STATE OF THE HYPOTHALAMIC-HYPOPHYSEAL NEUROSECRETORY SYSTEM IN THE EARLY STAGES OF EXPERIMENTAL MYOCARDIAL INFARCTION

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Much evidence of the influence of the hypothalamus on the state of the cardiovascular system has now accumulated [6, 8] but very little work has been done on the state of the neurosecretory nuclei of the hypothalamus in myocardial infarction [9]. Yet this is a subject "of deep therapeutic interest, demanding a shifting of the center of attention from the heart, as the 'target,' to the brain, as the triggering mechanism" [3].

The object of this investigation was to discover the relations between experimental myocardial infarction (EMI) in the early stages of its development and the state of the hypothalamic-hypophyseal neurosecretory system (HHNS).

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male albino rats weighing 180-200 g. A myocardial infarct was induced by ligating the descending branch of the left coronary artery. Changes in the hypothalamus, pituitary, and heart were studied during the fall and winter under normal conditions, and 1, 3, and 24 h after production of the infarct. ECG changes, the total lactate dehydrogenase (LDH) concentration, and histochemical changes in the myocardium (histochemical investigations were undertaken under Professor G. G. Nepryakhin's* guidance), served as parameters of the pathological process. The animals were decapitated in groups of 7-9 rats at a time under superficial ether anesthesia. The brain was removed from the cranial cavity, and with a razor the frontal part, at the level of the optic chiasma, the lateral parts, and half of the cerebellum were carefully cut off. The brain and pituitary were fixed in mercuric chloride–formol (9:1) for 24 and 4 h, respectively. The brain and pituitary were then rinsed for 2 days in running water and embedded, after dehydration in alcohols, in a paraffin wax mixture. Serial frontal sections through the brain (frontal sections are the best with which to study the hypothalamus [9]) and pituitary 5-6 μ thick were stained with cresyl violet by Nissl's method and with paraldehyde-fuchsine by the Gomori–Gabe method in Maiorova's adaptation [4]. As a result of staining the neurosecretory substance in the hypothalamic nerve cells assumed a purple-violet color, whereas that of the nucleus with the nucleolus was blue. Neurosecretory granules could be seen not only in the bodies of the nerve cells, but also in their processes. One aim of the investigation was to study the two large nuclei – paraventricular (PVN) and supraoptic (SON) – in the anterior division of the hypothalamus. The state of secretory activity of the neurosecretory cells of PVN and SON was analyzed by micrometric determination of the area of cross section of the neurons and their nuclei (50 measurements of each in the control and experiment), with an ocular micrometer with moving scale (MOV-1-15x), and with an objective giving a magnification of 40, in accordance with the equation

$$S = \frac{\pi AB}{4} E,$$

where A and B represent the greater and lesser diameters of the cell or nucleus, respectively, and E is the value of one division of the ocular micrometer scale.

*Deceased.

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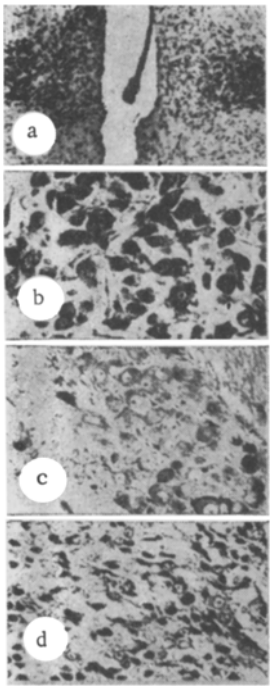

Fig. 1. Brain of healthy albino rat. a) Hypothalamus. PVN (frontal section), general view. Concentration of large polymorphic neurons in apex of conventional triangle most distant from walls of third ventricle. Paraldehyde-fuchsine, after Gomori, 25 ×; b) the same nucleus. Concentration of large neurons, varied in shape, depending on state and stage of their secretory activity; they contain a fairly large nucleus and clearly distinguishable nucleolus. Neurosecretory granules with a bead-like appearance can be seen in capillaries entwining thickly around the nerve cells of PVN, 100 ×; c) SON. Concentration of neurons characteristically dense, round, sometimes fusiform. Cells make contact with capillaries which contain neurosecretory material (NSM), 100 ×; d) pituitary of healthy rat. Uniform distribution of NSM, magnification 25 × 6.3.

EXPERIMENTAL RESULTS

Histological and histochemical investigations of PVN and SON of the hypothalamus were carried out on 43 rats, divided into three groups.

