

Lesions of rat skeletal muscle after local block of acetylcholinesterase and neuromuscular stimulation

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Mense, S., D. G. Simons, U. Hoheisel, and B. Quenzer. Lesions of rat skeletal muscle after local block of acetylcholinesterase and neuromuscular stimulation. *J Appl Physiol* 94: 2494–2501, 2003. First published February 7, 2003; 10.1152/jappphysiol.00727.2002.—In skeletal muscle, a local increase of acetylcholine (ACh) in a few end plates has been hypothesized to cause the formation of contraction knots that can be found in myofascial trigger points. To test this hypothesis in rats, small amounts of an acetylcholinesterase inhibitor [diisopropylfluorophosphate (DFP)] were injected into the proximal half of the gastrocnemius muscle, and the muscle nerve was electrically stimulated for 30–60 min for induction of muscle twitches. The distal half of the muscle, which performed the same contractions, served as a control to assess the effects of the twitches without DFP. Sections of the muscle were evaluated for morphological changes in relation to the location of blocked end plates. Compared with the distal half of the muscle, the DFP-injected proximal half exhibited significantly higher numbers of abnormally contracted fibers (local contractures), torn fibers, and longitudinal stripes. DFP-injected animals in which the muscle nerve was not stimulated and that were allowed to survive for 24 h exhibited the same lesions but in smaller numbers. The data indicate that an increased concentration of ACh in a few end plates causes damage to muscle fibers. The results support the assumption that a dysfunctional end plate exhibiting increased release of ACh may be the starting point for regional abnormal contractions, which are thought to be essential for the formation of myofascial trigger points.

dysfunctional end plate; contracture; contraction knots; myofascial trigger points

BLOCKING THE ACh ESTERASE (AChE) can have strong effects on the morphology of muscle fibers. As early as 1969, Ariens et al. (1) reported that systemic (intravenous) administration of sublethal doses of AChE inhibitors in rats caused necrosis of muscle fibers predominantly underneath the end plates. Subsequent histological studies in myasthenia gravis patients and rats also showed that the systemic application of small (therapeutic) doses of neostigmine led to marked changes in the arrangement of myofibrils underneath the end plates and in the morphology of the presynaptic portion of the end plate (5, 13, 30).

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The effects of systemic administration of AChE inhibitors and hence a global increase in neuromuscular ACh appear to differ from those of locally increased ACh concentration that affects only some end plates of a muscle. An increase of ACh in the cleft of an end plate has been shown to have a strong impact on the function of the end plate. In 1956, Liley (23) recorded miniature end-plate potentials (MEPPs) in rats and demonstrated that a weak pull at the nerve fibers supplying the end plate led to a strong increase in the frequency and amplitude of the MEPPs. Subsequent studies (4, 12) likewise showed that mechanical irritation of the end plate can cause the release of ACh. The mechanism of a stretch-mediated release of neurotransmitters from motor nerve terminals (probably by the opening of stretch-activated membrane channels in the nerve membrane) has been supported by more recent data (2). It is conceivable that a mechanical irritation of the end plate with ensuing ACh release occurs under many circumstances, e.g., during contusion of a muscle (29) and damage of muscle fibers during eccentric exercise (Refs. 6, 7; for review, see Ref. 34).

Simons (31) put forward the hypothesis that an increased ACh release in a limited number of end plates might contribute to the local abnormally contracted regions that are found in muscle fibers of a myofascial trigger point (32, 33), which is a frequent cause of chronic muscle pain. One possible mechanism for the abnormal contraction of single muscle fibers is that large amounts of ACh are released into the synaptic cleft of a damaged end plate by a quantal or nonquantal mechanism (8, 17). Both mechanisms would cause a depolarization of the postsynaptic membrane of the end plate with the quantal release inducing high-frequency MEPPs. The depolarization associated with the MEPPs may lead to a local release of Ca²⁺ from the sarcoplasmic reticulum. The resulting increase in cytoplasmic Ca²⁺ concentration may cause a local contracture that is restricted to the sarcomeres that are located underneath the end plate. In this case, the term contracture is used in the physiological sense, i.e., an activation of the actin and myosin filaments in the absence of action potentials propagating along the muscle membrane.

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Apparently, in contrast to a general increase in ACh concentration that, for instance in myasthenia gravis patients, is not associated with persistent pain in patients, such a local increase may lead to the formation of a trigger point that is often associated with chronic pain and dysfunction of the muscle harboring the trigger point.

