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Sympathetic Innervation of the Human Leg Arteries

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Summary. Sympathetic innervation of the human main leg arteries was morphologically investigated by the Formaldehyde-induced fluorescence method of Falck and the silver-impregnation method of Bodian. The following points were established:

1. There is a compact fine-meshed nerve-net directly on the surface of the media.
2. In the dorsal pedal, the anterior tibial, the peroneal, the posterior tibial, and the femoral artery, fine unmyelinated nerve fibers which run on the surface of the media penetrate constantly into the extreme outer layer of the media together with elastic and collagenous elements of the adventitia.
3. There is no difference in the innervation mode of the media of various leg arteries.
4. It seems that there is no correlation between the innervation of the arterial wall and the morbidity difference in the chronic arterial occlusive diseases of leg arteries.

Key words: Arterial spasm – Chronic arterial occlusive diseases – Innervation of the media.

Introduction

Arterial spasm is one of the most important factors in the pathogenesis of chronic arterial occlusive diseases (CAOD) such as arteriosclerosis obliterans, Buerger's disease and so on. In practice it is known that in CAOD of the lower extremities, some arteries, such as the profunda femoris and the peroneal artery, suffer less than other leg arteries from occlusive lesions (Watt, 1966; Hasse, 1970). Where does this difference originate from? Is there any correlation between the morbidity differences in CAOD and arterial innervation? We need to know the mechanism of arterial contraction to understand the development of, and the morbidity differences in CAOD.

The innervation of the arterial wall is still problematical. While it has been well known for some time that the adventitia of the artery has rich nerve supply (Busch, 1929), the innervation of the medial muscle cells is still controversial. At present there seems to be no general agreement regarding the existence of nerves in the media of human leg arteries.

It is the purpose of this paper to investigate the sympathetic innervation of human leg arteries morphologically by the Formaldehyde-induced fluorescence method of Falck (Falck, 1962) and a silver-impregnation method (Bodian, 1936), especially to determine (1) if there is a difference in the innervation mode and density among various leg arteries, and (2) if there are nerve elements in the media.

Materials and Methods

Two methods were applied: Formaldehyde-induced fluorescence (FIF) method of Falck and silver-impregnation method.

Fluorescence Histochemistry

Eight amputated legs were used. All legs were amputated because of the severe ischemic changes by arteriosclerosis obliterans (Grade IV after Fontaine). Immediately after the amputation, such arteries as the superficial femoral, the popliteal, the anterior tibial, the dorsal pedal, the peroneal, the posterior tibial, the sural artery etc were excised in the operating room. A part of calf- and thigh muscles and skin were also excised. After the excision, specimens were immediately frozen by immersion in liquid nitrogen, subsequently freeze-dried for 14 days at −35°C over phosphorous pentoixide in vacuo, warmed, and exposed to hot formaldehyde vapour at 80°C for 1 h. The specimens were then embedded in paraffin (mpt. 56–58°C, Merck) in vacuo at 60°C. Serial cross and oblique sections were made 10 μ thick, and mounted in a mixture of Entellan (Merck) and xylene. The fluorescence was examined in light from an OSRAM HBO 200 high pressure mercury lamp equipped with a Schott BG 12 (4 mm) as the primary filter. A Zeiss fluorescence microscope was used with a Zeiss 50 filter in the tube.

When the specificity of the FIF was questionable, it was checked with 0.1% sodium borohydride (Merck) in 90% isopropanol (Corrodi et al., 1964). Some of the neighbouring sections were also stained with Elastica-van-Gieson to differentiate the FIF of catecholamine from the nonspecific autofluorescence of elastic fibers.

In practice it is impossible to get completely fresh human leg arteries. How long after the end of blood flow adrenergic fibers continued fluorescence was therefore studied in normal rats. Forty and 60 min after sacrificing the rats, arteries were removed and the FIF method was carried out.

Silver Impregnation

Main leg arteries from 6 legs of 6 cadavers were excised within 24 h after death and Bodian's staining was carried out. The causes of death varied but all specimens were, at least macroscopically, without arteriosclerotic lesions. Ages of patients ranged from 14 to 63 years old. Serial cross, longitudinal and oblique sections were made. In one case, all excised arteries were cut open, flattened and sectioned serially from the intimal side. Sections were 10 to 12 μ thick. Hematoxylin-Eosin and Elastica-van-Gieson staining were also carried out to locate the nerve fibers.

