NEUROHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE ADRENERGIC INNERVATION OF RAT POSTERIOR VENA CAVA IN CHRONIC VENOUS HYPERTENSION INDUCED BY EXPERIMENTAL OLEOTHORAX

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Smooth muscle tissue of the great veins in mammals has a well-developed effector adrenergic innervation [3, 4]. Changes in this innervation under pathological conditions, such as in chronic venous hypertension, have been inadequately studied. Yet analysis of neuromuscular relations in the wall of veins during changes in the smooth muscle cells (SMC) induced by hypertension is important for the understanding of the trophic function of these nervous structures and to elucidate the mechanisms of development of chronic venous insufficiency. This paper examines the structure of the adrenergic innervation of the rat posterior vena cava during prolonged venous hypertension caused by experimental oleothorax.

EXPERIMENTAL METHOD

Venous hypertension was induced in rats by injection of the organosilicon oil polymethylsiloxane (PMS-100) – a biologically inert substance not evoking immune reactions [1, 2] – into the pleural cavities. The following scheme of administration was used: First, PMS-100 was injected in a volume of 5-6 cm^3 into each pleural cavity, after which an additional 1.5 cm^3 was injected on each side on the 13th, 26th, and 75th days. The venous pressure was measured in the femoral or jugular vein by means of a water or electric manometer. The abdominal portion of the posterior vena cava was used for neurohistological and electron-microscopic study, and full details of the method of processing the material were published previously [3]. All painful manipulations were carried out under ether anesthesia. Altogether 76 experimental and nine control noninbred male rats weighing 260-350 g were used. Four rats were used at each time for the neurohistological investigation, two or three for the ultrastructural study; altogether there were 10 groups of animals for the period from the 1st to the 210th day after induction of oleothorax. Before material for electron microscopy was collected from four control and six experimental rats of the number of animals indicated above, histochemical tests were carried out for catecholamines of adrenergic endings [5].

The results of the study of the adrenergic innervation were compared with data of karyometry, for which purpose total preparations of the vein from 32 rats (four at each time) were fixed in Carnoy's fluid, stained by the Feulgen method, after which the area of the nuclei of the myocytes was measured by means of a Leitz ACM semiautomatic apparatus for morphometric analysis.

EXPERIMENTAL RESULTS

As early as on the 3rd-4th days after the first injection of PMS-100, the venous pressure in the jugular and femoral veins was found to be raised (the system of the anterior and posterior venae cavae). The maximal increase (100-110 mm water) was observed on the 18th day after the second injection. Later, throughout the experiment the pressure in both the jugular and the femoral veins remained about 2.5-3 times higher than in the control animals. Starting from the 40th day of the experiments signs of hypertrophy of the wall of the right ventricle were observed.

On luminescence-histochemical investigation (Fig. 1a) no significant changes in the structure of the adrenergic nervous plexus were observed during the first 3 days after induction of oleothorax [3]. On the 5th-7th day the network of adrenergic nerve fivers adjacent to the muscular layer (medio-adventitial plexus) appeared less dense than normal (Fig. 1b). The nerve fibers were thin, free from varices, and the intensity of their luminescence was weakened. Ultrastructural investigation at this

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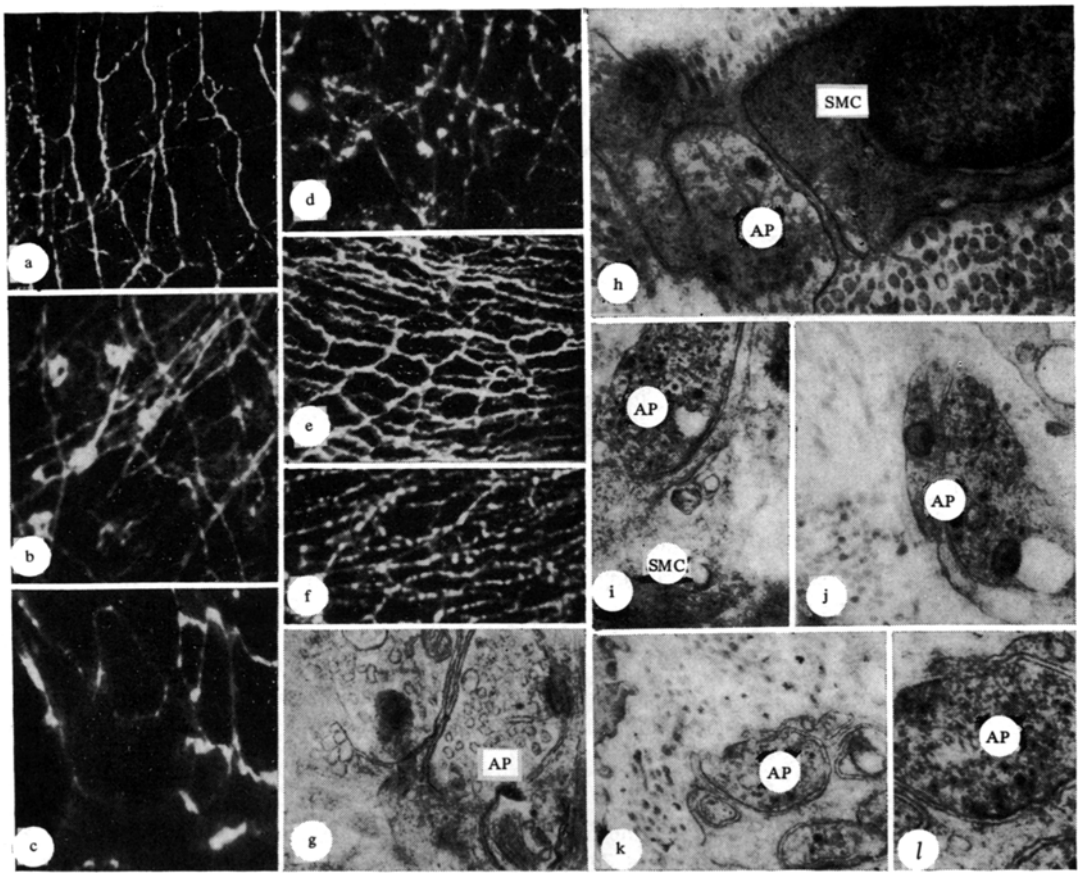

