REACTION OF SMOOTH MUSCLE CELLS OF THE POSTERIOR VENA CAVA OF ALBINO RATS TO INCREASED STRETCHING OF ITS WALL AND TO SHORT-TERM HORMONE ADMINISTRATION

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Increased streching of the wall of the posterior vena cava in rats is accompanied by the appearance in the wall of cells with increased diameter, length and volume of the nucleus, and the number of double nuclei is also increased. Cytophotometric examination reveals smooth muscle cells containing an increased quantity of nucleic acids in their nucleus, and autoradiographic studies show incorporation of thymidine-H^3. After adminstration of various hormones the diameter of the nuclei of the smooth muscle cells was changed, although the length and volume of the nuclei and also the quantity of gallocyanin absorbed by the nuclei showed little difference from the corresponding values in intact control rats.

There are indications in the literature of morphological adaptation of the components of the tunica media of blood vessels to regional changes in the hemodynamics. However, reports of hypertrophy of smooth muscle cells in response to changes in the hemodynamics are made on a priori grounds because no cytological investigations have been carried out in this field. This is because it is impossible to obtain a section through the wall of a blood vessel which is strictly parallel to the smooth muscle cells, an essential condition for karyometry [5]. On the other hand, methods based on isolation of smooth muscle cells are ineffective for blood vessels.

In this investigation we studied the possibility of hypertrophy of smooth muscle cells taking place in blood vessels during hemodynamic changes. Changes in volume of the nuclei of these cells during administration of various hormones were also studied.

EXPERIMENTAL METHOD

The posterior vena cava of the albino rat was used as test object. The wall of this vein when straightened out has a single layer of smooth muscle cells, which means that karyometry, cytophotometry, and other investigations can be carried out on total preparations of the film type.

The posterior vena cava of noninbred rats weighing 120-160 g was constricted to a measured degree by a silk ligature to about one-quarter of its lumen (to 1 mm in diameter) just above the point where it receives the right renal vein. The venous pressure measured in the proximal portion of the femoral vein thereupon rose during the first day to 10-12 times above its maximal normal value, and then fell slightly, although remaining at 3.5-4 times above normal throughout the experiment. The increase in diameter was thus also increased in accordance with the Laplace formula $T = R \times P$ (T represents tangential pressure or stress of the wall, R radius of the lumen, and P pressure).

We studied 16 preparations of the posterior vena cava (6 from healthy rats not undergoing operation, 4 from rats sacrificed 7 days after the operation, 3 from rats sacrificed 2.5 weeks after operation, and 3 from rats sacrificed 1.5 months after operation.

Experiments with adminstration of hormones were carried out on sexually immature female Wistar rats weighing 50-60 g, altogether 6 groups of animals with 12-15 in each group being used (group 6 consisted of intact rats). Hormones were administered for a short time (10 days) to rule out the possible effect of changes in the hemodynamics (DOCA and cortisone were given in doses of 5 mg per animal for 7 days and 10-15 mg for 3 days; testosterone in doses of 4 mg, folliculin of 400 units, and progesterone of 5 mg daily).

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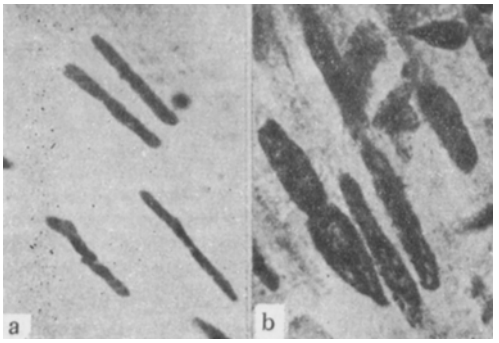

Fig. 1. Nuclei of smooth muscle cells of posterior vena cava of an albino rat. A) Vein of a healthy rat; B) vein of rat 1.5 months after constriction by ligature; nuclei increased in length and thickness; double nuclei are seen. Stained by Einarson's gallocyanin. 1350 × (immersion).

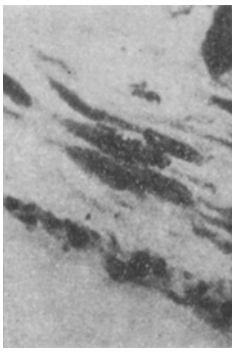

Fig. 2. Incorporation of thymidine-H^3 into nucleus of a smooth muscle cells of the posterior vena cava of an albino rat 7 days after constriction. Explanation in text. 1350 × (immersion).