Group 1 (control) included 11 animals killed under ether anesthesia. As Fig. 1 shows, PVN in frontal sections is shaped like an irregular triangle with compact concentration of large neurons in the apex most distant from the walls of the third ventricle. These cells vary in shape, depending on the state and stage of their secretory activity, and they contain a fairly large nucleus, most frequently arranged eccentrically, and a well-marked nucleolus (Fig. 1b). The mean area of cross section of the neurons was 238.452 ± 9.264 μ^2, and of their nuclei 86.426 ± 1.876 μ^2. Neurosecretory Gomori-positive granules in some neurons were grouped in the perinuclear zone, whereas in other neurons they were uniformly distributed throughout the cytoplasm. Neurosecretion often was found in capillaries which entwined the nerve cells of PVN fairly thickly, and also formed small concentrations in the intercellular space. SON was represented by a dense but variably shaped group of large neurons, circular as a rule but sometimes fusiform (Fig. 1c). Just like the neurons of PVN, cells of SON were in different phases of the functional activity, but most of them were pale cells with different numbers of Gomori-positive granules. The mean area of cross section of the neurons was 247.148 ± 4.516 μ^2, and of their nuclei 96.448 ± 0.505 μ^2. This pattern of varia-

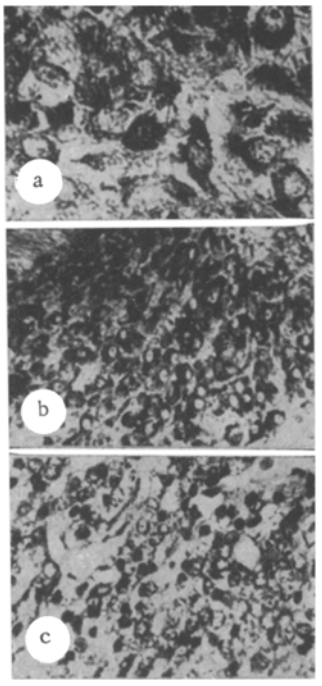

Fig. 2. Brain of rat with experimental myocardial infarction (1 h). a) Hypothalamus, PVN. Large neurons in different states of functional activity. Some of them contain increased quantity of NSM. Individual neurons have cytoplasm overloaded with NSM, with masking of nuclei. Commencing pericellular edema. Granules of NSM in processes and intercellular space, 200 ×; b) SON. Cytoplasm of neurons contains a somewhat increased amount of NSM. In individual cells NSM is seen as a dense mass with a clear "half-moon" at the periphery of the cell, or it occupies the whole cell. Intercellular space contains separate clumps of NSM. More numerous concentrations of NSM can been seen in large and small blood vessels. 100 ×; c) pituitary. Content of NSM a little reduced, its granules of different sizes, 100 ×.

tion in functional activity of PVN and SON neurons can be characterized as a state of "equilibrium" between synthesis and liberation of neurosecretion [5]. These two stages are active, but cells at these stages of the secretory cycle are approximately equal in size and differ mainly in the quantity of neurosecretion in their cytoplasm. In sections of the pituitary gland from the animals of group 1, a fairly intensive distribution of dust-like granules was found after staining by Gomori's method, evidence of sufficient outflow of neurosecretion into the neurohypophysis and of its utilization in the general blood flow (Fig. 1d).

Group 2 consisted of animals undergoing a mock operation (thoracotomy and closure of the chest), and served as the technical control. The histomorphological picture of PVN and SON in these animals differed only a little from that in the previous experiments, but morphometry of the neurons and their nuclei showed that in a state of neurosecretory activity of PVN and SON there was a tendency for their dimensions to increase. The mean area of cross section of the nuclei of PVN neurons was $87.082 \pm 0.309\ \mu^2$, and of the neurons themselves $239.290 \pm 3.632\ \mu^2$. Similar changes also were observed in the dimensions of the nuclei and cells of SON: 98.080 ± 0.991 and $253.106 \pm 5.848\ \mu^2$, respectively ($P < 0.05$ in every case). The neurosecretory substance in the pituitary of animals undergoing the mock operation consisted of the same granules as in the animals of group 1, and only the capillaries appeared rather more dilated. The HHNS of the animals in this series of experiments thus corresponded to the known descriptions of normal [5, 7, 10]. The histomorphological picture of the heart was characterized by the usual microstructure for these animals.

