THE EFFECT OF OVERSTRAIN OF DIFFERENTIAL INHIBITION ON THE REGENERATION OF SKELETAL MUSCLE IN WHITE RATS

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The problem of the influence of the functional state of the cerebral cortex on the complex morphophysiological processes taking place in repair is one of great theoretical and practical interest. We have previously [8, 9] studied the question of the influence of overstrain of the cortical process of excitation on the traumatic regeneration of skeletal muscle.

In the present communication we describe the results of experiments to show the influence of overstrain of the process of differential inhibition on the regeneration of skeletal muscle.

EXPERIMENTAL METHOD

For the experiments we used 48 white rats weighing 300-400 g (20 control and 28 experimental animals). As regards the type of nervous system of the experimental rats, 13 were excitable, and 15 were inhibited. A system of two positive and two inhibitory stimuli was produced in these rats. A continuous light from a 10 v electric lamp and a bell of medium strength were used as positive conditioned stimuli, and a winking light and buzzer were the inhibitory stimuli. After the production of this system the next step was to create overstrain of the process of inhibition, which was achieved by prolonging the time of action of the differential stimuli from 20 seconds to 2-3 minutes and by increasing their number in the course of one experimental day from two to 5-7. Ten days after the start of the experiments with overstrain of inhibition, a muscle in both hindlimbs of the rats was injured. The anterior tibial muscle was divided with scissors for two-thirds of its width in its middle part. The operation was carried out in strictly aseptic conditions under ether anesthesia. The process of healing of the muscle wound was studied by means of serial sections through the wound (thickness of the sections 6-8 μ). The material was fixed in Helly's mixture 12, 24 and 48 hours and 5, 8, 12, 16, 20 and 30 days after the wounding of the muscle. Paraffin wax sections were stained with suitable histological stains. The experiments with overstrain of inhibition continued after the operation, until the time that the material was taken for histological analysis. The process of regeneration in the muscle thus took place while the cortical activity of the animals was chronically disturbed.

EXPERIMENTAL RESULTS

In the group of excitable rats overstrain of differential inhibition after the first day of the experiment led to intensification of the motor food reaction. After a phase of general excitation, lasting about two weeks, there developed a short period (3-5 days) of relative localization of the processes of excitation and inhibition. Subsequently a sharp increase in excitation and a weakening of differential inhibition were observed, in which the food taking reaction was strengthened (the rats drew the feeding bowl towards them, and gnawed the floor and

the walls of the chamber). The signs of nervous difficulty increased. The number of times that disinhibition of differentiation took place increased, and during these times and in the intervals between them the rats performed training movements, which terminated by the fatigue of motor excitation. In the course of ten days the rats lost 20-30 g in weight. The state of sharply increased excitation then gave way to the ever increasing development of diffuse inhibition, in which 2-3 positive stimuli acting on different analyzers weakened it only for a short time.

The second group of rats (inhibited) possessed a weaker nervous system. During the introduction of differentiation the conditioned food reflexes in these animals disappeared, and were restored only one week later. The reaction of the cerebral cortex to the overstrain of differential inhibition also took place in these rats in the form of two principal phases: a state of strong food excitation and a state of food inhibition. In contrast to the excitable rats the first phase was shorter and the second deeper.

A description of the process of healing of the muscle defect in the control rats was given in our previous communication [9], and we shall not concern ourselves with it here.

