Destructive Reactions of Skeletal Muscles in Toxic Metabolic Injuries Caused by Bupivacaine in OXYS and Wistar Rats

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Universal types of muscle fiber injuries associated with characteristic changes in the myofibrillar system predominate in the spectrum of reactions to acute disorders of cell metabolism caused by bupivacaine. Quantitative parameters of the pathogenic exposure and structural and metabolic characteristics of the target fibers largely determine these changes: pronounced contractures involving primarily white muscle fibers, high incidence of disseminated and mixed forms, among which a special variant with predominant lytic processes ("cytolysis") was distinguished. Minimum changes in the contractile system were often paralleled by diffuse destruction of mitochondria, which attested to the role of these organelles in the development of muscle fibers alteration in this model.

Key Words: *metabolic injuries; skeletal muscles; bupivacaine; OXYS rats; polarization microscopy*

The capacity of some local anesthetics, primarily bupivacaine, to cause pronounced degeneration of muscle fibers is often used in studies of the regeneratory reactions of skeletal muscles [14]. However, the interest to this experimental model is also explained by some common aspects linked with the mechanisms of muscle cell damage [11].

Universal disturbances in mitochondrial metabolism, similar to those developing in some general pathological processes, underlie the myotoxicity of bupivacaine [13]. On the other hand, local and systemic exposure differ significantly by the intensity and dynamics of the damaging stimulus, which makes it possible to evaluate the role of these parameters in the formation of the spectrum of destructive reactions [2,6,7].

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The type of degenerative changes largely depends on structural and metabolic characteristics and function of the target fiber [2,9]. This was taken into consideration when choosing the material for experimental study: the structure of the anterior tibial muscle allows evaluation of the reactions of various types of muscle fibers to injury [10]; analysis of the pathological picture in senescence-accelerated OXYS rats is interesting, because it can reveal possible reduction of the adaptive potential of muscle fibers caused by progressive disorders in the structure and function of their mitochondria [1,4].

We studied the morphogenesis of acute injuries to muscle fibers caused by bupivacaine in Wistar and OXYS rats.

MATERIALS AND METHODS

Experiments were carried out on 38 OXYS and 38 Wistar rats aged 6 months and weighing 215-

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340 g. Intact animals of the same strains (5 per strain) and age served as the control.

Bupivacaine (Anecaine, Pliva; 0.5% solution) was injected (0.2 ml) throught a fine needle into the center of the anterior tibial muscle. The rats were sacrificed after 3 h, 1, 3, 7, and 14 days. The contralateral intact muscle (control) was examined in all animals; in two cases 0.2 ml sterile isotonic solution was injected into it at each term of the experiment.

Preparations of the muscles "stretched at rest" were made as described previously [2,9]. Muscle fragments for light microscopy were fixed in 10% neutral formalin and after standard processing embedded in paraffin with consideration for the muscle fiber orientation. Paraffin sections were stained with hematoxylin and eosin with Perls reaction, by the method of Van-Gieson with resorcin-fuchsin poststaining of elastic fibers after Weigert, and PAS reaction was carried out.

For electron microscopy the samples fixed in 4% paraformaldehyde were postfixed in 1% osmium tetroxide and after dehydration were embedded in epon-araldite mixture. Semithin (1 μ) sections were made on a Thesla ultratome and stained with Schiff reagent and 1% azur II solution. Ultrathin sections were made on an LKB III ultratome, contrasted with uranyl acetate and lead citrate after Reynolds, and examined under a JEM 1010 electron microscope.

In order to evaluate the status of the fibrillar system in muscle cells, paraffin sections (stained and not) with longitudinally oriented muscle fibers were examined in polarized light.

RESULTS

Two zones are distinguished in the anterior tibial muscle: outer zone consisting mainly of white muscle fibers and inner zone, where the greater part of muscle fibers has signs of red fibers [10]. Despite low dose of the drug, degenerative changes were disseminated and developed very rapidly.

