PATHOMORPHOLOGY OF ADRENERGIC AND CHOLINERGIC STRUCTURES OF SYMPATHETIC NERVE GANGLIA IN EXPERIMENTAL BURN TRAUMA

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KEY WORDS: burn trauma; sympathetic ganglia; adrenergic and cholinergic structures.

The study of the organization of adrenergic and cholinergic structures of sympathetic nerve ganglia in man under normal and certain pathological conditions, such as burns, is not only of scientific interest, but also of definite practical importance. Meanwhile this problem has not been adequately discussed in the literature, and available information on the possibility of studying the adrenergic and cholinergic innervation in autopsy material is contradictory [3, 8]. When data obtained by the study of such material is assessed, especially when histochemical methods of investigation are used, definite difficulties arise. These are concerned, primarily, with cadaveric changes, which may render difficult the interpretation of the structural disturbances observed. Some investigators have shown that enzymes in the tissues and hypothalamo-hypophyseo-neurosecretory system can be studied in autopsy material soon after death [1, 6, 7]. Meanwhile the use of experimental material to study histochemical changes in nerve structures enables post mortem changes, which are inevitable in the investigation of autopsy material, to be eliminated. Most experimental studies have been undertaken soon after infliction of burn trauma, for it is at this time that it resembles the clinical picture of burns in man [2, 4, 5]. Incidentally, although the study of experimental material almost completely rules out post mortem changes, it is only by its comparison with autopsy material that the true picture of changes in the organs and tissues in burns can be obtained.

EXPERIMENTAL METHOD

Sympathetic superior cervical, and celiac ganglia of the solar plexus were used as the test objects. After freezing with dry ice, frozen sections of the ganglia were treated with a 2% solution of glyoxylic acid and studied in the luminescence microscope. Cholinergic nerve structures were revealed by the method of Karnovsky and Roots. Experiments were carried out on 15 noninbred male albino rats weighing 200-250 g. The animals were fixed on a wooden bench, after which, under ether and oxygen anesthesia, the animal was shaved in the dorsal region. Burn trauma was inflicted by applying a metal plate measuring 4 × 9 cm, heated to 80-100°C, to the depilated skin. The exposure was 5-6 sec, and as a result, a burn of the IIIA and IIIB degree was produced on an area equal to 20-25% of the rats' body surface. The animals were killed by decapitation 3, 7, and 11 days after burn trauma, corresponding to the principal periods of burn healing. Material from five control animals was taken immediately after decapitation.

EXPERIMENTAL RESULTS

Four types of fluorescent structures were discovered in the sympathetic autonomic nerve ganglia (superior cervical, stellate and celiac ganglia of the solar plexus) under normal conditions, against a general background of nonspecific fluorescence of their stroma: the basic (postganglionic) neurons, small intensively fluorescent cells (SIF-cells), a network of adrenergic nerve fibers, and lipofuscin granules. In the principal neurons specific catecholamine fluorescence was observed

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Fig. 1. Brightly fluorescent groups of SIF-cells in rat superior cervical ganglion. Control. Incubation in 2% solution of glyoxylic acid. 120×.

in the cytoplasm and processes, and varied in degree. Meanwhile, neurons totally devoid of catecholamine fluorescence were found in the ganglia. The presence of different degrees of fluorescence or, indeed, its absence, evidently indicates the level of functional activity of the neurons.

Among neurons of the sympathetic nerve ganglia, SIF-cells were frequently distinguished and, as a rule, they were arranged in small groups. From 1-2 to 4-5 such concentrations were found in a mid-line section through the ganglia (Fig. 1). Solitary scattered SIF-cells or so-called highly intensively fluorescent cells also were found. Thick short processes of these cells can comparatively frequently be detected by the histofluorescence method. Incidentally, in the superior cervical and stellate sympathetic ganglia the SIF-cells were found more frequently than in the celiac ganglia of the solar plexus.

In the early stages of experimental burn trauma (until 3 days after infliction of the burn) some irregularity of fluorescence of the nerve cells of the sympathetic ganglia was observed, and there were some neurons with weakly fluorescent cytoplasm (Fig. 2). Fluorescent adrenergic nerve terminals between nerve cells were rare.

The study of the time course of repair in the sympathetic nerve ganglia 7-11 days after experimental burn trauma showed that fluorescence of catecholamines in adrenergic structures was quite distinctly visible, although in many areas, foci with a sharp decrease in the intensity of fluorescence or its complete absence could be distinguished against this background. Under these circumstances fluorescent adrenergic structures were more frequently found in the peripheral zones of the autonomic nerve ganglia which we studied (Fig. 3).