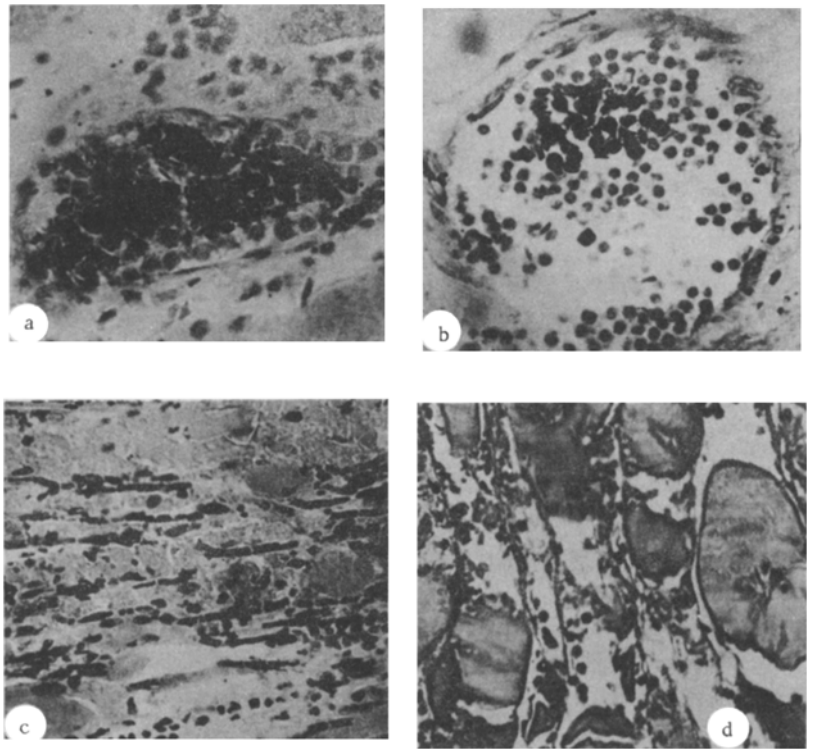
In the rats with an excitable type of nervous system the disturbance of cortical activity affected the course of healing of the muscle wound, especially in its early stages. The inflammatory reaction was accompanied by a very intensive leukocytic infiltration of the wound. Resolution of the fibrin threads and resorption of the necrotic cells and tissues took place more energetically than in the control rats. At the end of the first day the wound was completely cleansed of large fragments of destroyed muscle, which indicated the increased activity of the cells taking part in the cleansing of the wound. Judging by the considerable accumulation of fibroblastic cells in the focus of injury and by the large number of mitotic figures observed in many fields of vision in the sections, there was a more active connective tissue reaction in the experimental than in the control rats. The regenerative reaction of the muscle tissue on the 2nd-5th day of healing was also more intensive than in the control rats. The myosymplasts gave off more side branches, which were distinguished by their greater caliber and spread. The distal ends of many of these branches, however, showed signs of vacuolation and disintegration. On the eighth day the connective tissue showed proliferation of its fibers, which was especially marked in the central area of the wound, where it caused the death of many of the young muscle fibers (see figure).

Judging by the intensity of their staining by Mallory's method and by their density, the degree of development of the collagen bundles corresponded to the 12th day of regeneration in the control rats. Until the 16th day intensive development of collagen bundles and delay of the growth of young muscle fibers were observed. On the 20th day the regenerating wound presented a very complex histological picture. Collagen bundles and young muscle fibers ran about in different directions and were interwoven with each other. Among the young muscle fibers there were some which were fully differentiated and contained well defined structures, whereas others had no cross striation and contained collections of round nuclei; fibers were seen which were strongly contracting, fibers showing signs of disintegration and, among them, slender myosymplasts with a well marked basophilia. At the 30th day the defect was filled with a complex binding of regenerated muscle fibers and connective tissue septa. Many muscle fibers were deformed and had ill defined borders, and were distinguished by their degree of differentiation and their caliber.

The main feature of the process of regeneration in the excitable rats during overstrain of differential inhibition was thus an increased connective tissue reaction, and this was already observable in the early stages of healing of the muscle wound.

In the inhibited rats the course of the process of inflammation revealed a feeble leukocytic infiltration of the wound, due to delay in the dispatch of leukocytes from the blood vessels. Twelve hours after injury in sections through blood vessels collections of leukocytes could be seen close to the inner wall, and outside the walls of the vessels only isolated leukocytes were encountered. In the control rats, on the other hand, collections of leukocytes were found in the outer walls of the vessels, indicating that they had passed out of the blood stream. After 24 hours the wound in the inhibited rats contained much tissue fluid and large muscle fragments, with little or no sign of phagocytic activity. The newly formed blood vessels also were poorly developed.

