STRUCTURAL AND FUNCTIONAL STATE OF ADRENERGIC INNERVATION OF TERMINAL REGIONS OF THE CIRCULATION IN EXPERIMENTAL HYPERCHOLESTEREMIA

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Clinical and experimental studies have recently shown that in atherosclerosis lesions in the main vascular trunks and intramural vessels are preceded by changes in the terminal regions of the circulation [4, 5], which become involved in the pathological process in an early stage of hypercholesteremia which, in accordance with Myasnikov's classification, can be regarded as the preclinical period of atherosclerosis [9].

In this connection the study of the structural and functional state of innervation systems of the microcirculation assumes great importance in disturbances of lipid metabolism; this is particularly true of the sympathetic nervous system, for we know that innervation of the microcirculation is predominantly adrenegic [7, 10, 12, 15]. It has also been shown that noradrenalin, secreted by adrenergic terminals, not only has a vasoactive action, but, equally with other biologically active substances, it participates in the regulation of cholesterol metabolism in both animals and man [3, 6, 8, 11]. Yet the adrenergic innervation of the microcirculation in atherosclerosis has not been investigated from the structural point of view.

The aim of this investigation was to study the structural and functional state of the adrenergic component of innervation of the microcirculation in short-term experimental hypercholesteremia and also in a more prolonged and stable form of this condition.

EXPERIMENTAL METHOD

Experiments were carried out on 84 male rabbits weighing 2.0-3.0 kg. Experimental hypercholesteremia was induced with exogenous cholesterol (0.5 g/kg) by a modified Anichkov's method [13], and the severity of the condition was judged from the serum cholesterol level. In series I (32 rabbits) a single dose of cholesterol was given. The animals were killed by air embolism 3, 6, 15, and 24 h, and 4 and 12 days later and material was taken for investigation. In series II (32 rabbits) the animals were given an atherogenic diet daily for 60 days. Material was collected after 2, 4, 12, 20, 30, and 60 days. Intact animals, and also rabbits receiving reserpine intraperitoneally in a dose of 2.0 mg/kg 20 h before sacrifice, served as the control and enabled the specificity of the fluorescence-histochemical investigation of the sympathetic nervous system to be verified. The adrenergic innervation of terminal regions of the circulation was studied by the method of Falck and Owman [14], which reveals noradrenalin selectively in the sympathetic nervous system in total film preparations of serous membranes. The mesentery of the small intestine and the pericardium, which have a rich blood supply, were used so that the course of the sympathetic axons could be traced simultaneously to all components of the microcirculation. Film preparations were examined and photographed on the MBI-15 luminescence microscope. The intensity of catecholamine fluorescence was determined photometrically on the LYUMAM microscope and the numerical data obtained were expressed in conventional units (c.u.), indirectly reflecting the noradrenalin content in the adrenergic axons and, consequently, their functional activity [1, 2]. The numerical results were subjected to statistical analysis.

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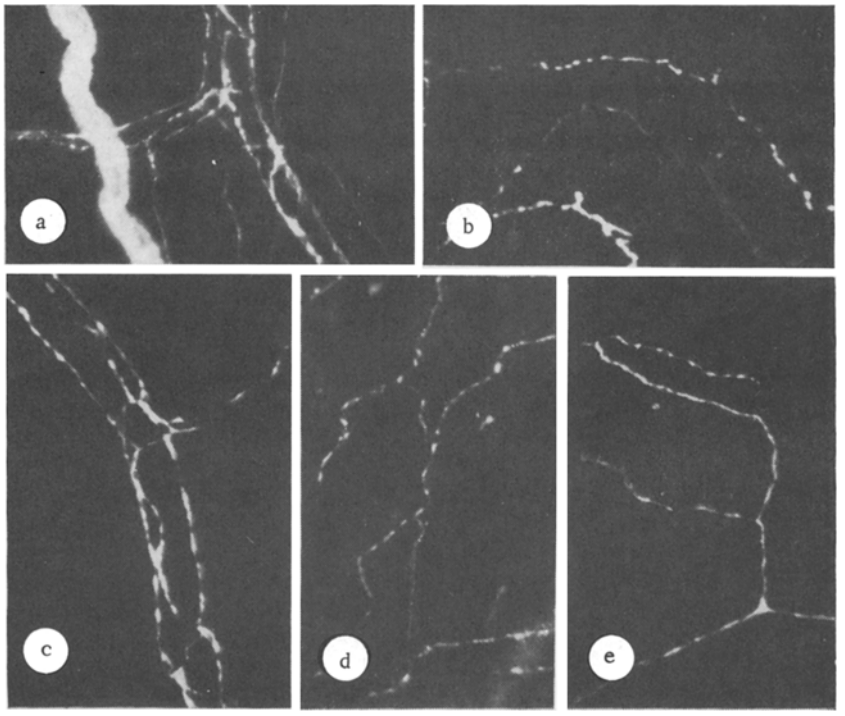