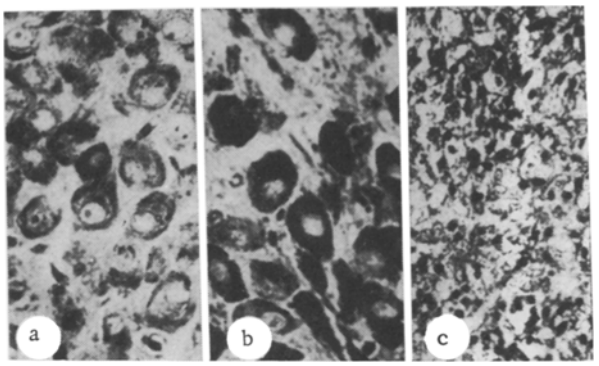

Fig. 3. Brain of rat with EMI (3 h). a, b) PVN and SON, respectively; quantity of NSM continues to rise. Dimensions of neurons and their nuclei increase and polymorphism is exhibited. Secretion of NSM into axons and intercellular spaces clearly visible, 200 ×; c) pituitary. Fairly large and distinct granules of NSM still present, density a little higher than that observed at the previous time. Areas of NSM in the form of concentrations were found in some preparations, 125 ×.

Group 3 consisted of animals with EMI. Mainly pale cells, often with a brightly outlined rim of neurosecretory material (NSM), predominated in PVN 1 h after production of EMI (nine animals). The cytoplasm of most neurons contained an increased number of neurosecretory granules, which filled its peripheral portions. Often the cytoplasm was overfilled with NSM, with masking of the nuclei and reduction of the tigroid substance. Processes of the nerve cells were widened and contained neurosecretion, which passed from them into the capillaries and could be traced for a long distance. NSM also was found in the periaxonal spaces of the axons, and in the intercellular space (Fig. 2a). The nuclei and cytoplasm of the cells were increased in size (89.059 ± 1.319 and 243.862 ± 8.114 μ^2, respectively; $P < 0.05$). These changes were seen more clearly still in SON. Their cytoplasm also contained an increased quantity of NSM, which often fused at the periphery of the cell into a dense mass, forming a clear rim, and sometimes it filled the whole cell. Separate clumps of NSM could be seen in the intercellular space. Its concentrations were particularly high in the large and small blood vessels distributed on the territory of SON (Fig. 2b). The quantity of NSM in the neurohypophysis was somewhat reduced, and the material consisted of granules of different sizes (Fig. 2c). At this stage of the investigation, tiny foci of edema of the interstitial tissue with loosening of the structure of the myocardium, with finely grained and clumped disintegration of the myocytes, and with a well-marked and irregular staining with acid stains (eosinophilia, picrinophilia) appeared in the potentially infarcted zone of the left ventricle. Only 1 h after production of the experimental myocardial infarct, the HHNS was thus characterized by commencing intensification of the neurosecretory function of neurons of PVN and, in particular, of SON, with perhaps slightly increased utilization of secretion.

The quanity of NSM continued to increase in the nerve cells of PVN and SON 3 h after EMI (nine animals). Polymorphism of the neurons was observed and was more marked in SON, an additional sign of their active function [2]. Almost half of the nerve cells contained large amounts of specific dark purple inclusions, consisting of distinct tiny grains. Sometimes neurosecretion occupied the whole cell, and these neurons appeared "dark" under the microscope. All the cells had clear boundaries, the origins of the axons of most of them were widened, and they contained different amounts of neurosecretion, which could be traced for a considerable distance from the cell bodies. Concentrations of neurosecretion or separate large granules of it often were found in the immediate vicinity of the endothelium of the blood vessels. Grains of NSM also could be seen in the lumen of the large vessels. This indicates that the neurosecretory substance can be liberated directly into the blood stream, by-passing the hypothalamic-hypophyseal tract. Other neurons were seen as large, pale cells (with pale cytoplasm, but with quite clearly distinguishable processes, especially in SON), with dust-like NSM in their neuroplasm (Fig. 3a, b). The mean area of cross section of the nuclei and neurons was increased, to 96.666 ± 1.380 and 259.233 ± 2.171 μ^2, respectively for PVN and to 109.202 ± 1.388 and 280.262 ± 1.633 μ^2 for SON (values statistically significant

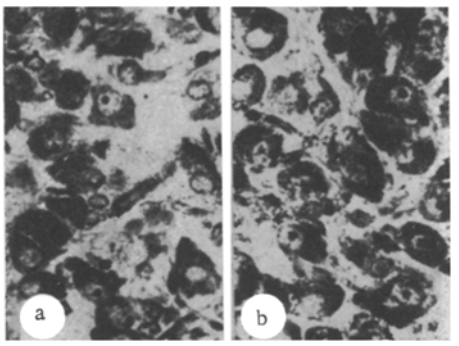

Fig. 4. Brain of rat with EMI (1 day). a, b) PVN and SON respectively. Polymorphism of neurons and different stages (depending on NSM content) of their functional activity remain. Dimensions of neurons and their nuclei continue to increase. Capillaries contain NSM, which can be traced for a long distance from the cells, 200 ×.

except for S cells of PVN, for which $P > 0.05$). Fairly large and clear granules still remained in the neurohypophysis, and its density was a little increased compared with that at the previous time of investigation (Fig. 3c).

