by changes in arterial blood pressure (1-4), and it has been postulated that these changes are elicited by stimulation of afferent nerve fibers in the contracting muscles (5, 6). Experimental support for this hypothesis has come from observations in the cat showing that direct stimulation of afferent nerve fibers from muscle causes changes in arterial blood pressure and in the resistance of several vascular beds (7-10).

The question to be answered is whether the contraction of skeletal muscles can cause reflex vascular changes similar to those seen during stimulation of afferent nerves from muscle. Recent studies in the cat have shown an increase in aortic blood pressure during tetanic muscular contractions, which is abolished by muscle paralysis (11) or section of the dorsal roots (11, 12). In the present experiments, we examined the reflex vascular responses in one hind limb of the dog during rhythmic and tetanic contractions of the opposite hind-limb muscles.

Methods

Preparation.—Twenty-four dogs (15-25 kg) were anesthetized with sodium thiopental and $\alpha$-chloralose (15 and 80 mg/kg body weight, respectively, iv) and artificially ventilated at 10-12 cycles/min. Additional doses of chloralose (10 mg/kg, iv) were administered to maintain an even plane of anesthesia. Heparin (3 mg/kg, iv) was given prior to cannulation of the vessels and hourly thereafter (1 mg/kg, iv). Arterial $P_{O_2}$ was maintained above 250 mm Hg by ventilation with oxygen, $P_{CO_2}$ was kept between 30 and 40 mm Hg by adjusting the tidal volume, and $pH$ was held between 7.30 and 7.40 by infusing bicarbonate as needed.

Both vagi were divided in the neck. Both carotid sinuses were isolated according to the Moissejeff technique (13). The pressure within the sinuses was monitored; it was set at the level of mean aortic blood
pressure at the beginning of the experiment and held constant throughout.

**Measurements.**—All pressures were measured with strain-gauge transducers (Statham P23De) and recorded on an ultraviolet Visicorder (Honeywell 1508).

Aortic blood pressure was measured through a catheter inserted in the right brachial artery. The left hind limb was perfused at constant flow via the left external iliac artery with a roller pump using autologous blood drawn from the terminal aorta. A depulsator and a heat exchanger were interposed in the perfusion line, and the temperature of the blood was maintained at 37°C. The perfusion pressure was measured just proximal to the point of insertion of the cannula in the artery. The pump speed was adjusted at the beginning of each experiment to provide a perfusion pressure similar to the mean aortic blood pressure. The changes in perfusion pressure reflected changes in vascular resistance.

To eliminate other sources of arterial inflow to the limb, all branches of the terminal aorta and the deep circumflex iliac and deep caudal epigastric arteries were ligated. In some dogs, the right iliac artery was cannulated and perfused in the same way as was the left.

**Stimulation of Muscle Afferents.**—Square-wave stimuli were provided for muscle stimulation by a Grass stimulator (type S4) and two pairs of electrodes; one pair was inserted into the right anterior thigh muscles, and the other was inserted into the right posterior thigh muscles. The two poles of each pair of electrodes were kept 5 cm apart. The applied voltage was measured directly on the stimulating electrodes. The voltage used was that which caused maximal contraction of the muscles of the thigh without causing contraction of other muscles. A train of stimuli lasting 1 minute was delivered, and the duration of each stimulus was 5 msec. Frequencies of 5 and 40 Hz were used; 5 Hz caused rhythmic muscular contractions and 40 Hz caused a sustained tetanic contraction.

In some experiments, the right femoral nerve was divided and the central end was stimulated at 1 v and 5 Hz (at the electrodes) or at 5 v and 40 Hz for 1 minute.

**Pharmacologic Blockade of Vascular Responses.**—The following drugs were used: atropine sulfate (0.2 mg/kg), propranolol (1 mg/kg), triprolidene (1 mg/kg), and phenoxybenzamine (3 mg/kg). To test the effectiveness of these antagonists, isoproterenol HCl (0.05 μg/kg) and histamine phosphate (0.05 μg base/kg) were injected before and after the corresponding blocking agent. The drugs were infused intra-arterially upstream from the pumps perfusing the vascular bed under study.

**Role of Muscle Contraction.**—The role of muscle contraction in causing the observed responses was investigated by stimulating the muscles before and after muscle paralysis induced by gallamine triethiodide (3 mg/kg). In some dogs, the effects of isometric and isotonic contraction were compared. For the isometric exercise, the thigh was fixed at the hip and the knee.