Fig. 1. Luminescence and electron-microscopic structures of adrenergic innervation of posterior vena cava of rats with chronic venous hypertension caused by oleothorax: a-f) network of adrenergic nerve fivers in animal after oleothorax for: a) 3 days, b) 10 days, c and d) 30 days, e and f) 75 days. Luminescence-histochemical method; b) reduction in density of network; d and e) "hyperneuria"; e and f) increase in density of network; g-*l*) electron micrograph of terminal segments of adrenergic nervous plexus; i-*l*) after Tranzer and Richards' histochemical reaction; g) 4 days after induction of oleothorax; vesicles, mainly "empty," can be seen in cross-sections of axons; h) formation of synaptic structure (crossed section of axon and SMC very close together, in region of approximation they share a common basement membrane, which is absent in places); i-*l*) well preserved adrenergic vesicles in late stages of venous hypertension: i) control, 0.5 ml of 0.2% adrenalin injected intramuscularly 30 min before sacrifice (to ensure saturation of vesicles); j, k) 6.5 months after induction of oleothorax; *l*) detail of previous photograph. AP) Axon profile, SMC) smooth muscle cell. Magnification: a, b, e, f) 100 X; c, d) 200 X; g) original magnification 10,000 X; h) original magnification 20,700 X; i, j, k, *l*) original magnification 19,200 X.

period showed that the varicose thickenings of the axons contained vesicles, mainly without dense cores (Fig. 1g). Only the presence of dense cores in isolated vesicles showed that these axon profiles belonged to adrenergic structures. The distance between these profiles and SMC was considerable, 200 nm or more. The electron-microscopic structure of the SMC at this period was indistinguishable from the structure of the cells of intact animals, and only an increased number of tetrapolyribosomes was found in the perinuclear zone of some SMC, evidence of activation of protein synthesis in the cell.

Starting with the 10th day of the experiments the first sign of an uneven "coarse" increase in the intensity of luminescence both of the varices and of the intervaricose regions appeared. These changes are usually interpreted as a manifestation of "hyperneuria." This reached a maximum by the 20th day (Fig. 1c, d). During this period "pools" and "spheres" appeared along the course of the terminal adrenergic structures. Electron microscopy at this time revealed long, cylindrical varices with numerous small pale vesicles (up to 100 to a fragment of a varix encountered in one section), and only single vesicles had a dense core. In SMC the rough endoplasmic reticulum was more highly developed, with dilatation of individual channels filled with fine granular contents.

On the 30th-40th day fibers of the terminal network appeared thicker, often twisted, and in some places no varicose thickenings could be seen. Karyometry at this time revealed marked hypertrophy of the SMC nuclei (mean area 43.6 $\pm$ 0.66 μ^2 compared with 28.7 $\pm$ 0.31 μ^2 in the control), and electron microscopy revealed coarse changes in the structure of SMC which can be interpreted as evidence of injury: marked translucency (emptying of the cytoplasm matrix with the

formation of "empty" plasmalemmal sheaths. This period, as was stated above, corresponds to involvement of the right heart in the process, i.e., it spread to a qualitatively new level.

In the following periods the outer structure of SMC and neuromuscular relations changed significantly. For instance, on the 75th-105th day of the experiment clear signs of activation were found in SMC: Round and oval concentrations of mitochondria appeared in the perinuclear zone, and hypertrophy of the rough endoplasmic reticulum was observed, with its lumen filled with finely granular contents. Neurohistochemical investigation showed a very dense network of adrenergic fibers (Fig. 1e, f). The varicose thickenings were round in shape, and there was little change in their frequency along the course of the nerve fibers (Fig. 1f). During this period "bare" axon thickenings, containing one or two large vesicles with a dense core as well as tiny vesicles, were seen very close to the SMC (Fig. 1h). These structures can undoubtedly be regarded as synaptic.

Later (180-210 days) sclerotic changes began to appear in the wall of the vein: thickening of the musculo-endothelial space and of the intermuscular spaces, in which finely granular material resembling plasma proteins was found together with collagen and elastic fibers. However, histochemically at the electron-microscopic level the adrenergic innervation remained well preserved at this time (Fig. 1i, j, k, *l*), indicating the presence of vesicles containing a dense core (the result of a positive reaction for catecholamines) in the varices.

During the development of venous hypertension in the posterior vena cava system structural changes thus take place in the adrenergic nervous apparatus. In the early stages of venous hypertension (until the 40th day) these changes are reactive in character (weakening of luminescence of the adrenergic network, a reduction in its density, followed by "hyperneuria"). After involvement of the right ventricle in the process (pulmonary hypertension, evidently connected with prolonged compression of the lungs by PMS) marked activation and hypertrophy of SMC develop and, at the same time, the structure of the neuromuscular relations changes correspondingly: The SMC become closer to the nerve endings (synapses appear), and on neurohistochemical investigation the density of the network of adrenergic fibers is increased. This points to the existence of a mechanism ensuring correlation between structural changes in the myocytes and adrenergic nervous apparatus, and it is also evidence that hypertrophied SMC "need" stronger nervous control. The sclerotic changes developing in the late stages of hypertension in the wall of the vein are unconnected with any changes in the adrenergic innervation, for they occur at a time when the latter is well preserved.

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