Calcitonin Gene-Related Peptide Prevents Disuse-Induced Sprouting of Rat Motor Nerve Terminals

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Calcitonin gene-related peptide (CGRP) coexists with acetylcholine (ACh) in motor nerve terminals. Externally applied CGRP has been shown to increase the synthesis of ACh receptors in cultured myotubes by a mechanism independent of muscle activity. Thus, CGRP is suggested to be a neurotrophic factor that may regulate the expression of several long-term events occurring at the neuromuscular junction. We have examined the effect of CGRP on the sprouting of motor nerve terminals induced by chronic block of nerve-muscle activity in adult rats. Daily treatment with CGRP suppressed the disuse-induced terminal sprouting in a dose-dependent manner, whereas the morphology of motor nerve terminals in active muscles was unaffected by CGRP. CGRP may be a possible candidate for an antisprouting agent which has been postulated to exist in nerve terminals. The disuse-induced outgrowth of terminal sprouts was accompanied by an increase in the mean quantum content of end-plate potentials, as well as in the frequency of spontaneous miniature end-plate potentials. This increased transmitter release was still maintained at the junctions in which disuse-induced terminal sprouting had been suppressed by CGRP. It is suggested that the formation of terminal sprouts per se is not responsible for the plastic change of transmitter release induced by prolonged disuse of the neuromuscular junction.

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide encoded by alternative processing of RNA transcripts from the calcitonin gene (Amara et al., 1982; Rosenfeld et al., 1983, 1984), and its immunoreactivity has been demonstrated in a variety of central and peripheral neurons (Rosenfeld et al., 1983; Gibson et al., 1984; Wiesenfeld-Hallin et al., 1984; Kawai et al., 1985; Goodman and Iversen, 1986; Ju et al., 1987). CGRP can be released spontaneously or by membrane depolarization from cultured neurons in a Ca^{2+} -dependent manner (Mason et al., 1984; also see Saria et al., 1986; Franco-Cereceda et al., 1987). At neuromuscular junctions, CGRP immunoreactivity is confined to the inside of the motor nerve terminal (Takami et al., 1985a, b). These results suggest that CGRP coexists with the transmitter in the terminal and that its release may exert a modulatory or trophic effect at the neuromuscular junction. In fact, externally applied CGRP has been shown to increase the synthesis of acetylcholine (ACh) receptors in cultured myotubes (Fontaine et al., 1986; New and Mudge, 1986; Fontaine et al.,

1987). This effect appears to be mediated by intracellular AMP, which increases in response to CGRP (Takami et al., 1986; Laufer and Changeux, 1987; Kobayashi et al., 1987). The synthesis of ACh receptors is increased by CGRP even after muscle activity is eliminated by tetrodotoxin (TTX), and the simultaneous application of CGRP and TTX shows additive increases in the receptor synthesis (Fontaine et al., 1986, 1987). Thus, CGRP is now suggested to be a neurotrophic factor that regulates the expression of several postjunctional proteins in a manner parallel to or independent of muscle activity (Changeux et al., 1987).

The purpose of the present study was to test whether the possible trophic regulation by CGRP can be demonstrated *in vivo*. Changeux et al. (1987) have classified the proteins present in muscle into 3 families. One family (family II) includes those proteins whose production increases following denervation, and it is suggested to be this family that may be subject to regulation by CGRP (Changeux et al., 1987). In addition to ACh receptors, a neurite-promoting factor extracted from muscle tissue is known to increase its activity following denervation (Henderson et al., 1983). For this reason, we have examined the effect of CGRP on sprouting of motor nerve terminals induced by chronic block of nerve-muscle activity with TTX in adult rats. The results show that CGRP suppresses disuse-induced terminal sprouting. Thus, a substance expected to be present in nerve terminals can hinder the terminals from sprouting. Phenomenologically, this coincides with a previous hypothesis that nerve terminals may contain an antisprouting agent which neutralizes the effect of a sprouting factor continually secreted from the target tissue (Aguilar et al., 1973; Diamond et al., 1976). If a similar mechanism were present at the neuromuscular junction, CGRP could be a possible candidate for the antisprouting agent.