Fig. 1. Structure of adrenergic innervation of terminal regions of the circulation in normal rabbits and rabbits with short-term experimental hypercholesteremia (series I). a) Mesentery of small intestine under normal conditions. Periarteriolar plexus, consisting of thin adrenergic axons with small varicosities; b) pericardium of intact rabbits. Avascular area. Thin terminal axons form spreading branches; c) mesentery of small intestine 3 h after administration of cholesterol; d) pericardium, avascular area, 6 h after administration of cholesterol; e) mesentery of small intestine 12 h after administration of cholesterol. Here and in Fig. 2, magnification 100.

TABLE 1. Intensity of Catecholamine Luminescence in Varicosities of Sympathetic Axons during Development of Experimental Hypocholesteremia in Rabbits after Administration of a Single Dose (series I) and Repeated Doses (series II) of 0.5 g/kg Exogenous Cholesterol (M ± m)

Series of experiments	Number of animals	Time	Blood cholesterol level, mg %	Intensity of catecholamine luminescence, c.u.
Control (normal)	10		40,5±3,7	4,4±0,25
I	7	3 h	48,8±10,6	4,8±0,36
	5	6 h	66,7±36,1	4,4±1,06
	5	15 h	85,0±9,2	2,3±0,58
	5	24 h	48,8±7,8	3,9±0,04
	5	4 days	55,5±8,2	2,6±0,1
	5	12 days	36,7±6,7	4,0±0,06
II	7	2 days	70,0±7,8	5,6±0,56
	5	4 days	155,0±19,3	4,0±0,33
	5	12 days	176,7±6,1	5,6±0,42
	5	20 days	425,0±67,9	6,5±0,29
	5	30 days	469,3±60,4	7,2±0,06
	5	60 days	811,7±72,8	6,5±0,37