To our knowledge, no study has been performed that specifically addresses the effects of a locally injected AChE inhibitor on the morphology of the muscle fibers in the vicinity of the injection site. The present experiments were undertaken to test the hypothesis that a local increase in ACh leads to abnormally contracted and otherwise damaged muscle fibers in the end-plate zone. In the long run, the experiments aim at developing an animal model for the induction of myofascial trigger points.

MATERIALS AND METHODS

The present report is based on data from 22 young adult (3–6 mo) male Sprague-Dawley rats. The study was carried out in accordance with the Guiding Principles for Research Involving Animals and was approved by the German State Authority for Animal Experimentation. For experiments with electrical stimulation of the muscle nerve, animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (80 mg/kg; Narcoren). The dose was supplemented with further intraperitoneal injections as necessary to maintain a deep level of anesthesia. The anesthesia was assumed to be deep if no withdrawal reflexes occurred on forced pinching of the toes or tail. The animals breathed room air spontaneously.

The gastrocnemius-soleus (GS) muscle of the left hindlimb was surgically exposed together with its blood vessels and nerves in the popliteal fossa. The skin flaps were sewn to a metal ring; thus a pool was formed that was filled with silicone oil prewarmed to 37°C. To increase the ACh concentration in the cleft of the neuromuscular end plates, two methods were used as shown in Fig. 1. 1) The AChE blocker diisopropylfluorophosphate (DFP) was injected into the proximal half of one of the heads of the gastrocnemius muscle. DFP inhibits the activity of AChE by binding to the catalytic center of the enzyme and forming an inactive conjugate. The lack of any histochemical AChE reaction in the DFP-injected region (cf., Fig. 2B) indicates that, with the concentrations of DFP used, the local AChE activity was largely blocked. 2) The GS nerves were electrically stimulated with a bipolar platinum hook electrode. The GS nerves supply both heads of the gastrocnemius muscle and the soleus muscle. The voltage

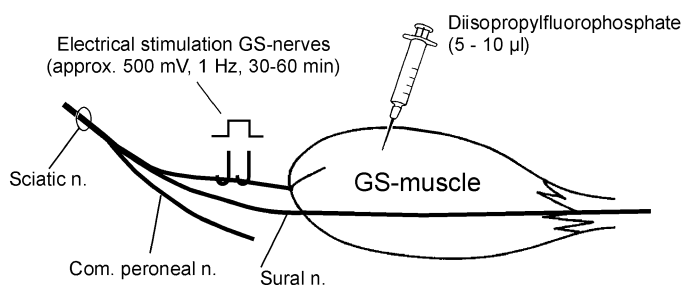


Fig. 1. Experimental arrangement showing injection of the proximal half of the gastrocnemius muscle and electrical stimulation of the muscle nerves. GS, gastrocnemius-soleus.

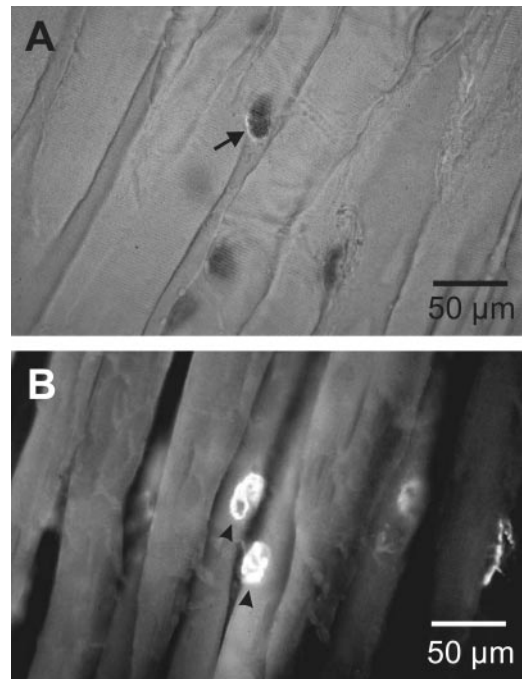


Fig. 2. Histological appearance of blocked and unblocked end plates with A bands visible. *A*: intact end plates showing a strong cholinesterase reaction (darkly stained) with a thin bright rim of fluorescent staining (arrow). Mixed fluorescence and brightfield illumination are shown. *B*: blocked end plates (arrow heads) showed only bright fluorescent immunostaining without any dark cholinesterase stain. In fluorescent illumination, unblocked end plates, as shown in *A*, exhibited only the thin fluorescent rim because the rest of the end plate is masked by the cholinesterase reaction product.

necessary to induce maximal muscle twitches was determined by using single rectangular stimulus of a duration of 0.1 ms. For stimulation of the muscle, 150% of that voltage (equivalent to ~500 mV in most experiments) was applied for 30 or 60 min at a frequency of 1 Hz. The foot of the stimulated leg could move freely; therefore, the muscle twitches were largely isotonic.

