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Stimulation of Denervated Rat Soleus Muscle with Fast and Slow Activity Patterns Induces Different Expression of Acetylcholinesterase Molecular Forms[1]

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Abstract

The relative amount and distribution of acetylcholinesterase (AChE) molecular forms were studied in slow soleus and (less extensively) in fast extensor digitorum longus (EDL) muscles of the rat before and after denervation and direct stimulation. Normal EDL muscles showed higher total and specific AChE activity than normal soleus muscles and contained essentially three different molecular AChE forms (G_1, G_4, and A_{12}) as opposed to six forms (G_1, G_2, G_4, A_4, A_8, and A_{12}) in the soleus. Denervation reduced AChE activity in both muscles. In the soleus direct stimulation starting 2 to 3 weeks after denervation increased the specific AChE activity markedly. The increase started 12 to 24 hr after the onset of stimulation, reached 3 to 5 times normal values after 2 to 7 days, and then declined gradually toward normal values over the next 2 weeks. Furthermore, the effect on the different molecular forms depended strongly on the stimulus pattern. Thus, intermittent 100 Hz stimulation (fast pattern) induced essentially the three forms typical of the normal EDL, whereas continuous 10 Hz stimulation induced the six forms characteristic of normal soleus muscles but with some differences in their relative proportions. In the EDL, 2 days of continuous 10 Hz stimulation (the only duration and pattern examined) failed to induce a similar increase in AChE activity.

Acetylcholinesterase (AChE) plays an essential role in cholinergic transmission, particularly at the neuromuscular junction, by rapidly hydrolysing acetylcholine (ACh) after its release into the synaptic cleft from nerve terminals (Katz and Miledi, 1973). AChE exists in a number of molecular forms which may be classified as globular forms (monomers G_1, dimers G_2, and tetramers G_4) and asymmetric or collagen-tailed forms which consist of assemblies of one, two, or three tetramers (A_4, A_8, and A_{12}) with a collagen-like element (Massoulié and Bon, 1982). The A-forms have received considerable attention because of suggestions that they play a special role in nerve-muscle interaction.These forms are present along the entire length of some embryonic and neonatal muscles (Sketelj and Brzin, 1980; Koenig and Rieger, 1981) but are highly concentrated at the endplate of some adult rat muscles where they essentially disappear after denervation and reappear following reinnervation (Hall, 1973; Vigny et al., 1976; Collins and Younkin, 1982; Younkin et al. 1982). More recently, however, the A-forms have been detected also outside the endplate in adult human muscles (Carson et al., 1979), and they have also been reported to increase markedly after denervation in a rabbit slow muscle (Bacou et al., 1982).

Our present understanding of the mechanisms underlying the regulation of AChE in muscles is limited. Several factors must be considered. First, early contact with the motor nerve appears to play an important role by leaving permanent instructions for later synthesis and localization of AChE at the endplate (Koenig and Vigny, 1978; Lømo and Slater, 1980; Weinberg and Hall, 1979). Second, muscle activity probably plays an essential role in the control of AChE because it has been shown that AChE activity falls after suppression of muscle activity by tetrodotoxin (Rieger et al., 1980; Brockman et al., 1984), blockage of neuromuscular transmission (Drachman, 1972; Rubin et al., 1980), or neural conduction (Butler et al., 1978; Cangiano et al., 1980). Conversely, AChE may be induced to appear at denervated ectopic endplates by direct muscle stimulation (Lømo and Slater, 1980). Third, there is strong evidence that substances released from motor nerve terminals contribute to the regulation of AChE at the neuromuscular junction. Thus, soluble extracts from motor nerves partly prevent the denervation-induced decay of AChE activity, including the A_{12} forms in rat muscles maintained in organ culture (Davey et al., 1979; Fernandez et al., 1980). These substances appear to be carried by axonal transport and are released in increased amounts when the nerve is stimulated (Davey et al., 1979).

In the present experiments we have abolished all evoked muscle activity and removed all direct neurotrophic influences by denervating the fast extensor digitorum longus (EDL) and slow soleus (SOL) muscles of the rat. After 2 to 3 weeks of denervation, when AChE content is low, we have started chronic stimulation of the muscles to examine the effect of evoked muscle activity on muscle AChE in the absence of direct neural influences. Further information was obtained by using two different patterns of stimulation: one resembling the impulse pattern normally present in a fast muscle such as the rat EDL (fast pattern) and one resembling the pattern in a normal slow muscle, such as the SOL (slow pattern) (Fischbach and Robbins, 1969; Hennig and Lømo, 1984). Recent work shows that the distribution of AChE molecular forms is different in rat EDL and SOL muscles (Gisiger and Stephens, 1982; Groswald and Dettbarn, 1983), and it is possible that this is related to the different firing patterns in fast and slow motor units. In this connection it is interesting that slow muscles become fast during stimulation with a fast stimulus pattern, whereas fast muscles become slow during stimulation with a slow stimulus pattern (Lømo et al., 1974; Salmons

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and Sreter, 1976). We were therefore interested in whether muscles show similar impulse pattern-dependent plasticity with respect to their content of different AChE molecular forms. If so, this may throw new light not only on the role of muscle activity in AChE regulation but also on the function of the different molecular forms.

