INVESTIGATION OF SYNAPTIC STRUCTURES OF INTRA- AND EXTRAFUSAL FROG MUSCLE FIBERS

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In a previous study [1], using a technique of recording miniature postsynaptic potentials (MPSP), three groups of intrafusal muscle fibers in the frog muscle spindle were distinguished: with single innervation, with multiple innervation, and with a combination of both types of innervation. On the morphological plane, the problem of nonhomogeneity of both nerve and muscle fibers of the spindles has been under consideration for a long time. It has been shown that the spindle tandems of the frog, when stained with methylene blue, revealed two types of motor nerve fibers and nerve endings [2]. A scheme for the innervation of the spindle with efferent fibers of large and small diameters, with corresponding endings of "twitch" and "grape" type, has been put forward. However, as Hess [3] points out, if methylene blue is used it stains both sensory and motor nerve fibers without differentiating them. As a result of the work of Karlsson and Andersson-Cedergren [5] it became clear that a zone of overlapping of nerve endings of the motor and sensory systems of the spindle exists. Accordingly, it is preferable to use a cholinesterase technique, whereby only motor endings can be stained, for it is there that cholinesterase is concentrated in quantities sufficient for its detection. This method, in Holmstedt's modification [4], was used by Ottoson [7] to study the spindle, but in most cases a negative cholinesterase reaction was found.

In the present writer's opinion, the use of the "direct" thiocholine method [6], by means of which the sites of distribution of cholinesterase can be revealed, could be a more adequate technique for a spindle equipped with a capsule, which prevents diffusion of substances toward motor nerve endings. An advantage of this method is that the end product of the current reaction is brightly colored, an essential feature for the extremely thin intrafusal fibers, where the cholinesterase concentration is inevitably low.

In the present investigation, besides spindle tandems, the whole muscle and its extrafusal muscle fibers also were investigated for the presence of cholinesterase. This allowed the particular features of innervation of intra- and extrafusal muscle fibers, previously studied by the writer by electrophysiological methods, to be judged.

EXPERIMENTAL METHOD

The m. extensor digiti longus IV of the frog Rana temporaria and isolated spindles of that muscle were investigated. Altogether 18 experiments were carried out on muscles and 10 experiments on spindles. The isolated muscle or spindle was kept in a cold place (5°C) for 1-1.5 h in an incubation medium of the following composition: 5 mg of the substrate acetylthiocholine iodide was dissolved in 6.5 ml of 0.2 M maleate buffer (pH 5.75-6.0), and 0.5 ml of 0.1 M sodium citrate, 1 ml of 30 mM $CuSO_4$, 1 ml water, and 1 ml 5 mM potassium ferricyanide were added. The thiocholine formed as a result of enzymic hydrolysis of the substrate acetylthiocholine iodide reduced the ferricyanide to ferrocyanide. The ferrocyanide combined with the copper ions present in the incubation medium to form a sparingly soluble brown precipitate in sites of localization of cholinesterase. The preparations were then washed in sucrose, dehydrated, and cleared in alcohols of increasing concentration and in xylol. The stained preparations were mounted in liquid balsam or polystyrene and photographed with the MBI-11 microscope. Control preparations were incubated in medium containing 0.1 mM eserine.

