changes in the fine structure of these multiple endings or in the extraocular muscle fibres even 21 days after the operation (Figure 2).

These results demonstrate that in the rat the neurones of the ciliary ganglion do not innervate the extraocular muscle fibres. The contraction of the extraocular muscles observed by some observers after electrical stimulation of the ciliary ganglion[4] must have been due to other mechanisms, possibly concomitant excitation of the oculomotor nerve close to the ganglion. No atrophy of the extraocular muscles was observed such as has been reported earlier after removal of the ganglion ciliare[3]. A follow-up period of 21 days should be long enough for such atrophy to occur, if the endings were connected with cells of fibres in the ganglion ciliare. It is concluded that the small multiple endings originate outside the ciliary ganglion.

Zusammenfassung. Die motorischen Endplatten der nicht myelinhaltigen Nervenfasern in den Augenmuskeln der Ratte wurden elektronenmikroskopisch untersucht. Strukturelle Veränderungen treten nach Entfernen des Ganglion ciliare nicht auf.

H. Teräväinen and K. Huikuri

Department of Anatomy, University of Helsinki, Helsinki 17 (Finland), 23 December 1968.

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Localization of Acetylcholinesterase Activity in Myotendinous and Myomyous Junctions of the Striated Skeletal Muscles of the Rat

The attachment of a striated skeletal muscle fibre in a tendon has received special attention since Couteaux[1] demonstrated histochemically that increased acetylcholinesterase (AChE) activity was present in this region of the muscles of the frog and mouse. This differentiated part of the muscle fibre is called the myotendinous junction (MTJ). Locally increased AChE activity seen in very thorough light microscope studies of the MTJ has since been reported in other animals, such as the rat[2], the goat[3], birds[4], the cat and dog[5], and man[6]. Electron microscope studies have changed the older theories of the structure of the MTJ (e.g.[7]), showing that the myofibrils are separated from the tendon by the sarcolemma and that no other special structures, such as nerves, are present[5, 8, 9].

In mammals, the fibres of the skeletal muscle usually run from end to end of the muscle. However, in the human sartorius and gracilis muscles, muscle fibres have been described which do not continue to the tendon but join another muscle fibre[10]. These fibres have been thought always to have a tendon-like connective tissue component connecting the muscle fibres[10], and accordingly it has been held that true fibre-to-fibre junctions like the intercalated disc of the heart muscle do not exist in striated skeletal muscles. The present report describes such a myomyous junction.

Materials and methods. The diaphragm and the rectus superior, medialis and lateralis of the extraocular muscles of adult Sprague-Dawley rats were used in the experiments. The muscles were removed under ether anaesthesia and fixed in toto for light microscopy at 4 °C with 3.5% formol-calcium for 4–12 h. The method used for light microscopic localization of cholinesterases was based on the Gömöri[11] modification of the Koelle[12] thiocholine technique with minor modifications (Teräväinen[13]). Tetra-isopropylpyrophosphoramide (iso-OMPA; L. Light & Co. Ltd., Colnbrook) and 1:5-*bis*-(4-allyl dimethylammoniumphenyl)pentan-3-one diiodide (284C51; Burroughs and Wellcome, London) were used to discriminate between other cholinesterases (E.C. 3.1.1.8) and acetylcholinesterase (E.C. 3.1.1.7). Acetylthiocholine iodide (Fluka AG., Buchs) was used as a substrate for AChE and butyrylthiocholine iodide (Fluka AG, Buchs) for ns. ChE (for inhibitors see e.g. Teräväinen[13]).

The pieces of muscle were immersed for 20–60 min in 3% glutaraldehyde buffered to pH 7.2 with phosphate, rinsed for 2–3 h in the phosphate buffer and incubated according to the method of Karnovsky[14] for about 1 h to enable the distribution of AChE to be studied electron microscopically. The muscles were then sectioned longitudinally at approximately 200–300 µ with a razor blade and the area required was separated, using injection needles as knives, under an ordinary light microscope. The separations were postfixed with 1% osmium tetroxide in the phosphate buffer. After dehydration in a graded ethyl alcohol series, the tissue blocks were embedded in Epon 812[15], sectioned and stained with lead citrate stain[16].

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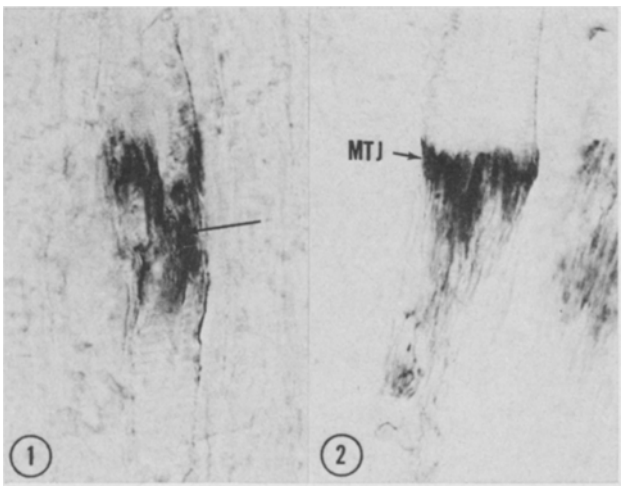

Fig. 1. AChE activity is seen apparently in the middle of one muscle fibre, which is thicker on either side of the transversely folded area where enzyme activity (arrow) begins. The transverse striations continue up to the beginning of AChE activity. Compare the structure with the distribution of AChE activity in the myotendinous junction (Figure 2). Extraocular muscle. Gomori reaction. × 2000.