In this article, two sets of experiments are described.

In *group A*, five rats were treated with an injection of 5 µl of 1×10^{-3} M DFP dissolved in tyrode solution (corresponding to a dose of 0.76 µg DFP) into the proximal half of the left medial head of the gastrocnemius muscle, followed by 30 or 60 min of electrical stimulation of the GS nerves (Fig. 1). The injections (20-gauge needle) were aimed at the geometrical center of the muscle half. In three animals, the muscle nerves were stimulated for 30 min and in two animals for 60 min. Because the morphological data of the two subgroups exhibited no differences in the semiquantitative evaluation (see below), the data were pooled. At the end of the stimulation period, the rats were transcardially perfused under deep anesthesia.

In *group B*, no electrical stimulation was used and the lateral head of the gastrocnemius muscle was injected. After the injection, the animals moved freely in their cages for 24 h. Four rats were treated with an injection of 10 µl of 5×10^{-3} M DFP, two animals with 10 µl of 1×10^{-3} M DFP, and two rats with 10 µl of 1×10^{-2} M DFP. Recovery from anesthesia and survival period were uneventful, and the rats did not guard the injected muscle. The injections were made through the intact skin into the proximal half of the lateral head of the gastrocnemius muscle during a short-lasting volatile

anesthesia with Isofluran. This injection procedure was chosen to minimize trauma to the animals. Because the thin medial head is difficult to inject through the intact skin, the larger lateral head was used in this group. Because of the larger size of the lateral head, a greater volume was injected. Again, no morphological differences were detected between the experiments using various DFP concentrations; therefore, the data were pooled. Nine additional rats were used for pilot experiments to test the effects of very high concentrations of DFP, injections of vehicle, and electrical stimulation without DFP injection.

For histological evaluation, the animals were transcardially perfused with 4% paraformaldehyde. The injected head of the gastrocnemius muscle was taken out and cut transversely in two halves, with the proximal half containing the injection site. The distal half was also evaluated; in *group A*, this part of the muscle was not injected with DFP but had performed the electrically induced contractions. In *group B*, the contralateral GS was taken out as additional control tissue that was not injected with DFP and had not performed electrically induced contractions.

The tissue was postfixed in 4% paraformaldehyde overnight because sometimes the perfusion fixation of the exposed muscle was not sufficient. All parts of the muscles were cut longitudinally on a freezing microtome at a thickness of 40 μm . To obtain an overview of the entire muscle, the tissue was sectioned in the following way. The first eight sections were discarded, the next two sections were mounted on a slide, the next eight sections discarded, and so on until processing of a head was completed. This procedure yielded ~ 15 sections/animal.

Staining of end plates was performed as double staining histochemically with the conventional AChE reaction and immunohistochemically with antibodies to AChE that were marked with a fluorescent marker. For AChE immunohistochemistry, the sections were rinsed in phosphate-buffered saline plus thimerosal for 10 min, blocked with 10% horse serum plus 0.3% Triton X-100 for 60 min, incubated with the first antibody (anti-AChE from mouse 1:1,000; source: Alexis, Grünberg, Germany) for 24 h, rinsed three times in buffer, incubated with the second antibody (biotinylated anti-mouse IgG from horse 1:100; source: Alexis) for 2 h, rinsed three times in buffer, incubated with streptavidin-Cy2 (1:1,000; source: Bio-Trend, Cologne, Germany) for 2 h, and rinsed three times. The processing was continued with the AChE histochemical reaction in which the sections were incubated in acetylthiocholine iodide solution for 3 min, rinsed three times in Tris buffer, and mounted on slides with floursave.

In muscle regions that were not injected with DFP, the double staining showed end plates that were heavily stained enzymatically and had a small rim of fluorescent stain around them (Fig. 2A). In muscle regions injected with DFP, the enzymatic staining was missing, and now the fluorescent staining gave a complete picture of the blocked end plate (Fig. 2B). Thus muscle regions with DFP-blocked end plates could be distinguished from noninjected areas. The sections of *groups A* and *B* were stained the same. Because staining was performed with hematoxylin-eosin and trichrome impaired fluorescence, the sections were not counterstained. The end plates of both heads of the gastrocnemius muscle formed a thin line that ran diagonally through almost the entire length of the muscle.

