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Pharmacology and Physiology of Perivascular Nerves Regulating Vascular Function

Sympathetic Cholinergic Vasodilation of Skeletal Muscle Small Arteries

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ABSTRACT—Recently we have studied the direct vasomotor response of the hindlimb extramuscular large arteries (internal diameter, 500 – 1400 μm) and intramuscular small arteries (internal diameter, 50 – 500 μm) of in vivo thick skeletal muscle during activation of sympathetic cholinergic nerve in anesthetized cats. The hypothalamic defense area was electrically stimulated so as to induce a profound increase in femoral blood flow mediated by sympathetic cholinergic fibers. To visualize the vascular arrangement from the extramuscular large feeding arteries to small arteries in the triceps surae muscle, we developed a new X-ray TV system. The internal diameter, flow velocity, and volume flow of arterial blood vessels were directly measured before and during stimulation of the hypothalamic defense area. The major new finding is that the hypothalamic stimulation causes an intense increase in the internal diameter of small arteries in skeletal muscle, which is abolished either by cholinergic blockade or by the section of the sciatic nerve, but not by combined α- and β-adrenergic blockade. In contrast, the internal diameter of the extramuscular larger arteries does not change during the hypothalamic stimulation, but their flow velocity and volume flow increase. These findings indicate that sympathetic cholinergic vasodilation occurs at intramuscular small arteries with internal diameter of 50 – 500 μm, which in turn increases flow velocity and volume flow of upstream blood vessels.

Keywords: Vasodilation, Skeletal muscle, Sympathetic cholinergic nerve, X-ray angiography

Introduction

Sympathetic cholinergic nerve fibers innervate blood vessels of skeletal muscle in the cat, dog, sheep, fox, mongoose, jackal and human (1, 2). When a localized area in the hypothalamus and the midbrain periaqueductal gray matter that is called a defense area is stimulated, sympathetic cholinergic vasodilation in muscle is selectively activated in concert with vasoconstriction in the kidney and skin and increases in heart rate, cardiac output, arterial blood pressure and respiration (3 – 8).

Regarding the location of sympathetic cholinergic vasodilation of skeletal muscle vessels, it has been postulated from measurement of the pressure of small muscle artery that sympathetic cholinergic fibers may dilate precapillary resistance vessels of skeletal muscle, but not other consecutive vascular sections, and that the more proximal vessels seem to be involved in the sympathetic cholinergic vasodilation unlike in the case of adrenergic vasoconstriction or muscle exercise (9). Bolme and Fuxe (1) reported acetylcholinesterase-containing nerve terminal-like structures in the adventitia of intramuscular arteries 30 – 100 μm in diameter and fewer such structures in arteries larger than 100 μm and in veins. However, when the direct changes in the diameter of skeletal muscle microvessels (<50 μm in diameter) during stimulation of the lumbar sympathetic nerve trunk or paravascular sympathetic nerve were studied using intravital microscopy, vasodilation of the microvessels was never seen during the sympathetic stimulation regardless of the presence of atropine (10). The questions...
as to the vascular site where sympathetic cholinergic vasodilation occurs in skeletal muscle and the extent to which the internal diameter of the muscle blood vessels increases during activation of sympathetic cholinergic nerve fibers, therefore, remained to be studied. Our hypothesis is that small arteries of skeletal muscle (50 – 500 μm in diameter), larger than the microvessels observed using intravital microscopy, are the major focus of action of the sympathetic cholinergic nerves. This review will attempt to answer these questions regarding a physiological role of the sympathetic cholinergic nerve.

**X-ray angiography**

Recently, we have developed a new X-ray TV system to visualize small arteries (internal diameter, 50 – 500 μm) of in vivo thick skeletal muscle and extramuscular larger feeding arteries (internal diameter, 500 – 1400 μm) of the cat hindlimb (11, 12). Along the vascular arrangement from extramuscular large arteries to small arteries in the triceps surae muscle, the internal diameter, flow velocity, and volume flow of arterial blood vessels were directly measured during activation of sympathetic cholinergic nerve fibers (11). An X-ray sensitive 1-inch Vidicon (Hamamatsu Photonics, Hamamatsu) or 9-inch Image-Intensified camera (Toshiba, Tokyo) was placed underneath the triceps surae muscle. Under exposure of softer X-rays emitted from the specially designed X-ray generator with a focal spot of 8 or 100 μm (Osaka Hitex, Osaka), a warm contrast medium was injected into the terminal aorta via the contralateral femoral artery. The angiographic image detected by either X-ray sensitive camera was transformed into a video signal at a speed of 30 frames/s. To measure the internal diameter of arteries, 4 to 6 serial frames, in which arteries were fully filled with the contrast medium, were digitally averaged and printed out with a magnification of 2 – 30. To measure the flow velocity of arteries, movement of the leading edge of the contrast medium was serially analyzed.