After 2-4 h, the signs of edema of muscle fibers and endomysium, more pronounced in the external zone of the anterior tibial muscle, were observed in all groups. Limited sites along the muscle fibers became homogeneous in the same zone, some of these sites looked as "contracture nodes". Polarization and electron microscopy revealed contrast changes in the main "marker" structure of muscle fibers, the myofibrillar system, in these foci and in the adjacent segments. The contracture node (III degree contracture) [2] was presented by anisotropic conglomeration formed from compact hypercontracted sarcomers (Fig. 1, *a*) delineated from other fibers by zones of their drastic overstretching or sites of complete dissociation of myofilaments.

Less "contrast" changes involved muscle fibers at an appreciable length and were mixed: sites of hypercontractures transferred into zones of overstretching and disordered cross-striation (Fig. 1, *b*). Examination in transmitted light often showed finely dispersed grains in the A-disc material in these zones, the discs acquired a "dotted" look (with preserved contours) and were characterized by pronounced diastasis; the strips of the I disk looked "empty", some groups of sarcomers looked disintegrated. Further evolution of these changes was seen in the next segments of the same fiber, where false orderliness of anisotropic discs was lost and the "grains" of contractile material formed an amorphous mass.

Electron microscopy showed that selective degradation of fibrillar elements of I-discs and Z-strips (Fig. 1, *c*) was responsible for this picture, to some extent resembling descriptions of "discoid degradation" [6]. Thick myofilaments retained their characteristic configuration and arrangement in A-discs, which were in a state of diastasis. Individual small vesicles of the sarcoplasmic reticulum and large or small round mitochondria with diffuse or focal matrix clarification, crist destruction, and signs of outer membrane destruction were seen in the focus of injury.

Diffuse total destruction of mitochondria with the formation of electron transparent vesicles was often noted in the intermyofibrillar spaces even if the ultrastructural organization of myofibrils was preserved.

In contrast to contracture changes associated with disseminated and heterogeneous signs of disorganization of the contractile compartment, manifestations of small focal lysis of fibrillar structures ("empty" isotropic foci with clearly seen interface without cross-striation), more characteristic of the deep area of the anterior tibial muscle, were usually seen in the fibers without any other appreciable changes. In some cases, when the myocytolysis foci were numerous, the muscle fibers in polarized light looked like a "moth-eaten" tissue (Fig. 1, *d*). With a certain degree of carefullness we can say that early focal lytic injuries were somewhat more often seen in OXYS than in Wistar rats.

All forms of irreversible contracture injuries were detected in damaged zones after 24 h; the major part of lesions were in the phase of lumpy degradation (Fig. 2, *a*). Fragmentation in large buckled fragments corresponding to "contracture nodes" and formed (according to electron microsco-