Materials and Methods

Preparation. Adult Wistar rats weighing 200–240 gm were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). A glass capillary (750 μm i.d., 280 o.d.) filled with a TTX solution was inserted beneath the epineurium of the right sciatic nerve at the midhigh level in the manner described by Mills and Bray (1979). One end of the capillary was tapered by an electrode puller and fire-polished to restrict its inner diameter to 10–15 μm . The total length of the capillary was 6–10 mm. The capillary was then filled with an 80 mM TTX (Sankyo Co.) solution dissolved in citrate buffer, and the unrestricted end was sealed with hematocrit tube sealant. Immediately after recovery from anesthesia used for the operation of capillary implantation, paralysis of the right hindleg was already evident by the absence of the toe-spreading reflex. Paralysis was examined twice daily, using this reflex, and the conduction block was maintained for 10 d. If the limb recovered before this, the animal was discarded. On the contralateral side, a capillary filled with the vehicle solution alone (citrate buffer without TTX) was similarly implanted to the sciatic nerve. The implants filled with citrate buffer alone did not

Received Nov. 27, 1987; revised Jan. 11, 1988; accepted Jan. 22, 1988.

This work was supported by research grants from the ministry of Education, Science and Culture of Japan.

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Figure 1. Photomicrographs of motor nerve terminals on the control (*A*) and nerve-blocked (*B*) sides in a saline-treated rat. Scale bar, 20 μ m. The heminodal region of a motor nerve fiber can be seen below the terminal with a round shape at a position of 5 o'clock in *A*. No sprouts are observed in the terminal on the control side. Note the capillary vessel crossing the muscle fiber from the lower right corner and extending toward the terminal in *A*. In *B*, one sprout emerges from the upper middle edge of the terminal and another sprout with a bifurcation from the lower middle edge.

affect the toe-spreading reflex, and this unblocked side served as a control.

During the 10 d postoperative period, rat CGRP (Peptide Institute, Inc.) dissolved in a mammalian saline was subcutaneously injected every day in one group of rats, whereas another group of rats was daily treated with the saline alone.

Morphology. At the end of the 10 d period, the extensor digitorum longus (EDL) muscle was excised from each hind limb and fixed for 40 sec with 2.5% glutaraldehyde in 0.1 M PIPES buffer (pH adjusted to 7.3 by Tris). The motor nerve terminals were then stained for about 6 min with nitro blue tetrazolium (1.0 mg/ml) and phenazine methylsulfate (0.1 mg/ml) as described by Letinsky and DeCino (1980). The overall end-plate size was occasionally determined by a combined staining for acetylcholinesterase (AChE; Karnovsky and Roots, 1964). However, the amber AChE reaction product tended to make the contour of fine terminal sprouts obscure. Therefore, the demarcation of Schwann cell processes identified with Nomarski optics was usually used to define the end-plate zone. After the terminal staining, single muscle fibers were dissected and mounted in glycerol on glass slides. For each junction, several photomicrographs were taken at different focal planes, and the entire terminal profile was reconstructed. The length of individual sprouts and the total terminal length were then measured by digitizing tablet attached to a computer.

Electrophysiology. The EDL muscles and attached nerves excised from the nerve-blocked and contralateral, control sides were placed side by side in the same recording chamber and superfused with a solution equilibrated with 95% O₂–5% CO₂. The perfusion fluid had the following composition (in mM): NaCl, 136.8; KCl, 5.0; CaCl₂, 1.0; MgCl₂, 8.0; NaH₂PO₄, 1.0; NaHCO₃, 12.0. The bath temperature was kept at 25.0

$\pm$ 1.0°C. The muscle nerves were prepared for stimulation separately with suction electrodes. Intracellular recordings were made alternately from disused and control EDL muscles with glass microelectrodes filled with 3 M KCl. The electrode resistances were between 10 and 25 M Ω . At each junction, 160–220 end-plate potentials (e.p.s.) were recorded at a stimulus frequency of 0.5 Hz. This procedure was followed by recording a series of about 100 spontaneous miniature end-plate potentials (m.e.p.s.). The mean quantum content (*m*) was determined from the ratio of the mean e.p.p. amplitude to the mean m.e.p.p. amplitude. Since the *m* values were <5 except for a few junctions, no correction was attempted for nonlinear summation of the unit e.p.s. (Martin, 1955). Recordings were made at 6–9 junctions from each of the disused and control EDL muscles. Morphometric observations on the motor nerve terminals were carried out on the same EDL muscles in 6 out of 7 animals that had been subject to the electrophysiological analysis of e.p.s.