The following morphological features were assumed to represent altered fibers and were evaluated.

Contraction Disks

Contraction disks consisted of a sudden bulging of the muscle fiber with a protruding center that typically was without any discernible structures and was flanked on either side by an abnormally contracted area in which the sarcomere length was reduced (Figs. 3 and 4A). An area was defined as abnormally (or regionally) contracted when only part of a muscle fiber was contracted and particularly if the sarcomeres became progressively shorter until the A bands were closer together than expected in a maximum physiological contraction (cf., Ref. 28). For evaluation purposes, a sarcomere length of 40–60% of that of neighboring normal fibers was accepted as abnormally contracted (14). In the central portion of the contraction disk, sarcomeres could no longer be identified, and the area appeared hyalinized with no cross striations. The protruding center of the contraction disk had sharp borders and extended for only a short length in the longitudinal direction of the muscle fiber (mostly less than its diameter). Sometimes the disks occurred as a contraction-disk complex, i.e., as a pair or multiple disks separated by an appreciable segment of abnormally contracted sarcomeres.

Torn Fibers

Torn muscle fibers (Fig. 5, small arrow) could be distinguished from cut fibers ending on the margin of the section by

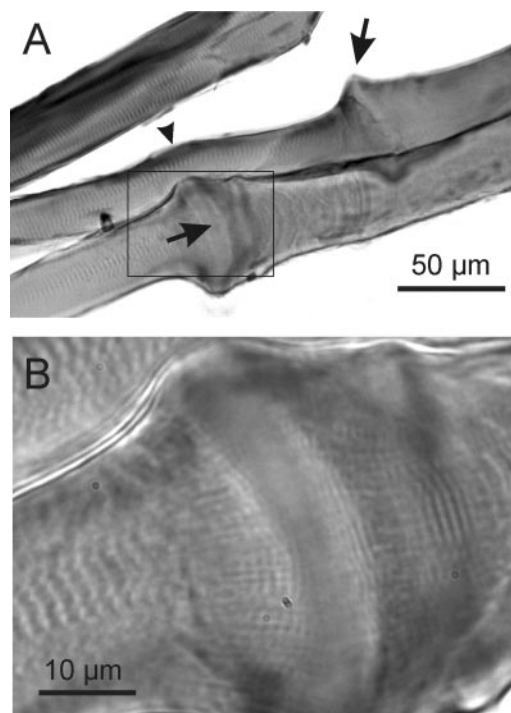


Fig. 3. Contraction disks in an area of the muscle where end plates were blocked as evidenced by a lack of cholinesterase stain [injection of 1×10^{-3} M diisopropylfluorophosphate (DFP), electrical stimulation for 60 min]. The blocked end plates were located outside the area shown. A: contraction disks (arrows) cause marked bulging of the sarcolemma that can impinge on adjacent muscle fibers and distort their sarcomere pattern (arrowhead). The widely spaced curved lines to the right of the lower contraction disk show that the arrangement of sarcomeres, which is normal to the left of the disk, is completely out of register. B: enlarged view of the boxed area in A. Note the abnormally contracted regions flanking the hyaline center of the disk compared with the normal A band spacing seen in the uppermost fiber in A.

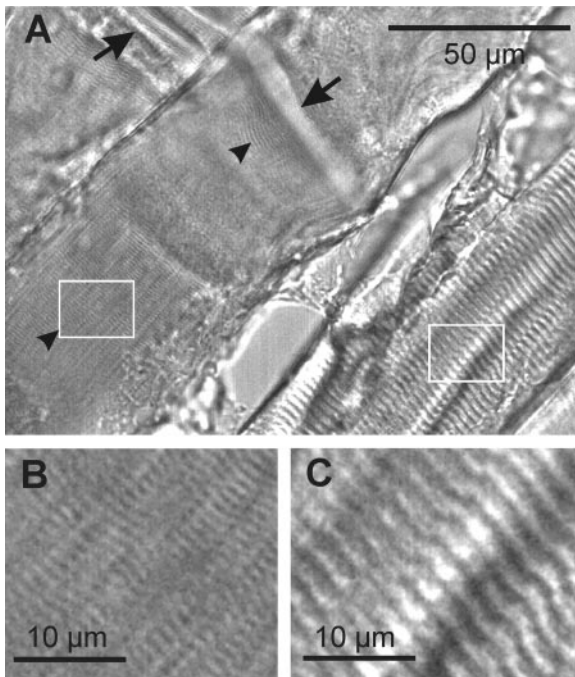


Fig. 4. Contraction-disk complexes (injection of 1 M DFP, electrical stimulation for 6 min at 1 Hz). *A*: 2 muscle fibers crossing the upper left of the figure show multiple contraction disks whose centers appear as white bands without any discernible structures (arrows). Areas with abnormally contracted sarcomeres are marked with arrowheads. The fiber to the lower right shows undisturbed sarcomere length. *B*: left boxed area in *A* at a higher magnification to show the abnormally contracted sarcomeres. *C*: right boxed area in *A* exhibiting normal A band spacing.

the lack of cross striation and uneven contours at the site of the tear.