Materials and Methods

Denervation and chronic stimulation. Under ether anesthesia a 5 to 10-mm-long segment of the sciatic nerve was removed from the thigh on one or both sides of young male Wistar rats weighing 200 to 250 gm. Ten to 15 days later, two Teflon-coated multistranded steel wires (outer diameter, 0.011 mm, AS 632, Cooner Sales, Chatsworth, CA) were implanted into the right leg under anesthesia (Equithesin, 0.4 ml/100 gm of body weight). The distal ends of the wires with the insulation removed were placed across the SOL or EDL, one end proximally and posteriorly and the other end distally and anteriorly. Both wires were run under the skin to the head and into flexible silicon tubes (outer diameter, 6 mm) which were fixed to the skull with screws and dental cement. The wires and the protecting tubes were connected to a rotating contact approximately 1 m above the rat, which moved freely in a wide bucket. Stimulation usually started 2 to 3 weeks after denervation and lasted for 6 hr up to 21 days. The stimulation was either continuous at 10 Hz (slow pattern) or 60 pulses at 100Hz every 60 sec (mean frequency, 1 Hz; fast pattern). Each stimulus was bipolar with a duration of 0.2 msec and an intensity of 5 to 10 mA in each direction.

Acute experiments. At the end of the stimulation period, the muscles were exposed under ether anesthesia and the nerve trunk was stimulated. No contraction was seen with the dissection microscope, showing that no reinnervation had occurred, except in two muscles which were not included. The muscles were then removed and, in one series of experiments, pinned out in a bath with oxygenated saline. An endplate-containing central region and an endplate-free proximal region of about equal length (3 to 4 mm) were cut from each muscle and frozen in liquid nitrogen for subsequent biochemical analysis. In two other series, the tendons were trimmed away and the muscles were frozen whole in liquid nitrogen.

Biochemical analysis. The muscles were homogenized in a detergent saline buffer containing anti-proteolytic agents (Tris-HCl, pH 7, 10 mM; EDTA, 10 mM; NaCl, 1 M; Triton X-100, 1%; benzamidine, 1 mM; Zymofren (aprotinin) 25 units/ml; bacitracin, 1 mg/ml). The AChE activity was determined by the spectrophotometric method of Ellman et al. (1961), and the proteins were assayed by the method of Lowry et al. (1951) with bovine serum albumin as standard. AChE activities are expressed as nanomoles of acetylthiocholine hydrolyzed per minute at 28°C, either per muscle or per milligram of protein. Molecular forms of AChE were analyzed by centrifuging an aliquot of the muscle extract in a sucrose gradient (5 to 20% sucrose in 1 M NaCl, 50 mM $MgCl_2$, 10 mM Tris HCl, pH 7, 1 mg/ml of bacitracin, 1% Triton X-100) in a Beckman SW 41 rotor at 4°C, 40,000 rpm for 20 hr. Sedimentation coefficients were determined by comparison with β-galactosidase from *Escherichia coli* (16 S) and alkaline phosphatase from calf intestine (6.1 S).

Results

AChE in normal and denervated SOL and EDL

Activity. The effect of denervation on SOL muscles was examined in three different series of experiments. In the first two series (Figs.

