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Acetylcholinesterase of the Motor Endplate and Its Response to Muscle Denervation*

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Abstract. In innervated and denervated sternohyoid muscles of adult mice the AChE with a pH optimum at 7.2 was shown to occur in all three fiber types in two separate structural areals located: extrafibrillarly (synaptic cleft, postsynaptic folds, subsarcolemmal vesicles, T-tubules, interfibrillar space) and intrafibrillarly (perinuclear cisternae, SR including SR cisternae). There is not a stable connection between the two areas. The functional significance of the intrafibrillar AChE, in particular, is unknown. After muscle denervation, intrafibrillar AChE of the A and B fibers disappeares more quickly than that of C fibers. This phenomenon not only suggests a general, but possibly also a fiber-specific neurotrophic effect.

Key words: Muscle fiber types – Acetylcholinesterase – Sole-plate nuclei

Introduction

Light and electron microscopic studies on skeletal muscle of adult mammals have revealed acetylcholinesterase (AChE) at the motor endplates (for references see Couteaux 1972) as well as in the region of the musculotendinous junction (Couteaux 1963; Lubinska and Zelena 1967). Whereas the function of this enzyme at the motor endplates is known – AChE hydrolyzes acetylcholine and thereby limits its transmitter properties – the physiological significance of AChE at the musculotendinous junction has not yet been clarified. Precise understanding of the origin of the enzyme is also lacking.

Enzyme activity has been demonstrated in the synaptic cleft in skeletal muscle in studies on motor endplate development of the rat (Brzin and Kiauta 1979; Toth and Karcsu 1979), rabbit (Tennyson et al. 1971; Tennyson et al.

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1973), and chicken (Wake 1976). In the above studies, activity was also found in the adjacent subsarcolemmal vesicles and the sarcoplasmic reticulum of myoblasts and myotubes – indications that the developing muscle fiber could be viewed as a site of acetylcholinesterase production.

It is thus assumed, that the muscle fiber of adult animals is directly involved in AChE production. The present investigation was designed to test this notion.

Materials and Methods

Adult, male NMRI mice were anesthetized with nembutal, the sternohyoid muscles dissected, the nerve cut unilaterally as it entered the muscle and removed, and the wound subsequently stitched. Fixation was performed under deep ether anestesia 4, 7, and 14 days later. Initially, Rheomacrodex (10%, salt-free, Fa. Knoll, Ludwigshafen, FRG) was warmed up to body temperature and perfused via the aorta; subsequently a 2% solution of glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) was applied. Thereafter the same fixation solution was used for in situ immersion for 3 h, and subsequent fixation of the individual muscles for the same period of time. The preparations were stored overnight in 0.1 M cacodylate buffer (pH 7.2) and sliced into 150 μm-thick vibrotome sections on the following day.

The method of Lewis and Shute (1966) was used for AChE staining. A slight modification was employed because biochemical investigations (Pilz 1974) have shown that AChE consists of a mixture of two enzymes with respective pH

Fig. 1. Detail from the vicinity of a motor endplate of a C fiber. Positive enzyme reaction in the synaptic cleft and postsynaptic folds (*SC*), sarcoplasmic reticulum (*SR*), perinuclear cisternae (*PC*) and the intrafibrillar cleft (*IC*). Negative magnification 12,800 : 1; total magnification 25,600 : 1

