REACTION OF SMOOTH MUSCLE CELLS OF BLOOD VESSELS TO AN INCREASE IN FUNCTIONAL LOAD

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Changes in the muscle tissue of the caudal vena cava of rats and its branches after disturbance of the blood drainage were studied by the methods of isotope biochemistry, autoradiography, immunomorphology, and cytophotometry. Enlargement and polyploidy of the DNA-synthesizing muscle cells with activation of protein synthesis in them and the formation of intimal layers resembling the muscular layers were found in the main trunk of the vein which was dilated the most. Activation of DNA and protein synthesis in the less distended femoral vein was much less marked than in the main trunk. Activation of protein synthesis occurred after a delay compared with the activation of DNA synthesis but both processes were phases of development of hypertrophy of the muscle tissue of the vessel wall in response to increased functional loading.

KEY WORDS: smooth muscle of blood vessels; protein synthesis; tissue hypertrophy.

One way of imposing a functional load on the smooth muscle cells of the blood vessel wall is by strongly stretching it [5]. If this process is reproduced in vivo, for example by interfering with the drainage of blood from a vein and increasing the lateral pressure, the mass of smooth muscle cells in the vessel wall increases [2]. This increase is based on an increase in the number of DNA-synthesizing muscle cells and intensification of protein synthesis in the smooth muscles [2]. It is not clear whether these processes are interconnected, nor is it clear what is the nature of their dynamics or the specific contribution of each of them to the increase in the muscle mass.

In this investigation the problems were studied by methods of isotope biochemistry, histoautoradiography, cytophotometry, and immunomorphology.

EXPERIMENTAL METHOD

Constriction of the caudal vena cava was produced as described previously [2] in noninbred female rats weighing 280-320 g.

In the experiments of series I the veins of 5 rats after stenosis for 4 days, the veins of 9 rats after stenosis for 6, 17, and 30 days, and the veins of 2 intact animals were studied. To study DNA and protein biosynthesis, 0.1 μCi/g thymidine-H^3 (specific activity 15.3 Ci/mmole) and 0.01 mCi/g leucine-C^{14} (specific activity 120 mCi/mmole) were injected intraperitoneally into the rats at the same time. The duration of exposure was 4 h. The rats were decapitated, the caudal vena cava was freed from the adventitia, and the inner surface of the vessel was wiped with gauze. Pieces of the femoral veins and the cranial and caudal portions of the posterior vena cava were fixed in Carnoy's fluid. The remaining parts of the posterior vena cava were used to study incorporation of the precursors into DNA and protein with the aid of a Mark 2 (USA)

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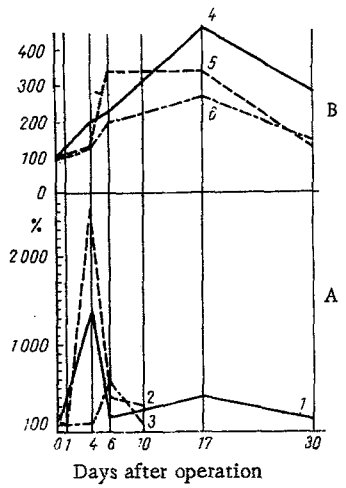

Fig. 1. Intensity of DNA (A) and protein (B) synthesis in smooth-muscle cells of dilated part of rat vena cava. Ordinate, intensity of synthesis (in percent of control); abscissa, days after operation; 1) total intensity of incorporation of thymidine-H^3 into DNA; 2) frequency with which DNA-synthesizing muscle cells were found in the vena cava; 3) the same parameter for the femoral veins of animals undergoing the operation; 4) total intensity of incorporation of leucine-C^{14} into protein; 5) number of grains of silver in C^{14}-tracks per 10 μ^2 section of muscle cells (intensity of protein synthesis); 6) the same as 5, but for femoral veins of animals undergoing the operation.

scintillation counter programed for the double label. The material was prepared in accordance with the approved scheme [6]. Sections 4 μ in thickness were cut from the fixed segments of the blood vessels, coated with "M" emulsion, and exposed for 21-60 days at 5°C. By examination of the developed histoautoradiographs, stained with 0.1% cresyl violet solution, the number of grains of silver in the tracks of the C^{14} β-particles was determined per 10 μ^2 area of the muscle coat without distinction between karyoplasm and cytoplasm.