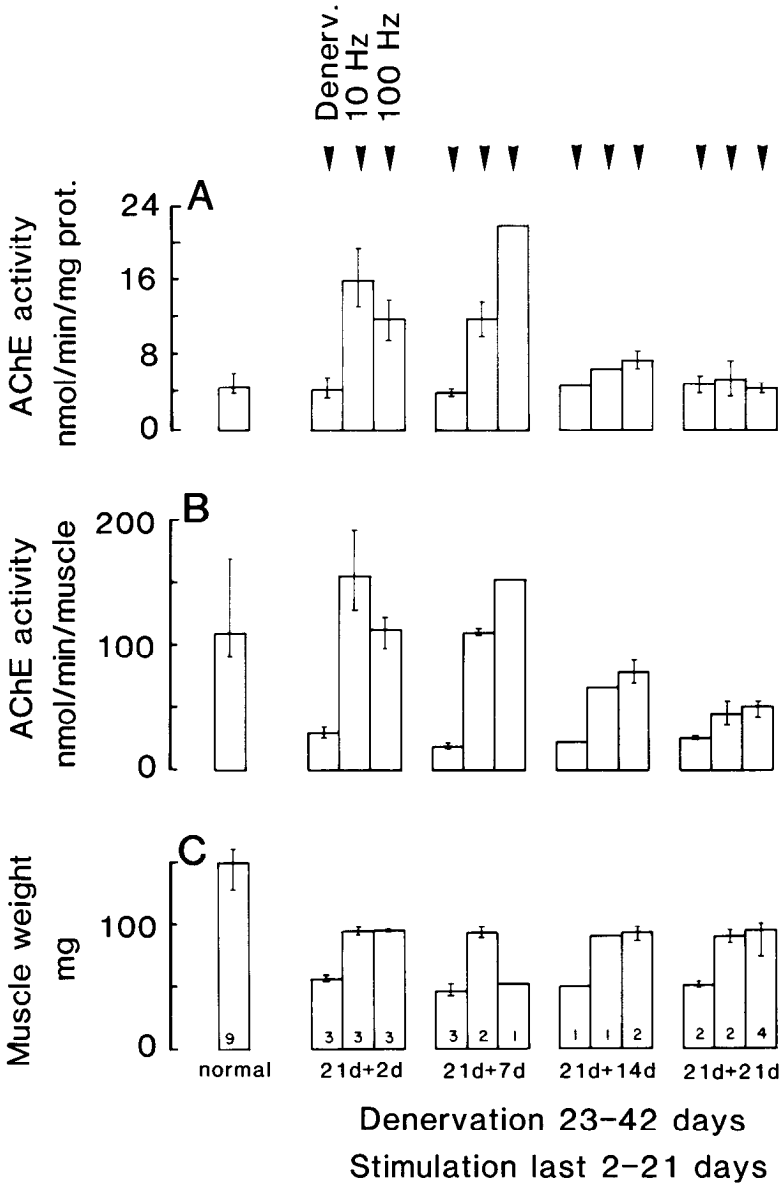

Figure 1. Stimulation increases specific AChE activity (*A*), total AChE activity (*B*), and muscle weight (*C*) in denervated SOL muscles. Intermittent 100 Hz or continuous 10 Hz stimulation (see "Materials and Methods") started 21 days after denervation and lasted 2, 7, 14, or 21 days as indicated. For each point in time (group of *three bars*) *arrowheads* on *top* indicate denervation only or denervation + stimulation at 10 or 100 Hz. The height of each *bar* indicates mean values, the *narrow bars* give the total range of all observations, and the *numbers* in the *bars* give the number of muscles.

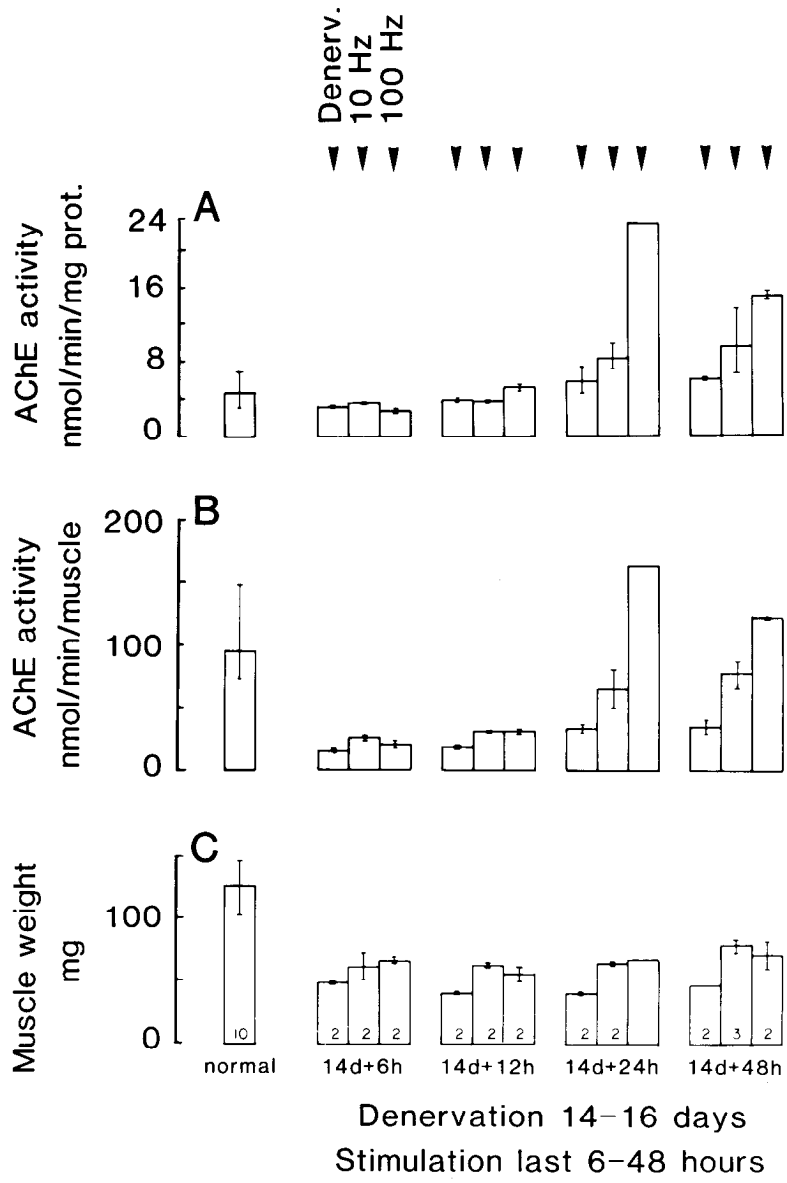

Figure 2. Stimulation increases specific AChE activity (*A*), total AChE activity (*B*), and muscle weight (*C*) in denervated SOL muscles. Similar to Figure 3 except that stimulation started after 14 days of denervation and lasted for 6, 12, 24, or 48 hr.

