Downhill running preferentially increases CGRP in fast glycolytic muscle fibers

DARLENE A. HOMEMKo AND ELIZABETH THERIAULT
The Toronto Hospital Research Institute, Toronto Western Division, Toronto, Ontario, Canada M5T 2S8

Received 12 February 1999; accepted in final form 6 June 2000

Homonko, Darlene A., and Elizabeth Theriault. Downhill running preferentially increases CGRP in fast glycolytic muscle fibers. J Appl Physiol 89: 1928–1936, 2000.—Calcitonin gene-related peptide (CGRP) is present in some spinal cord motoneurons and at neuromuscular junctions in skeletal muscle. We previously reported increased numbers of CGRP-positive (CGRP+) motoneurons supplying hindlimb extensors after downhill exercise (Homonko DA and Theriault E, Inter J Sport Med 18: 1–7, 1997). The present study identifies the responding population with respect to muscle and motoneuron pool and correlates changes in CGRP with muscle fiber type-identified end plates. Twenty-seven rats were divided into the following groups: control and 72 h and 2 wk postexercise. FluoroGold was injected into the soleus, lateral gastrocnemius, and the proximal (mixed fiber type) or distal (fast-twitch glycolytic) regions of the medial gastrocnemius (MG). Untrained animals ran downhill on a treadmill for 30 min. The number of FluoroGold/CGRP+ motoneurons within proximal and distal MG increased by 72 h postexercise ($P < 0.05$). No significant changes were observed in soleus or lateral gastrocnemius motoneurons postexercise. The number of α-bungarotoxin/CGRP+ motor end plates in the MG increased exclusively at fast-twitch glycolytic muscle fibers 72 h and 2 wk postexercise ($P < 0.05$). One interpretation of these results is that unaccustomed exercise preferentially activates fast-twitch glycolytic muscle fibers in the MG.

neuropeptides; neuromuscular plasticity; activity; rat

IMMUNOCYTOCHEMICAL (23, 35) and in situ hybridization (6) studies in control animals have shown that motoneurons supplying fast-twitch muscles (e.g., extensor digitorum longus) show higher levels of calcitonin gene-related peptide (CGRP) staining than do motoneurons innervating muscles of slow-twitch fiber type [e.g., soleus (Sol)]. A similar pattern of CGRP expression is observed in the muscle, with CGRP found predominantly at the motor end plates of fast-twitch muscle fibers (23, 24). However, none of these studies has correlated CGRP expression patterns in identified (i.e., retrogradely labeled) motoneurons with motor end plates identified according to muscle fiber type.

Whereas the role of CGRP in the normal adult motor system is not entirely clear, the present framework of evidence suggests that it is associated with presynaptic sprouting and postsynaptic structural changes at the neuromuscular junction (21, 22, 33, 39, 45). Any form of experimental intervention that disrupts the connection between the motor nerve and the neuromuscular junction, either surgically (3, 36) or pharmacologically (39, 45), results in an upregulation of CGRP peptide and/or its mRNA. CGRP expression also increases after spinal cord transection (2, 36) or androgen deprivation (37, 38). To further investigate the role of CGRP in the normal, intact adult neuromuscular system, our approach was to develop a “noninterventional” experimental paradigm, which provided a physiological challenge to the motoneuron and its target. We previously demonstrated that CGRP expression in rat hindlimb motoneurons increased after an acute bout of downhill running exercise in sedentary animals (27). The results showed that CGRP expression remained elevated over a 2-wk period, returning to baseline by 4 wk, in motoneurons of the knee extensors (triceps surae; e.g., muscles performing mostly lengthening contractions while loading) but not in the knee flexors (anterior crural; e.g., muscles performing mostly shortening contractions).

We now identify the responding motoneurons, their fiber type association, and the time course of change in CGRP expression after unaccustomed downhill exercise. Intramuscular injections of FluoroGold were used to retrogradely identify motoneurons supplying the Sol, lateral gastrocnemius (LG), and the proximal (PMG) and distal regions (DMG) of the medial gastrocnemius (MG). Changes between control and experimental groups were quantified by using double-labeling immunofluorescence techniques, identifying the MG as the muscle within the triceps surae with a significant increase in the numbers of CGRP-positive (CGRP+) motoneurons after exercise. Interestingly, in the MG muscle, there was a significant elevation in CGRP levels at fast-twitch glycolytic (FG) motor end plates exclusively.