Results

Prevention of disuse-induced terminal sprouting by CGRP

Figure 1 illustrates motor nerve terminals observed in the EDL muscles on the control and nerve-blocked sides in a rat treated with the saline alone. On the control, unblocked side, the motor nerve terminals had a round or oval configuration, and their borders delineated the end-plate zone (Fig. 1*A*). In agreement with previous reports (Brown and Ironton, 1977; Pestronk and Drachman, 1978; Betz et al., 1980; Harris, 1981), chronic con-

Figure 2. Suppression of disuse-induced terminal sprouts by CGRP. Photomicrographs of motor nerve terminals on the disused side in a saline-treated rat (*A*) and a CGRP-treated rat (*B*; 50 $\mu\text{g/d}$). Arrowheads indicate terminal sprouts. Note the extensive sprouts in *A* and a short sprout in *B*. *C*, Mean total length of sprouts that emerged from each terminal in disused muscles (filled circles) as a function of the amount of CGRP injected daily. Open circles, sprout length measured on the control side. Sprout length was measured at 22–34 junctions in each muscle. The points at 0 and 50 μm show the mean and SD (vertical lines) obtained from 3 rats. Other points represent the results from one animal. The curve shows the relation expected from a 50% inhibition dose of 10 $\mu\text{g/d}$.

duction block of the sciatic nerve with TTX resulted in the outgrowth of one or more ultraterminal sprouts that extended beyond the end-plate zone in the majority of the EDL neuromuscular junctions (Fig. 1*B*). Occasionally, a long sprout showed many fine branches near its tip (Fig. 2*A*, upper-right corner), some of which ended with a structure similar to growth cones (Harris, 1981). In the animals treated with CGRP (50 $\mu\text{g/d}$), the formation of terminal sprouts on the nerve-blocked side was markedly reduced (Fig. 2*B*). In each animal, 22–34 junctions were randomly sampled from the EDL muscle on the control side as well as on the nerve-blocked side. In 3 saline-treated rats, terminal sprouts were seen, on the average, in 86.0% of the terminals ($n = 93$) examined on the nerve-blocked side. This ratio was significantly ($p < 0.001$) diminished in 3 CGRP-treated rats (31.5%, $n = 93$). The degree of suppression of disuse-induced terminal sprouting by CGRP was quantified in terms of the mean total length of sprouts emerging from each terminal, including those terminals without sprouts. As shown in Figure 2*C* (filled circles), the mean total length of sprouts on the nerve-blocked side in 3 saline-treated rats (at 0) was 55.9 ± 5.5 (SD) μm , whereas this value in 3 CGRP-treated rats (50 $\mu\text{g/d}$) was 10.1 ± 3.7 μm . Since the SDs of the mean total lengths of sprouts were relatively small (vertical bars at 0 and 50 in Fig. 2*C*), the dose dependence of the CGRP effects was examined, using only one animal for each of 4 other doses in the range of 0.5–20 $\mu\text{g/d}$. These points distributed reasonably well along the curve expected from a 50% inhibition dose of 10 $\mu\text{g/d}$ (Fig. 2*C*, filled

circles). The peak extracellular CGRP concentration expected after an injection of 10 μg CGRP is estimated to be about 20 nM. Since the fate and stability of CGRP *in vivo* after a single injection are not known, the half-maximal dose of CGRP effects (20 nM) evaluated here may well be an overestimate.

In the mammal, a small population of motor nerve terminals shows sprouting even in normal muscles (Barker and Ip, 1966; Tuffery, 1971; Brown and Irton, 1977; Betz et al., 1980; Harris, 1981). In the present study, terminal sprouts were observed in 3.0% of the junctions ($n = 96$) examined on the control side in saline-treated rats. This ratio was unaffected by 50 $\mu\text{g/d}$ CGRP (3.1%, $n = 96$). The mean total lengths of sprouts measured on the control side in saline- and CGRP-treated rats are shown in Figure 2*C* (open circles).