1 and 2) the AChE activity was examined in whole muscles, whereas in the last series (Fig. 3) it was examined in endplate-containing (*E*) and endplate-free (*NE*) regions. In the first two series, 14 to 42 days of denervation reduced total AChE activity to about 25% of normal (Figs. 1*B* and 2*B*). Muscle weight was similarly reduced (to about 35% of normal, Figs. 1*C* and 2*C*), and the specific AChE activity was little affected (Figs. 1*A* and 2*A*). In the last series, 19 to 29 days of denervation reduced not only the total, but also the specific AChE activity in endplate-free as well as endplate-containing regions (Fig. 3*B*). As expected from earlier studies (Hall, 1973; Vigny et al., 1976), the activity was higher in endplate-containing than in endplate-free regions of normally innervated muscles. The specific AChE activity of whole muscles appeared higher in normal EDL (Table I) than in normal SOL (Figs. 1 and 2) muscles (7.14 ± 0.53, $n = 2$ versus 4.71 ± 0.94, $n = 19$; mean ± SD, nmol/min/mg of protein). Furthermore, there was a large reduction in specific AChE activity after 13 to 16 days of denervation in EDL (Table I), whereas in the SOL the specific activity was either essentially unchanged (Figs. 1*A* and 2*A*) or less markedly reduced (Fig. 3).

Molecular forms. Rat SOL and EDL muscles contain several AChE molecular forms in different relative proportions, as shown in Figure 4, *A* and *C*. The different forms are identified by sedimentation coefficients (Bon et al., 1979). Closely sedimenting molecules such as G_4 (9.9 S) and A_4 (8.8 S) partially overlap but can be safely recognized, when present in sufficient proportions.

In the normal EDL, the A_{12}, G_4, and G_1 forms predominate (Fig. 4*C*). Normal SOL muscles present a more complex pattern, with significant proportions of the smaller collagen-tailed forms, A_8 and A_4 (Fig. 4*A*). The asymmetric forms are barely detectable in non-endplate regions of two muscles (not shown). After denervation (2 to 3 weeks), they decrease markedly in SOL and EDL (Fig. 4, *B* and *D*). A_8 and A_4 then appear to be more abundant than A_{12}, and are present both in endplate and non-endplate regions (see Fig. 6, *A* and *C*).

Chronic stimulation of the denervated SOL muscle. In the first series of experiments on the SOL we started stimulation after 21 days of denervation and continued stimulation for 2 to 21 days. The stimulus frequency was either 10 Hz (slow pattern) or 100 Hz (fast pattern). Both patterns markedly increased specific AChE activity measured in the whole muscle (Fig. 1*A*). The specific activity was highest (about 3 times normal) after 2 and 7 days and then declined toward normal values during the following 2 weeks. The total AChE activity, which was very low in denervated control muscles, was transiently increased by stimulation to normal or above normal values (Fig. 1*B*). With continued stimulation, however, the activity fell to subnormal values, because stimulation failed to restore normal

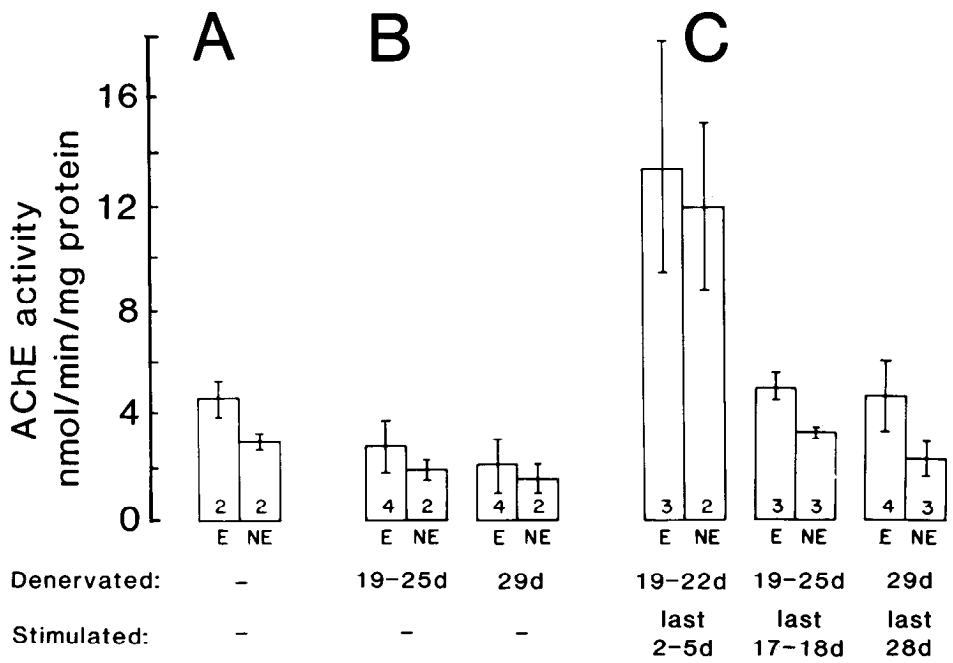

Figure 3. Specific AChE activity in endplate-containing (*E*) and endplate-free (*NE*) regions of SOL muscles (*A*) is decreased by denervation (*B*) and increased by stimulation (*C*). Denervation lasted 19 to 25 days and 29 days in *B* and 19 to 22 days, 19 to 25 days, and 29 days in *C*. Stimulation in *C* occurred during the last 2 to 5 days, 17 to 18 days, and 28 days as indicated. Each *bar* in *C* represents pooled results from stimulating either intermittently at 100 Hz or continuously at 10 Hz (see "Materials and Methods"). The *height* of each *bar* indicates mean values, *narrow bars* give total range of all observations, and *numbers* in the *bars* give the number of muscles.

