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Is Fast Fiber Innervation Responsible for Increased Acetylcholinesterase Activity in Reinnervating Soleus Muscles?

Running Head: Specific Reinnervation of Fast and Slow Muscle

Karl E. Misulis & Wolf-D. Dettbarn

Department of Pharmacology &
The Jerry Lewis Neuromuscular Disease Research Center
Vanderbilt University
Nashville, TN   37212

Send reprint requests to:

   Karl E. Misulis, M.D., Ph.D.
   Vanderbilt University
   Medical Center South
   2100 Pierce Avenue
   Nashville, TN   37212
   (615) 322-2989

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Abstract

During reinnervation of the rat hindlimb we had observed a temporary overproduction of acetylcholinesterases (AChE) in the soleus (SOL) but not in the extensor digitorum longus (EDL) muscle. In the present study, we have investigated whether the predominantly slow SOL, which is low in AChE activity, is initially reinnervated by axons that originally innervated fast muscle fibers with high AChE activity, such as those of the EDL. Local denervation of the rat SOL was performed to eliminate reinnervation by axons destined for other muscles. This produced an overshoot in AChE activity that was qualitatively similar to that observed with high sciatic crush. Local denervation of the SOL in the guinea pig was performed because this muscle is composed solely of slow (type I) fibers; thereby virtually eliminating the possibility of homologous muscle fast fiber innervation. The overshoot in this preparation was qualitatively similar to that seen with distal denervation in the guinea pig and local and distal denervation in the rat. Thus, initial fast fiber innervation is <u>not</u> responsible for the patterns of change in AChE activity seen with reinnervation in the SOL. It is concluded that the neural control of AChE is different in these two muscles and may reflect specific differences in the characteristics of AChE regulation in fast and slow muscle. How these neural influences are translated into muscle synthesis and degradation remains unknown.

Introduction

Denervation of muscle results in a variety of morphological, physiological, and biochemical changes, including a marked loss of acetylcholinesterase (AChE) activity (2,8,11,13,14). Although the mechanisms responsible for these changes are complex and as yet not well understood, two factors, trophic agents and muscle activity, seem to play major roles in the control of this enzyme (1,4,12,15,16,17,21,23). This is evidenced partly by the fact that the predominantly slow soleus (SOL) had approximately half the AChE activity of the predominantely fast extensor digitorum longus (EDL). We previously reported (3) that following crush of the rat sciatic nerve in the mid thigh region AChE activity of the SOL and EDL was reduced to about 40% of control within three days and 15% of control in two weeks. Following the second week, with onset of reinnervation, enzyme activity began to recover in both muscles; however, the rate of recovery in the SOL was much faster than in the EDL. By the end of the fourth week, AChE activity in the SOL had risen by 150 to 250% of control while the activity in the EDL had recovered to only 80% of control. By weeks 5 and 6 the SOL AChE activity returned towards its own control level, and the EDL AChE activity continued to recover. Immediately following denervation, decreases in the three AChE molecular forms from EDL (4, 10, and 16S) were evident, while the SOL exhibited both rapid increases (4 and 10S) and decreases (12S and 16S). In both muscles the 4S form reappeared before the 16S and 10S molecular forms, suggesting that the light form, 4S, may be a precursor of the heavier molecules. Transient increases

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during reinnervation occurred in the 16S AChE form in both
muscles; however, they were approximately five times greater in
the SOL than in the EDL. In SOL all other molecular forms showed
similar transient increases, while none were seen in the EDL (7).

The gradual increase of AChE in EDL during reinnervation may
reflect either of two mechanisms: a) AChE synthesis in a single
reinnervated muscle fiber could be a rapid process and the slow
increase per whole muscle then due to the slow addition of newly
innervated muscle fibers. Alternatively, b) the rapid
innervation of all muscle fibers followed by the slow synthesis
of AChE in each fiber could give similar results (12). The
majority of the crushed nerve fibers reach their termination
within two weeks, at a time when the first electrical and
mechanical signs of reinnervation became evident (3). Thus, the
slow increase probably reflects the rate of synthesis rather than
the gradual addition of newly innervated fibers.