**Afferent and Efferent Pathways.**—The afferent pathway responsible for the reflex changes in the perfused hind limbs was studied by stimulating the muscles before and after dividing the femoral nerve high in the groin and the obturator and sciatic nerves in the pelvic cavity. The efferent pathway was studied by removing the left sympathetic chain from L4 to L6. This procedure eliminates most of the sympathetic supply to the hind limb (14). In some dogs, prior to the left sympathectomy, the right sympathetic chain was removed from L4 to L6.

**Localization of Receptors.**—To eliminate the cutaneous afferents, the thigh was skinned from the hip to below the knee. To eliminate the influence of stretch receptors in the tendons, the muscles were (1) stimulated after the tendons had been cut at the musculotendinous junctions and (2) separated from the femur. These procedures also prevented movement at the knee.

**Results**

**Effect of Stimulation of Right Hind-Limb Muscles on Perfusion Pressure in Left Iliac Artery.**—In 22 dogs, stimulation at 5 Hz and 4 v caused a mean (± se) decrease in the perfusion pressure of 76 ± 6 mm Hg; the aortic blood pressure decreased 48 ± 5 mm Hg. In the same dogs, stimulation at 40 Hz and 4 v caused an increase in perfusion pressure of 28 ± 3 mm Hg and an increase in aortic blood pressure of 35 ± 5 mm Hg. An example is shown in Figure 1. In some dogs, these increases were preceded and followed by transient decreases in aortic and perfusion pressures.

Depressor and pressor responses also were obtained by stimulation of the central end of the right femoral nerve. In four dogs, nerve stimulations at 5 Hz and 1 v caused a decrease of 73 ± 9 mm Hg in perfusion pressure in the left iliac artery and of 30 ± 7 mm Hg in aortic blood pressure. At 40 Hz and 5 v, the perfusion pressure increased 31 ± 11 mm Hg and the aortic blood pressure increased 15 ± 3 mm Hg. In the same dogs, muscle stimulation at 5 Hz caused a decrease of 78 ± 10 mm Hg in the perfusion pressure and of 43 ± 5 mm Hg in the aortic blood pressure; at 40 Hz the increases were 30 ± 7 and 27 ± 3 mm Hg, respectively.

**Role of Muscle Contraction.**—In 14 dogs, the effects of muscle stimulation were investigated before and after muscle paralysis with gallamine. An example is shown in Figure 2. In the control condition, stimulation at 5 Hz caused a mean (± se) decrease in perfusion pressure in the hind limb of 72 ± 10 mm Hg and a decrease in aortic blood pressure of 42 ± 5 mm Hg. Muscle paralysis did not abolish these responses, although the amplitudes were somewhat reduced: the decreases were 49 ± 7 mm Hg for hind-limb perfusion pressure and 36 ±
Changes in left hind-limb perfusion pressure during stimulation of right hind-limb muscles at 5 Hz (left) and 40 Hz (right). The hind limb was perfused at constant flow. Stimulation at 5 Hz caused rhythmic muscle contractions, and stimulation at 40 Hz caused a sustained tetanic contraction. IA = iliac artery, Ao = aorta.

At 5 Hz, a mean (± SE) pressor response of 27 ± 4 mm Hg in perfusion pressure and of 43 ± 8 mm Hg in aortic blood pressure was observed. After sympathectomy, this stimulation caused a mean decrease of 23 ± 4 mm Hg in aortic blood pressure and of 37 ± 4 mm Hg in perfusion pressure. At 40 Hz, before sympathectomy, muscle stimulation caused an increase of 47 ± 9 mm Hg in aortic blood pressure and of 33 ± 7 mm Hg in perfusion pressure. After sympathectomy, this stimulation caused an increase of 35 ± 5 mm Hg in aortic blood pressure and of 45 ± 9 mm Hg in perfusion pressure.