TABLE I

Effects of denervation and continuous 10 Hz stimulation on the EDL

Treatment	Muscle Weight	AChE Activity	AChE Activity
	mg	*nmol/min/ muscle*	*nmol/min/mg of protein*
Normal	139, 143	174, 206	6.8, 7.5
Denervated 13–16 days	71.3 ± 8.5 ($n = 4$)	25.5 ± 11.6 ($n = 4$)	2.5 ± 0.9 ($n = 4$)
Denervated 16 days, stimulated last 2 days	78, 89	32, 36	2.8, 3.0

muscle weight. After 2 days of stimulation the denervated muscles had recovered about 40% of their previous weight loss, but continued stimulation did not further increase muscle weight (Fig. 1*C*).

In the second series of experiments we examined in more detail the early stimulation-induced changes in AChE activity. We now started stimulation 14 days after denervation, expecting better responsiveness at this time. However, the results were similar to those already described. In addition, they showed that both muscle weight and AChE activity began to rise as early as 12 hr after the onset of stimulation (Fig. 2).

In the third series of experiments, some of the muscles were denervated for 3 weeks (19 to 22 days) and then stimulated for the last 2 to 5 days of this period. The stimulation caused a marked increase in specific AChE activity to levels which were about 5 times higher than in denervated control muscles and about 3 times higher than in normal muscles (pair of *bars* to the *left* of Fig. 3*C*). The activity was equally high in endplate-free and endplate-containing regions, suggesting that the newly synthesized enzyme was not associated with endplates. Other SOL muscles were denervated for 19 to 29 days but stimulated during most of this time. In these muscles the specific activity was essentially normal both in regions containing the denervated endplates and outside (*bars* to the *right* of Fig. 3*C*). This indicates that prolonged stimulation causes a preferential increase in AChE activity at endplates in addition to restoring normal activities along the rest of the fiber.

The number of muscles that we have examined at each point in time is small. However, taken together, the results of the three series of experiments just described form a consistent picture which may be summarized as follows. (*1*) Stimulation of a 2- to 3-week denervated rat SOL induces a rapid transient increase in the AChE activity to several times normal values. (*2*) When chronic stimulation is continued for longer periods, the activity progressively returns to normal or near-normal values. (*3*) Initially, the stimulation appears not to affect junctional regions specifically. Later, AChE activity is preferentially localized at the denervated endplates.

Different stimulus patterns have different effects on AChE molecular forms

Two different stimulation patterns were used (intermittent 100 Hz and continuous 10 Hz), and both patterns had similar effects on total and specific AChE activity (Figs. 1 and 2) in endplate and non-endplate regions (Fig. 3, see legend). No consistent quantitative differences were observed, but more data are required to settle this point.

Qualitatively, however, the two patterns produced markedly different results. During intermittent 100 Hz stimulation (fast pattern) the proportions of the various molecular forms in denervated SOL muscles approached those in normal EDL muscles (cf. Fig. 5, *A* to *C* and Fig. 4*C*). As in the EDL, the molecular forms A_{12}, G_4, and G_1 were usually prominent, whereas the A_8 and A_4 forms normally present in the SOL were poorly represented. The increase in A_{12} forms was observed both in endplate and in non-endplate regions (Fig. 6, *B* and *D*).

Different results were obtained with continuous 10 Hz stimulation (slow pattern). After 2 to 21 days of stimulation (Fig. 5, *D* to *F*), the sedimentation patterns resembled those of normal SOL muscles (Fig. 4*A*). All globular and asymmetric forms were represented, including A_8 and A_4, normally absent in EDL. However, the relative amount of collagen-tailed forms was lower than in normal SOL muscles, but higher than in EDL. The content of G_4 was also relatively high. These muscles are therefore better described as having characteristics intermediate between those in normal SOL and EDL muscles. Possible reasons for the failure of the slow stimulus pattern to maintain normal SOL properties with respect to AChE activity are discussed below.

The differential effects of the two stimulation patterns on the relative proportions of molecular AChE forms developed gradually during the first 2 days of stimulation and then remained roughly stable. It is concluded that in the denervated SOL muscle a fast stimulus pattern induces the appearance of AChE molecular forms