While this explanation may hold for the EDL, it cannot
explain the rapid recovery and transient overshoot of AChE in SOL
during reinnervation. This marked increase in SOL AChE activity
may reflect any of several mechanisms including: a) initial
innervation by fast fibers conveying higher intrinsic AChE
activities, b) hyperneurotization by homologous (i.e. SOL)
fibers, or c) fiber type conversion independent of innervation by
fast fibers.

In this study we have examined the first possibility, i.e.
that with sciatic nerve crush, nerve fibers originally
innervating fast muscle (type II) may reinnervate slow SOL fibers

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(type I) during the early phase and induce a change in AChE
activity. This possibility is suggested by the facts that both
the nerve and muscle fibers of type II motor units possess higher
AChE and ATPase activities than those of type I motor units, and
that axons innervating fast muscle regenerate faster than those
innervating slow muscles (3,7,9,10,19).

To reduce the chance of reinnervation of the SOL and EDL
muscle fibers by nerves destined for other muscles, these muscles
were denervated locally, in the rat, by crushing the specific
nerves at their entry into the muscles. At intervals of 1 day to
5 weeks later the muscles were assayed for AChE activity. These
data were then compared to those obtained from the rat with a
high sciatic nerve crush.

However, this protocol does not completely eliminate the
possibility of an initial preferred innervation of the rat SOL
muscle by nerve fibers which normally innervate some of the few
type II fibers in this muscle. In a rat SOL of the age group used
here, about 68% of the fibers are type I and 32% are type II.
Therefore, the guinea pig, which has only slow fibers in the SOL,
was additionally used for experiments examining the effects of
both local and distant denervation.

Methods

Experiments were performed on male Sprague-Dawley rats,
180-250 g, and male guinea pigs, 350-450 g, maintained with ad
lib access to food and water under a controlled day-night cycle.
The animals were anesthesized with ether, and all surgical

procedures were carried out under a hood where rigorous aseptic conditions were maintained. The preparations chosen were the EDL and SOL, as well as their nerves.

For sciatic nerve denervation, thigh muscles were dissected along fascial plains revealing the joined peroneal and tibial nerves at the sciatic notch. These were crushed over a 3 mm segment for 40 sec with a small hemostat modified such that it had a smooth crushing surface. This method of denervation allows for interruption of the nerve fibers but leaves the fascial sheath relatively intact, thus facilitating regrowth of nerve fibers.

For local denervation, either the SOL or EDL muscle was exposed, carefully dissected free from adjacent tissues, and the neurovascular bundle isolated. Then the innervating nerves were crushed in a manner similar to that described above, 2 mm before their entry into the muscles. After surgery, incisions were closed with sterile stainless steel wound clips. There was no post-operative morbidity or mortality. Nerves and muscles of unoperated animals served as controls. The SOL and EDL nerve crushes were performed in separate animals.

At intervals of 1 to 5 weeks after nerve crush, the animals were sacrificed by decapitation and the SOL and EDL muscles removed for AChE assay (5). Prior to removing the muscles, the status of innervation or the progress of reinnervation was tested by observing the effects of electrical stimulation of the sciatic nerve above and below the site of the crush on the SOL and EDL muscles. Muscles were trimmed and homogenates pre-incubated for

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30 min in iso-OMPA (5×10^{-5} M), a specific irreversible inhibitor of BuChE. Subsequently, hydrolysis of acetylthiocholine was measured colorimetrically with a Beckman Model DU8 spectrophotometer (5). Data analysis was facilitated by an Apple II+ computer.