SIF cells were invariably found, both immediately after burn trauma and at definite time intervals after the experiment. Their fluorescence was not appreciably reduced in the various groups of experiments. SIF cells are the most stable of formations, and their biogenic amine content correspondingly remains unchanged immediately and also at various times after burn trauma.

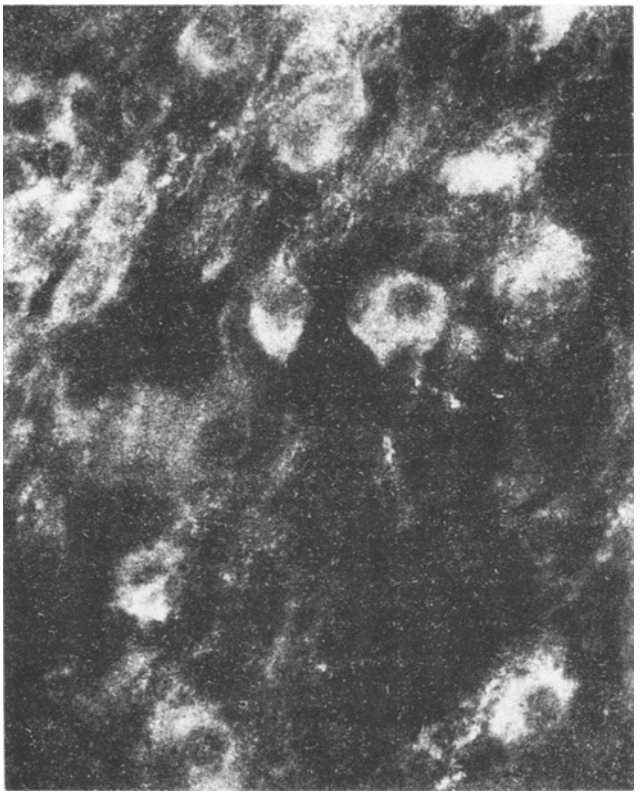

Fig. 2. Uneven decrease in brightness of fluorescence of neuron of rat superior cervical sympathetic ganglion. Three days after burn trauma. Incubation in 2% solution of glyoxylic acid. 120×.

Investigation of sympathetic nerve ganglia of the experimental rats by the Karnovsky–Roots histochemical method showed that the cytoplasm of neurons in the control group stained brown, and in some cells even dark brown. Nuclei of the nerve cells appeared pale and contained vesicles. Thick nerve fibers, discovered in the stroma of the ganglia, also stained the characteristic dark brown color of this reaction. This was probably because the structural elements of the sympathetic nerve ganglia give a positive reaction for acetylcholinesterase (AChE).

In the early stage (until 3 days) after experimental burn trauma, individual groups of nerve cells distinguished by an irregular concentration of AChE in their cytoplasm were seen in the sympathetic ganglia of the rats. At the same time, AChE-positive nerve fibers could be seen running in the composition of a nerve bundle in the stroma of the ganglion. In the late period (after 7-11 days) after burn trauma, increased AChE activity could be detected in the rats simultaneously in the cytoplasm of neurons of the sympathetic ganglia and in nerve fibers of thick and medium caliber Fig. 4). These findings agree on the whole with the mosaic pattern of the lesion affecting the nerve ganglia and their catecholamine content described above.

To conclude, sympathetic ganglia are complex formations for the nervous regulation of the various systems of the body. They include a variety of adrenergic and cholinergic structures, which undergo considerable changes when pathological processes take place in the body. A sharp decrease in the content of catecholamines in structures of sympathetic nerve ganglia is observed during the first day of burn trauma (the period of burn shock). Some recovery of mediator reserves in them takes place in the subsequent periods of burn healing. In our view, the fluorescence-histochemical method can be used to shed light on some mechanisms of development of burn healing and also with the aim of substantiating a number of therapeutic procedures when complications involving the cardiovascular system are present.