In three of the five dogs, the femoral, sciatic, and obturator nerves on the right side were cut. The reactions to muscle stimulation at 5 and 40 Hz were very much attenuated in two and abolished in the third dog. An example is given in Figure 3.
Efferent Pathway.—The depressor responses to low-frequency stimulation of the muscles were not influenced by atropine sulfate, propranolol, or tripelennamine. In eight dogs, before atropine administration, stimulation at 5 Hz caused a decrease of $84 \pm 6$ mm Hg in perfusion pressure; after atropine administration, this stimulation caused a decrease of $76 \pm 10$ mm Hg. In three dogs, stimulation at 5 Hz caused decreases in perfusion pressure of 80, 100, and 60 mm Hg before propranolol administration and of 60, 100, and 90 mm Hg after it. In three dogs, stimulation at 5 Hz caused decreases in perfusion pressure of 100, 60, and 50 mm Hg before tripelennamine administration and of 80, 60, and 60 mm Hg after it. In four other dogs, muscle stimulation at 5 Hz was performed before and after phenoxybenzamine administration. During the control period, stimulation at 5 Hz caused decreases of 60, 100, and 25 mm Hg in perfusion pressure; after phenoxybenzamine administration, the response to stimulation at 5 Hz was very much attenuated or abolished: 10, 0, 0, and 5 mm Hg. The vessels were still capable of vasodilatation, since glyceryl trinitrate (0.5 mg/kg, ia) caused a decrease in perfusion pressure of 15-20 mm Hg. Left sympathectomy (five dogs) abolished the response to stimulation at 5 and 40 Hz. An example is shown in Figure 4. At 5 Hz in the control condition, blood pressure decreased $28 \pm 7$ mm Hg and perfusion pressure decreased $54 \pm 10$ mm Hg. After left sympathectomy, stimulation at 5 Hz still decreased aortic blood pressure $27 \pm 4$ mm Hg, but perfusion pressure no longer showed any significant change ($-1 \pm 1$ mm Hg). At 40 Hz in the control condition, blood pressure increased $38 \pm 9$ mm Hg and perfusion pressure increased $43 \pm 11$ mm Hg. After left sympathectomy, blood pressure increased $45 \pm 9$ mm Hg, but perfusion pressure did not show any significant change ($1 \pm 1$ mm Hg).

Site of Receptors in Limb.—In three dogs, muscle stimulations at 5 and 40 Hz were performed after the thigh had been skinned. Stimulations at 5 Hz caused decreases in the perfusion pressure in the hind limb of 30, 50, and 100 mm Hg before and of 30, 40, and 90 mm Hg after skinning. Stimulations at 40 Hz caused increases in the perfusion pressure of 40, 40, and 40 mm Hg before and of 80, 30, and 50 mm Hg after skinning.

In the same three dogs, the muscles were also stimulated after the tendons had been cut at the distal musculotendinous junction. Stimulations at 5 Hz caused decreases in the perfusion pressure in the hind limb of 20, 30, and 30 mm Hg before and of 30, 70, and 100 mm Hg after the section. Stimulations at 40 Hz caused increases in the perfusion pressure of 80, 30, and 50 mm Hg before and of 80, 30, and 50 mm Hg after the section. An example is shown in Figure 5.
Discussion

To study the effects of electrical stimulation of somatic afferents from muscle, the carotid sinuses were isolated and the vagi were cut. This procedure eliminated reflex changes generated by alterations in activity of the high-pressure receptors in the carotid sinus and the aortic arch as well as those from the heart and the lung.

Both anesthesia and decerebration have been shown to decrease the amplitude of the pressor responses to somatic nerve stimulation (15). In the present experiments, the dogs were anesthetized with chloralose, and this procedure may have decreased the magnitude of the pressor responses.

Muscle contraction was caused by stimulation of the efferent motor fibers, because it was blocked by gallamine. To obtain direct muscle cell stimulation, a much higher intensity of stimulation is necessary; in that condition, spread of electricity and subsequent contraction of muscles elsewhere is observed.

Stimulation of the right hind-limb muscles at 5 Hz caused a decrease in aortic blood pressure and perfusion pressure in the left hind limb; an opposite response was obtained with stimulation at 40 Hz. The afferent pathway of these responses is in the somatic nerve fibers from the muscles, since the reactions were markedly attenuated after the sciatic, femoral, and obturator nerves were cut but were unaffected by right sympathectomy. Moreover, similar responses could be obtained by stimulating the femoral nerve, which is considered to be mainly a muscle nerve (16).

The efferent pathway is mediated by the sympathetic nerves, because the responses in the hind limb were abolished by left sympathectomy. The vasodilatation was not due to activation of cholinergic fibers (17), because the response was not prevented by atriphenamine and propranolol. Phenoxybenzamine abolished or markedly attenuated the vasodilatation, suggesting that it was caused by a withdrawal of sympathetic vasoconstrictor tone. This finding is in agreement with experiments in the cat in which a decrease or an increase in sympathetic nerve activity, according to the type of fibers, has been recorded during stimulation of somatic afferent fibers from muscle (18-21). Sympathetic changes were also noticed in the stimulated limb; the decrease in perfusion pressure caused by muscle stimulation at 5 Hz after muscle paralysis was abolished by sympathectomy on that side.