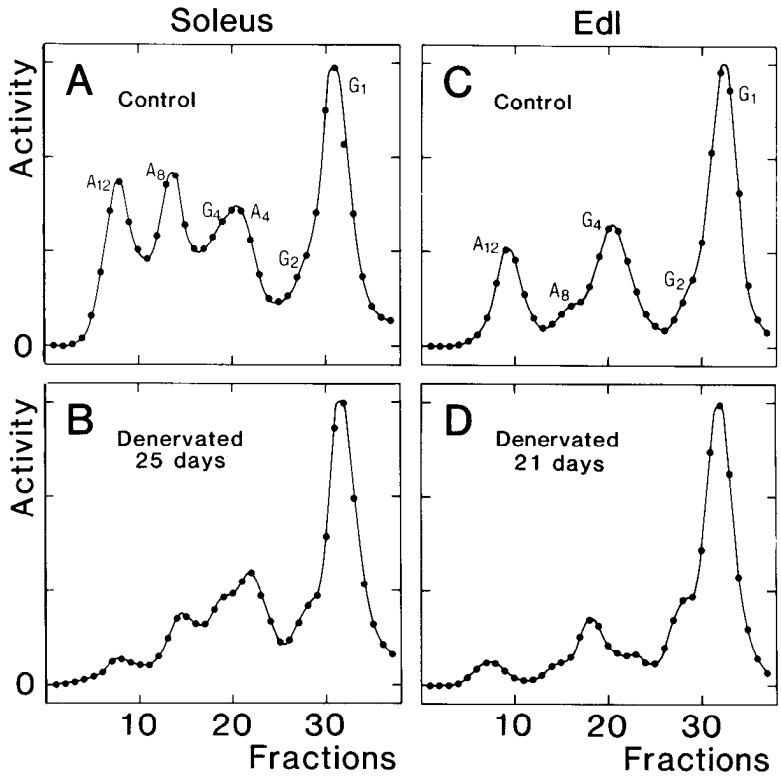

Figure 4. Different molecular forms of AChE in SOL (*A*) and EDL (*C*) muscles of rats, and effects of denervation (*B* and *D*). The sedimentation patterns were obtained after AChE was solubilized quantitatively by homogenizing muscle samples with 20 vol of extraction medium (see "Materials and Methods").

characteristic of fast muscle, whereas a slow stimulus pattern maintains the forms normally present in the soleus.

Chronic stimulation of the denervated EDL

The EDL was denervated for 14 days and then stimulated continuously for 2 days at 10 Hz (slow pattern). In contrast to the SOL, the EDL responded with only a small (or possibly no) increase in AChE activity (Table I). The sedimentation pattern of the different molecular forms was also apparently unaffected (data not shown). In experiments reported by Groswald and Dettbarn (1983) the specific AChE activity of the EDL remains low during early stages of reinnervation, whereas in the SOL it rises rapidly to several times normal values before it gradually declines to normal, just as it does during stimulation (see above). This suggests that EDL and SOL muscles may respond differently to activity because of intrinsic differences. However, more extensive stimulation experiments on the EDL are needed to pursue this question further.

Discussion

One main result of this work is that stimulation has a marked enhancing effect on the AChE activity of denervated rat SOL muscles. The increase was largest during the first few days of stimulation. Then followed a gradual decline in specific activity toward normal values. At least one of the stimulus patterns (fast pattern) was well within the physiological range of normal fast motor unit activity both with respect to frequency (100 Hz) and amount (mean frequency, 1 Hz; Hennig and Lømo, 1984). Therefore, these results confirm a large body of evidence (see Massoulié and Bon, 1982) that muscle activity plays an important role in the regulation of muscle AChE.

Mechanisms underlying stimulation-induced increase in AChE activity. The AChE activity of the denervated SOL started to rise 12 to 24 hr after the onset of stimulation. This could be due to reduced release of AChE from the muscle, increased rate of synthesis, or reduced rate of AChE degradation. Denervation increases rapidly the release of AChE from rat diaphragm (Carter and Brimijoin, 1981). The possibility must therefore be considered that stimulation enhances AChE activity in the muscle by suppressing its release. Both the sarcolemma and the basal lamina are likely to be involved in AChE release from muscle. It is therefore interesting that the properties of these structures are markedly affected by muscle activity (Lømo and Westgaard, 1976; Sanes and Lawrence, 1983). Muscle activity probably stimulates AChE synthesis. From a low basal level of synthesis in completely inactive muscle cultures, fibrillatory activity increases AChE activity primarily through an effect on synthesis (Brockman et al., 1984). The regulation of AChE levels may also involve changes in the degradation rate. For example in cultures of a murine cell line, T 28, and in primary cultures of avian muscle cells, only a minor fraction of rapidly degraded AChE monomers or inactive precursors reaches the external cellular surface or is secreted in an active form (Lazer et al., 1984; R. L. Rotundo, personal communication). Finally, denervation activates proteases in muscle (McLaughlin et al., 1974; Fernandez and Duell, 1980), and this could reduce AChE activity either by increasing the rate of degradation or by affecting the surface membranes of the muscle so that more AChE is released.

Regulation of AChE in junctional and extrajunctional regions. The specific AChE activity was higher in endplate-containing than in endplate-free regions of innervated SOL muscles in agreement with the high concentration of AChE at neuromuscular junctions (Hall, 1973; Vigny et al., 1976). Denervation reduced AChE activity, whereas stimulation substantially increased it, initially to the same degree in both endplate-containing and endplate-free regions. Later, when the specific activity declined toward normal values, the normal difference betwen the two regions was re-established (Fig. 3). The simplest explanation of these results is that muscle activity quickly induces the appearance of AChE all long the fiber. Then, after a delay, AChE begins to accumulate at the denervated endplates, owing to earlier local instructions laid down by the nerve. Present and earlier evidence (Lømo and Slater, 1980) suggest that such "instructions" remain for at least some weeks after removal of the nerve. It is not known, however, how long they can be maintained. These results strengthen the conclusion that muscle activity may be responsible for the normalizing effect of ectopic innervation on the AChE content of denervated endplates (Weinberg and Hall, 1979).