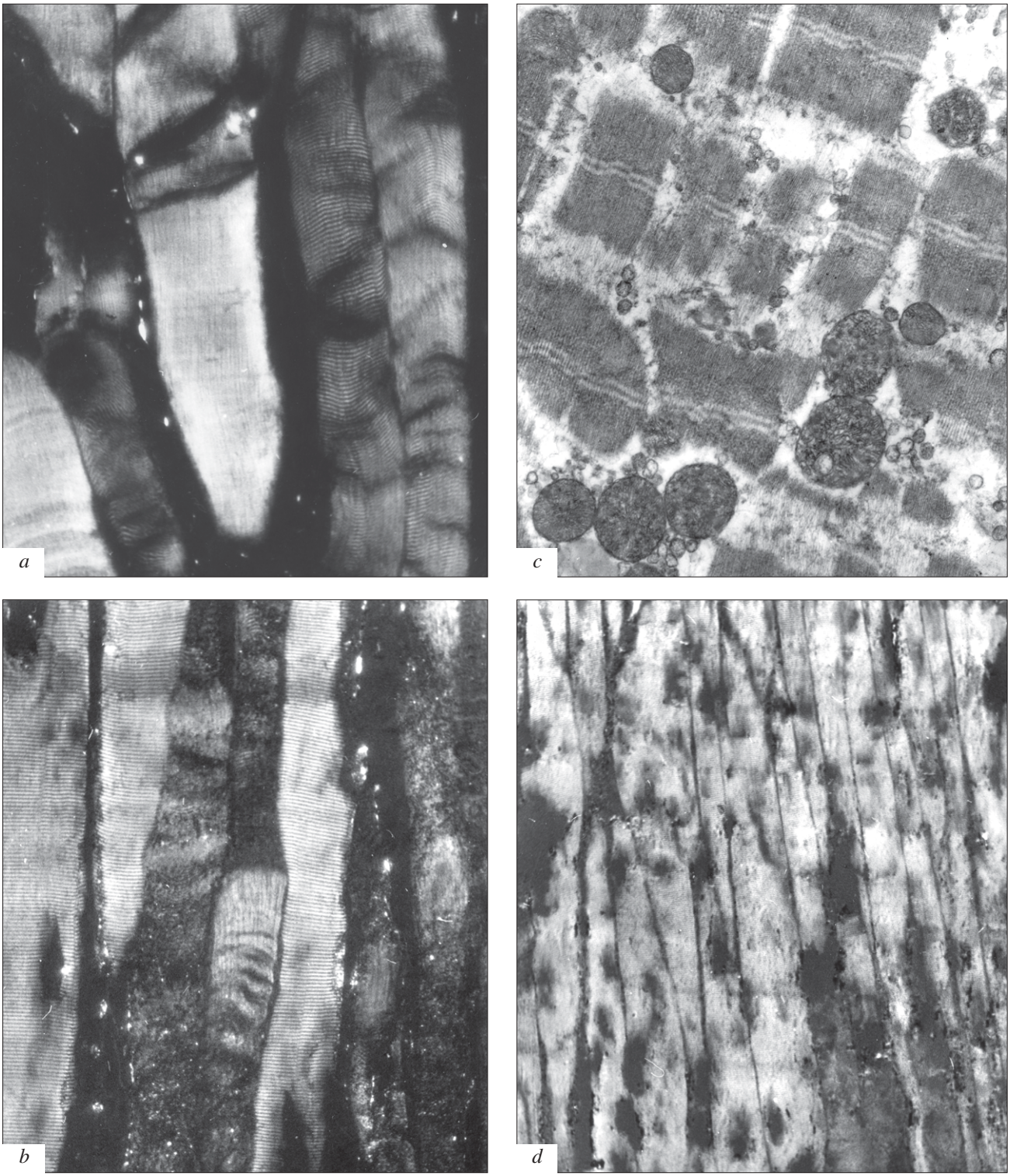

Fig. 1. Acute toxic and metabolic injuries to the skeletal muscles of OXYS (*a*, *d*) and Wistar (*b*, *c*) rats 3 h after injection of bupivacaine. *a*) III-IV degree contractures of myofibrils. Alternation of sites of hypercontracture and hyperstretching of sarcomers; *b*) combination of pronounced destructive changes (III-IV degree contractures of myofibrils, disaggregation of anisotropic structures) and signs of normal structure in adjacent muscle fibers; *c*) muscle fiber "cytolysis": disappearance of Z-strips, disaggregation and lysis of fine filaments, retention of A-disc configuration, destructive changes in mitochondria; *d*) multiple foci of myocytolysis. *a*, *b*, *d*: photographs in polarized light; *a*, *b*: ×500; *d*: ×250; *c*) electronogram, ×10,000.