The depressor response to a 5-Hz stimulation of the muscles was not prevented by muscle paralysis; thus, it is likely that the electrical stimulation...
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caused a direct activation of afferent nerve fibers in the muscles. Similar results could be obtained by direct stimulation of the femoral nerve, and these results are in agreement with those obtained during direct stimulation of somatic nerves in the cat (7-10) and the dog (22). The present experiments did not make it clear whether, in the normal condition, regular muscular contractions could cause a depressor response. As the frequency of stimulation was increased, the depressor response changed to a pressor response. In most dogs, the pressor response commenced at about 20 Hz and was marked at 40 Hz. The pressure increase was often preceded or followed by a transient decrease. In contrast with the depressor response, which was not altered by muscular paralysis, the pressor response was abolished or changed to a depressor response. That the dogs were still capable of vasoconstriction after muscle paralysis was shown by increasing the intensity of the stimulation, which caused a tetanic contraction and an increase in blood pressure and perfusion pressure in all dogs. An increase also occurred with stimulation of the central end of the femoral nerve at 40 Hz and 5 v. Recent studies in the cat have shown an increase in the aortic blood pressure during tetanic contraction caused by stimulation of the ventral roots; this increase is abolished by gallamine (11) or by section of the dorsal roots (11, 12). Our experiments confirmed in the dog that stimulation of the muscles at 40 Hz, which elicits a tetanic contraction, caused an increase in aortic blood pressure. They also showed that this increase in blood pressure was accompanied by a reflex increase in perfusion pressure in the hind limb. All these changes were abolished after muscle paralysis. Thus, the muscle contraction appears to be necessary for activation of the afferent fibers leading to an increase in the sympathetic activity to the vessels.

Cutaneous receptors are not involved, since the depressor and pressor responses were unchanged after the limbs were skinned. Section of the tendons at the musculotendinous junction and separation of the muscles from the femur, preventing at the same time all movement in the knee and stretch of the ligaments, did not decrease these responses, indicating that receptors in tendons and joints are not necessary for the responses. This finding was anticipated, because the afferent fibers from the Golgi organs in tendons belong mainly to group I (see below), and stimulation of these fibers does not change aortic blood pressure (8). Thus, the primary cause of the pressor response is in the contracting muscles; whether the receptors concerned are activated mechanically or as a consequence of the metabolic changes in the contracting muscles, or both, is not known.

Myelinated afferents of muscle nerves fall into three groups according to the diameter of their nerve fibers and their electrophysiological characteristics (23). The fibers of group I (12-21μ in diameter) arise from muscle spindles and Golgi organs in tendons. Those of group II (6-12μ in diameter) also arise from muscle spindles, probably from the secondary endings of the spindles, but not from tendon organs. The majority of group III fibers (1-6μ in diameter) arise from pressure receptors, and a very few arise from stretch receptors, although in some of these fibers no impulses can be aroused by mechanical stimuli (24). In addition to these three groups of myelinated afferents, there also are nonmyelinated group IV (or C) fibers which are not activated by passive stretching of the muscle but are known to fire during muscle contraction under ischemic conditions (25).

It is unlikely that the muscle spindles are involved, because they decrease their firing during muscle contraction (23) unless the gamma efferents are stimulated. However, the primary and secondary afferent fibers from the spindles belong to groups I and II. These fibers do not influence blood pressure (8); this phenomenon has recently been confirmed by electrophysiological studies (26, 27). Similar conclusions were drawn by McCloskey and Mitchell (12) using anodal block of the dorsal roots in the cat. Thus, it is likely that the present reflex responses are caused by activation of group III and possibly group IV afferent fibers.

For a long time it has been postulated that the changes in aortic blood pressure observed during muscular exercise are mainly due to stimulation of receptors in the contracting muscles (3-6, 28). The present experiments in the dog demonstrate that muscles made to contract by electrical stimulation can activate afferent fibers within these muscles with a resultant increase in activity of the noradrenergic fibers to resistance blood vessels. It is likely that this mechanism plays a role in the changes in sympathetic activity seen during normal muscular exercise.

Acknowledgment

We thank Gary F. Burton for his technical assistance and Mrs. Joan Y. Troxell for typing the manuscript.
References
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Circ Res. 1973;33:386-392
doi: 10.1161/01.RES.33.4.386

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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