Clearly, neural influences other than evoked muscle activity are responsible for the precise localization of AChE at neuromuscular

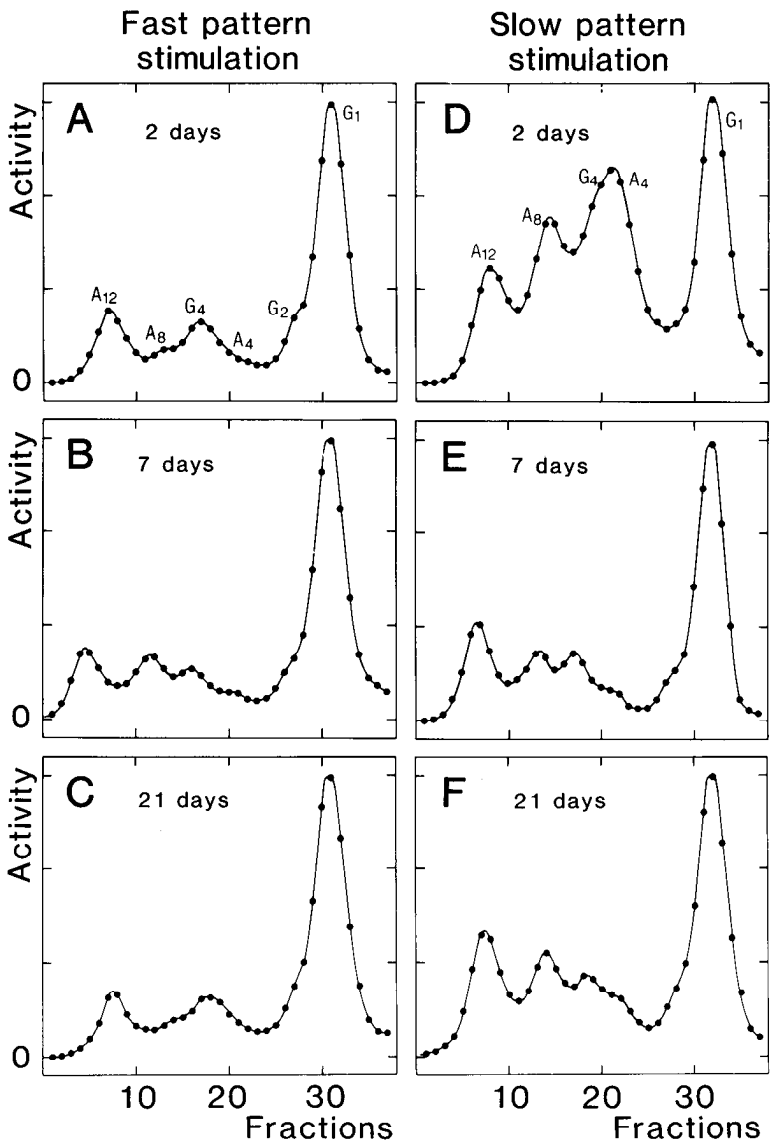

Figure 5. Stimulus pattern influences the distribution of AChE molecular forms in denervated SOL muscles. Stimulation was intermittent at 100 Hz in *A* to *C* and continuous at 10 Hz in *D* to *F*. Durations of denervation and of stimulation were, respectively, 23 days and the last 2 days in *A* and *D*, 22 days and the last 7 days in *B* and *E*, and 22 days and the last 21 days in *C* and *F*.

junctions. Several lines of evidence indicate that neural substances cooperate with evoked muscle activity in the regulation of junctional properties, particularly AChE. Substances present in extracts from neural tissues partially prevent the loss of AChE activity at denervated endplates (Davey et al., 1979; Fernandez et al., 1980) and also induce AChR clustering in noninnervated muscle fibers (Jessel et al., 1979). However, normal maturation of motor endplates, including the appearance of postjunctional folds, AChR channel complexes with short open times, and AChE, appears to require evoked muscle activity (Brenner et al., 1983; Lømo et al., 1984b).

The function and regulation of AChE in extrajunctional regions are less clear. In some muscles extrajunctional AChE activity decreases after denervation (Fig. 3) whereas, in others, it increases (Bacou et al., 1982). Regardless of the direction of the response to denervation, however, evoked muscle activity returns the AChE activity of noninnervated muscles toward normal values (Fig. 3; Walker and Wilson, 1975). Nerve extracts do not appear to influence extrajunctional AChE activity in denervated muscle (Davey et al., 1979). Furthermore, there is an apparent dissociation between the regulation of AChE and ACh receptors (AChRs) in extrajunctional regions since denervation stimulates the synthesis and appearance of AChRs regardless of whether the AChE activity simultaneously increases or decreases. In some muscles, the A_{12} molecular form of AChE is located highly concentrated at the endplate, suggesting that it may have a specific role in nerve-muscle interaction. In the rat SOL, however, this form is detected also outside the endplate even after several weeks of denervation and stimulation increases its activity both in endplate-containing and endplate-free regions. It appears from these varied observations that evoked muscle activity plays an important role in the regulation of both extrajunctional and junctional AChE. In the extrajunctional region, however, certain features of the regulation depend dramatically on the species and type of muscle. Moreover, in this region there is, so far, little evidence that activity-independent neurotrophic influences play an important role.