In the experiments of series II 0.3 μCi/g thymidine-H^3 was injected intraperitoneally into rats on the 1st, 4th, 6th, and 10th days after the operation (2 rats at each time) and into 2 intact animals. The rats were decapitated 1.5 h later and parts of the posterior vena cava taken from the cranial and caudal portions (relative to the site of stenosis) in order to prepare histoautoradiographs. The percentage of labeled smooth-muscle cells (in 1000-2000 counted) was calculated.

In the experiments of series III cryostat sections through the wall of the caudal portion of the posterior vena cava of 7 rats 30 days after the operation were treated by the indirect Coons' method [7] using monospecific serum against smooth muscle myosin [8].

The results of this series were compared with the observations of light microscopy and with electron micrographs obtained previously [3].

In the experiments of series IV the caudal and cranial portions of the vena cava from rats on the 8th and 30th days after the operation (3 at each time) and 2 intact animals were stained by Feulgen's method. Films of splenic lymphocytes from intact rats were stained as diploid cells. The DNA content was determined in total preparation of the veins and in films of the lymphocytes by means of the microcytophotometer constructed by Morozov [4]. The relative percentages of diploid, tetraploid, and octaploid muscle cells were calculated.

EXPERIMENTAL RESULTS

The curves in Fig. 1A show that on the 4th day after interference with the drainage of blood the intensity of DNA synthesis increased sharply in the smooth-muscle cells of the dilated part of the posterior vena cava. For instance, the total incorporation of thymidine-H^3 (continuous line) was increased by 14 times, whereas the number of DNA-synthesizing smooth-muscle cells was increased by 26 times (from 0.3 to 7.8%; $P < 0.01$). By the 6th day these values were lower but were still higher than in the control.

In the slightly dilated femoral vein the number of DNA-synthesizing cells was not increased on the 4th day; a slight increase in this index was observed only on the 6th day (from 0.3 to 1.7%); later it returned to its initial level. In addition, on the 6th day single DNA-synthesizing muscle cells began to appear in the undilated cranial portion of the vein (0.8%), possibly in connection with diffusion of the products of injury from the distended segment.

DNA-synthesizing smooth-muscle cells in the dilated portion of the vein were found very frequently close to the ligature, but even at a considerable distance from it they were still seen both singly and in groups. No smooth muscle cells dividing by mitosis could be found.

The cytophotometric investigations showed the appearance of polyploid cells from the 8th day after the operation in the dilated segments of the vein. At that time the number of tetraploid cells was 30%, rising to 33% on the 30th day. The number of octaploid cells on the 30th day was 6%. These results indicate conversion of some of the DNA-synthesizing smooth-muscle cells into polyploid cells.