Results

With high crush in the rat, for both SOL and EDL muscles, there was a rapid decline in AChE activity within the first few days following denervation. These data are illustrated in figure 1. Figure 2a shows the results of the same experiments as figure 1 except that the data are expressed as total activity per muscle rather than as per gram of muscle tissue. Enzyme activity expressed in units of whole muscle reflects both changes in weight and in specific activity and, therefore, may be more reliable in experiments where manipulations induce changes in weight and protein. The drop in AChE with sciatic nerve crush was similar in the SOL and EDL, although the control activities were markedly different, that of the EDL being approximately twice that of the SOL. During reinnervation, however, these muscles showed distinctly different patterns of AChE recovery. With high sciatic nerve crush, the first signs of functional reinnervation are observed after 7 to 14 days, depending on the location of the crush site. From 2 weeks and thereafter, SOL AChE activity increased rapidly to levels greater than those present in the control SOL and indeed, even higher than in the

control EDL. This overshoot eventually subsided with a decrease of activity to control levels. The EDL exhibited a slow recovery of AChE gradually approaching control, but never exceeding it.

With low crush in the rat functional reinnervation occurred sooner than with high crush. The pattern of change in SOL AChE activity was the same as that seen with the high crush except that the reduction in activity was not as great, and the pattern of overshoot was slightly altered (figure 2b). The former is likely due to the shorter distance for reinnervation. The latter may be due to a change in the temporal relation between denervation and reinnervation effects.

The results of the experiments performed on guinea pigs reinforce the data obtained from the rat. Crush of the sciatic nerve in mid thigh region produced a loss, overshoot, and recovery of AChE activity in the SOL equal to that observed in the rat (figure 3a). Also, the decay and recovery of EDL AChE activity had a time course and magnitude very similar to that occurring in the rat EDL. The critical test, however, was the crush of the SOL nerve close to the muscle (figure 3b). This elicited an overshoot of AChE activity comparable to that shown in figures 2a and 2b for crush of rat sciatic or SOL nerves.

As mentioned above, we believe that comparison of total muscle AChE activity is more physiological than AChE activity per gram tissue. Nevertheless, weight changes were recorded and compared. Weight lots were less with local denervation than with sciatic crush in both rats and guinea pigs, we would be expected from the shorter distance for reinnervation. Therefore, with

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activity expressed as per gram tissue the overshoot with sciatic crush was graphically amplified. However the overshoot with local denervation was still quite marked and was qualitatively similar to that displayed for rat sciatic crush in figure 1.

Discussion

The results of these experiments indicate that there is a distinct difference in AChE recovery following nerve crush between the SOL and EDL. Reinnervation following sciatic or SOL nerve crush causes a rapid recovery and overshoot of AChE activity in the SOL which is not observed in the EDL even under conditions when only the nerve to the EDL is crushed. The fact that in SOL there is still the overshoot with low nerve crush shows that reinnervation by non-SOL motor axons, which have different intrinsic fiber type specificities, is not responsible for the overshoot. In addition, the increase in SOL AChE reaches significantly higher levels of enzyme activity than EDL control levels. The possibility exists, however, that there is a differential effect of reinnervation by axons of fast and slow motor units. Since about 32% of the fibers of the rat SOL are fast (type II), one could postulate that in view of the fact that fast nerve fibers reinnervate more rapidly than slow nerve fibers, the former may innervate the majority of slow muscle fibers first (8). This could produce a transformation of slow muscle fibers to fast fibers, and an increase in AChE activity to a level much greater than SOL control, followed by a gradual decline as the larger population of slow fiber axons re-establish

innervation. However, the peak of the reinnervating SOL AChE
activity is greater than that in EDL at any time before or during
denervation. This suggests that on the basis of the data from
the rat, alone, initial fast fiber innervation is probably not
responsible for the overshoot of AChE activity in the SOL.

The guinea pig data support this conclusion. Since the
guinea pig SOL is one of only a few mammalian muscles to be
composed exclusively of slow fibers, it was ideally suited for
testing the effects of reinnervation by fast and slow motor
axons. With SOL nerve crush close to the muscle where there is
no possibility for any reinnervation by fast fibers, the same
pattern of AChE recovery is obtained as with high crush.
Therefore, the overshoot in activity occurs also with homonymous
fiber type reinnervation.