Prolonged block of nerve conduction is known to produce general enlargement of the motor nerve terminals, in addition to the formation of sprouts, in the paralyzed muscle (Duchen and Strich, 1968; Brown and Irton, 1977; Pestronk and Drachman, 1978; Betz et al., 1980; Harris, 1981). Therefore, the total terminal length, including sprouts, was also measured. Figure 3*A* illustrates the relation between the total terminal length and the muscle fiber diameter for junctions on the control and nerve-blocked sides in a rat treated with the saline alone. As shown in a variety of vertebrates (Harris, 1954; Cole, 1957; Anzenbacher and Zenker, 1963; Nyström, 1968; Kuno et al., 1971; Harris and Ribchester, 1979; Eldridge et al., 1981), the total terminal length was positively correlated with the muscle

Figure 3. Relation between the total terminal length and the muscle fiber diameter in (A) a saline-treated rat and (B) a CGRP-treated rat (50 $\mu\text{g}/\text{d}$). In each rat, about 30 junctions were examined on the nerve-blocked side (filled circles) and on the control side (open circles). Arrows, mean values for the control side; arrowheads, mean values for the nerve-blocked side. A decrease in muscle fiber diameter (disuse atrophy) can be seen on the nerve-blocked side in both the saline- (A) and CGRP-treated (B) rats, whereas increased terminal length on the disused side is not observed in CGRP-treated rats.

fiber diameter on the control side (Fig. 3A, open circles). In 3 saline-treated rats, the mean wet weight of the EDL muscle on the nerve-blocked side was 75.0% of that on the control side. This apparent disuse atrophy was also manifested by a decrease in muscle fiber diameter (Fig. 3A, filled circles and arrowhead on the abscissa). Also, there was a clear increase in the total terminal length on the disused side (Fig. 3A, filled circles and arrowhead on the ordinate). In 3 saline-treated rats, the mean ($\pm$ SD) total terminal length on the control side was $150.1 \pm 7.8 \mu\text{m}$, whereas that on the nerve-blocked side was $259.0 \pm 54.2 \mu\text{m}$. This 1.72-fold increase in terminal length was highly significant. The difference in the mean total terminal length (about $109 \mu\text{m}$) between the control and nerve-blocked sides might be attributed in part to formation of terminal sprouts (about $56 \mu\text{m}$; see above) and in part to general enlargement of the terminal on the disused side.

Figure 3B shows a similar relationship on the control and nerve-blocked sides in a CGRP-treated rat (50 $\mu\text{g}/\text{d}$). Again, muscle fiber diameters were reduced on the disused side compared with those on the control side. However, an increase in the total terminal length expected on the disused side was not observed in CGRP-treated animals. In 3 CGRP-treated rats (50 $\mu\text{g}/\text{d}$), the mean total terminal length on the disused side was not significantly different from that on the control side, the former being only 1.02 times the latter, while the mean diameter of disused muscle fibers was 77.4% of the control value. The mean total terminal length ($150 \mu\text{m}$) on the control side in saline-treated rats was similar to that ($148 \mu\text{m}$) on the control side in CGRP-treated animals. Therefore, it seems reasonable to conclude that CGRP suppresses disuse-induced sprouting without affecting the configuration of active terminals. Furthermore, the mean muscle fiber diameter ($53 \mu\text{m}$) on the control side in saline-treated rats was also comparable to that ($55 \mu\text{m}$) on the control side in CGRP-treated rats. Thus, CGRP does not seem to affect the general metabolic activity of muscle fibers. This agrees with previous observations that CGRP increases the synthesis of ACh receptors without affecting general protein synthesis in cultured myotubes (Fontaine et al., 1986, 1987; New and Mudge, 1986).

Effects of CGRP on transmitter release at neuromuscular junctions

The amount of transmitter released by a nerve impulse at a neuromuscular junction is proportional to the total length of its