Results

Fluorescence Histochemistry

It was extremely difficult to get fluorescence of the catecholamine in the arterial wall of the amputated legs. FIF of the catecholamine was observed in 2 specimens only. In one specimen from the thigh muscle, fluorescent adrenergic fibers were present at the boundary between the media and the adventitia. This artery was an intramuscular artery (Figs. 1 and 2). Cross-sectioned adrenergic fibers were observed in another specimen at the extreme outer layer of the media. This was a direct branch of the anterior tibial artery (Fig. 3).

In supplemental experiments with rats, it was found that a satisfactory demonstration of the adrenergic fibers was consistently obtained when intra-abdominal blood vessels were excised within 60 min of death.

Silver Impregnation

1. Adventitia. There were many layers of nerve nets in the adventitia of the leg arteries. In the outer part of the adventitia, numerous large nerve trunks, which ran parallel to the axis of the artery, were seen. From these nerve trunks, thin nerve-bundles divided and anastomosed, forming the plexus adventitialis. Beside making large anastomoses, the nerve-bundles gave off many branches with free nerve terminals. In the inmost part of the adventitia, e.i., directly on the surface of the media, fine unmyelinated nerve fibers ran longitudinally at similar intervals, making a fine-meshed nerve-net (Fig. 4). This nerve-net surrounded the media compactly (Fig. 5). These appearances were, on the whole, identical in all leg arteries investigated. There was no difference in the innervation mode and density in the adventitia of main leg arteries.

Smaller arteries (precapillary arteries and capillaries) had only one layer of nerve-net or only a few unmyelinated fine nerve fibers.

2. Media. In the dorsal pedal, the anterior tibial, the peroneal, the posterior tibial and the femoral artery, fine unmyelinated nerve fibers running directly on the surface of the media, constantly penetrated into the extreme outer layer of the media (Figs. 6 and 7). These nerve fibers entered into the media together with a small amount of adventitial elements such as elastic fibers, collagenous fibers etc. Nerve fibers alone did not penetrate into the media. No nerve fibers were found in the inner part of the media and in the intima.

There were no nerve fibers in the media of the arterioles, precapillary arteries and of capillaries.

We would point out that elastic fibers fluoresced strongly when sections stained by Bodian's or Hematoxylin-Eosin techniques were observed by a fluorescence microscope under the same filter conditions described above. A correlation between the nerve fibers and the elastic fibers could be readily assessed by this method. All sections examined with Bodian's staining were checked by this procedure.

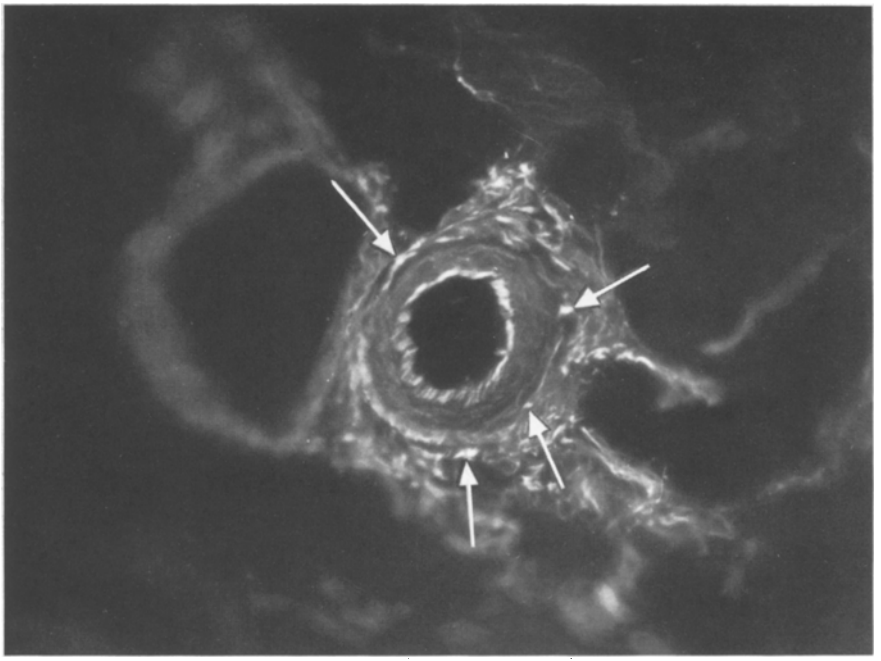

Fig. 1. Four fluorescent adrenergic fibers at the boundary between the adventitia and the media (*arrows*). The accompanying vein has no fluorescent fibers. 67 years old. male. Intramuscular artery. Cross section, Fluorescence microphotograph. ×100