Longitudinal Stripes

These changes (Fig. 5, area around large arrow in the top middle) consisted of multiple dark lines that ran parallel to the long axis of the muscle fiber. Often, they were associated with the absence of cross striations, but in some cases they occurred together with distinguishable A bands.

All sections were evaluated by using a fluorescence microscope at a magnification of $\times 100$ – 200 with a dim brightfield illumination added so that both the stain of the end plates and the striation pattern could be recognized simultaneously. The frequency of occurrence of the above features per tissue section was evaluated for each section in a semiquantitative way by using a score with 0 meaning “no lesion,” 1 meaning “lesions at one or a few sites,” 2 meaning “several lesions,” 3 meaning “lesions at multiple places or in a large area,” and 4 meaning “lesions in all parts of the section.” In each animal of group A, at least six sections were evaluated for the above-mentioned lesions (contraction disks, torn fibers, longitudinal stripes) and the two experimental conditions of 1) injected and contracted tissue (proximal half of the injected muscle head) and 2) noninjected but contracted tissue (distal half of the injected head). To calculate the mean occurrence of one of these features, the frequency scores of the feature were added for all slides and then divided by the number of slides read. To avoid counting artifacts that were commonly present at the borders of the tissue blocks, the outer 200 μm of the sections were discarded. The person evaluating the sections was blinded to the experimental condition. The statistical

evaluation was performed by using a two-sided Mann-Whitney's *U*-test. A probability level of $<5\%$ ($P < 0.05$) was considered significant.

RESULTS

General Observations in Both Experimental Groups A and B

The abnormal features and lesions were not uniformly distributed over the sections but were focal, i.e., they affected only a limited number of muscle fibers that often were not adjacent but interspersed between apparently normal fibers. Moreover, the lesions often did not extend over the entire length of the fibers. The location of the damaged fibers did not always coincide with the area injected with DFP, as evidenced by the presence of blocked end plates. In most cases, the location of the lesions was in the middle of the section rather than at the borders. Because longitudinal sections were evaluated, the lesions occurred close to the muscle belly. The various lesions described above were often present in the same section, i.e., lesions that apparently represented different levels of severity occurred together.

Even in animals with severe damages in many fibers, the macroscopic appearance of the injected head was completely normal, and the muscle performed the twitches for 30–60 min without problems. There were great differences among animals of the same treatment group; therefore, with the limited number of animals tested so far, it was not possible to quantitatively determine the influence of different amounts of DFP or different lengths of electrical stimulation.

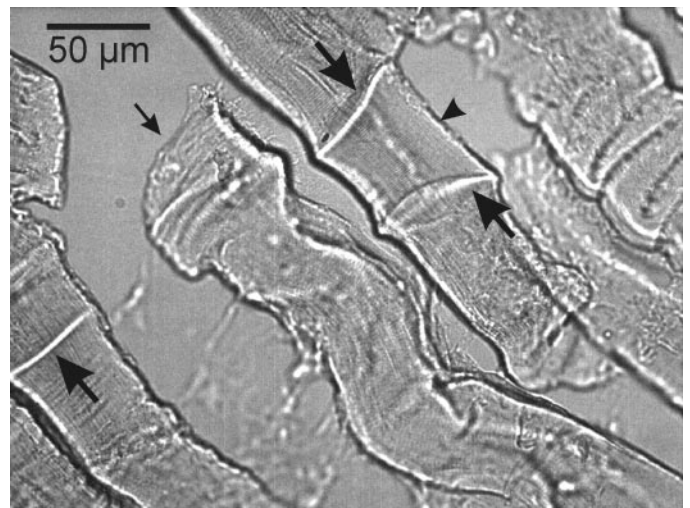


Fig. 5. Contraction-disk complexes and torn fibers (same muscle as in Fig. 4). The fiber to the upper right is torn and shows 2 contraction disks (arrows) with an intermediate region of abnormally contracted sarcomeres (arrowhead), and the fiber on the left margin shows 1 contraction disk (single arrow). On both sides of the contraction disks of the upper right fiber, the cross striation is partly replaced by longitudinal stripes. The fiber in the middle is torn (thin arrow) and has several contraction disks close to the torn end.