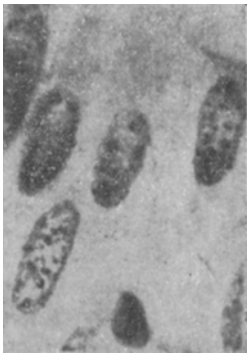

Fig. 3. Nuclei of smooth mucle cells of posterior vena cava of an albino rat after injections of 5 mg testosterone daily for 10 days, showing thickening. Einarson's gallocyanin, 1350 × (immersion).

The action of the hormones was verified by weighing the genital apparatus of the rats and the adrenals. In every case total preparations of the posterior vena cava were fixed in Carnoy's fluid, stained with gallocyanin by Einarson's method [6], passed through alcohols and xylol, and embedded in balsam. The length and diameter of the nucleus was measured on the graduated scale of the ocular. The volume of the nucleus was calculated as the volume of a cylinder with diameter equal to the diameter of the nucleus and height equal to its length [5]. In each preparation karyometry was carried out on 100 nuclei of smooth muscle cells. Cytophotometry of these nuclei, stained with gallocyanin, was carried out on a scanning microspectrophotometer*. In the preparation of the posterior vena cava of intact rats and of rats sacrificed 1.5 months after the operation cytophotometry was carried out on 100 nuclei, and in all other experiments on 50 nuclei. The relative density of gallocyanin in the nuclei was recorded through the microspectrophotometer on an electronic potentiometer. The number of double nuclei, evidence of amitotic division of the smooth muscle cells [3], was counted. Double nuclei were counted in each of 16 cases in 36 fields of vision under a magnification of 1350 times (immersion), and their relative proportion among single nuclei counted in the same 36 fields of vision was then calculated. Esterase hydrolyzing α-naphthylacetate was determined. Pieces of the posterior vena cava taken from a rat 7 days after constriction were incubated in a medium containing thymidine-H^3 (5 μCi/ml, specific activity 120 μCi/mg) or uridine-H^3 (2.5 μCi/ml, specific activity 500 μCi/ml) for 1 and 2.5 h, and then embedded in paraffin wax. The sections were coated with type M NII Khimfoto emulsion. Exposure continued for 7, 14, and 21 days at 4°.

All numerical data were analyzed by appropriate statistical methods.

* For details of the method, see: Yu. E. Morozov and V. V. Rogozhin. Mechanical Analysis of Microscopical Objects [in Russian], Moscow (1968), p. 47.

TABLE 1. DNA Content in Nuclei of Smooth Muscle Cells after Hormone Administration

Hormone	Conventional cytophotometric index		
testosterone	5,7±0,26	σ-1,8	*n*-50
folliculin	6,8±0,29	σ-2,0	*n*-50
cortisone	4,4±0,29	σ-2,0	*n*-50
DOCA	4,6±0,29	σ-2,0	*n*-50
progesterone	7,2±0,37	σ-2,5	*n*-50

EXPERIMENTAL RESULTS

Under normal conditions in sexually mature albino rats the nuclei of smooth muscle cells of the posterior vena cava have a diameter usually (82% of cells) of 2 μ and length from 20 to 35 μ. The volume of the overwhelming majority of the cells (77 %) varies from 50 to 100 μ^3. On the 7th day after constriction the number of cells with diameter 3-4 μ is increased (67%), and so also is the relative number of cells with a nucleus more than 30 μ in length (53%). Correspondingly, the proportion of cells in which the volume of the nucleus is 200-400 μ^3 at this period is 57% (compared with a normal 10%). The results of karyometry 2.5 weeks after the operation were not significantly different from those obtained after 1 week. The number of nuclei with a diameter of 4 μ 1.5 months after the operation was 43% of the total number of cells, the number with a diameter of 6 μ was 33%, 72% of nuclei were more than 30 μ in length (more than 40 μ in nearly half of these), 75% of nuclei exceeded 350 μ^3 in volume, and 33% exceeded 800 μ^3 (Fig. 1).

