The Journal of Neuroscience, May 1990, 10(5): 1444–1454

Regulation by Exercise of the Pool of G_4 Acetylcholinesterase Characterizing Fast Muscles: Opposite Effect of Running Training in Antagonist Muscles

Bernard J. Jasmin[1,a] and Victor Gisiger[2]

[1]Sciences de l'Activité Physique, [2]Département d'Anatomie et Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Québec, Canada H3C 3J7

Fast muscles of rodents characteristically differ from their slow-twitch counterparts by exhibiting high levels of G_4, i.e., the tetrameric acetylcholinesterase (AChE) molecular form. Converging evidence suggests that this additional G_4 pool is specifically regulated by the type of activity actually performed by the muscle. This hypothesis was tested by studying the effect of a chronic increase in neuromuscular activity on the AChE content and distribution of molecular forms of functionally antagonist rat hindlimb muscles. They included the fast ankle extensors gastrocnemius (GAST) and plantaris (PL), the fast ankle flexors tibialis anterior (TA) and extensor digitorum longus (EDL), as well as the slow-twitch soleus (SOL). Neuromuscular activity was enhanced by subjecting the rats to a 12-week training program consisting of repeated sessions of prolonged endurance running on a rodent treadmill. This exercise regimen preferentially affected the G_4 pool characterizing fast muscles which exhibited marked and opposite changes according to the functional role of the muscles. The amount of G_4 was increased by more than 50% in the ankle extensors GAST and PL, which play a dynamic role, and reduced by about 40% in the ankle flexors TA and EDL, which exhibit a predominant tonic activity during running. The asymmetric forms A_{12} and A_8 were slightly elevated in the fast muscles. In the case of the slow-twitch SOL, running training resulted in a small, nonspecific decrease in AChE content which affected most of the molecular forms. These data indicate that the size of the G_4 pool characteristic of fast muscles depends on the type, dynamic or tonic, of activity actually performed. The present results support the conclusion that this G_4 pool fulfills a specific and essential function, distinct from that of A_{12}.

Muscle acetylcholinesterase activity (AChE; EC 3.1.1.7) has been shown to be significantly altered following chronic modifications of neuromuscular function using models entailing intact motor innervation, such as prolonged endurance running training (Crockett et al., 1976; Gardiner et al., 1982a), limb immobilization (Snyder et al., 1973; Butler et al., 1978; Gardiner et al., 1982a), hindlimb suspension (Gupta et al., 1985), and compensatory hypertrophy (Guth et al., 1966; Granbacher, 1971; Snyder et al., 1973). Insights into the regulatory mechanisms presiding over muscle AChE plasticity have been provided by studies that took into account that AChE is a polymorphic enzyme. AChE exists as a family of molecular forms that corresponds to a series of oligomers of diverse structural complexity.[3] The various molecular forms are located at different subcellular sites and are subject to separate regulations, suggesting that they fulfill distinct physiological functions (Massoulié and Bon, 1982; Brimijoin, 1983; Toutant and Massoulié, 1987). The best-documented case is that of the asymmetric forms A_{12}, A_8, and A_4, which have been identified as the synaptic molecular forms. In muscles of rodents, the asymmetric forms are preferentially located at the neuromuscular junction where they are believed to fulfill the only function recognized so far for AChE, i.e., the rapid termination of the action of ACh on junctional cholinergic receptors.

A series of studies recently performed in rodents (rat, mouse, and rabbit) has revealed that G_4 is closely related to the dynamic state of the skeletal muscle. Indeed, whereas slow-twitch muscles contain only a minimal amount of tetramer, fast muscles characteristically exhibit high levels of G_4 (Bacou et al., 1982; Gisiger and Stephens, 1982, 1982–1983, 1983; Groswald and Dettbarn, 1983). Furthermore, according to a recent report (Gisiger and Stephens, 1988), this additional pool is concentrated at the perijunctional sarcoplasmic reticulum, where it forms a cojunctional compartment embedding the neuromuscular junctions in an AChE-rich environment. These facts are consistent with the view that the additional pool of G_4 plays a specific role, distinct from that of A_{12}. The nature of this function, however, remains to be elucidated.

Nevertheless, converging lines of evidence strongly suggest that the G_4 pool characterizing fast muscles bears an important physiological significance: (1) fast muscles definitively acquire

Received April 25, 1989; revised Nov. 8, 1989; accepted Nov. 14, 1989.

We are especially grateful to Dr. P. F. Gardiner for his support, stimulating discussions, and helpful comments on the manuscript. This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Fonds pour la Formation de Chercheurs et l'Aide à la Recherche (FCAR) (Team Grant). B.J.J. was the recipient of a postgraduate scholarship from NSERC.