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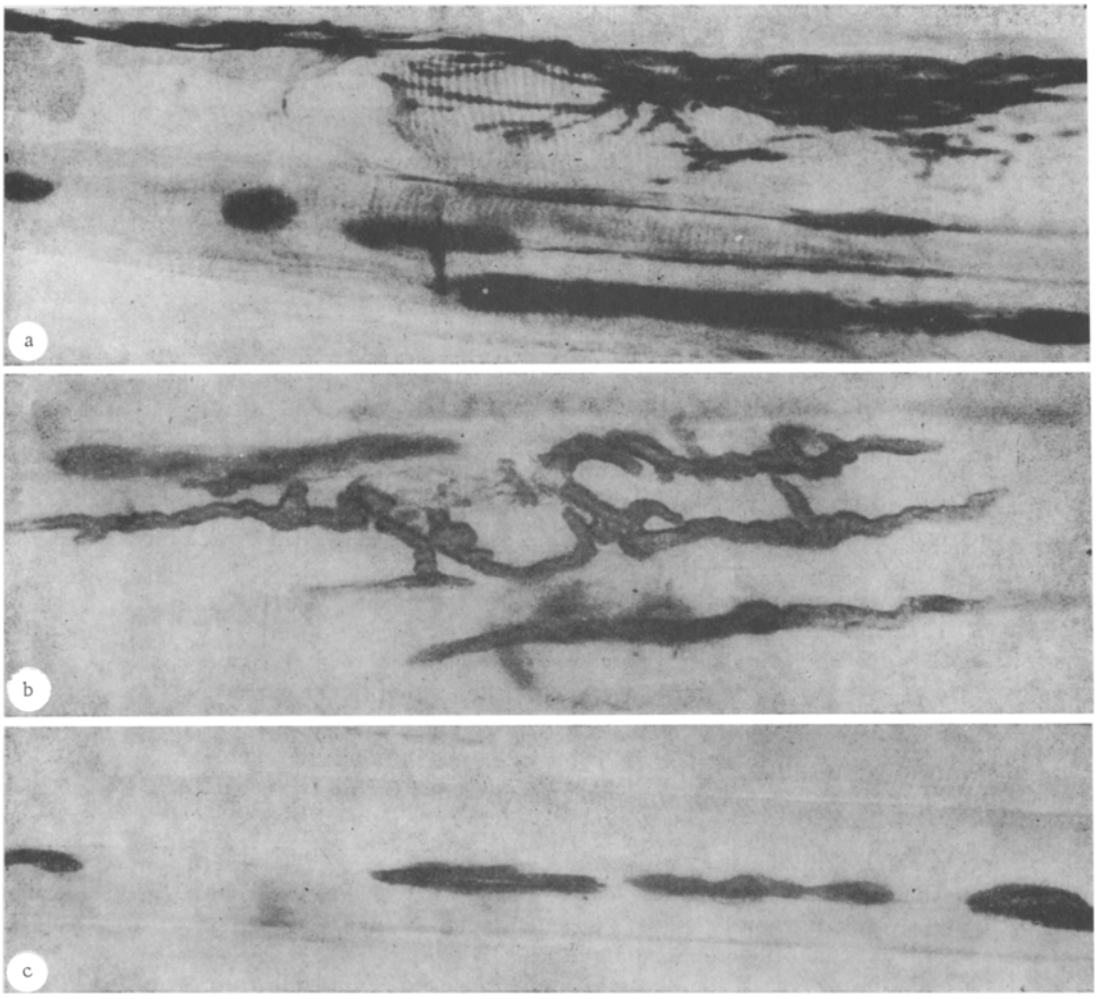

Fig. 1. Types of nerve endings of extrafusal muscle fibers of m. extensor digiti longus IV of the frog. a) One part of the muscle; b) ending of "twitch" type; c) ending of "grape" type. "Direct" thiocholine method. Magnification: a, c) 100, b) 200×.

EXPERIMENTAL RESULTS

Total staining of the muscle evidently revealed two types of localization of cholinesterase and, correspondingly, two types of innervation of the extrafusal muscle fibers (Fig. 1a). The fibers most frequently found were those on which the zone of cholinesterase activity was concentrated in one place of the fiber, on a relatively large area of it, forming terminal "clusters" (Fig. 1b). The length of one such motor zone is about 350 μ. Features such as these are usually associated with fast muscle fibers, innervated by single nerve fibers of large diameter, and possessing endings of the "twitch" type. Muscle fibers in which cholinesterase was distributed as small clumps, scattered unevenly along the fiber, were much less frequent (Fig. 1c). The length of these individual motor zones was less than in the first case, namely 20-180 μ, with gaps between 10 and 300 μ between them. This type of distribution of cholinesterase is characteristic of slow muscle fibers, with multiple innervation by fibers of small diameter and with nerve endings of the "grape" type.

The intrafusal muscle bundle of the spindle (Fig. 2a) also was found to contain fibers heterogeneous as regards the mode of distribution of the enzyme. However, the motor zones of both types discovered here were smaller than those on the extrafusal fibers. Fibers with endings of "twitch" type, in which single zones of cholinesterase activity were concentrated in the form of terminal "clusters" on an area approximately one-third as large as on the extrafusal muscle fibers, occupying 100-120 μ along the length of the fiber (Fig. 2b), were found in the muscle bundle of the spindle. Nerve endings of "grape" type, 1-15 μ long, also were present in some fibers of the intrafusal bundle (Fig. 2c). The arrangement of the motor zones along these fibers was uneven, with intervals of between 1 and 7 μ between the loci.

