EFFECT OF ACETYLCHOLINE ON NORMAL AND DENERVATED MUSCLE RECEPTORS

R. S. Arutyunyan

UDC 612.74+612.885

The action of acetylcholine on normal and denervated muscle receptors was studied in the gastrocnemius muscle. After intra-arterial injection of acetylcholine (50 μg/kg) a transient increase in frequency of discharges from muscle receptors took place. After denervation, the sensitivity of the denervated muscle receptors took place. After denervation, the sensitivity of the denervated muscle receptors increased sharply and intra-arterial injection of acetylcholine in a dose of 0.05 μg/kg also caused an increase in the frequency of their discharges. It is concluded that the excitatory action of acetylcholine on muscle receptors is based on contraction of intrafusal muscle fibers.

* * *

Muscle receptors (MRs) are highly specialized sensory structures controlling the relative length of the muscle and the rate of change of this length, and sending information concerning these changes in coded form through modulated nerve impulses.

MRs consist of groups of intrafusal muscle fibers, lying parallel to extrafusal fibers, which are innervated by both sensory and motor axons.

Since intrafusal muscle fibers are a variant of extrafusal fibers, in this investigation the effect of acetylcholine (AC) was studied on normal and denervated muscle fibers.

EXPERIMENTAL METHOD

Experiments were carried out on cats anesthetized by intraperitoneal injection of a mixture of urethane (500 mg/kg) and chloralose (50 mg/kg). Both limbs were completely denervated except for the gastrocnemius muscle on the side of investigation, and were fixed by metal nails in the region of the pelvis, thigh, and leg.

After laminectomy the anterior and posterior roots were dissected at the level of segments L_5-S_2. MRs were stimulated by stretching the muscle with a weight of 170 g, acting throughout the experiment. Afferent fibers arising from the investigated MRs were looked for in the posterior roots. Single posterior root bundles were dissected in warm mineral oil. The isolated thin posterior-root bundle containing the fiber from the investigated MR was placed on an Ag–AgCl electrode connected to the input of an ac amplifier. Muscle contractions were recorded by means of the UTS-1-VT-12 universal tensometric apparatus. The two processes were photographed on film from the screen of a dual-beam CRO. Acetylcholine was injected intra-arterially via a polyethylene catheter through the contralateral femoral artery, the tip of the catheter lying in the region of bifurcation of the abdominal aorta. In the course of the experiment the animal was kept warm (the body temperature was maintained at 37-38°).

Laboratory of Development of Motor Activity, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR S. V. Anichkov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 12, pp. 14-17, December, 1969. Original article submitted March 3, 1969.

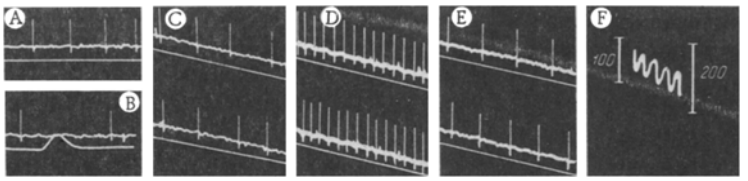

Fig. 1. Effect of acetylcholine on muscle receptors of gastrocnemius muscle. Top beam gives MR discharges, bottom beam muscle contraction. A) MR discharges evoked by stretching muscle with load of 170 g; B) MR response to contraction of muscle evoked by single stimulation of corresponding anterior root. Curves A and B obtained by a single sweep of the beam, others during continuous scanning; C) MR discharges before injection of AC, frequency of discharges 8/sec; D) the same 5 sec after injection of AC (50 μg/kg), maximum frequency of discharges 28/sec; E) the same 14 sec after injection of AC, frequency of discharges 9/sec; F) calibration: 100 μV, time 50 Hz, 200 g.

EXPERIMENTAL RESULTS

During stretching the muscle by a weight, a regular flow of afferent activity was recorded in the isolated posterior-root bundle (Fig. 1A). In order to identify the discharges, a contraction of the muscle was produced (Fig. 1B). Since the group of intrafusal fibers is parallel to the extrafusal group, contraction of the latter must reduce tension on the spindle, and must therefore lead to the cessation of its spike discharges [4, 13]. Intra-arterial injection of acetylcholine (AC) (50 μg/kg) led to a transient increase in frequency of MR discharges followed by a return to their initial level (Fig. 1C-E). The results obtained were in agreement with those reported by other workers [7, 14]. To study whether "primary" and "secondary" MRs respond equally to intra-arterial injection of AC, the conduction velocity of impulses during stimulation of the nerve to the gastrocnemius muscle was measured and action potentials of the isolated posterior-root bundle were recorded. It was found that the "primary" and "secondary" endings respond to AC injection identically. No reactions on the part of the Golgi apparatus of the tendon could be detected under these circumstances.

The sensitivity of structures to physiologically active substances is sharply increased after prolonged denervation. This increase in sensitivity after denervation has been described as the law of denervation [2]. It is seen particularly clearly in striated muscles. It was decided to examine whether this law applies also to extrafusal and intrafusal muscle fibers. For this purpose, under sterile conditions the anterior roots were divided at the level L_5-S_2. As a result both extrafusal and intrafusal fibers developed atrophy, and this was most marked in the former [1]. As Fig. 2 shows, 30-35 days after the operation, in response to intra-arterial injection of AC (0.05 μg/kg), the extrafusal muscle fibers contracted, and this was followed by a period of rest. The same pattern was observed so long as the intrafusal muscle fibers did not respond to intra-arterial injection of AC. The action of the intrafusal muscle fibers began to appear 12 sec after the beginning of its injection; it took the form of the appearance of spikes the background of muscular contraction, reaching a maximum after 15 sec. Afferent activity returned to its initial level after 22 sec. Intra-arterial injection of AC (0.05 μg/kg) in earlier stages (for example, 20-25 days after denervation) had no effect on MRs. Responses in these cases were limited to muscle contraction and cessation of spike activity.