These findings raise some interesting questions regarding
the control of AChE activity in muscle. What causes the greatly
increased AChE activity in the SOL muscle? The possibility
exists that a large proportion of the muscle AChE is transported
down the axon, released at the neuromuscular junction, and taken
up by the muscle fibers. However, calculations based upon AChE
transport rates suggest a turnover time of 200 days for AChE in
muscle (22). This is not consistent with the rapid accumulation
of AChE observed in SOL. Indeed, it would preclude even the
relatively slow recovery rate of AChE seen in the EDL.
Therefore, neural innervation must regulate intrinsic muscle AChE
synthesis. The response to reinnervation may depend on an
endogenously programmed event which may be different in fast and

slow muscle. In the presence of decreased or altered
stimulation, as well as prolonged disuse as seen after crush with
beginning reinnervation, there may be a reversion of the soleus
to a more immature state, characterized by a very high AChE
activity. This is comparable to the initial postnatal period
when the SOL exhibits a very high AChE activity, higher than that
seen in the EDL during this or any other period (6). This
suggests to us that soleus AChE development is more dependent on
innervation and induced muscle activity than the EDL and that
neural input actually down-regulates AChE synthesis in the fully
innervated and functional soleus. Preliminary data from our
laboratory has shown that nonsurgically induced hypokinesia
stimulates AChE synthesis in soleus (20). The muscle specific
portions of AChE change are not due to an effect of difference in
time of restoration of neural or mechanical activity, since the
return of muscle function occurs almost simultaneously in the SOL
and EDL. The dependency of AChE on neural innervation is
reinforced by the observation that no recovery of AChE activity
is seen in either muscle when reinnervation is prevented
(unpublished observations). We have established that there is an
innervation-dependent "signal" capable of regulating AChE and
differentiating between different muscle types. How the muscle
translates the message carried by neural impulses, trophic
factors, or both into synthetic activity remains to be studied.
If the mechanisms of this control can be determined they may
prove to be fundamental to neural control of muscle biochemistry
and neurotrophic regulation in general.

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Figure Legends

Fig. 1.   Change in AChE activity of rat SOL and EDL after
          sciatic nerve crush. Each point is an average of 5
          animals and is expressed as mean ± standard deviation.
          Enzyme activity is calculated as micro-moles
          acetylcholine hydrolyzed per gram muscle. Data are
          expressed as percentage of control muscles from
          unoperated animals. Abscissa represents time in weeks
          after nerve crush.

Fig. 2.   AChE activity with high and low crush in the rat.

          a.   Change in AChE activity in rat SOL and EDL after
               high sciatic nerve crush. Data displayed in this
               figure are analogous to those illustrated in
               figure 1 except that they are expressed as micro-
               moles/muscle/h. Enzyme activity per whole muscle
               reflects both changes in weight and changes in
               AChE/mg protein.

          b.   Change in AChE activity in rat SOL and EDL after
               specific low denervation. Data are expressed as
               in figure 2. The changes are qualitatively
               similar to those shown in figure 2. The SOL and
               EDL nerve crushes were not performed in the same
               animals.

Fig. 3.   AChE activity in the high and low crush in the guinea
          pig.

     a.   Change in AChE activity in guinea pig SOL and EDL
          after sciatic nerve crush. See figure 2a for
          further explanation.