**Extramuscular large arteries**

Femoral blood flow velocity and femoral vascular conductance markedly increase during stimulation of the hypothalamic defense area, which is abolished by intraarterial (i.a.) injection of atropine (13). As shown in Fig. 1, the femoral artery (FA) bifurcates, back of the knee joint, into the distal caudal femoral artery (DCFA) and the popliteal artery (PA). A descending branch of DCFA, called the sural artery, feeds the triceps surae muscle, while the branches of PA partly supply to skeletal muscles in the tibial and sural area but are extensively distributed to the foot. The internal diameter (500 – 1400 μm) of the extramuscular large arteries around the knee shows no significant increase during hypothalamic stimulation. In contrast,
the flow velocity and volume flow of the extramuscular arteries, in particular DCFA, increase in an atropine-dependent manner during the hypothalamic stimulation. Therefore, sympathetic cholinergic nerve fibers may not innervate the extramuscular large arteries. Stimulation of sympathetic cholinergic nerve due to the hypothalamic stimulation is likely to cause vasodilation in downstream smaller arteries in skeletal muscle, resulting in increases of volume flow and flow velocity in the upstream extramuscular large arteries.

**Vascular site of action of sympathetic cholinergic nerve: intramuscular smaller arteries**

Figure 2 shows an example of the angiograms of small arteries in the triceps surae muscle before and during stimulation of the hypothalamic defense area. The sural artery branches off in the triceps surae muscle and the first-, second-, third- and forth-order arterial branches of the artery are traced using X-ray angiography. The branches usually run straight and have an internal diameter of approximately 50–500 μm. During the hypothalamic stimulation (Fig. 2), the internal diameter of all arterial segments clearly increased. This vasodilation was distributed along the entire length of the segments. On average, the internal diameter of the small arteries was increased 65% from 249 ± 24 to 410 ± 45 μm and the cross-sectional area was increased 171% accordingly. The mean flow velocity of the segments was increased threefold from 2.0 ± 0.21 to 5.9 ± 0.94 cm/s during the hypothalamic stimulation, and the volume flow was tremendously increased by 647%.

The next question is whether the profound increase in the internal diameter of the small arteries is a primary direct effect of sympathetic cholinergic nerve or a secondary flow-dependent effect due to a large increase in volume flow. It has been reported that blood vessels are sensitive

![Fig. 2. Vidicon angiograms of small arteries in the triceps surae muscle were taken in the following four conditions: A, at control; B, during hypothalamic stimulation; C, at control in the presence of atropine; D, during hypothalamic stimulation in the presence of atropine. The sural artery branches off in the triceps surae muscle. The first- (denoted S), second- and third-order branches of the artery were seen in the control angiogram. The internal diameter of all arterial branches increased during hypothalamic stimulation. Furthermore, new arterial branches became observable in panel B (as indicated by †). In the presence of atropine, the increase in internal diameter of the small arteries was abolished and newly detected arterial branches disappeared as well. An arrow (†) shows a position-mark bar. Adopted from Ref. 11 with permission.](image)
Neurogenic mechanism of vasodilation

The vasodilation of the small arteries is abolished by either cholinergic blockade or by the section of the sciatic nerve, although the increase in arterial blood pressure during the hypothalamic stimulation is enhanced or remains unchanged. It is suggested that the vasodilation of the small arteries is independent of the increase in arterial blood pressure. On the other hand, a combined injection of phentolamine and propranolol does not impair the vasodilator response of the small arteries. The vasodilation is mediated by a neural mechanism through sympathetic cholinergic nerve but not passive factors and/or humoral factors such as catecholamines.

Newly detected arterial segments during hypothalamic stimulation

It is noted that the number of the arterial segments observable was increased by 42% from 111 to 158 segments during the hypothalamic stimulation (11). The newly detected arterial branches, which had an internal diameter of 50–500 µm and came out at a site downstream just from the ramification points of the vessels, disappeared in the presence of atropine. The same characteristics of the vasomotor pattern have been reported in parasympathetic cholinergic vasoconstriction of small pulmonary arteries in the rabbit (16). Such cholinergic neural control of vasomotion localized at the downstream site near a branching point may play an important role in regulating the number of open arterial vessels.

Differential innervation of muscle small arteries by sympathetic cholinergic and adrenergic nerves

Previous studies using vital microscopy (10, 17–19) showed that adrenergic vasoconstriction by direct stimulation of sympathetic nerve or vasodilation by a baroreflex inhibition of sympathetic activity is seen in arterial microvessels mostly less than 50 µm. The primary site of either action is arterioles with an internal diameter of approximately 10–30 µm. On the other hand, vasodilation of arterial microvessels (mostly <50 µm) in response to sympathetic trunk stimulation is never seen regardless of the presence of atropine (17, 18). Based on these previous results and our findings (11–13), it is considered that the sympathetic cholinergic vasodilation evoked by hypothalamic stimulation occurs in the small arteries of skeletal muscle, while the sympathetic adrenergic vasoconstriction occurs in more distal vessels than in the case of sympathetic cholinergic vasodilation.

Conclusion

To examine the neurogenic vasomotor response of skeletal muscle small arteries induced by stimulation of the hypothalamic defense area, we visualized the vascular arrangement from the extramuscular large feeding arteries to small arteries in the triceps surae muscle using a new X-ray TV system. The hypothalamic stimulation caused an intense increase in internal diameter of small arteries in skeletal muscle, which was abolished either by cholinergic blockade or by section of the sciatic nerve, but not by combined α- and β-adrenergic blockade. In contrast, the internal diameter of the extramuscular larger arteries did not change during the hypothalamic stimulation but their flow velocity and volume flow increased. It is concluded that sympathetic cholinergic vasodilation occurs at intramuscular small arteries with internal diameter of 50–500 µm, which in turn increases flow velocity and volume flow of upstream blood vessels.

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