MATERIALS AND METHODS

Retrograde labeling of motoneurons. To identify the responding population(s) of motoneurons, we used a total of 34 female Wistar rats (250–275 g) for this study. All animals,
with the exception of the animals used in the glycogen depletion study, were given intramuscular injections of 4% FluoroGold (Fluorochrome, Inglewood, CA). Identification of the three-dimensional topographic locations of the Sol, LG, and MG motor nuclei in the spinal cord was completed in a series of retrograde labeling experiments, which have been described previously (27). Briefly, FluoroGold (10 μl) was injected into the belly of the left Sol muscle of 11 animals and into the right LG muscle (15 μl; belly portion) of 9 animals. The pMG contains a mixture of fiber types [10% slow-twitch oxidative (SO), 10% fast-twitch oxidative glycolytic (FOG), 35% FG; cf. Ref. 10], whereas the dMG is composed of FG muscle fibers (80% FG; Ref. 10; Fig. 1). Therefore, in 14 animals, the proximal-medial region of the MG (pMG) of the left leg and the distal-medial region of the MG (dMG) of the right were injected with 15 μl of FluoroGold (Fig. 1). A single injection of tracer was delivered into each muscle with the use of an adapted 100-μl Hamilton syringe (Fisher, Mississauga, ON). PE-20 Silastic tubing was placed onto the syringe needle with the other end of the tubing supporting a 30G1/2-gauge needle (Baxter Canlab, Mississauga, ON) attached to a micromanipulator. Care was taken during these injections to prevent leakage of tracer into other muscles by using a localized microsurgical approach and by using petroleum jelly and tiny gauze pads to isolate each muscle (27). The injection site was then sealed with a drop of cyanoacrylate glue (Baxter Canlab). Three days after the muscle injections, animals were exercised. All experimental procedures were completed according to the Canadian Council on Animal Care Guidelines on the Use of Animals in Research, with ethics approval granted by the Toronto Hospital Animal Care Committee.

**Exercise protocol and tissue processing.** Animals were randomly placed into three groups: control (nonexercise) and 72 h and 2 wk postexercise. These time points were selected based on the results of our previous study (27). Untrained (i.e., sedentary) animals ran continuously on a motor-driven treadmill at a speed of 12 m/min for one 30-min period on a −20° slope. After exercise, runners from each group were returned to their cages where they were given food and water ad libitum.

At the time of death, animals were administered an overdose of pentobarbital sodium (1.0 ml; 60 mg/ml). Before fixation perfusion and under anesthesia, the MG was quickly excised, and the pMG and dMG were separated, sliced into 1-mm-thick cross sections, mounted in optimum cutting temperature embedding media on cardboard, and then snap frozen in a 2-methylbutane (Baxter Canlab) bath immersed in liquid nitrogen. The spinal cord was harvested after transcardial perfusion with 500 ml of 4% paraformaldehyde (pH 7.35–7.45; BDH, Oakville, ON). The lumbar region of the spinal cord (L3–L5) was removed intact, postfixed in 4% paraformaldehyde for 18 h, and cryoprotected overnight in 20% sucrose in 0.1 M phosphate buffer, pH 7.2 (BDH). Tissues were then frozen in 2-methylbutane at −80°C.

**Immunocytochemistry of the spinal cord.** The immunocytochemical methodologies have been previously reported (27) and are briefly described here. Frozen spinal cord lumbar regions (L3–L5) were serially sectioned at 10 μm. In an attempt to reduce interexperimental variability, spinal cord cross sections from each experimental group were placed on the same slide (i.e., control, 72 h, 2 wk). Sections were then washed in 0.1 M PBS, pH 7.1, blocked with 10% normal goat serum (Gibco, Baxter Canlab), and then incubated overnight at 4°C with a polyclonal antibody to CGRP (rabbit anti-CGRP (Rat, 1–37); Genosys, The Woodlands, TX) at a 1:2,000 dilution. Tissues were then processed with the use of the avidin-biotin complex kit with a goat anti-rabbit secondary antibody (Vectastain, Vector, Mississauga, ON) followed by incubation with avidin-conjugated Texas red fluorophore (Vector). Slides were coverslipped with Mowiol.