Besides an increase in the dimensions of the nuclei, the number of double nuclei was also increased. In the normal posterior vena cava, for instance, in 184 fields of vision 9 double nuclei were found for 3419 nuclei of smooth muscle cells, while 7 days after the operation in 99 fields of vision there were 7 double nuclei in 1496 cells, and 1.5 months after the operation 46 double nuclei to 907 cells in 76 fields of vision.

Autographic studies showed that by the 7th day after operation thymidine-H^3 was incorporated into the nuclei of individual smooth muscle cells (Fig. 2), indicating synthesis of DNA in these cells. Cytophotometrically an increase in the proportion of cells whose nuclei contained relatively more DNA was found 2.5 weeks after the operation. Whereas under normal conditions the cytophotometric index for the majority of cells (75%) was 4-10 conventional units, and only 10% of nuclei had a cytophotometric index greater than 10 units, 2.5 weeks after the operation there were 30% of such nuclei. The same number of smooth muscle cells with nuclei having a high DNA content than usual was still found 1.5 months after the operation. In individual nuclei this increase was due not only to an increase in volume of the nucleus, but also to an increase in its relative DNA concentration. In connection with the appearance of many nuclei with a high DNA content, the mean gallocyanin content per nucleus also increased. Whereas nuclei of smooth muscle cells of the posterior vena cava of the intact rats had a mean DNA content of 7.3± 0.2 conventional units, 1.5 months after the operation the DNA content had increased to 8.5± 0.3 ($P < 0.001$). No incorporation of uridine-H^3 into smooth muscle cells of the posterior vena cava was found at the periods studied.

Changes in the dimensions and shape of the nuclei of smooth muscle cells of the posterior vena were observed following hormone administration, notably thickening of the nuclei (Fig. 3). This process was most marked after administration of sex hormones (especially testosterone). In that case, for example, 30% of cells had a nucleus with diameter 5 μ, 13% a diameter of 6 μ, and 12% a diameter of 7 μ. Lengthening of the nuclei was less marked. Correspondingly, after hormone administration, cells with a larger nuclear volume than normally appeared, but this process was not so marked as after constriction of the posterior vena cava by a ligature. For instance, after administration of sex hormones up to 30% of cells had a volume exceeding 400-500 μ^3, and nuclei with volumes exceeding 650-700 μ^3 were practically never found in the preparations. The distribution of nuclei by gallocyanin content in them, which presumably is proportional to their DNA content, showed that the curves for different hormones were basically similar to each other and to the curves for the posterior vena cava of the normal rat. This similarity was shown by the fact that no nuclei could be found with cytometric indices exceeding 10 conventional units, whereas after constriction of the posterior vena cava there was up to 30% of such cells. After administration of corticoids there was a shift toward cells with a slightly reduced DNA content (cytophotometric index 2-4 conventional units), and after administration of sex hormones a moderate shift toward cells with a higher DNA content (Table 1).

No double nuclei were seen among the smooth muscle cells of the posterior vena cava after administration of hormones.

After cortisone administration a considerable weakening of esterase activity of the smooth muscle cells of the posterior vena cava was observed, while after administration of DOCA moderate weakening took place, and testosterone and folliculin caused a moderate increase in esterase activity.

After increased stretching of the wall of the posterior vena cava, cells thus appeared in which the nucleus was greater in length, diameter, and volume than the normal nucleus. These characteristics are regarded by some workers as evidence of hypertrophy of cells [1], although analogous changes can take place in certain types of cell reproduction (endoreproduction, initial stages of amitosis, polyploidy [2]). The signs of DNA synthesis which we found in individual nuclei of the smooth muscle cell, the mosaic pattern of the process, and the increase in number of double nuclei in association with increased stretching of the wall of the posterior vena cava indicates that the changes taking place under these circumstances in the system of smooth muscle cells are complex in their genesis. The possibility cannot be ruled out at present that the appearance under these conditions of smooth muscle cells with a nucleus of larger than normal volume may be connected with stages in the reproduction of smooth muscle cells (in particular, with polyploidy). Following short-term administration of hormones, changes also take place in the shape and volume of nuclei of the smooth muscle cells of the posterior vena cava, while changes in the length and volume of the nuclei under these circumstances are slight. Signs of reproduction of smooth muscle cells, such as the appearance of smooth muscle cells with a double nucleus, or with a large increase in the content of nucleic acids in the nucleus, also are absent. These factors distinguish the effect of short-term hormonal action from adaptation of the smooth muscle cells to constriction of the posterior vena cava.

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