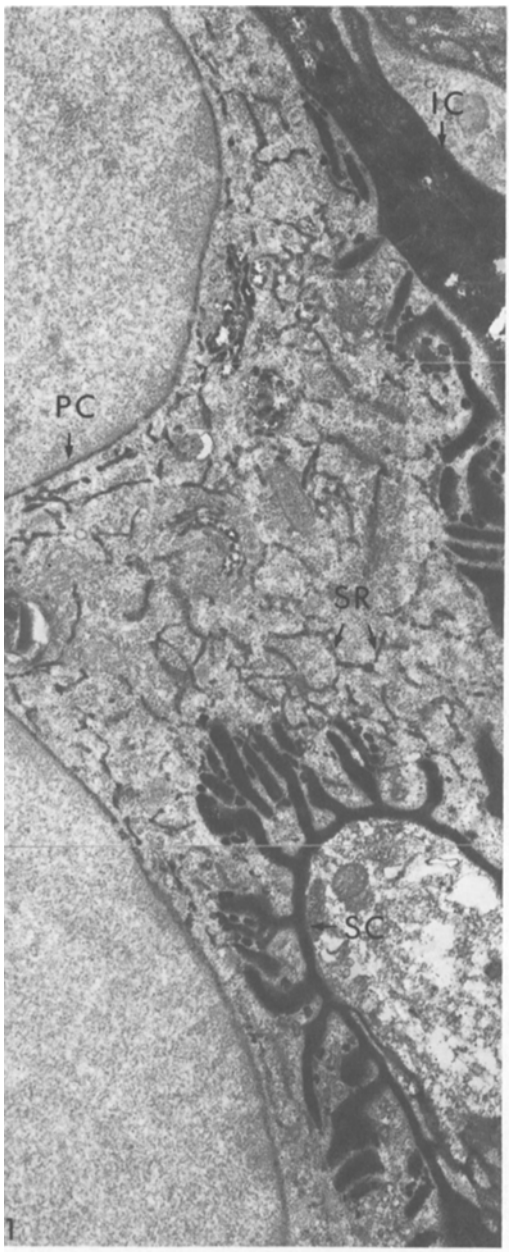
Fig. 2. Detail of postsynaptic folds of a motor endplate of a muscle fiber. A postsynaptic fold (*SC*) and the connected postsynaptic vesicles (*PV*) reveal a positive enzyme reaction. Negative magnification 16,000 : 1; total magnification 60,000 : 1

Fig. 3. Longitudinal section of a muscle fiber with adjacent intrafibrillar cleft. The intrafibrillar cleft (*IC*) and the closely associated subsarcolemmal vesicles (*SV*) reveal positive acetylcholinesterase reaction. Negative magnification 32,000 : 1; total magnification 64,000 : 1

Fig. 4. Longitudinal section of a muscle fiber. Positive enzyme reaction in sarcoplasmic reticulum (*SR*) with cisternae (*SRC*). The distribution of AChE varies in the T-tubules (*TS*). Negative magnification 16,000 : 1; total magnification 32,000 : 1

Fig. 5. Longitudinal section of a B fiber. Enzyme reaction in the intrafibrillar cleft (*IC*) and T-tubules (*TS*). The SR cisternae (*SRC*) are free of AChE. Negative magnification 13,000 : 1; total magnification 26,000 : 1

Fig. 6. Detail from the region of a motor endplate of a C fiber 7 days after denervation. The postsynaptic folds (*SC*) and their vesicles (*PV*) do not reveal enzyme reaction; positive reaction in the sarcoplasmic reticulum (*SR*). A stable connection between the positively and negatively reacting structures is lacking. Negative magnification 32,000 : 1, total magnification 64,000 : 1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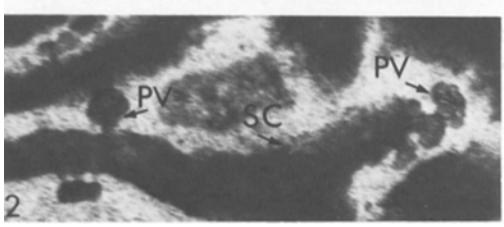
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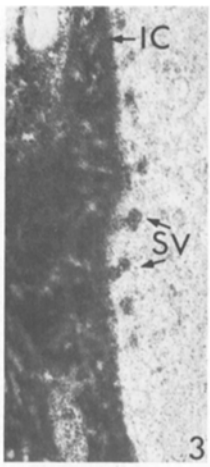
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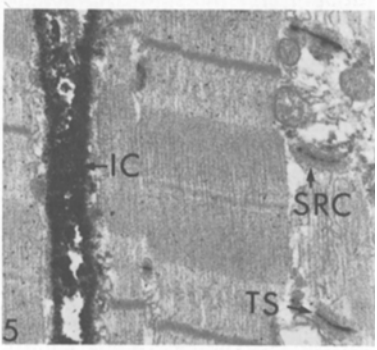
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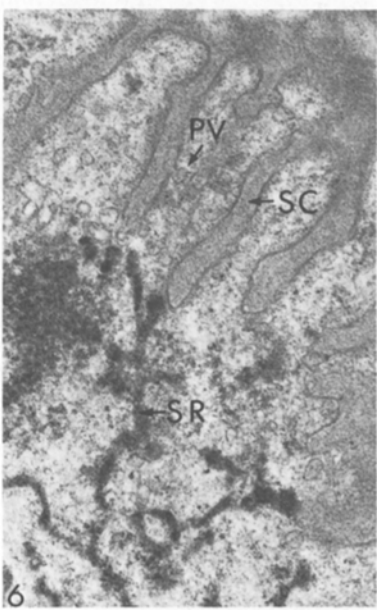
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optima of 7.2 and 8.6. Demonstration of AChE was limited in the present study to the enzyme with a pH optimum at 7.2.