**Acetylcholinesterase histochemistry and immunocytochemistry of muscle tissue.** Motor end plates were identified by acetylcholinesterase (AChE) histochemistry to determine the innervation pattern and location of neuromuscular junctions in the two regions of MG. Frozen, unfixed muscle tissue was serially sectioned at 12 μm. Three series of samples were collected every 200 μm and placed directly onto slides. The first series was processed for AChE histochemistry, the second series for immunocytochemistry, and the third series for myofibrillar ATPase (myosin ATPase) determination (see Myofibrillar ATPase histochemistry below). Briefly, for AChE histochemistry (34), cross sections on slides were incubated in 20% sodium sulfate (BDH) for 3 min followed by a wash in deionized water, incubated in reaction solution [pH 7.2; 5-bromoindoxyl acetate, ethanol, K3Fe(CN)6, K4Fe(CN)6, 3H2O, Tris-HCl, Tris base, and CaCl2; Sigma Chemical] for 15 min, washed in deionized water, quickly dipped in eosin (Sigma Chemical), and then defatted and coverslipped with Entellan (BDH).

For immunocytochemistry, frozen serial sections from unfixed muscle tissue were collected on slides as described above. Slides were washed in 0.1 M PBS, pH 7.1, and immersed in 4% paraformaldehyde fixative (pH 7.4; BDH) for 30 min. Slides were then washed in 0.1 M PBS, pH 7.1, blocked with 10% normal goat serum (Gibco, Baxter Canlab), and incubated overnight at 4°C with the CGRP antisera at a 1:1,000 dilution. Tissue sections were processed by using the avidin-biotin complex kit with a goat anti-rabbit secondary antibody (Vectastain, Vector) followed by incubation with avidin-conjugated Texas red fluorophore (Vector). After a washing in 0.1 M PBS, the tissues were incubated overnight in fluorescein-
**Glycogen study: tissue sampling and analysis.** To evaluate whether the exercise protocol may have preferentially activated the pMG or the dMG, we examined the pattern of glycogen depletion in both regions of the muscle after downhill running. Two groups of animals were divided into control (n = 4) and downhill runners (n = 4). The running group completed the exercise protocol and was killed 20 min later. The MG (2 per animal) were quickly dissected out and snap frozen as described above. Frozen control and exercise pMG and dMG muscle were serially sectioned, placed on the same slide, histochemically analyzed for glycogen content using the periodic acid Schiff (PAS) stain (14), and placed on separate slides for myosin ATPase.

**Myofibrillar ATPase histochemistry.** Muscle fibers were identified and classified as SO, FOG, and FG from the ATPase stain (8). Briefly, frozen muscle sections mounted on slides were placed in coplin jars containing acid medium (NaC₂H₃O₂ and KCl, pH 4.6; BDH) for 4 min, washed in basic medium (C₂H₅NO₂, CaCl₂, NaCl, and NaOH, pH 9.4; BDH) for 30 s, and placed in incubation medium (basic medium + ATP, pH 9.4, 30 min, 37°C; Sigma Chemical), followed by a series of washes in CaCl₂ (BDH), rinses in CoCl₂ (Fisher), and rinses in distilled H₂O, ending with a 1-min incubation in 20% (NH₄)₂ (Fisher). Slides were then cleared and coverslipped with Entellan (BDH).

**Quantitation of CGRP+ motoneurons and motor end plates.** The quantitation of CGRP+ motoneurons and motor end plates was completed by using a Leica DM/DR fluorescence microscope. Counts of motoneurons staining positively for both CGRP and FluoroGold were obtained by selecting only those somata with observable nuclei; profiles not containing a nucleus or nucleolus were not quantitated (cf., Ref. 46). The motor nuclei of the Sol, LG, pMG, and dMG were topographically located in the medial-ventral region of the spinal cord, beginning at the L₄ ventral root entry zone and extending rostrally ~2,000 µm (27). FluoroGold-labeled motoneurons exhibited cell bodies and dendrites filled with granules of bright gold fluorescence (Fig. 2C). CGRP-Texas red immunofluorescence was characterized as cytoplasmic and punctate, with the fluorescent granules preferentially located in the soma and in the proximal parts of the major dendrites (Fig. 2D). All motoneurons with FluoroGold labeling were counted, and their CGRP+ or CGRP-negative (CGRP−) status was determined. MG muscles injected with Fluorogold were analyzed for Fluorogold content by examin-

**Table 1. Number of muscle fibers analyzed per fiber type in the proximal and distal regions of MG for glycogen depletion studies**