Contraction Disks and Abnormally Contracted Regions

Contraction disks could appear repeatedly in one muscle fiber and in various combinations that may represent different degrees of severity of the same process. Figure 3 shows samples from a muscle region that was injected with 10 μl of 1×10^{-3} M DFP and performed twitches for 60 min at 1 Hz. Two contraction disks with a strong protrusion of the sarcolemma are discernible (Fig. 3A). The center of the disks appears hyalinized (hypercontracted) and is sandwiched between two regions of regional contractions (Fig. 3B). To the right of the disk, the lower muscle fiber lost its cross striation and showed an irregular pattern of curved lines at a very wide spacing. This part of the muscle fiber appeared to be stretched. However, because we do not know whether these curved lines really represent A bands, another explanation for the curves would be interference patterns.

Figure 4 illustrates a contraction-disk complex (several disks in close proximity) from a muscle region that was injected with a high concentration of DFP (1 M, 5 μl , see below) and contracted for 6 min at 1 Hz. The figure shows abnormally contracted regions (arrowheads) between disk-like hyaline structures (arrows). The latter structures are associated with a discoidal protrusion of the sarcolemma. The *left* boxed area with abnormally contracted sarcomeres is enlarged in Fig. 4B compared with a neighboring fiber that shows normal, undisturbed sarcomere spacing (*right* boxed area, Fig. 4C).

Figure 5 shows additional variations of the contraction-disk complex from the same muscle. Here, a more widely spaced pair of discoidal structures with sarcolemmal bulging (two large arrows pointing to each other) is separated by a considerable region of uniform and closely spaced A bands (arrowhead). Beside the arrowheads, on either side of a complex, longitudinal stripes are discernible.

Torn Fibers

Frequently, the contraction disks occurred together with tearing of the fibers, i.e., the fibers that exhibited one or more contraction disks were torn (Fig. 5, small arrow). Sometimes, the fibers had the appearance of an empty sarcolemmal tube close to the site of the tear. Normal fibers leaving the section because they followed an oblique course could easily be distinguished from torn fibers because the former always had smooth cut borders and exhibited cross striation up to the cut end.

Group A (48 Sections Evaluated)

As shown in Table 1, the three evaluated abnormal features (contraction disks, torn fibers, longitudinal stripes) were significantly more frequent in sections from the proximal half of the muscle, which was both injected with DFP and electrically contracted. In the distal half of the injected head, which was contracted but not injected with DFP, a few torn fibers were found.

Table 1. Mean frequency scores of occurrence of morphological changes

Morphological Changes	Test (DFP-injected, Contracted Tissue)	Control (Noninjected, Contracted Tissue)
Contraction disks	$0.67 \pm 0.87^*$	0.00
Torn fibers	$1.89 \pm 1.54^\dagger$	0.13 ± 0.36
Longitudinal stripes	$0.89 \pm 1.36^*$	0.00

Values are means \pm SE. Semiquantitative evaluation of tissue sections of *group A* in which the medial head of the gastrocnemius muscle was treated with injections of diisopropylfluorophosphate (DFP) and electrically induced contractions. Test, tissue from the DFP-injected proximal half of the injected muscle head that also performed electrically induced contractions; control, tissue from the distal half of the same muscle head (outside the injected area) that was not injected but performed contractions. Significant differences between test and control condition: $*P < 0.05$; $^\dagger P < 0.01$ (2-sided Mann-Whitney *U*-test).

Therefore, with few exceptions, all damaged fibers occurred in that portion of the muscle that was treated with DFP and contracted.

Most of the damaged fibers were located in that area of the muscle where the end plates were blocked, but the abnormal features were often located not directly under the end plates. However, the damaged fibers were still relatively close to the end-plate zone. In three animals, the distance between the damaged fibers and the end plates was determined to vary from 500 to 900 μm .

Group B (114 Sections Evaluated)

The abnormal features observed in *group B* were the same as those induced by DFP plus electrical stimulation (contraction disks, torn fibers, longitudinal stripes) but occurred at a lower frequency. In these animals, a suggestion of dependency of the severity of the lesions on the amount of the injected DFP was apparent. The two animals that received 1×10^{-3} M DFP showed lesions that scored 0–1 (corresponding to 0–2 damaged fibers per section). The four rats that were injected with 5×10^{-3} M DFP exhibited lesions that scored 0–2 (corresponding to 0–5 damaged fibers per section). The two animals that received 1×10^{-2} M DFP showed lesions with a score of 2 (corresponding to ~5 damaged fibers per section).