It is now known that stretching a muscle leads to the appearance of a generator potential in sensitive endings, and when this reaches the critical level it evokes spike activity which persists throughout the period of stretching [8, 9, 12].

A similar phenomenon evidently arises during contraction of the intrafusal muscle fibers, observed during selective stimulation of the fusimotor fibers [3, 5, 6, 10, 11]. The discharge frequency of the MRs in these cases may therefore to some extent act as a criterion of contraction of the intrafusal muscle fibers. The observed increased in frequency of discharges of the muscle receptors in response to intra-arterial

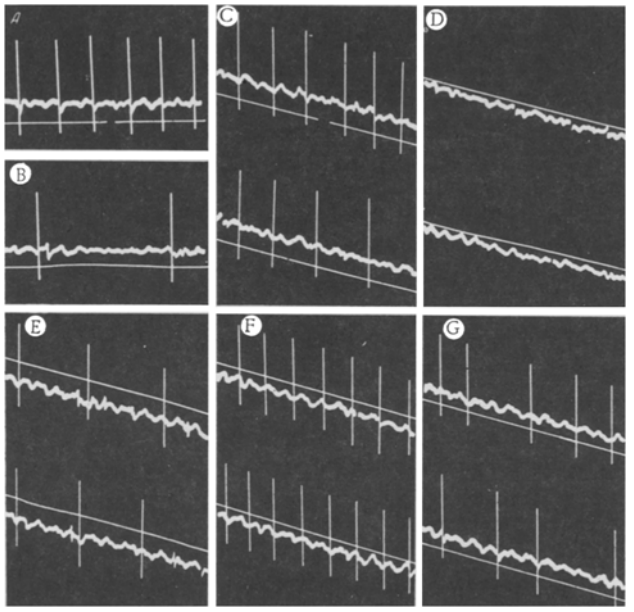

Fig. 2. Effect of acetylcholine on denervated muscle receptors of gastrocnemius muscle. A) Discharges of MRs of denervated gastrocnemius muscle during stretching of muscle by load of 170 g; B) responses of denervated MRs to muscle contraction. Muscle contraction produced by direct stimulation of muscle; C) discharges of MRs before injection of AC. Frequency of discharges 10/sec; D) the same, 3 sec after beginning of injection of AC (0.05 μg/kg). Muscle contracted and period of rest started; E) the same 12 sec after injection of AC. Discharges of MR at 6/sec appeared against the background of muscle contraction; F) the same 15 sec after injection of AC. Maximum discharge frequency 15/sec; G) the same 22 sec after injection of AC. Discharge frequency 9/sec.

injection of AC could evidently be explained by its depolarizing action on the membrane of the end plate of the intrafusal muscle fiber. Probably under these circumstances the intrafusal fiber itself contracts, leading to increased afferent activity.

Evidence in support of this hypothesis is given by the results of experiments on denervated MRs. After division of the anterior roots the sensory endings remain intact [1]. It is therefore difficult to suggest that a dose of AC 1000 times smaller than that acting on intact MRs could excite sensory endings of denervated MRs. Evidently after intra-arterial injection of AC, not only the denervated extrafusal, but also the intrafusal fibers began to contract. As a result, against the background of contraction of the extrafusal fibers, which should have stopped to receive spikes from the MRs, a considerable afferent activity was recorded. The cessation of activity from the muscle observed during the first few seconds after injection of AC could be explained by a more rapid response of the extrafusal fibers to the injected drug.

LITERATURE CITED

1. J. A. Boyd, Phil. Trans. Roy Soc., B, 245, 81 (1952).
2. W. B. Cannon and A. Rosenblueth, The Supersensitivity of Denervated Structures New York (1950).
3. C. Eyzaguirre, J. Physiol. (London), 150, 186 (1960).
4. J. F. Fulton and J. Pi-Suner, Am. J. Physiol., 3, 554 (1928).
5. C. C. Hunt and S. W. Kuffler, J. Physiol. (London), 113, 283 (1951).
6. C. C. Hunt and S. W. Kuffler, J. Physiol., (London), 113, 298 (1951).
7. C. C. Hunt, Fed. Proc., 11, 75 (1952).
8. B. Katz, J. Physiol. (London), 111 , 248 (1950).
9. B. Katz, J. Physiol. (London), 111, 261 (1950).
10. S. W. Kuffler, C. C. Hunt, and J. P. Quilliam, J. Neurophysiol., 14, 29 (1951).
11. L. Leksell, Acta Physiol. Scand., 10, Suppl. 31 (1945).
12. O. C. Lippold, J. G. Nicholls, and J. W. Redfearn, J. Physiol. (London), 153, 209 (1960).
13. B. H. Matthews, J. Physiol. (London), 38, 1 (1933).
14. P. M. Rack and D. R. Westburry, J. Physiol. (London), 186, 698 (1966).