<table>
<thead>
<tr>
<th>Region of MG</th>
<th>Fiber Type</th>
<th>No. of Animals</th>
<th>No. of Fibers Analyzed per Animal</th>
<th>Average No. of Fibers per Cross Section</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal</td>
<td>ST</td>
<td>8</td>
<td>100</td>
<td>2,900</td>
</tr>
<tr>
<td></td>
<td>FOG</td>
<td>8</td>
<td>100</td>
<td>2,900</td>
</tr>
<tr>
<td></td>
<td>FG</td>
<td>8</td>
<td>100</td>
<td>2,900</td>
</tr>
<tr>
<td>Distal</td>
<td>FG</td>
<td>8</td>
<td>500</td>
<td>4,000</td>
</tr>
</tbody>
</table>

MG, medial gastrocnemius; ST, slow-twitch; FOG, fast-twitch oxidative glycolytic; FG, fast-twitch glycolytic.
ing the center of the muscle at the midline to determine whether diffusion had occurred as a result of the injection (27).

CGRP staining at the motor end plate was evaluated in 12 animals that were randomly divided into three groups: control and 72 h and 2 wk postexercise (Fig. 3). Motor end plates were identified in transverse section by AChE staining. Based on the results from a separate series of experiments in which we evaluated AChE-stained longitudinal muscle sections taken from the belly of the MG (see MATERIALS AND METHODS), we were able to determine that the average length of a MG motor end plate was ~200 μm. This method permitted us to establish a sampling distance within the muscle region that would not result in duplicate counts of motor end plates that were evaluated for α-BuTx. In subsequent adjacent sections, motor end plates were identified with α-BuTx fluorescence by using a blue filter (525 nm; 10× PL FLUOTAR objective) for fluorescein detection, followed by a green filter (625 nm; 100×/1.25 N Plan Oil) for detecting Texas Red immunofluorescence, to colocalize the CGRP+ signal. CGRP immunofluorescence was clearly detected as a punctate staining pattern visible in regions of the junctional folds (see Fig. 6D), overlapping the α-BuTx labeling that filled the junctional area (see Fig. 6C). Approximately 140 end plates were quantitated per muscle sample (Table 2). Slides with CGRP+ motor end plates were then compared with adjacent serial sections stained for myosin ATPase to determine the fiber type of the identified neuromuscular junction.

Importantly, all tissue analyses were done blinded to the experimental condition throughout all the procedures described in this study. The identity of the experimental groups was subsequently decoded after completion of data collection to permit statistical analysis. Statistical significance was determined by Student’s t-test, ANOVA, and, where necessary, the Kruskal Wallis test for nonparametric distributions (SigmaStat version 1.0, Jandel Scientific). Data are presented as means ± SE. Differences were considered to be significant at P < 0.05.

RESULTS

CGRP response in retrogradely labeled motoneurons. FluoroGold-labeled motoneurons within the motor nuclei of the Sol, LG, pMG, and dMG were readily identified under ultraviolet light (Fig. 2). Double-labeled Texas red-conjugated CGRP+ cells had a punctate, red staining pattern (Fig. 2D), making this easily discernible from the more homogeneous, finely granular, white FluoroGold signal (Fig. 2C). Motoneurons that were labeled with FluoroGold and identified as “CGRP−” were stunningly clear in their lack of CGRP (Fig. 2B). Numbers of double-labeled motoneurons in the identified Sol motor nucleus did not change after downhill exercise over the experimental time period (Fig. 3A). A similar observation was true for the double-labeled motoneurons of the LG motor nucleus (Fig. 3B).

In both regions of the MG, however, downhill exercise resulted in a significant increase in the number of double-labeled CGRP+ motoneurons 72 h after exercise compared with control (pMG: P = 0.003; dMG: P = 0.03; Fig. 3C). Significant differences in the number of CGRP+ motoneurons were not observed between control and 2 wk postexercise groups in pMG or dMG (P > 0.05), indicating that CGRP expression in these motoneurons had returned to baseline levels by this time and confirming our previous results (27).