On the fifth day the injured muscle fibers became intensively basophilic, and partially lost their structure. The cross striation disappeared, their fibrillary structure was preserved at the periphery of the fiber, and chains of nuclei showing amitotic division occupied a central axial position. On the eighth day these fibers underwent complete dedifferentiation and were converted into long myosymplastic bands. The regeneration of the connective tissue was less marked than in the control rats and much less than in the excitable rats. Mitoses were rarely



Inflammatory process in the muscle in the control and experimental (inhibited) rats. a)Control rat 12 hours after operation. Transverse section through a blood vessel. In the outer walls of the vessel groups of leukocytes are seen. b)The same, after 24 hours. In the wound may be seen highly fragmented and disintegrating muscle, groups of phagocytes and newly formed capillaries. Stained with azure II–eosin. Magnification: objective 40 ×, ocular 5 ×. c) Overstrain of differential inhibition. Inhibited rat, 12 hours after operation. Transverse section through a blood vessel. In the vessel may be seen leukocytes, mainly situated near the inner wall, only a few leukocytes being present in the outer walls of the vessel. d) Overstrain of differential inhibition after 24 hours. In the wound may be seen large fragments of disintegrating muscle and much tissue fluid. A few feebly developed newly formed blood vessels. Small groups of phagocytes. Stained with azure II eosin. Magnification: objective 40 ×, ocular 5 ×.

seen in the fibroblasts (5th day) and on the eighth day fine collagen bundles were formed. Until the 20th day collagen formation was feeble, which provided favorable conditions for the regeneration of muscle fibers. In fact numerous myosymplasts appeared in the regenerating muscle tissue, and the muscle tubes contained an enormous number of nuclei, which indicated an increase in their proliferative activity. At the same time the differentiated structures in the muscle tubes were ill defined. On the 30th day the quantity of connective tissue in the wound increased appreciably, and the collagen bundles proliferated and grew thicker (staining an intense blue with Mallory's stain), while the newly formed muscle fibers lagged behind those in the control rats in their degree of differentiation (feeble cross striation, many nuclei) but their diameter did not differ from that in the controls. The impression was created that, besides a weakening of the processes of collagen formation, which was observed until the 20th day, the coordination between the growth of the muscle elements and their differentiation was disturbed.

The connective tissue reaction is of great importance to the regeneration of muscle tissue. Connective tissue is an essential supporting component of the regenerating muscle, accompanying the growth of the young muscle fibers [16]. We have shown [9] that in the course of regeneration the relationship between muscle and

connective tissues may be changed by influencing cortical excitation. In the present investigation a similar result was obtained by influencing the process of internal (differential) inhibition. In both cases differences clearly emerge in the healing of the muscle defect in rats with different types of nervous system. During overstrain of the process of inhibition, regeneration of muscle took place in association with alternate states of increased and diminished motor food excitation in the cerebral cortex. In the phase of the maximum level of motor food excitation (until the 16th-20th day) increased regenerative activity of the connective tissue was observed, which restricted regeneration of the muscle tissue. After the 20th day healing took place in association with inhibited cortical activity. At this period a reduction in the amount of connective tissue in the regenerating muscle was observed by comparison with the 16th day, but it was still greater than in the controls. In the inhibited rats the early period of regeneration was accompanied by a weakened connective tissue reaction, and at this time a state of inhibition dominated the cortical activity of the rats. In the phase of increased motor food excitation (12th-20th days) the collagen fibers in the regenerating muscle as before were more feebly developed than in the control rats, and only on the 30th day was there an appreciable increase in the quantity of connective tissue. These comparisons reveal how extraordinarily complex are the relationships between the cortical and regenerative processes. The changes in the reaction of the connective and muscle tissues are the result of disturbance of the trophic influences of the nervous system, which are connected with the active working of the cortical cells. In reaching these conclusions we have taken into consideration the facts discovered by several workers during disturbances of the activity of the cerebral cortex [6, 13, 16, 18, 19, 21, 22].

SUMMARY

Overstraining of differential inhibition renders significant effect upon the process of traumatic regeneration in the skeletal muscle. In rats with the excitatory type of the nervous system the inflammatory reaction is very vivid. Healing of the muscular wound is associated with intensive growth and connective tissue differentiation, inhibiting growth of the young muscle fibers and provoking atrophic changes in them. In rats with the inhibitory type of the nervous system the process of wound cleaning is delayed. The collagen formation is reduced up to the 20th day, and not much connective tissue is seen in the wound. The muscular tissue regeneration in this case is characterized by an intensive formation of myosymplasts and their retarded differentiation.

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