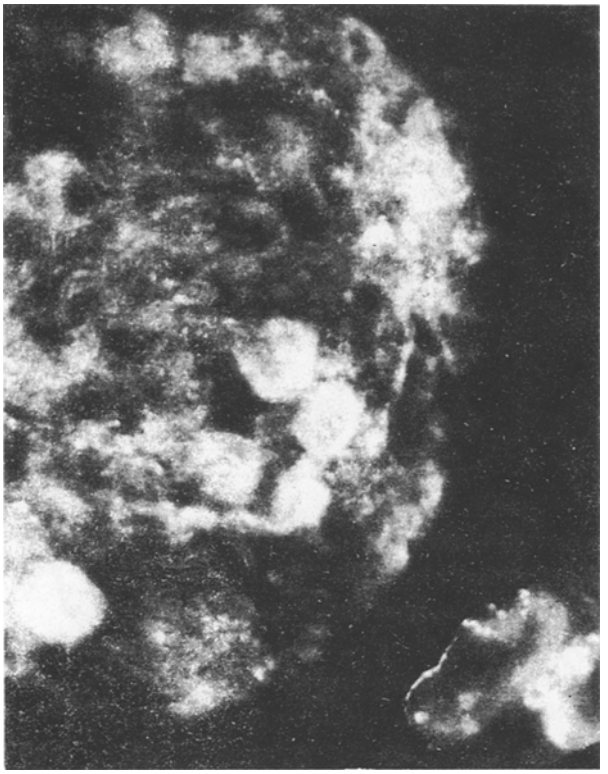

Fig. 3. Bright fluorescence of adrenergic structures in peripheral parts of rat stellate ganglion. Eleven days after burn trauma. Incubation in 2% glyoxylic acid solution. 120×.

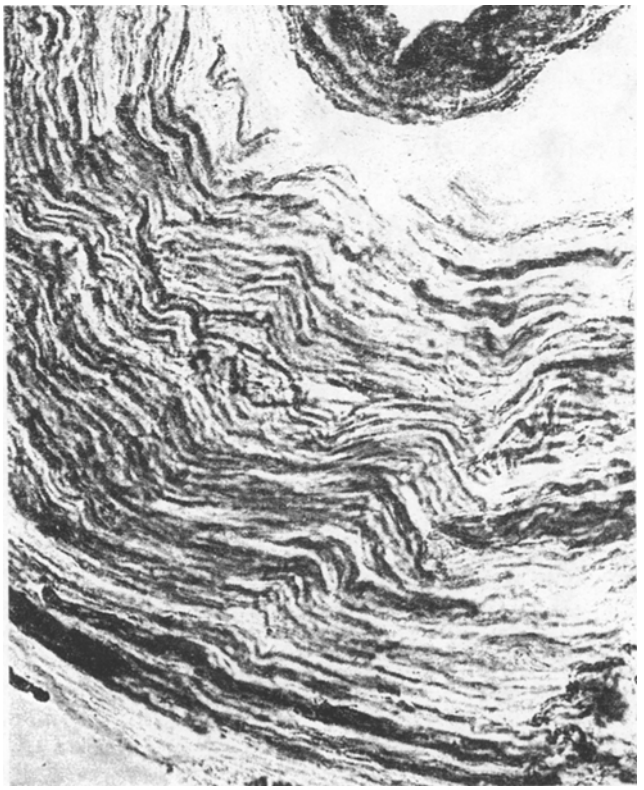

Fig. 4. Increase in AChE concentration in nerve fibers of thick and medium caliber in stroma of celiac ganglion of rat solar plexus. Seven days after burn trauma. Karnovsky–Roots method. 200×.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF CYTOCHROME P-450

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Synthesis and production of steroid hormones are observed in many organs and tissues: the adrenal cortex, ovaries, testes, and placenta [6, 7]. These synthetic processes are varied and quite complex and they include a complete cascade of reactions of hydroxylation of the side chains of the steroid molecules. However, the limiting stage in these conversions is oxidation of cholesterol into pregnenolone. This process is catalyzed by cytochrome P-450 [1].

The study of the morphological and functional organization of enzyme systems of cytochromes of the adrenals, ovaries, and testes has been pursued quite actively. For reasons which will be understood, most attention has been paid to hormone synthesis in the adrenals. The immunochemical characteristics and structural localization of this cytochrome have been investigated. It has been shown that cytochrome P-450 is located on the inner cristae of the mitochondria of the adrenal cortex [9]. A study of the distribution of the mixed monooxygenase system in the ovaries has shown that cytochrome P-450 is located in ripe follicles [8]. In the testes P-450 is located in the Leydig's cells [3].

Much less attention from this standpoint has been paid to the placenta. It has been shown that in man and animals oxidation of cholesterol into pregnenolone takes place in the mitochondrial fraction of the placenta [4]. As yet, however, there has been virtually no research into the structural localization of cytochrome P-450 in the placenta. The aim of this investigation was accordingly to demonstrate cytochrome P-450 (forms C, D, and B) in sections of the human placenta with the aid of specific antibodies.

EXPERIMENTAL METHOD

The placenta was taken immediately after normal birth (12 deliveries) into 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The placenta was fixed for 4 h, and then taken through a series of alcohols and xylol and embedded in paraffin wax. Sections (4 μ thick) of the placenta were mounted on a slide, dewaxed in xylol (for 10 min twice), rehydrated in alcohols, and carried through to phosphate buffer. Specific antibodies against cytochrome P-450 of the C, D, and B

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