Glycogen depletion experiments. Based on our observation of the enhanced expression of CGRP in MG

Table 2. Total number of motor end plates quantified in the proximal and distal regions of the MG in the control and experimental groups

<table>
<thead>
<tr>
<th>Region of MG</th>
<th>No. of Animals</th>
<th>Total No. of FITC-α-BuTx End Plates</th>
<th>Total No. of CGRP+ Motor End Plates</th>
<th>Fiber Type of CGRP+ End Plates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ST</td>
</tr>
<tr>
<td>Proximal</td>
<td>12</td>
<td>1,700</td>
<td>150</td>
<td>1</td>
</tr>
<tr>
<td>Distal</td>
<td>12</td>
<td>1,700</td>
<td>400</td>
<td>0</td>
</tr>
</tbody>
</table>

FITC-α-BuTx, fluorescein-conjugated α-bungarotoxin; CGRP+, calcitonin gene-related peptide positive.
motoneurons, it was of interest to ascertain whether both pMG and dMG were equally recruited by the downhill running protocol. Differences in the activity patterns of glycogen depletion would permit us to qualitatively interpret the physiological state of the muscle. Therefore, the glycogen content of both pMG and dMG was determined by PAS histochemistry (Fig. 4). Relative optical density measurements (Fig. 5) showed that both regions and all fiber types in the pMG (SO, \( P = 0.00002 \); FOG, \( P = 0.0002 \); FG, \( P = 0.0005 \)) and dMG (FG, \( P = 0.0006 \)) were significantly depleted of glycogen stores after this eccentric exercise paradigm. While indicating that both pMG and dMG were active, these results did not allow us to quantitate the relative amounts of glycogen breakdown in the different muscle fiber types.

CGRP response at motor end plates in the pMG and dMG after eccentric exercise. When viewed in transverse section, the motor end plates in the MG were easily identified by FITC-\( \alpha \)-BuTx staining as they followed a visible pattern throughout the belly of the muscle. The junctional folds of the end plate region were consistently and clearly labeled with bright green FITC-\( \alpha \)-BuTx (Fig. 6). Texas red CGRP immunofluorescence staining was similar to that observed in the motoneuron, presenting with an irregular, punctate distribution that overlapped portions of FITC-\( \alpha \)-BuTx immunoreactivity in the junctional folds (Fig. 6C). This distinction in staining pattern made it easy to distinguish between CGRP\(^+\) and CGRP\(^-\) end plates and to identify end plates that were not CGRP\(^+\) as being affected by bleed through of the \( \alpha \)-BuTx signal. Interestingly, CGRP\(^+\) end plates were observed most often in regions in which clusters of neuromuscular junctions were found, although not all neuromuscular junctions within a cluster were CGRP\(^+\) (Fig. 6D), with the majority being CGRP\(^-\) (Fig. 6B).

Elevated numbers of immunofluorescent CGRP\(^+\) motor end plates were observed in both pMG and dMG after downhill exercise. In pMG, a mixed fiber type region, a significant increase in the numbers of CGRP-immunoreactive neuromuscular junctions was observed at 72 h postexercise (10%; \( P = 0.03 \)) and re-

---

**Fig. 4.** Photomicrographs of myosin ATPase and periodic acid Schiff (PAS)-stained muscle fibers from the proximal region of the MG describing the glycogen depletion pattern before and after downhill exercise. Frozen serial cross sections of the MG stained for myosin ATPase (A and C) and PAS (B and D) in the control condition (B) and 20 min after downhill running exercise (A, C, and D). ST, slow-twitch fibers. Calibration bars, 50 \( \mu \)m.

**Fig. 5.** Glycogen depletion profiles of ST, FOG, and FG muscle fiber types in the pMG (A) and dMG (B) 20 min postexercise, as indicated by the relative optical density (ROD) measurement. *Significant difference compared with control, \( P < 0.001 \) (t-test).
mained significantly elevated 2 wk later (9.9%, \(P = 0.03\); Fig. 7B). In the dMG, composed entirely of FG fibers, a 25% increase in the number of CGRP\(^+\) motor end plates compared with control was observed at 72 h postexercise (\(P = 0.002\)) (Fig. 7B). Interestingly, this percentage continued to be significantly elevated in the dMG compared with control, increasing to 33% by 2 wk after exercise.

CGRP immunoreactivity and muscle fiber type. Analyses of serial cross sections of pMG and dMG stained histochemically for myosin ATPase content showed that CGRP\(^+\) motor end plates colocalized almost exclusively to FG muscle fibers (Fig. 8). Out of ~3,000 end plates examined in this study, CGRP\(^+\) end plates at FOG muscle fibers and SO fibers were rarely observed (Table 2).