Other Experimental Conditions (Pilot Experiments)

Increased concentration of DFP. In the two animals in which the DFP concentration was increased to 1 M, the histological picture showed muscle fibers torn into small pieces and many fibers with contraction-disk complexes (cf., Fig. 4). In cross sections, myofibrils were present only in the periphery of the fibers, whereas their center had the appearance of empty cytoplasm. In these rats and in the two animals that received 10 μl of 1×10^{-2} M DFP [see *Group B (114 Sections Evaluated)*], there were signs of a systemic effect in that the contralateral (noninjected and nonstimulated) muscle showed similar lesions as the injected one, albeit at a lower frequency. In animals that were injected with 10 μl of 1×10^{-3} M DFP, no lesions

were found on the contralateral side. The animals treated with 1 M DFP died shortly after the injection.

Injection of vehicle. The contraction disks observed after DFP injection do not appear to be due to the lesion caused by the injection procedure because pilot experiments with injections of tyrode showed that along the trace of the needle the muscle fibers were cut or damaged (with hypercontracted segments at the cut edge), but no contraction disks in the sense of this report were present.

Electrical stimulation without DFP injection. In three rats, the GS nerves were electrically stimulated for 60 min, but no DFP injections were given. These animals exhibited frequencies of contraction disks that were lower than those of the tissue that was contracted and injected with DFP (mean value 0.23 against 0.67; cf., Table 1).

All muscles were digitally palpated directly before perfusing the animal; in no case were bandlike structures or palpable nodules found.

DISCUSSION

General Observations

The finding that the lesions occurred focally and were not evenly distributed in the injected tissue is in accordance with descriptions of other authors of lesions in muscles subjected to eccentric contractions, forced lengthening, or electrical stimulation for days. In experiments employing electrical stimulation, only a few percent of the muscle fibers (maximally 14% after stimulation at 5 or 10 Hz for 9 days) showed histological signs of damage (21, 38), and the variability between animals subjected to the same stimulation protocol was great (22). Laskowski et al. (18) likewise described focal morphological changes after systemic administration of an irreversible AChE inhibitor with normal end plates interspersed between altered ones. From these data, the general assumption appears to be justified that, even under identical experimental or pathophysiological conditions, the lesions in the affected muscles can be expected to occur focally and with great variability. This makes a quantitative evaluation of the morphological data and of differences between different experimental conditions difficult.

The reason for the focal occurrence of the lesions is obscure. Possible explanations are that the damaged muscle fibers are of the same type (type I or II) or had unfavorable working conditions and, therefore, were more susceptible to contraction-induced damage (similar to the focal lesions in a muscle after eccentric contractions). As stated in MATERIALS AND METHODS, the injections were aimed at the geometrical center of the muscle half (deep into the muscle). In the medial gastrocnemius, both type I and type II fibers are present in this area. In contrast to other studies that examined the morphological sequelae of muscle strain and found the lesions to occur predominantly at the muscle tendon junction (9, 10, 25), the lesions in our study were located mainly close to the belly of the muscle. The lesion caused by the injection needle and the injected

volume does not appear to be of importance for the observed changes because injections of tyrode solution did not induce contraction disks.

Contraction Disks and Abnormally Contracted Regions

Contraction disks are probably contractures in the physiological sense, namely, an activation of actin and myosin filaments without electrical activity in α -motoneurons. Abnormally contracted focal areas seem to be a general and unspecific reaction of muscle fibers to lesions, and they have also been found in heavily strained muscle 60 min after injury (27), after immobilization in a shortened position (2), after micropunctures of muscle fibers with a needle (3), in perfused heart muscle tissue (35), and after eccentric exercise (26). The authors used the terms "hypercontractions," "hypercontracted filaments," "supercontracted regions," or "contraction bands" to describe their findings. The contraction bands of Van der Heide et al. (35) come close to the contraction disks of the present study. Ogilvie et al. (26) reported regions of Z-line lesions flanked by hypercontracted sarcomeres after eccentric exercise. These structures are similar to what we termed contraction-disk complexes. However, such sharply delineated disklike structures as observed in the present study have not been reported before.