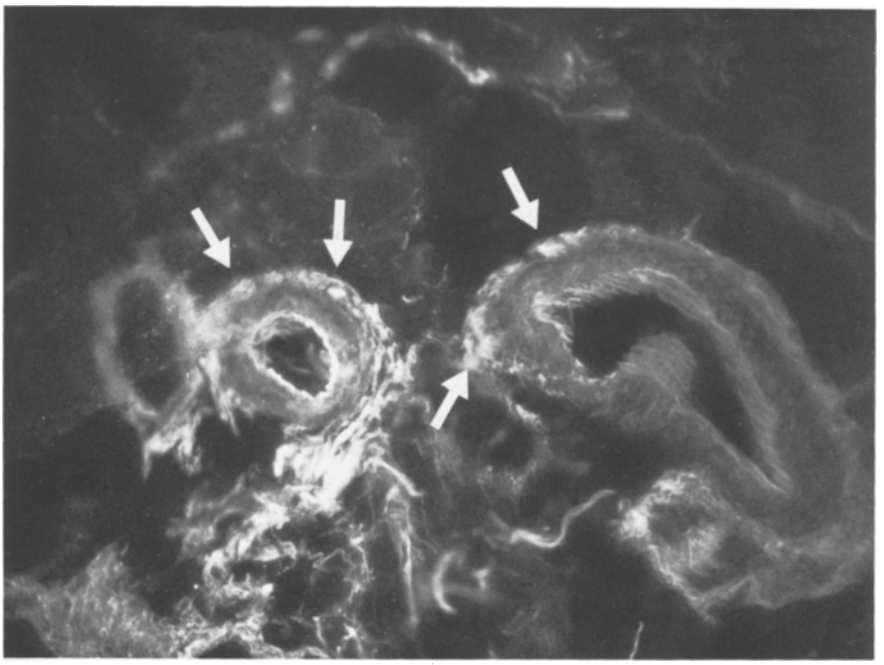

Fig. 2. Fluorescent adrenergic nerve fibers at the boundary between the adventitia and the media (*arrows*). 67 years old. male. Intramuscular artery of the thigh. Fluorescence microphotograph. ×72

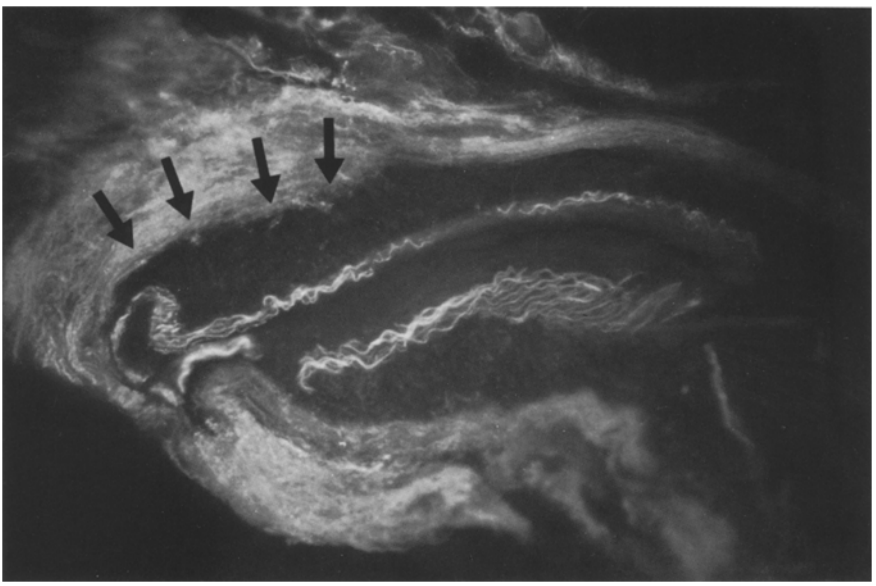

Fig. 3. Fluorescence of catecholamines is seen at the extremely outer layer of the media (*arrows*). 65 years old. male. A direct branch of the anterior tibial artery. Cross section. Fluorescence microphotograph. ×72