The sections were incubated in calcium-free solution at this pH (7.2) and 37° C for 30 min and washed (according to modification of Lewis and Shute 1969). On the following day, the sections were postfixed for 2 h in 1% osmium tetroxyde, dehydrated, and embedded in Epon. Uranyl acetate/lead citrate was used for contrasting.

Results

In the vicinity of the motor endplates of innervated sternohyoid muscles of adult mice, AChE with a pH optimum at 7.2 has been detected in the three muscle fiber types A, B, and C (Dauber 1978) (Fig. 1). The specific enzymatic endproduct fills both the synaptic cleft, as well as the postsynaptic folds, and occasionally is spread to the adjacent fiber surface. Furthermore, AChE also appears in the perinuclear cisternae of the sole-plate nuclei and in the sarcoplasmic reticulum (SR). Thus, there is an additional intrafibrillar AChE reaction, as is known to occur in other tissues (for references see Villani et al. 1977).

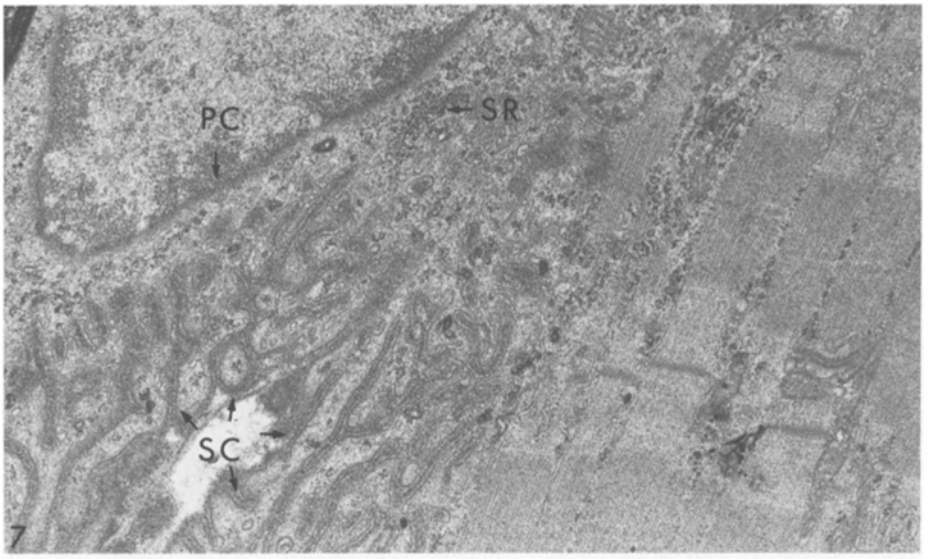
As can also be seen in Fig. 1, some of the SR is closely associated with postsynaptic folds and postsynaptic vesicles which are frequently positioned between the folds. Depending on the plane of section, higher magnification reveals connections between the postsynaptic vesicles and the postsynaptic folds (Fig. 2). It is not really apparent, however, whether the postsynaptic vesicles are portions of the SR or invaginations of the postsynaptic membrane and thereby comparable with subsarcolemmal vesicles (Fig. 3) of the rest of the fiber surface.

Whereas the SR – including the cisternae – situated between the myofibrils regularly reveals AChE, the T-tubules are not always filled with the enzymatic endproduct (Fig. 4). Interestingly AChE activity in the SR decreases with increasing distance from the endplates, whereas the activity in the T-tubules remains constant, provided the enzyme is also detected in the intrafibrillar space (Fig. 5).