**DISCUSSION**

We previously reported (27) that one 30-min bout of downhill running resulted in increased numbers of CGRP\(^+\) motoneurons in hindlimb extensor but not flexor motor nuclei. Those results were the first to demonstrate increased CGRP levels as a result of physiological neuromuscular activity rather than a lack of activity, i.e., as induced by surgical or pharmacological paralysis or by hormone deprivation. Our present report demonstrates that CGRP expression was exclusively elevated in MG motoneurons and that, within the muscle, this response was specifically localized to motor end plates on FG muscle fibers.

The increased CGRP levels in MG motoneurons, and subsequently at motor end plates in the muscle, could be due to a variety of factors. For example, the exercise regime may result in frank histopathological damage to the muscle, thereby initiating repair and regenerative mechanisms that would require new end plates on de novo myofibrils. Alternately, unaccustomed exercise may induce growth-related morphological alterations within the individual myofibrils and subsequently at their neuromuscular junctions, leading to growth of the motor end plates. A more subtle process could be that the particular demands of downhill exercise may differentially affect muscle fibers within a motor unit, leading to remodeling events at the affected neuromuscular junctions.
Downhill running produces changes in MG motoneurons in the absence of muscle damage. Although a detailed biomechanical analysis of downhill running in rats has not yet appeared in the literature, in this downhill running model the ankle flexors are assumed to be performing concentric exercise (shortening while loaded), whereas the ankle extensors are similarly assumed to undergo eccentric contractions (lengthening while loaded). Previous studies that have used chronic and/or extended bouts of downhill running activity in rats report SOL muscle damage with indexes of pathology clearly observed 3–5 days postexercise (1, 32). Although Smith et al. (42, 43) detected the formation of new muscle satellite cells in the rat SOL after downhill running, followed by significant increases in developmental myosin isoforms and the appearance of new myofibrils (42), neither the MG nor the LG showed any significant trends, thus making the need for denovo end plates in the MG appear unlikely. Because there is no significant histopathology or inflammation in the MG, compared with the SOL, after either chronic or acute eccentric exercise protocols (27, 42), it seems unlikely that the changes in CGRP levels we reported to cannot be attributed to myofibrillar damage and repair processes. In addition, the relative lack of baseline CGRP immunoreactivity in SOL motoneurons and the absence of change in CGRP levels in this slow-twitch muscle after exercise (despite the histopathological data) argue against the idea that CGRP may be related to extensively used or easily recruited (e.g., SOL) motoneurons (4). Our results, however, do support the view that motoneurons innervating FG fibers (e.g., larger and faster motor units (5, 25)) contain more CGRP.

CGRP and sprouting at the neuromuscular junction. Sprouting of the motor nerve terminal in the adult has been correlated with changes in CGRP peptide and mRNA expression in motoneurons after either a significant injury to the motor nerve or a substantial pharmacological interruption of neuromuscular connectivity (3, 36, 39, 45). In our study, however, no damage is incurred by the MG motor nerve, and overt muscle damage is absent. Furthermore, it is unlikely that the intramuscular injection of FluoroGold produced a nerve injury that initiated a sprouting response and an upregulation in motoneuronal CGRP expression. Data from our first study demonstrated elevated numbers of CGRP+ motoneurons after eccentric exercise; these muscles were not injected with FluoroGold (27). Other investigators have also reported no changes in CGRP immunoreactivity and α-CGRP mRNA in the bulbo cavernous muscle in sham and vehicle-treated groups with the use of a multiple injection protocol (38).

Chronic exercise has been shown to effect morphological changes at the neuromuscular junction (12, 13, 48, 49), in motoneurons, and in axons (16). It seems unlikely to us that a single 30-min bout of downhill running would elicit a measurable sprouting response; whether chronic exercise elicits a sustained CGRP response and growth or sprouting at the neuromuscular junction remains to be examined.

A neuromodulatory role for CGRP at the motor end plate after exercise. Perhaps the elevated expression of CGRP in MG motoneurons and their end plates on FG muscle fibers may be correlated with more subtle remodeling events at the neuromuscular junction. Apart from its “fast” neurotransmitter-like actions on the nicotinic acetylcholine receptor (AChR), CGRP also exerts longer lasting trophic functions at the neuromuscular junction, mediated by specific CGRP receptors localized to the postsynaptic membrane (reviewed in Ref. 4). In cultured chick myotubes, CGRP has been shown to upregulate the appearance, number, and insertion of nicotinic AChRs into the muscle membrane (22, 31). Other in vitro studies have shown that CGRP increases the mRNA coding for the stabilizing α-subunit in the AChR complex (21, 22, 33). Whether CGRP elicits such changes at the adult neuromuscular junction has not yet been examined.