nerve terminal (Kuno et al., 1971; Harris and Ribchester, 1979; Grinnell and Herrera, 1980, 1981). Prolonged block of the sciatic nerve with TTX has been shown to increase transmitter release at the junctions in the paralyzed muscle as evidenced by an increase in the mean quantum content (m) of e.p.s., as well as in the frequency of m.e.p.s (Snider and Harris, 1979; Harris, 1981). Thus, increased transmitter release has been suggested to be a physiological correlate of disuse-induced sprouting at the neuromuscular junction (Snider and Harris, 1979; Harris, 1981). We have recorded e.p.s and m.e.p.s alternately from the control and disused EDL muscles placed side by side in the same recording chamber (see Materials and Methods). As exemplified in Figure 4, the mean amplitude of e.p.s and the frequency of m.e.p.s were markedly enhanced in disused muscle fibers compared with those on the control side in saline-treated animals. Figure 5A summarizes the results obtained from 2 saline-treated rats. It is clear that both the mean quantum content and m.e.p.p. frequency are significantly higher at disused junctions than at the junctions on the control side in these animals. In 3 saline-treated rats, the m value and the m.e.p.p. frequency at the disused junctions ($n = 27$) were, on average, 2.7 and 2.8 times higher, respectively, than the values observed on the control side ($n = 27$). This is in good agreement with the previous observations that enlargement of motor nerve terminals at disused junctions is associated with an increase in transmitter release (Snider and Harris, 1979; Harris, 1981). To our surprise, however, essentially the same phenomena were also observed in the rats in which disuse-induced terminal sprouting had been suppressed by CGRP (50 $\mu\text{g}/\text{d}$), as shown in Figure 5B. In 4 CGRP-treated rats, the mean m value and the mean m.e.p.p. frequency were 2.3 and 2.5 times higher, respectively, on the disused side ($n = 30$) than on the control side ($n = 30$). The magnitude of increased transmitter release on the disused side in saline-treated rats was not significantly ($p > 0.4$) different from that in CGRP-treated animals. Thus, alterations in the transmitter release process and in the terminal size following prolonged disuse of the neuromuscular junction were dissociated in the presence of CGRP.

The mean m value (1.9) and the mean m.e.p.p. frequency (2.2 Hz) on the control side in saline-treated rats were not significantly different from those (1.6 and 2.5 Hz) observed on the control side in CGRP-treated rats. Therefore, chronic treatment with CGRP did not seem to alter the transmitter release process

Figure 4. Sample records of e.p.p.s and m.e.p.p.s obtained from EDL muscle fibers on the control (*A*) and nerve-blocked (*B*) sides in a saline-treated rat. Several e.p.p.s are superimposed in each record; note the occasional failures in *A* but not in *B*. Two traces of m.e.p.p.s in *A* or *B* are a continuous recording.

at active terminals. Takami et al. (1985b) have reported that isometric twitch tensions of the mouse diaphragm evoked by nerve stimulation or by direct stimulation of the muscle increase by about 10% within 10 min after the application of 100 nM CGRP. We have examined the possible acute effects of CGRP on neuromuscular transmission in the EDL muscles excised from 5 normal, unoperated rats. In these experiments, the *m* value or the amplitude or frequency of m.e.p.p.s showed no significant alterations during observation periods of up to 3 hr after superfusion with 100 nM CGRP. Thus, there was no evidence that CGRP has any modulatory action on neuromuscular transmission. This is in contrast to results obtained from the rat spinal cord, in which CGRP appears to enhance the nociceptive flexor reflex in the presence of substance P (Woolf and Wiesenfeld-Hallin, 1986) or to potentiate the release of substance P (Oku et al., 1987).

As reported by Bray et al. (1979), the mean resting membrane potential of TTX-paralyzed muscle fibers (-62 mV, $n = 27$) was significantly lower than that on the control side (-71 mV, $n = 27$) in saline-treated rats. A similar difference in the resting potential of muscle fibers between the control (-66 mV, $n = 30$) and TTX-blocked (-60 mV, $n = 29$) sides was also observed in CGRP-treated animals. Since the present study was concerned primarily with changes in the transmitter release (*m* values and the frequency of m.e.p.p.s), the alterations in resting potential of muscle fibers were not further pursued. In acute experiments, CGRP (100 nM) did not affect the resting potential of muscle fibers.

Discussion

There are two primary findings of this study. First, sprouting of motor nerve terminals induced by chronic block of nerve-muscle activity can be suppressed by CGRP. Second, the sprout

Figure 5. Relation between the frequency of m.e.p.p.s and the mean quantum content of e.p.p.s measured at neuromuscular junctions on the nerve-blocked (filled circles) and control (open circles) sides. The EDL muscles excised from each side were placed side by side in the same recording chamber and superfused with a mammalian saline containing low Ca^{2+} (1 mM) and high Mg^{2+} (8 mM). *A*, Results from 2 saline-treated rats in each of which 9 disused and 9 control junctions were examined. *B*, Results from 3 CGRP-treated rats (50 $\mu\text{g/d}$) in each of which 6–9 disused and 6–9 control junctions were examined.

outgrowth and an increase in transmitter release seen at disused junctions do not necessarily occur concomitantly. The implications of these 2 findings will be discussed below.