Fig. 7. Longitudinal section in the region of the motor endplate of an A fiber, 7 days after denervation. Postsynaptic folds (*SC*), sarcoplasmic reticulum (*SR*), and perinuclear cisternae (*PC*) are free of enzyme reaction. Negative magnification 8,000 : 1; total magnification 16,000 : 1

Fig. 8. Longitudinal section in the region of the motor endplate of a C fiber, 7 days after denervation. Postsynaptic folds (*SC*) free of enzyme reaction. Sarcoplasmic reticulum (*SR*) and perinuclear cisternae (*PC*) reveal enzyme reaction. Negative magnification 8,000 : 1; total magnification 16,000 : 1

Fig. 9. Longitudinal section in the region of the motor endplate of a C fiber, 14 days after denervation. Postsynaptic folds (*SC*) and perinuclear cisternae (*PC*) are free of enzyme reaction. The sarcoplasmic reticulum (*SR*) is discernible as small pieces of membrane in the cytoplasm. Negative magnification 8,000 : 1; total magnification 16,000 : 1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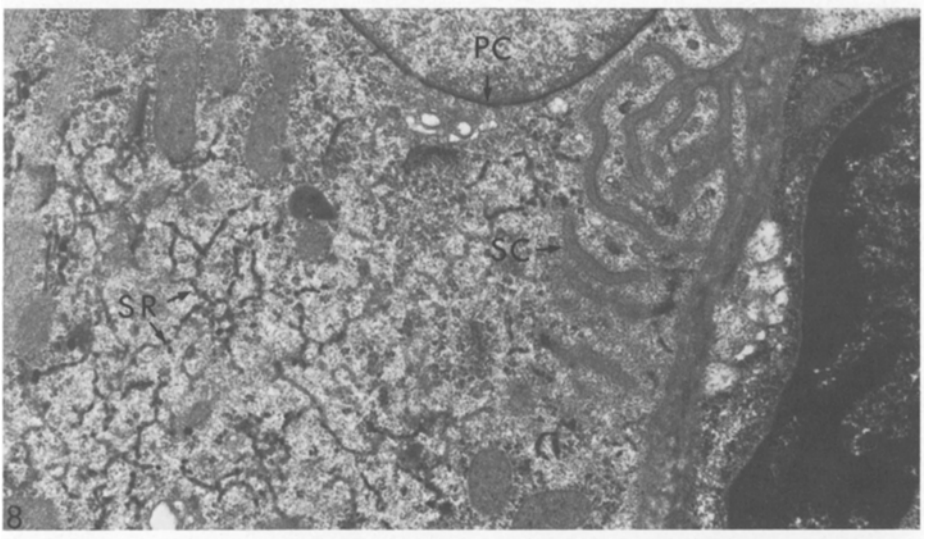
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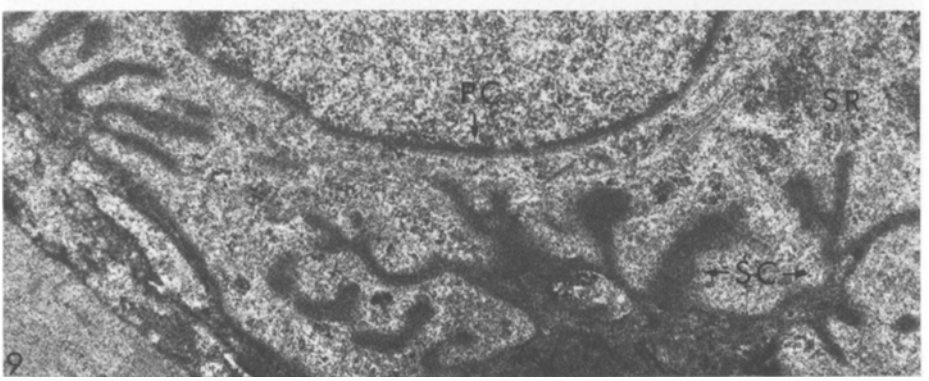
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On the 7th day after muscle denervation, staining of AChE reveals that enzyme activity in the region of the motor endplates is not uniform among the three fiber types.

Whereas the A and B fibers reveal similar activity, both differ with respect to C fibers. AChE is lacking in the A and B fibers, both in the synaptic cleft and the postsynaptic folds and vesicles – i.e., extrafibrillarly – and in the perinuclear cisternae and the SR – i.e., intrafibrillarly (Fig. 7). In the C fibers, only the synaptic cleft and the postsynaptic folds lack AChE activity, whereas distinct staining persists in the interior in the perinuclear cisternae and the SR (Fig. 8).