The modulation of synaptic efficiency may also involve changes in the relative proportions of AChE enzymes. One of the subunits, the asymmetric form G4, located at the neuromuscular junction, is involved in the fast clearance of ACh at junctional receptors (20) and is known to be upregulated by chronic treadmill exercise (26, 28) with the greatest response observed in fast-twitch muscles (18, 26). Mouse myotubes treated with CGRP displayed a 2.5-fold increase both in (G4) AChE and in AChR α-subunit mRNA, further implicating CGRP as a trophic factor regulating the gene expression of integral postsynaptic molecules at the intact adult neuromuscular junction (7). Intramuscular injection of exogenous CGRP, however, has been reported to reverse the exercise-induced increase in G4.

Fig. 8. CGRP immunoreactivity is present at motor end plates of type IIB muscle fibers in the MG after downhill exercise. A: muscle cross section from the pMG identified as a type IIB muscle fiber by myofibrillar ATPase histochemistry. Serial cross section identifying a FITC-α-BuTx-labeled neuromuscular junction that is CGRP+ (B) as detected by Texas red immunofluorescence (C). Calibration bars: 30 μm (A) and 10 μm (B and C).
in rat gracilis muscle (19). Whether CGRP regulates $G_4$ AChE and AChR subunit expression at the neuromuscular junction after physiological exercise is not known.

**Elevated CGRP expression as a function of motor unit recruitment.** Both the LG and MG motor nuclei have equivalent baseline levels of CGRP: 60% of motoneurons are CGRP+ in the sedentary animal. The fact that there are no changes in CGRP expression in LG motor nuclei after downhill running may suggest a subtle effect of this exercise paradigm on the MG that is not elicited in the LG. There is evidence to suggest that the LG is less active than the MG during locomotion (15, 44). Studies by Duyssens et al. (15) describe the reduced activation of the LG in humans during walking or running, whereas Smith and Carlson-Kuhtas (44) observed a similar lack of LG activation in felines during slope walking. The functional or biomechanical implications of our findings need to be more rigorously investigated using electrophysiological techniques.

The rat MG is a highly compartmentalized muscle with distinct fiber type distributions. Morphologically, the pMG, innervated by the proximal and lateral branches of the MG nerve, is a mixture of slow- and fast-twitch fibers (SO, FOG, FG), whereas the distal region innervated by the distal branch of the MG nerve is exclusively FG (9, 47). These compartments appear to be preferentially activated in the performance of different motor tasks (11, 47). There is growing evidence to suggest that the complex morphological structure (i.e., compartmentalization or regionalization) of a muscle influences muscle fiber properties and intramuscular activity (reviewed in Ref. 29). Our PAS staining describes glycogenolysis in all fiber types of both MG regions, suggesting that the expression of CGRP in FG fibers reflects a difference in the activity response of this fiber type within the MG after downhill running. Perhaps the unaccommodated activity in our model preferentially perturbs neuromuscular junctions in FG fibers because of their higher susceptibility to transmission failure (41). This altered use may then initiate morphological adaptation and repair of the affected neuromuscular junctions for which CGRP is required (39).

Based on human studies, motor control strategies for eccentric work (e.g., downhill running) are suggested to differ from those of concentric work (e.g., predominantly uphill running) in that fast- rather than slow-twitch motor units are recruited first (17, 30). To date, motor unit recruitment strategies for downhill running have not yet been addressed in animal models. To determine whether enhanced CGRP expression at FG motor end plates is associated with selective recruitment of fast-fatiguable or fast-fatigue-resistant motor units, intracellular recordings from identified motoneurons (cf., Refs. 10, 40) will need to be done.

In conclusion, we have shown that, after downhill running in the rat, CGRP expression is elevated in MG motoneurons and at MG motor end plates on FG muscle fibers. Our results may indicate a preferential response of FG fibers after unaccustomed exercise, resulting in synaptic reorganization. This model provides a novel system in which to further investigate whether physiological exercise, specifically downhill exercise, preferentially recruits FG motor units and whether exercise-induced increases in CGRP expression play a role in the remodeling of the postsynaptic junction in the intact, adult neuromuscular system.

**REFERENCES**