There is a controversy in the literature concerning the possibility that abnormally contracted regions in a muscle might be artifacts caused by the biopsy procedure (for a review, see Ref. 28). In our experiments, this possibility can be ruled out for two reasons. 1) The data were obtained in a comparison between DFP-injected and noninjected parts of the same muscle that were removed from the animal by using the same technique. 2) In the tissue sections, the outer 200 μ m were not used for evaluation to exclude abnormal contractions caused by cutting the muscle in half. Thus all the evaluations were done in tissue where muscle fibers were not cut before fixation.

The most valid argument against biopsy artifacts is assumed to be the presence of leukocyte infiltrations (28). In the present investigation, no infiltrations were found, but because no specific staining for leukocytes was performed, accumulations of a few leukocytes could have been overlooked. On the other hand, the histological methods used apparently were sufficient to detect larger numbers of leukocytes because, in some of the animals that were not stimulated electrically and survived for 24 h, small infiltrates of granulocytes were found in the muscle around torn fibers.

An interesting aspect of the neostigmine-induced regional contractions reported by Hudson et al. (13) is that changes in the presynaptic portion of the end plate also occurred. There were morphological alterations of the endoplasmic reticulum in the motor nerve terminal indicative of an increased ACh release, the frequency of MEPPs was increased (18), and, in the motor nerve, antidromic action potentials were recorded (19).

The contraction disks and abnormally contracted areas of this study may correspond to the contraction knots observed in biopsy material of myofascial trigger points in vivo (32) and postmortem in human material (36). The main difference between the data of the present study and the assumptions of the dysfunctional end-plate hypothesis is that regional contractions were often observed not directly underneath but in the vicinity of the end plates. One explanation for this finding is that the lesion started under the end-plate and then spread along the muscle fiber (18). Another mechanism would be that the abnormally contracted area under the end plate caused leaks in the membrane of the muscle cell at a distance from the endplate. Through these leaks, Ca^{2+} could enter the cell and lead to local contractures. The mechanism causing such leaks may be similar to those leading to plasma membrane disruptions described by McNeil and Khakee (24) in rats performing eccentric contractions. The extracellular Ca^{2+} concentration of ~ 2.5 mM is more than sufficient to cause activation of actin and myosin filaments (the highest cytoplasmic concentration during contraction is $\sim 10^{-2}$ mM).

Torn Fibers

After systemic administration of high (sublethal) doses of pyridostigmine (11) or paraoxon (19) and survival times of several days, complete necrosis of muscle fibers with leukocyte infiltration was described. In the present study, tearing of fibers, which was often associated with a contraction disk or a contraction-disk complex, could of course be due to mechanical stress caused by the disk that simply ruptured the fiber. However, there is another mechanism that might be involved in this phenomenon, namely, the influx of extracellular Ca^{2+} through L-type channels that are located in the T tubules of the sarcolemma. The increased intracellular Ca^{2+} may activate intracellular enzymes [e.g., a protease that digests the Z band (20)] and lead to necrosis of the muscle fiber (37).

Longitudinal Stripes

At the light microscopical level, these stripes looked like longitudinal separations (slits) between bundles of neighboring myofibrils. The bundles still exhibited cross striation. In severe cases, the separations between the myofibrils were so large that in cross sections the muscle fibers exhibited holes in their cytoplasm. Such holes were observed in the two animals that were treated with the high concentration of 1 M DFP. Why the myofibrils separated under the experimental conditions of the present study is unclear at present. Possibly, a mechanism similar to that described for swellings (e.g., activation of enzymes by Ca^{2+}) was involved.

Theoretically, DFP may increase the action of ACh on the microvasculature and thus cause local vasodilation followed by tissue swelling. The intensity of electrical stimulation used in the experiments was not sufficient for activation of postganglionic autonomic

fibers, but the ACh released from cholinergic sympathetic vasodilator fibers in muscle (16) could have a stronger action under DFP, and the muscular activity itself could cause vasodilation. However, it seems unlikely that this effect influenced our data because in the microscope no edema-like swelling could be recognized in the (small) injected muscle volume compared with the rest of the muscle.

Collectively, the data of the present study show that an increase of ACh in a small fraction of the end plates of a skeletal muscle, which may occur after light mechanical trauma in everyday life, leads to marked morphological changes in the affected muscle fibers. The most conspicuous phenomena were spatially restricted contractures (contraction disks) that may represent the starting point for the development of a myofascial trigger point. However, the results of the present study suggest that so far only the very beginning of the formation of trigger points could be induced if the lesions found in the experiments are related to trigger points at all. Nevertheless, the data of this study support one of the basic assumptions of the dysfunctional end-plate hypothesis of trigger point formation (31), namely, that an increased ACh concentration in the cleft of the neuromuscular end plate induces local contractures.

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