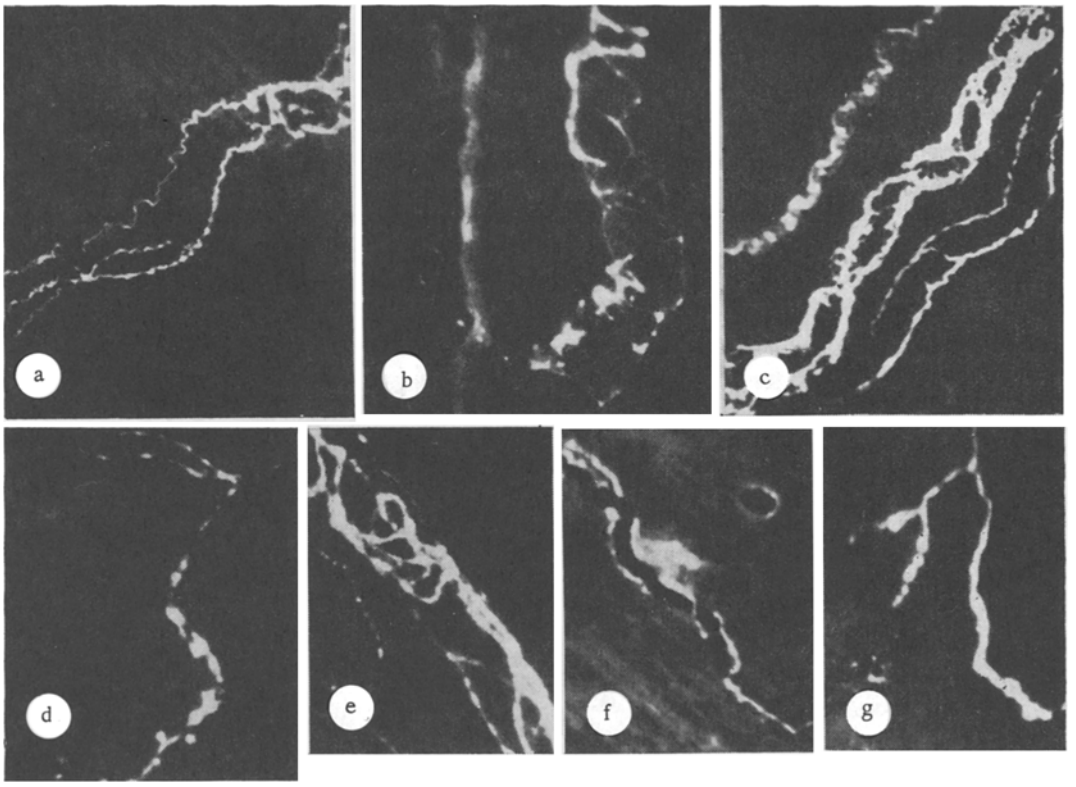

Fig. 2. Structure of adrenergic innervation of terminal microvessels of rabbits during long-term administration of cholesterol (series II). a, b) Atherogenic diet for 4 days; c, d) atherogenic diet for 12 days; e-g) atherogenic diet for 60 days: a) mesentery of small intestine. Increased tortuosity of axonal plexus along course of lymphatic microvessel; c) mesentery of small intestine. Second adrenergic axons in periarteriolar and perivenular plexuses; d) avascular area of mesentery of small intestine. Terminal axons with large varicosities; e) mesentery of small intestine. Periarteriolar plexus consists of grossly thickened axons with large varicosities; f) mesentery of small intestine. Venule, entwined by thickened axons; g) avascular region of pericardium. Terminal axons have large varicosities and pools.

EXPERIMENTAL RESULTS

Consecutive microscopic examination of film preparations of the pericardium and mesentery of the small intestine in intact rabbits showed that sympathetic nerve fibers were distributed topographically mainly along the course of the arterioles and precapillaries. Thin adrenergic axons, forming a perivascular framework, contained small varicosities (Fig. 1a), in which the intensity of luminescence was 4.4 ± 0.25 c.u. Collaterals branching from the perivascular nervous plexuses had a structure of varied complexity and penetrated into the avascular region of the serous membranes, where they formed localized or spreading terminal branches. Along the course of the axons longitudinal areas with weak luminescence alternated with varicosities with brighter luminescence, giving the axon the appearance of a broken line (Fig. 1b). Along the course of the venules of the microcirculation, by contrast with the arterioles, no adrenergic nerve fibers were found or they were present only in the form of single terminal adrenergic axons. In the microcirculation of rabbits, the arterioles thus have the best developed adrenergic innervation, which coincides structurally and topographically with the adrenergic innervation of the microcirculation as established in other species of animals [7, 12, 15]. In the control group fluorescence in the axons disappeared after preliminary injections of reserpine.

To study the response of the adrenergic nervous system innervating the terminal regions of the circulation when lipid metabolism was disturbed, in the experiments of series I the animals were given a single dose of cholesterol. The blood cholesterol level in these animals was 1.6 times higher than initially after 6 h and twice as high after 15 h. However, the

hypercholesteremia was of short duration, for the cholesterol level fell rapidly during the 24 h after the experiment began and returned to normal (Table 1). Microscopic examination of film preparations at all times of short-term hypercholesteremia and its regression showed adrenergic nerve fibers to be present predominantly along the course of the arterioles of the microcirculation and in avascular areas of serous membranes and there were no visible structural or topographical differences from the innervation of the microcirculation in intact rabbits (Fig. 1: c-e).

Meanwhile quantitative analysis of the intensity of catecholamine luminescence showed a decrease in luminescence in the axons after 15 h by 52% compared with normal, although the blood cholesterol during this period was twice as high as initially. However, this phenomenon was transient in character, for as the blood cholesterol level was restored to normal the intensity of luminescence increased, and by the end of the experiment it was within normal limits (Table 1).