Significance of pattern of muscle activity. A second main result of this work is that stimulation affects the distribution of AChE molecular forms in the SOL in a stimulus pattern-dependent manner. First, it is noteworthy, however, that regardless of the pattern, stimulation increased the relative proportion of the A-forms, providing new evidence of the muscular origin of these forms and of the capacity of the muscle to synthesize the A-forms in the absence of the nerve. The fast pattern resulted in a distribution of AChE molecular forms that was similar to that in normal EDL muscles: the G_1, G_4, and A_{12} forms were prominent components, whereas A_4 and A_8 were only present in minor proportions. The slow pattern, on the other hand, did not suppress the A_8 and A_4 forms but produced a sedimentation pattern similar but not identical to that of normal slow SOL muscles. The failure to recover a completely normal SOL pattern may be due to the particular stimulation pattern used. We now know that normal SOL motor units discharge around 18 to 20 Hz (and not

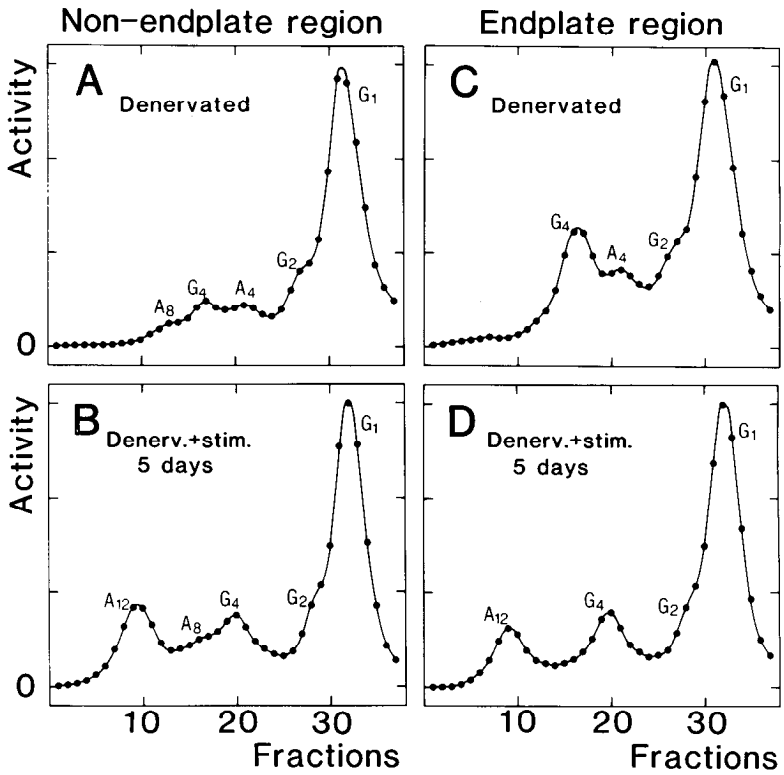

Figure 6. Denervation decreases (*A* and *C*) and stimulation increases (*B* and *D*) the content of asymmetric AChE molecular forms, especially the A_{12} form, in endplate-free (*A* and *B*) and endplate-containing (*C* and *D*) regions of SOL muscles. The sedimentation patterns were obtained from two 23-day denervated SOL muscles from one rat. One muscle had been stimulated intermittently at 100 Hz for the last 5 days.

10 Hz) and are intermittently active for about 20 to 35% of the time, rather than being continuously active (Hennig and Lømo, 1984). The answer to whether chronic stimulation can maintain a normal distribution of AChE molecular forms in the denervated SOL must therefore await stimulation with patterns more typical of normal SOL motor units.

The functional significance of the different AChE molecular forms is not known. One possibility is that effective neuromuscular transmission requires different junctional properties depending upon the rate of impulses arriving at the junction. In normal rat SOL and EDL motor units, the impulse rates vary around 20 and 50 to 90 Hz, respectively (Hennig and Lømo, 1984). This could explain why motor endplates in fast muscles, such as the EDL, have higher AChR densities (Sterz et al., 1983), deeper and more extensive postjunctional folds (Padykula and Gauthier, 1970), higher AChE activity (this work), and different distributions of AChE molecular forms (Fig. 4) compared to the endplates in slow muscles.

Although the catalytic activity of the different AChE molecular forms appears to be the same, their physical properties differ and this may well influence their localization and hence their function at the motor endplate (Vigny et al., 1978). If effective transmission of fast and slow impulse rates requires different junctional properties, then the cellular processes determining these properties are likely to be modified by changes in impulse rates. It has been shown elsewhere that the twitch contraction speed of denervated rat SOL muscles becomes much faster during direct stimulation with a fast stimulus pattern, whereas a slow contraction speed is maintained during stimulation with a slow pattern. As a result, effective rate modulation of muscle force output can be maintained despite even large changes in imposed impulse patterns (Lømo et al., 1984a). The present experiments indicate that the different AChE molecular forms at the neuromuscular junction also adapt to varying impulse patterns.

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