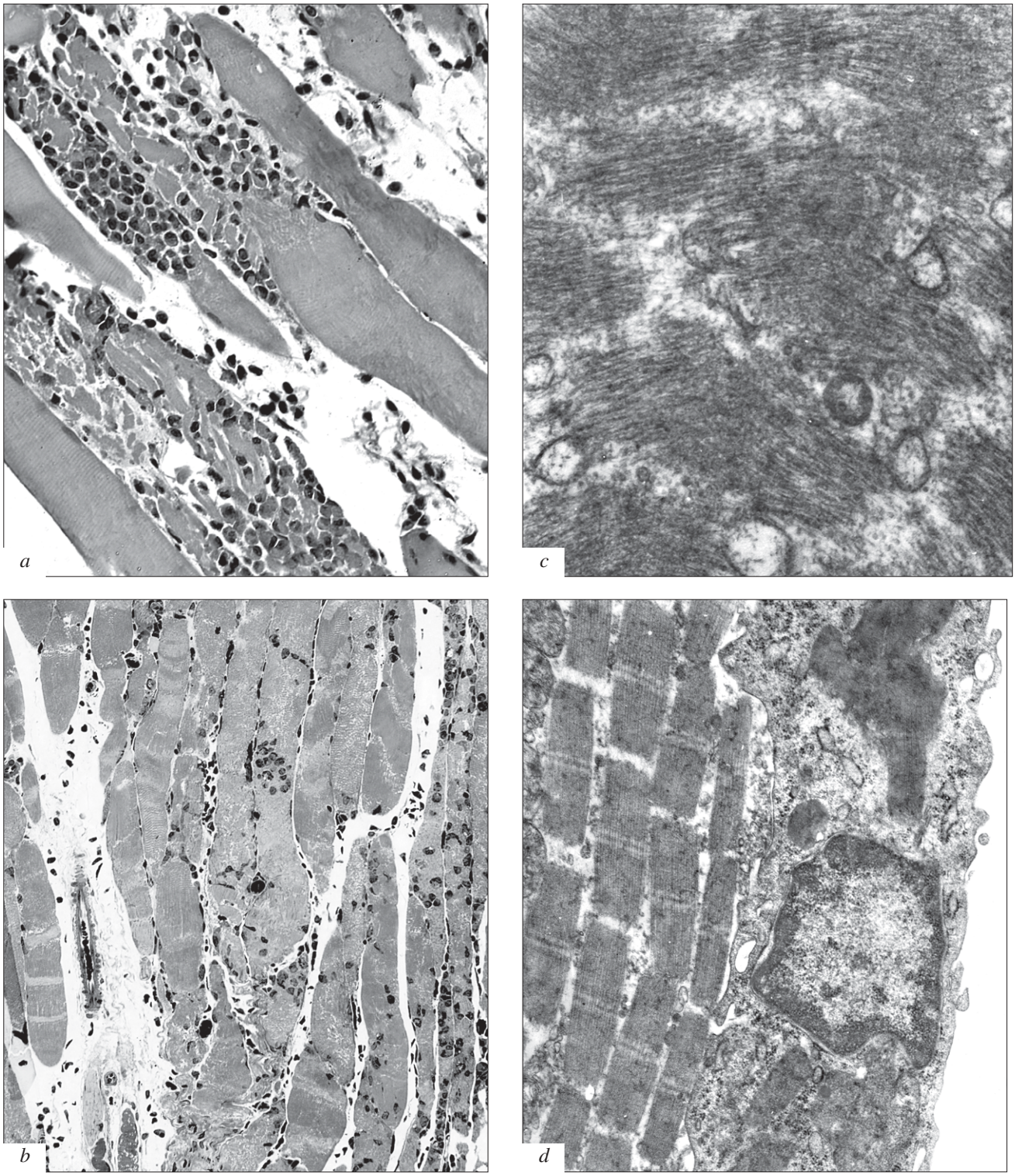

Fig. 2. Acute toxic and metabolic injuries to skeletal muscles in OXYS (*a*, *b*, *d*) and Wistar (*c*) rats 24 h after injection of bupivacaine. *a*) cell infiltration in focus of damage in the lumpy degradation phase. Hematoxylin and eosin staining, ×500; *b*) disseminated necrobiotic changes in muscle fibers, lumpy degradation, and cell infiltration. Semithin section, azur II staining, ×250; *c*) IV degree contracture of myofibrils, myofilament disaggregation, destruction of cytoplasmic organelles, ×20,000; *d*) a macrophage in the zone of muscle fiber destruction. Phagocytosis of large fragments of myofibrillar structures, ×10,000. *c*, *d*: electronograms.

py) by condensed mass of disaggregated and disoriented protofibrils, most often started at the periphery of the focus; small accumulations of the infiltrate cells were also found here (Fig. 2, *b*).