The extrafibrillar and intrafibrillar enzyme areas remain separate from each other. "Retrograde" filling of the postsynaptic folds via the SR, which would be expected if there were an open connection between both areas, was not observed (Fig. 6). As can be seen in the cytoplasm directly adjacent to the nucleus in Fig. 7, portions of the C fiber SR can be free of endproduct 7 days after denervation. Morphology alone does not clearly indicate whether this may be due to an empty time interval between two "episodes" of enzyme production, or "permanent" degradation. Fourteen days after operation, AChE was no longer detected in the C fiber (Fig. 9).

Discussion

The present findings indicate that the AChE with a pH optimum at 7.2 can be detected both extrafibrillarly and intrafibrillarly.

Extrafibrillar activity occurs in the synaptic cleft, in the subsarcolemmal vesicles, and in the T-tubules (Fig. 1). The latter two structures form a morphological unit in which the vesicles possess openings oriented toward the fiber surface, and, in the form of T-tubules, extend towards the interior of the fiber (Franzini-Armstrong et al. 1975). In the same manner, the vesicles between the postsynaptic folds could also communicate with the T-tubules.

Furthermore, the existence of the above structural unit would explain the present results showing the occurrence of AChE activity in the triads of the T-tubules when AChE occurs intrafibrillarly. It is not clear, however, why the activity in the SR decreases with increasing distance from the motor endplate (Fig. 5).

AChE appears intrafibrillarly in the perinuclear cisternae of the sole-plate nuclei and –as in myoblasts and myotubes (see Introduction) – in the sarcoplasmic reticulum including SR cisternae (Fig. 1). In the present study, the other muscle fiber nuclei do not reveal AChE activity within perinuclear cisternae, which is an indication of the special status of sole-plate nuclei.

There is a close localization of the structures with extrafibrillar and intrafibrillar AChE. The notion that the two enzyme areals are connected in the vicinity of the motor endplate, which would consequently permit continuous exchange of AChE, cannot be confirmed.

Whereas the extrafibrillar AChE does not reveal differences between the three fiber types A, B and C subsequent to muscle denervation (Dauber 1978),

there are clear differences in the time course of intrafibrillar enzyme depletion in the C fiber, on the one hand, and A and B fibers on the other hand.

In the latter two fibers, intrafibrillar AChE, similar to the extrafibrillar enzyme, disappeared early – within merely 4–7 days in the present experiments. In the C fiber, AChE activity is still detectable 7 days after denervation and does not disappear until after 14 days. The slower decrease in activity may be related to a higher number of sole-plate nuclei in the C fiber. Perhaps also the fiber-specific structure of the SR system plays a role (Dauber 1978).

The decrease in AChE in all three fiber types subsequent to muscle denervation confirms the notion of a neurotrophic effect on the muscle (Guth 1968) and the observed pattern of the decrease even suggests the fiber-specificity of the neurotrophic effect.

A satisfactory explanation for such different morphological localization of AChE has not yet been forthcoming. The AChE of the synaptic cleft – i.e., extrafibrillar AChE – is considered to be a muscle enzyme, the production of which is subject to neurotrophic regulation (Rathbone et al. 1979). This notion, as already mentioned in the introduction, stems from observations of the ontogenetic development of motor endplates. The present investigation of adult animals, however, does not yield any indication of a detectable morphological structure which would permit release of AChE from the muscle fiber into the synaptic cleft.

Another notion holds that AChE in the synaptic cleft originates from the peripheral nerve. Biochemical investigations have shown that three molecular forms of AChE occur in the mammalian muscles, of which only the 16S form is detected at the motor endplate (Hall 1973; Vigny et al. 1976). The same molecular form occurs in the axon of the peripheral nerve and is transported between the axolemma and Schwann cells through a "secretion" process (Kasa and Raconczay 1982). It is not yet clear, however, whether this intraaxonal AChE can be transported along the axolemma to the motor endplate.

Solution of the problems revealed in the present paper, as well as clarification of the functional significance of the enzyme activities in the T-tubules and sarcoplasmic reticulum, will not be achieved by morphological means alone. Supplementary physiological, biochemical and pharmacological investigations are also neccessary.

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