Fig. 4. Fine-meshed nerve-net directly on the surface of the media. In this photograph, nerve fibers run transversely. In the background, medial muscle cells which are crossing the nerve fibers, are seen. *M* media muscle cells. The femoral artery. 50 years old. Male. Flat section. Silver impregnation method. ×416

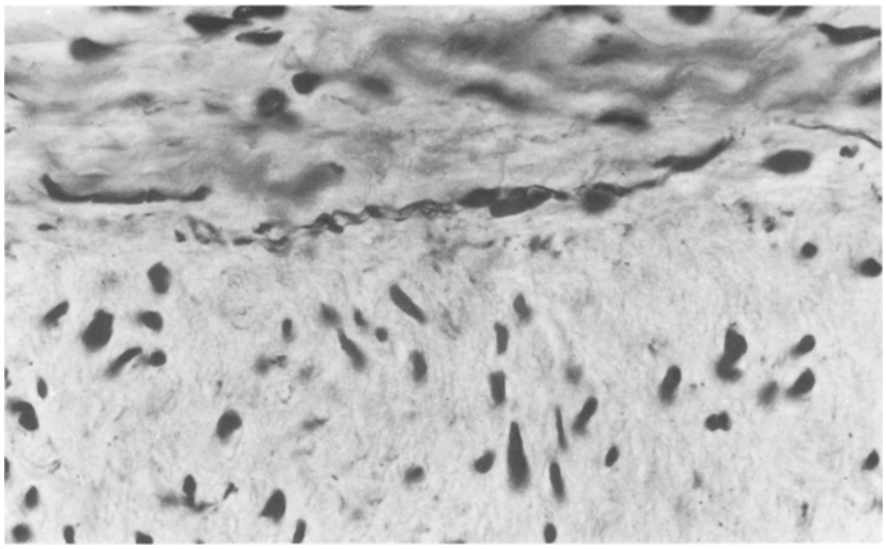

Fig. 5. Nerve fibers just outside the media. The posterior tibial artery. 56 years old. Female. Longitudinal section. Silver impregnation method. ×416

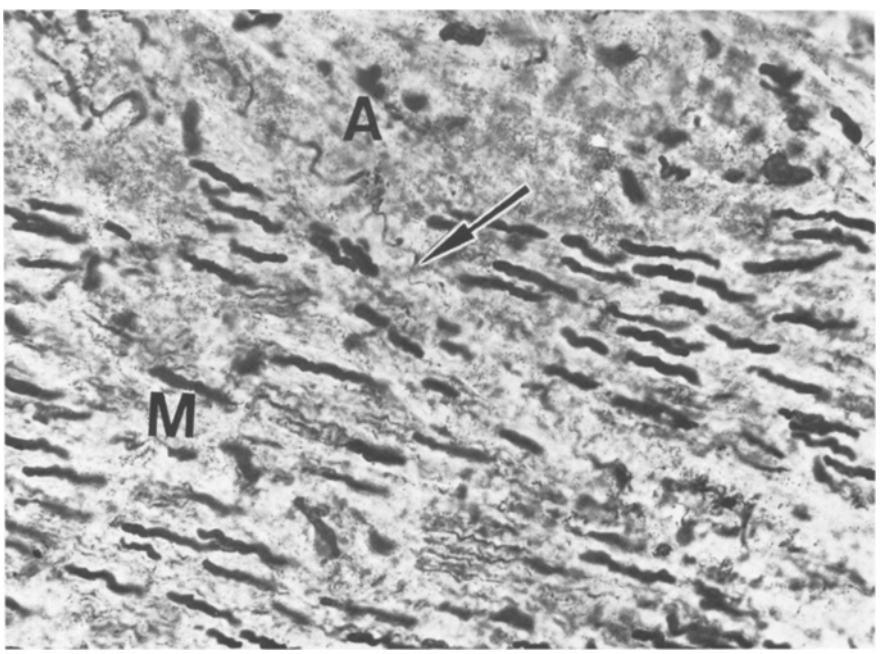

Fig. 6. A fine unmyelinated nerve fiber enters into the media (*arrow*). *A* adventitia. *M* media. The femoral artery. 14 years old. Female. Slight oblique section. Silver impregnation method. ×416