Suppression of terminal sprouting by CGRP

Sprouts from intact nerve terminals were first observed following partial denervation of the muscle (Hoffman, 1950; Brown and Ironton, 1978). Under this condition, however, sprouts occur only from those intact terminals located within 200 μm from denervated muscle fibers (Slack and Pockett, 1981; Pockett and Slack, 1982). Thus, the stimulus for terminal sprouting appears to be a diffusible substance. This is consistent with the observation that when the conduction of part of a muscle nerve is chronically blocked with TTX, active terminals in the partially paralyzed muscle can also sprout (Betz et al., 1980). This substance probably emanates from paralyzed or denervated muscle fibers since direct stimulation of a partially denervated muscle (Brown and Holland, 1979) or a botulinum-poisoned muscle (Brown et al., 1980) can inhibit the formation of terminal sprouts. In fact, a neurite-promoting factor has been extracted from muscle tissue, and its activity has been shown to increase after denervation (Henderson et al., 1983). In the salamander, normal cutaneous sensory fibers have been reported to sprout into the territory innervated by an adjacent skin fiber whose axoplasmic transport had been blocked with colchicine (Aguilar et al., 1973; Diamond et al., 1976). It has then been postulated that nerve terminals may contain an antisprouting agent normally transported by axoplasmic flow, which neutralizes the effect of a sprouting factor continually secreted from the target tissue (Diamond et al., 1976). Our results may be interpreted in a similar way. Thus, CGRP normally released from the motor nerve terminal may neutralize the effect of a sprouting factor secreted from the muscle fiber, but terminal sprouting may be induced if the sprouting factor is excessively produced by elim-

ination of muscle activity. This assumption is consistent with the results that externally applied CGRP prevents disuse-induced terminal sprouting, whereas the morphology of terminals in the active muscle is not affected. How CGRP neutralizes the effect of a sprouting factor is a matter of speculation. From the absence of CGRP effects on active terminals, the action of CGRP may be assumed to inhibit the synthesis of the sprouting factor in muscle fibers. However, at present, this possibility is nothing more than speculative. The presence of CGRP in motor nerve terminals and its antisprouting effect could be a fortuitous coincidence. Also, it should be noted that while CGRP appears to be synthesized in the cell bodies of spinal motoneurons (Gibson et al., 1984), a subpopulation of motoneurons lacks CGRP immunoreactivity (New and Mudge, 1986).

Sprouting and functional plasticity induced by prolonged disuse

From the general correlation between terminal size and transmitter release (Kuno et al., 1971; Harris and Ribchester, 1979; Grinnell and Herrera, 1980, 1981), one might assume that an increase in transmitter release observed following prolonged disuse of the neuromuscular junction results from the concomitant formation of terminal sprouts (Snider and Harris, 1979; Harris, 1981). Therefore, it was rather unexpected to find that CGRP suppresses disuse-induced sprouting but fails to prevent an increase in transmitter release. However, the formation of terminal sprouts and the formation of new release sites and ACh receptors may be 2 different processes. The fact that the 2 processes induced by disuse can be dissociated, at least in the presence of externally applied CGRP, implies that the stimuli for the 2 processes may be different or that in response to the same stimulus, the "threshold" for triggering the formation of new release sites and their postjunctional counterparts may be lower than that for the sprout outgrowth. In fact, even without CGRP, a 2.7-fold increase in transmitter release induced by disuse was not quantitatively proportionate to a 1.7-fold increase in terminal size. Harris (1981) has shown that the increase in transmitter release at disused junctions cannot be attributed to any alterations in the Ca^{2+} dependence of transmitter release or in the process of depolarization-release coupling. Herrera et al. (1985) have suggested that the variability in the relation between terminal size and transmitter release may be the result of differences in the size and number of active zones (also see Fukunaga et al., 1982; Ko, 1984; Propst and Ko, 1987) and in the number of mitochondria within the terminal. Recently, it has also been shown that the sprout outgrowth of motor nerve terminals elicited by botulinum toxin is associated with insertion of new ACh receptors and redistribution of preexisting receptors (Yee and Pestronk, 1987). It is then likely that functional plastic changes in transmitter release induced by disuse are related to alterations in active zones, the number of mitochondria, and distribution of ACh receptors. At present, it is not known whether the functional plastic change precedes or follows the formation of terminal sprouts after chronic block of nerve-muscle activity. The time courses of these 2 changes should be determined.

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