     b.   Change in AChE activity in guinea pig SOL and EDL
          after specific low denervation. See figure 2b for
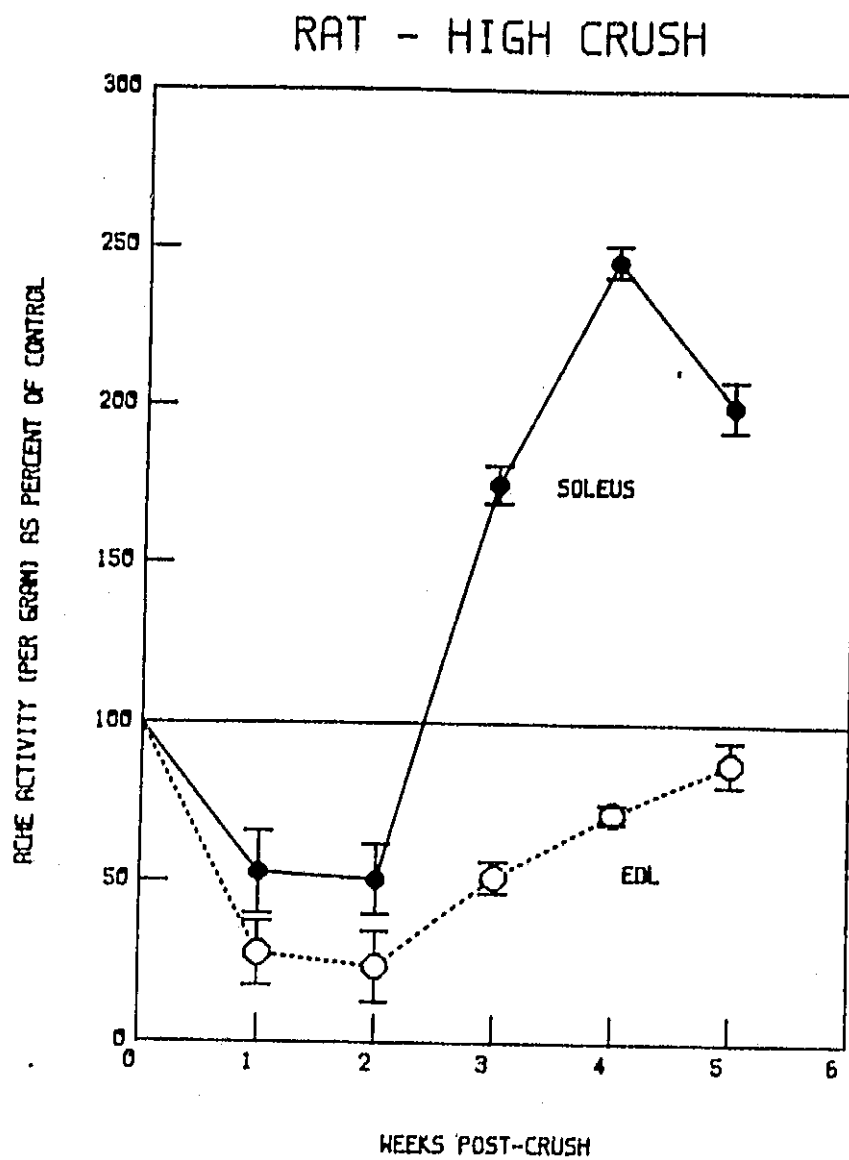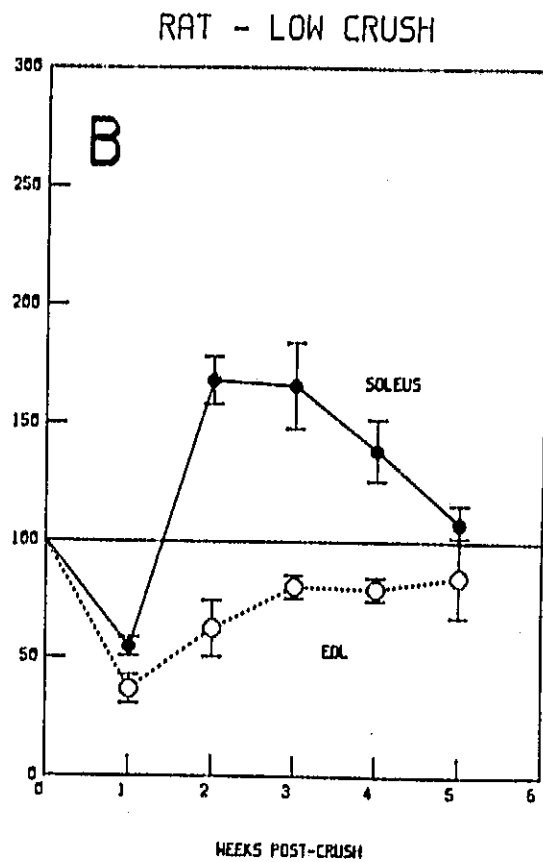