After 24 h (nine animals) the dimensions of the neurons and their nuclei continued to increase: the area of cross section of a neuron in PVN was $268.770 \pm 2.462\ \mu^2$ and of the nucleus $96.393 \pm 1.183\ \mu^2$; the corresponding values of SON were 292.784 ± 3.387 and $112.097 \pm 1.781\ \mu^2$ ($P < 0.05$). At the same time the concentration of neurosecretion in the neurons of PVN and SON differed. In PVN most cells were pale and polymorphic, as a rule with dust-like Gomori-positive substance. A higher content of neurosecretion was found in the axons of the cells than at the previous time of investigation. PVN is shown in Fig. 4a, where capillaries with large granules of neurosecretion and polymorphic neurons in different stages of functional activity are clearly visible. A similar picture, but with more intensively neurosecreting neurons, also was characteristic of the cells of SON (FIG. 4b). Cells which had lost their outlines (having undergone plasmolysis and karyolysis) were found in some preparations. Neurosecretion in them either had disappeared or was found in the form of a half moon at the periphery of the residual outline of the cell. Nevertheless, such "underfilled" neurons or neurons completely free from neurosecretion were infrequently seen. Otherwise the whole morphological picture both of the hypothalamic cells and of the state of NSM in the pituitary, corresponded approximately to the changes observed at the previous time of investigation. In the zone of the infarct in the left ventricle tiny foci of anuclear necrosis and disintegration were seen: myolysis with productive inflammation characterized by numerous cells and granulations.

Comparison of data on the functional state of the neurosecretory cells of PVN and SON, their morphometry, and the presence of NSM in the neurohypophysis with morphological changes of experimental myocardial infarction thus reveals correlation between the state of neurosecretion and the time course of changes due to the pathological process and indicates that in the early stages of its development EMI is not a condition which involves the cardiovascular system alone.

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ISCHEMIC AND POSTISCHEMIC DISTURBANCES OF THE PULMONARY MICROCIRCULATION

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KEY WORDS: lung; ischemia; microcirculation; biomicroscopy.

Temporary ischemia of the lung leads to functional disturbances in the pulmonary circulation [2, 7]. Considering that the microcirculation of any organ is a vital factor providing for its nutrition and function [4, 9-12], it was decided to study the pulmonary microcirculation during and after various periods of ischemia of the lung.

EXPERIMENTAL METHOD

In 40 experiments on five groups of dogs (eight animals in each group) and under combined anesthesia and artifical ventilation of the lungs with a mixture of air and oxygen, left-sided thoracotomy was performed and the pulmonary artery, the main bronchus, and pulmonary veins of the left lung were isolated. Ischemia was induced by successive application of atraumatic clamps to all structures in the root of the left lung for 30, 60, 90, 120, and 180 min in each group, respectively. The main bronchus was clamped at the height of expiration. The pulmonary microcirculation was studied by means of the LYUMAN K-1 biological contact microscope, mounted on the stand of an MLK-1 microscope [3]. The dark field method of investigation was used. Observations were made and photographs taken with contact 10 × 0.30 and 20 × 0.75 epiobjectives and an MFN-12 photomicrographic attachment. The contact objective was moved up to the lung until it touched the visceral pleura. To prevent movement of the region examined relative to the objective a vacuum fixer fitted to the contact objective was used. Observations were made after thoracotomy, during temporary exclusion of the lung from the circulation and ventilation, and during the 30 min after ischemia. The pressure in the pulmonary trunk was recorded at the same time by direct manometry and the cardiac output was determined by the thermodilution method.

EXPERIMENTAL RESULTS

On biomicroscopy of the normal lung the alveoli appeared as round or oval structures with thin and even walls and they were clearly separated from one another by interalveolar spaces. A many-looped network of capillaries could be seen against the background of the alveoli. Along the outer borders of the capillaries and interalveolar spaces there was a band of increased illumination because of reflection of light from their walls (Fig. 1). Arterial and venous microvessels of the interalveolar spaces differed in the direction of the blood flow at points where the microvessels divided or joined together. Blood cells in the stream occupied a central axial postion; a paler, thin layer of plasma could be identified near the walls of the vessels.

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