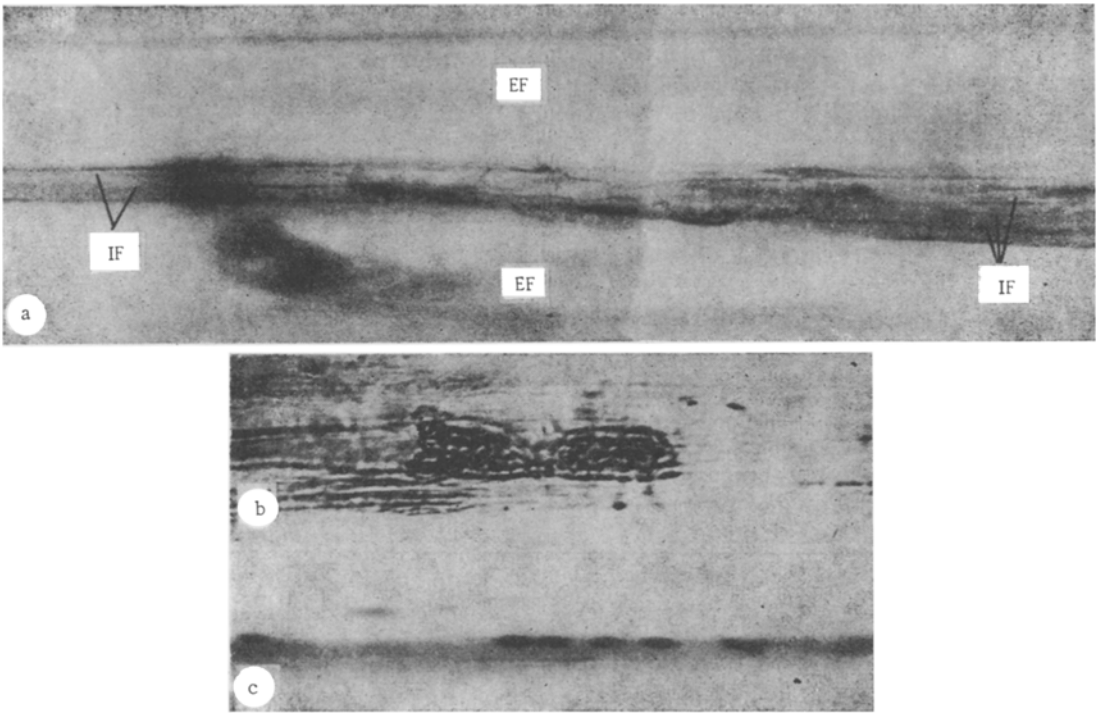

Fig. 2. Types of nerve endings of intrafusal muscle fibers of m. extensor digiti longus IV of the frog. a) General view of spindle; b) ending of "twitch" type; c) ending of "grape" type. EF) Extrafusal fiber; IF) intrafusal fiber. "Direct" thiocholine method. Magnification: a) 100, b, c) 200×.

The presence of two types of nerve endings in one layer of the intrafusal bundle, evidently on the same intrafusal fiber, discovered in one of the preparations, is interesting. It suggests the presence of two types of nerve endings here and, correspondingly, of two types of innervation of this muscle fiber.

A characteristic feature of the spindle is that cholinesterase was concentrated only in the polar regions of the bundle of muscle fibers, and was entirely absent in the equatorial zone, which had a purely sensory innervation.

The observations described above explain the variety of distributions of amplitudes of MPSP which were observed previously in intrafusal muscle fibers [1]. It might be supposed that it was in fibers with a single innervation and with nerve endings of "twitch" type that MPSP whose amplitude lay within the normal distribution were recorded. In fibers with multiple innervation and with nerve endings of "grape" type, however, skew distributions of amplitudes of MPSP with a high degree of asymmetry were found. The fiber in which the distribution was complex in form (as the result of superposition of normal and skew distributions) from the morphological point of view evidently had a mixed innervation with endings of "twitch" and "grape" types.

Extrafusal muscle fibers, possessing nerve endings of either "twitch" or "grape" type, are characterized by normal or skew distributions of amplitudes of MPSP respectively [1].

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