Different results were obtained in the experiments of series II, with a long-term atherogenic diet. The blood cholesterol level on the 2nd and 4th days of the experiment was 1.7 and 3.8 times higher than initially respectively (Table 1). Under these conditions, during the study of total film preparations attention was directed to the increase in the number of adrenergic structures revealed both along the course of the arterioles of the microcirculation and also in avascular areas of serous membranes, which was evidently connected both with activation of the functions and changes in the structure of the adrenergic axons. The periarteriolar plexuses showed increased tortuosity (Fig. 2a). In a few cases bright luminescence was observed along the course of the dilated lymphatic microvessels (Fig. 2b), evidence that the walls of these vessels have an adrenergic innervation. The intensity of catecholamine luminescence in the varicosities along the axons was 27% above normal on the 2nd day, but it fell to its initial level on the 4th day (Table 1).

At the next stage of the investigation (12th day) the blood cholesterol level was 4.3 times higher than initially. Adrenergic fibers were discovered in total film preparations not only in the arterial part of the microcirculation, as at the previous times, but also along the course of the venules (Fig. 2c). Thickened axons with large varicosities and increased tortuosity were predominant in the perivascular plexuses. The intensity of catecholamine luminescence also were observed along the course of the terminal axons (Fig. 2d).

On the 30th day and, in particular, on the 60th day of the experiment the blood cholesterol level was 11.3 and 20 times higher than initially respectively. Even more marked changes occurred in adrenergic nerve fibers at these times compared with previously. The periarterial plexuses consisted mainly of grossly thickened axons with large varicosities and bright catecholamine luminescence could be detected clearly along the course of the venules (Fig. 2f). In the avascular areas of the serous membranes large varicosities in terminal adrenergic axons merged to form large pools, distinguished by intense catecholamine luminescence (Fig. 2g).

Under these conditions of persistent and steadily increasing hypercholesteremia the intensity of catecholamine luminescence in the adrenergic structures remained high until the end of the experiment (60 days). This finding may evidently be linked with increased biosynthesis and accumulation of noradrenalin in adrenergic axons as a result of increased functional activity of the sympathicoadrenal system as a whole, which is known to develop in the presence of disturbances of lipid metabolism [1-3, 6].

Comparison of the results of the fluorescence-histochemical study of the adrenergic nervous system in total film preparations of the serous membranes in rabbits showed that in experimental hypercholesteremia structural and functional changes whose intensity is closely linked with the duration and level of disturbance of cholesterol metabolism develop in the peripheral zones of the sympathetic nervous system of the experimental animals.

In the case of short-term hypercholesteremia, for instance, transient disturbances of function took place in adrenergic nervous structures, manifested as a fall in the intensity of catecholamine luminescence, could be detected mainly along the course of the arteriolar component of the microculation [1, 2, 15]. Meanwhile persistent and steadily increasing hypercholesteremia was accompanied by marked structural and functional changes in the adrenergic component of innervation of the arterioles and also of the venules, as well as in avascular areas of the serous membranes. The presence of a high intensity of catecholamine luminescence under these conditions is evidently the histochemical reflection of increased axonal noradrenalin biosyntheseis [1, 2] in response to the action of a risk factor such as hypercholesteremia [2, 8, 10].

The results thus suggest that disturbance of the adrenergic innervation in the terminal zones of the circulation modifies their adaptive and trophic properties and contributes to the development of changes in the microcirculation detectable in the preclinical period of atherosclerosis.

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RESPONSES OF PERITONEAL MESOTHELIAL CELLS IN RATS WITH ASEPTIC AND BACTERIAL PERITONITIS

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Contractions of nonmuscular cells (endotheliocytes, fibroblasts, leukocytes) play an important role in physiological and pathological processes [1, 11, 13, 14]. There is evidence in the literature to suggest that active contractions of the peritoneal mesothelial cells are possible, for example in inflammation [3, 9, 12], although this problem requires further study in order to elucidate the regulatory and structural mechanisms of the contractile responses of mesotheliocytes (MC). In the investigation described below this problem was studied by means of transmission and scanning electron microscopy (TEM and SEM respectively), in a step by step analysis of responses of MC in different forms of experimental peritonitis.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 280-320 g were used. There were four series of experiments. In series I aseptic peritonitis was induced by intraperitoneal injection of 3% aqueous solution of amylodextrin (15 animals). In series II aseptic peritonitis was induced by intraperitoneal injection of a suspension of edible starch [11]. In series II (18 rats) and IV (10 rats) the ascending part of the large intestine was constricted [4] and an incision

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