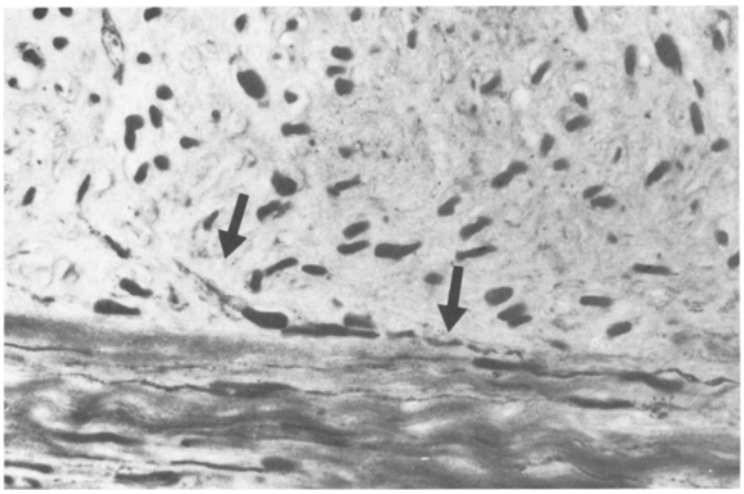

Fig. 7. Unmyelinated fine nerve fiber enters into the media (*arrows*). The posterior tibial artery. 56 years old. Female. Longitudinal section. Silver impregnation method. ×320

Discussion

In chronic arterial occlusive diseases of the muscular arteries, arterial spasm plays an important role in the pathogenesis and the development of the arterial wall lesions. But all arteries do not suffer simultaneously and similarly from the lesions. There is a marked morbidity difference among arteries. For example, the profunda femoris and the peroneal artery are occluded infrequently and act as important collaterals (Watt; Hasse). Other leg arteries (e.i., the superficial femoral, the anterior tibial, the dorsal pedal and the posterior tibial artery) suffer more from occlusive lesions. Is there any difference in the innervation of the arterial wall amongst these various arteries? There is basically still a controversy about the innervation of the media, in particular whether there is direct innervation by nerves in the media, or humoral innervation from nerve endings in the adventitia, or both? Many investigators have denied the existence of nerve fibers or nerve endings in the media by light-microscopic (Lang, 1965), electron microscopic (Seifert, 1963; Appenzeller, 1964; Verity et al., 1966; Lever et al., 1966), or by FIF methods (Doležel, 1966; Owman et al., 1971). Some investigators found that only rarely did nerve fibers penetrate into the media (Brettschneider, 1964; Fux et al., 1965; Ehinger et al., 1966). On the other hand, in animals, the existence of nerve fibers in the media of the aorta (Cheng, 1957; Ábrahám, 1961) and of the dorsal pedal artery (Tsunekawa et al., 1967; Gerova et al., 1967) has been reported. In human material, a few authors have found nerve fibers in the media (Busch; Reiser, 1933; Knoche, 1952; Hagen, 1955; Hachisuka, 1958). Aronson described the existence of rich nerve terminals in the media of the fetal ductus arteriosus (Aronson et al., 1970). However, with regard to the existence of nerve fibers in the media of human leg arteries, there seems to be no definite systematic report.

In the present study it was found that in human leg arteries, apart from an outer plexus adventitialis, there is a compact fine-meshed nerve-net directly on the surface of the media, and that in the dorsal pedal, the anterior tibial, the posterior tibial, the peroneal and the femoral artery, nerve fibers penetrate into the extreme outer layer of the media together with elastic and collagenous elements of the adventitia. The medial muscle cells of these arteries are innervated by these nerve endings in the media. No difference in the innervation mode exists in the media of various main leg arteries. The differences in the innervation density of the media between large arteries, such as the femoral artery, and the more peripheral leg arteries was not resolved by the present investigation. Arterioles and precapillary arteries are innervated by nerve endings at the boundary between the adventitia and the media.

From these findings it seems that the innervation of the arterial wall has no influence on the morbidity difference in CAOD of human leg arteries. FIF method is a specific and a very good method for the study of catecholamines, but specimens to be exammed in this way must be extremely fresh. In practice it is impossible to get extremely fresh human leg arteries, and amputated legs are already "old" in this sense, even immediately following amputation.

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