Fig. 2. Acetylcholinesterase activity in the myotendinous junction (MTJ). Extraocular muscle. Gömöri reaction. × 2000.

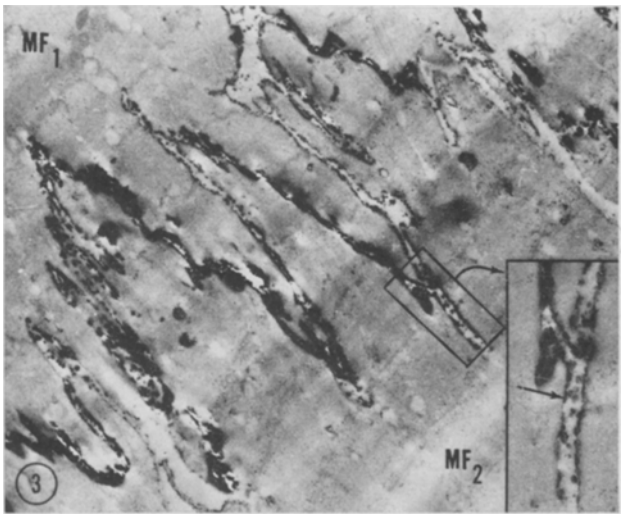

Fig. 3. Electron micrograph cut longitudinally through the structure seen in Figure 1. Note that there are 2 muscle fibres, MF_1 and MF_2, which are separated only by their plasmalemmas without any intervening tendon of connective tissue. No special sarcoplasmic structures, contact regions or nerves are present. The AChE activity partially fills the space between the 2 sarcolemmas. At higher magnification the reaction granules can be seen adjacent to the extracellular side of the sarcolemma (arrow). Diaphragm. Karnovsky reaction. × 10,000 (insert × c. 20,000).

Results. In a very few muscle fibres AChE activity was observed outside the zone of the myoneural junctions in a peculiar band apparently across the middle of one muscle fibre (Figure 1). In light microscopy the histochemical distribution of AChE activity had a folded appearance (Figure 1), structurally somewhat resembling the AChE activity of the MTJ (Figure 2). In these junctions, no ns. ChE activity was observed. The muscle fibre was thicker on either side of the zone of increased AChE activity and the cross striations of the muscle fibre appeared to continue in a normal manner up to the level at which the enzyme activity was visible (Figures 1 and 2). No special structures could be seen with the light microscope.

The peculiar distribution of AChE activity in the middle of 'the muscle fibre' was seen to be a junctional area between 2 separate apposed muscle fibres (Figure 3). The 2 sarcolemmas of the separate muscle fibres showed deep invaginations (Figure 3), which have also been observed with the electron microscope in the MTJ[5,8,9]. In contrast to the MTJ, however, there was no connective tissue between the 2 apposed plasmalemmas. Myofibrils continued close to the sarcolemma and no special sarcoplasmic structures or changes in the content of muscle mitochondria or sarcoplasmic reticulum were present (Figure 3). The structure of this junction is thus totally different from that of the intercalated disc of the heart muscle, in which a disc network and special regions of cell contacts can be observed (SJÖSTRAND et al.[17]).

Under the electron microscope, AChE activity was seen to be located between the invaginations of the 2 apposed plasmalemmas of the muscle fibres (Figure 3). The cupric ferrocyanide granules filled the space between the 2 plasmalemmas at sites of extensive hydrolysis of actylthiocholine iodide. However, at sites of somewhat weaker activity, the reaction product was located adjacent to the extracellular side of the 2 sarcolemmas (Figure 3 insert).

Discussion. The present work demonstrates that, in contrast to the accepted view, 2 muscle fibres of striated skeletal muscle can be apposed to each other without the intermediation of a connective tissue tendon, although the phenomenon appears to be very rare. The structural differentiation of this kind of myomyous junction (MMJ) in striated skeletal muscle fibres appeared to be wholly comparable to the structure of the MTJ. The local increase of AChE activity in the muscle plasma membrane of the MMJ is interesting. The enzyme is presumably located on the extracellular side of the 2 sarcoplasma membranes, since true extracellular location of the enzyme in the muscles seems unlikely[13]. Its function in the muscle terminal has yet to be explained.

Some speculations can be made, however. Suggestions have been made earlier that AChE activity in the muscle has some bearing on the conduction of excitability in the muscle fibre[14]. According to KARNOVSKY[14], the intercalated disc of the heart muscle, which is capable of spreading the impulse, is devoid of AChE activity, unlike the MMJ, as observed in the present work. It thus seems possible that the AChE activity of the MTJ and MMJ may be functionally connected with limiting the spread of impulse conduction in skeletal striated muscle fibres.

Zusammenfassung. Mit licht- und elektronenmikroskopischen Methoden wird demonstriert, dass die quergestreiften Muskelfasern sich ohne kollagenes Bindegewebe an andere Muskelfasern anschliessen können. An den gegenüber liegenden Sarcoplasmamembranen konnte Azetylcholinesterase nachgewiesen werden.

H. TERÄVÄINEN

University of Helsinki, Department of Anatomy, Helsinki (Finland), 10 December 1968.

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