Compact zones were alternating with zones of complete disorganization of contractile material (sometimes with "false" cross-striation; Fig. 2, *b*) in muscle fibers damaged at an appreciable length. Ultrastructural analysis showed disaggregation of sarcomers in the majority of fragments of these fibers; Z-discs in these sites were completely destroyed and separate fragments of fine filaments aggregated with each other were seen instead of I-discs. Scraps of thick filaments lost their orderliness, but sometimes remained at the sites of the former A-discs (Fig. 2, *c*). In other zones, the myofilaments were completely disaggregated and their remnants were left in complete disorder.

Numerous macrophages were seen in the foci of muscle fiber damage. Macrophages inculcated in the damaged foci, their cytoplasm forming numerous protrusions and containing fragments of muscle cells: mitochondria, scraps of myofibrils, and sometimes large fragments, in which myofibrillar cross-striated structures could be discerned (Fig. 2, *d*).

Contractures and cell infiltrates sometimes disseminated in the form of "cords" involving bundles of muscle fibers, but were more often zone-dependent (subtotal injuries and diffuse cell infiltration in the external zone were adjacent to a visually intact area formed by darker fine fibers).

Further evolution of the morphological picture (after 3, 7, and 14 days) reflected regeneratory processes, which started to predominate. However, even at the stage of maximally pronounced proliferative phase of the regeneratory reaction (after 3 days), secondary contractures and pictures of small focal myocytolysis were seen in the muscle fibers adjacent to fields of cell infiltration and resorption.

Hence, intramuscularly injected bupivacaine caused a rapid development of disseminated destructive changes predominantly in the white muscle fibers (pronounced contracture-type injuries) or in fibers of both types (foci of myocytolysis and moderate contractures). One of the causes of this selective damage can be a great compensatory reserve of the mitochondrial compartment in type I muscle fibers [11]. The minimum changes in the contractile system were often associated with diffuse destruction of the mitochondria, which probably indicated the primary role of these organelles in the development of muscle fibers alteration in this model.

An important feature in the spectrum of structural reaction, heretofore not observed in more "benign" total exposure [2], was detection of myofibril disaggregation zones, resembling the picture of "discoid degradation", in the studied material [8]. This form of damage, described for cardiomyocytes in occlusive myocardial infarction and vast infarction-like metabolic injuries, was called "cytolysis" (life-time autolysis) [5].

Experiments with bupivacaine showed no signs of significant disorders in blood supply; signs of cytolysis were observed in just separate muscle fibers and, as a rule, not over their entire length, alternating with contractures of different severity. It seems that drastic disorders in oxidative phosphorylation during local treatment with high concentrations of the damaging factor in the presence of a certain local physiological context "simulated" by their intensity the state of acute hypoxia with rapid development of deep energy deficiency, anticipating activation of lytic processes and total lysis of primarily cytoskeleton elements and actin filaments in the isotropic disc area. Long retention of A-disc structures in these zones can be due to resistance of myosin heavy chain to calpain-dependent proteolysis [12] and to the formation of transverse links between the myosin and actin filaments in the absence of ATP [3].

On the whole, comparative analysis of acute toxic metabolic injuries to the skeletal muscles in some experimental and clinical pathological processes [2,6] demonstrated an important role of quantitative parameters of the destructive factor and of the structural and metabolic characteristics of muscle fibers in the formation of the spectrum of destructive reactions. The absence of appreciable differences in this aspect between OXYS and Wistar rats can be due to rapid and massive restructuring of muscle tissue under extreme dysmetabolic conditions. When exposure to the destructive factor is over, these changes, causing a threshold strain of the skeletal muscle reparative reserve, create the base for full-value regeneratory reaction. Presumably, this explains the fact that, despite wide use of local anesthetics, clinically significant muscle dysfunction is observed mainly after prolonged blocking of the peripheral nerves and is, as a rule, completely reversible [15].

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