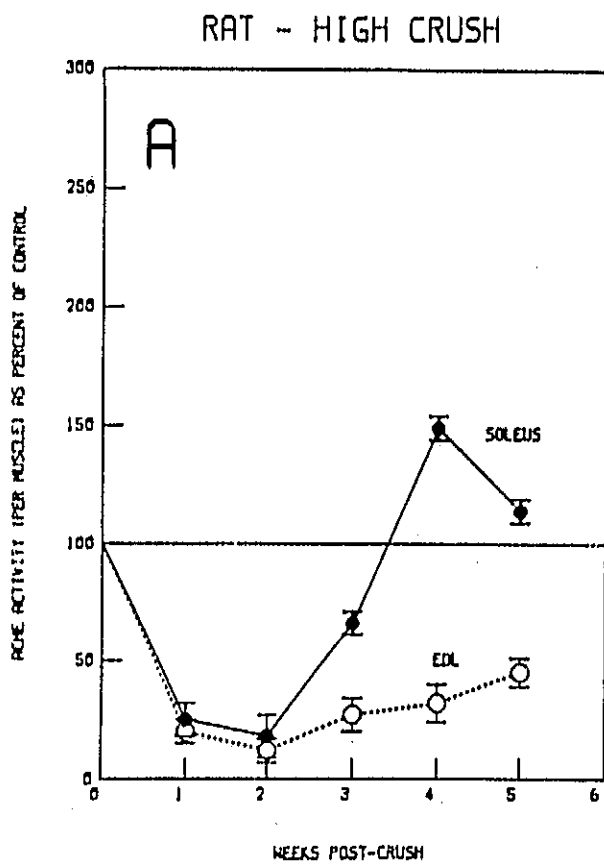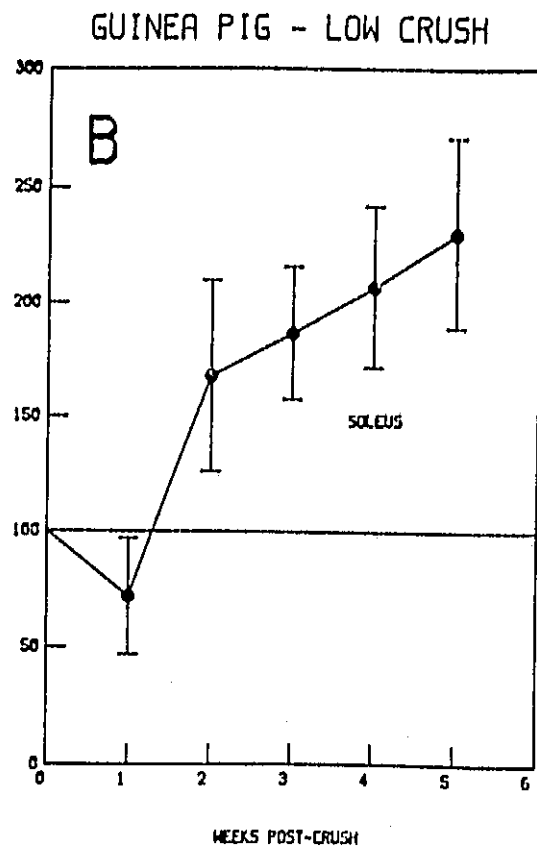   further explanation.

RAT - HIGH CRUSH

Fig. 1

RAT – HIGH CRUSH

RAT – LOW CRUSH

Fig: 2

Fig. 3