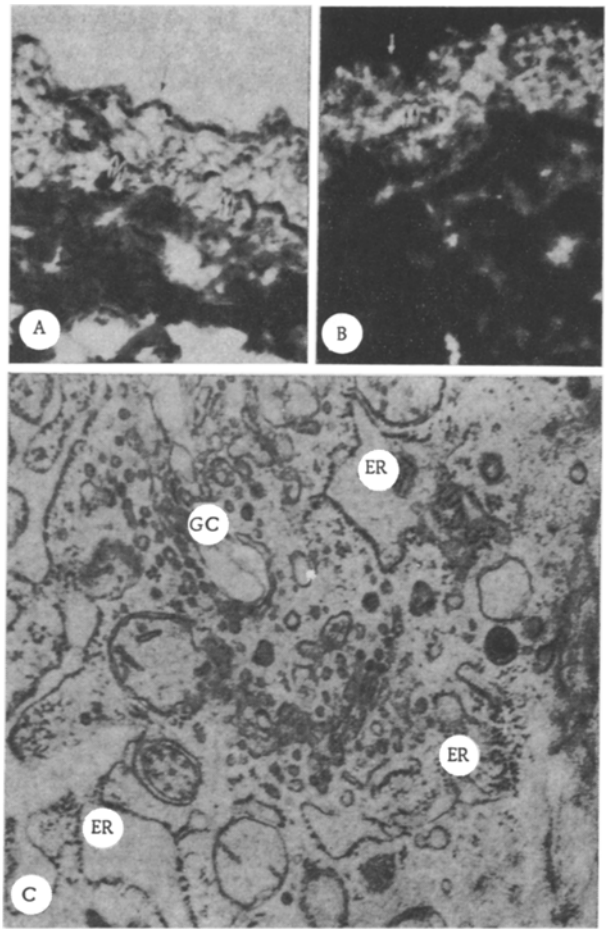

Fig. 2. Structure of newly formed intimal (identified by arrows) muscular layer in the dilated portion of the posterior vena cava: a) 10 days after operation a fibrocellular intimal layer equal in thickness to the media can be seen in the survey sections. The outer boundary of the layer with the inner elastic membrane (↑↑) is clearly visible. Combined staining with fuchselin and by VanGieson's method, 200×; b) the cells of this layer contain antigen similar to smooth muscle myosin (luminescent on treatment of the sections by the indirect Coons' method), 200×; c) fragment of electron micrograph of intimal cell in which the dilated rough endoplasmic reticulum (ER) and a well-defined Golgi complex (GC) can be seen, 7700×.

In the light microscope the formation of new muscular layers, arranged longitudinally within the media (Fig. 2a), could be seen in the areas occupied by the DNA-synthesizing smooth-muscle cells. Immunomorphologically, smooth muscle myosin was detected in the cells of this layer (Fig. 2b). On the electron micrographs these cells closely resembled fibroblasts; they were distinguished by a well-marked rough endoplasmic reticulum, a hyperplastic Golgi complex (Fig. 2c), and also a reduced filamentary apparatus. These cells can be classed as activated smooth-muscle cells [9, 11, 12]. After various types of injury to the vessel wall they migrate into the intima and give rise to an intimal muscular layer [9-11].

In portions of the caudal vena cava that are strongly distended there is thus an increase in the number of DNA-synthesizing smooth-muscle cells, subsequently undergoing polyploidization, with the appearance of newly formed muscular layers.

Besides activation of DNA synthesis, activation of protein synthesis is also observed in the smooth-muscle cells of the distended portions of the vein. It will be clear from the diagram (Fig. 1B) that the ac-

tivation of protein synthesis is delayed compared with the activation of DNA synthesis and reaches its maximum on the 6th-17th day, after which it starts to fall. The activation of protein synthesis was smaller in degree (not more than 4 times); the labeled amino acid was distributed relatively uniformly among the muscle cells.

Appreciable activation of protein synthesis was also observed in the smooth muscles of the femoral vein (Fig. 1, curve 6), i.e., where the increase in the number of DNA-synthesizing muscle cells was small. However, in the strongly dilated main trunk of the posterior vena cava, where marked activation of DNA synthesis and polyploidization of the muscle cells occurred, the activation of protein synthesis was about twice as high as in the femoral vein ($P < 0.01$). It is in those parts, as was shown previously [2], that the hypertrophy of the muscle tissue reaches its maximum. The hypertrophy of this tissue starts to appear at the time of marked activation of protein synthesis (the 3rd-4th week after the operation). These results agree with those obtained by Brodskii on the role of polyploidy for tissue nutrition [1].

Although the activation of DNA and protein synthesis in the smooth muscles of the distended wall of the caudal vena cava thus does not coincide in time, it nevertheless reflects the phases of the same process – working hypertrophy of the muscle tissue of the blood vessel in response to an increase in the functional load.

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