Correspondence should be addressed to Dr. Victor Gisiger, Département d'Anatomie, Faculté de Médecine, Université de Montréal, C.P. 6128, Succursale A, Montréal, Québec, Canada H3C 3J7.

[a] Present address: Laboratoire de Biologie Cellulaire des Membranes, C.N.R.S., Institut Jacques Monod, Université Paris VII, Tour 43, 2 Place Jussieu, 75251 Paris Cedex 05, France.

[3] Six AChE oligomeric states have been characterized to date: 3 globular (monomer G_1, dimer G_2, and tetramer G_4) and 3 asymmetric forms (A_4, A_8, and A_{12}), which consist of 1, 2, or 3 tetramers attached to a collagen-like tail (Bon et al., 1979; Massoulié and Bon, 1982).

their characteristic G_4 pool only at the end of the postnatal maturation period of the neuromuscular system (Brzin et al., 1981; Skau, 1983; Yeakley et al., 1987); (2) in murine muscular dystrophy (Skau and Brimijoin, 1981; Gisiger and Stephens, 1983) or motor endplate disease (Rieger et al., 1983; Yeakley et al., 1987), the AChE content of fast muscles is specifically changed into that of the slow type, characterized by a low level of G_4, while the synaptic form A_{12} is only marginally affected; (3) imposing a fast stimulation pattern on denervated rat soleus induces a shift of its slow AChE content toward that typical of fast muscles (Lomo et al., 1985); (4) the AChE activity of the soleus muscle cross-innervated with a fast motor nerve, after exhibiting a transitory increase, returns to a low level typical of the normal soleus (Müntener and Zenker, 1986); (5) chronic elevation of the level of natural muscle activity, as achieved by a mild exercise program, selectively affects the pool of G_4 characteristic of fast muscles (Fernandez and Donoso, 1988). The ensemble of these facts has led to the proposal that the G_4 content of a muscle depends more on the type of activity it actually performs than on the influence of its motor nerve (Gisiger and Stephens, 1988).

In the present study, we tested this hypothesis by examining the effect of a chronic elevation in motor activity on the content in AChE molecular forms of functionally antagonist rat muscles. Neuromuscular activity was enhanced by an endurance running training program which meets the requirement for preservation of the integrity of the neuromuscular system when studying the regulation of the G_4 pool characteristic of fast muscles. A brief account of this work has been reported previously (Jasmin et al., 1989).

Materials and Methods

Animal care and training program. Female Sprague-Dawley rats (180–200 gm) were obtained from Charles River (St.-Constant, Québec). The animals were housed in groups of 5 in a temperature-controlled room at 20°C and maintained on a 12 hr/12 hr light-dark cycle. They were provided with water and food (Purina rat chow) *ad libitum*. One week following arrival in our animal facilities, a group of rats was randomly selected to undergo an endurance running training program. These rats were exercised on a motor-driven treadmill (Quinton Instruments, Seattle, WA) for approximately 12 weeks. The training program consisted of running 3 d out of 4, at a speed of 27 m/min with a 10% grade. Initially, rats ran for 10 min. Running time was progressively increased by 10 min every fourth day so that by the seventh week, the animals ran 120 min per exercise session. Control rats were cage-confined.

Tissue preparation and homogenization. Twenty-four hours following the last training session, trained rats and their controls were anesthetized with sodium pentobarbital (35 mg/kg, i.p.) and the following 5 muscles were removed: gastrocnemius (GAST), plantaris (PL), soleus (SOL), extensor digitorum longus (EDL), and tibialis anterior (TA). Upon excision, the muscles were immediately frozen in melting isopentane precooled with liquid N_2 and subsequently kept at −80°C.

Whole frozen muscles were later homogenized in 2.5 ml (PL, SOL, and EDL), 5 ml (TA), or 10 ml (GAST) of the following high-salt, detergent buffer containing antiproteolytic agents: 10 mM Tris-HCl, pH 7.0; 10 mM EDTA; 1 M NaCl; 1% Triton X-100; 1 mg/ml bacitracin (Sigma); 25 units/ml aprotinin (Sigma) (Lomo et al., 1985). Two different techniques of homogenization were used during the course of this study, with no significant difference in yield of total AChE activity and distribution of molecular forms. In one procedure, the frozen muscles were crushed in a mortar cooled with liquid N_2, and the powder was subsequently homogenized in a Teflon-glass homogenizer kept on ice. In the second procedure, the muscles were homogenized on ice with a Polytron, at a setting of 6, for 30–75 sec, depending on the muscle's size. Supernatants, obtained by low-speed centrifugation (20,000 × *g* for 15 min), were kept at −80°C until further analysis.

Biochemical analysis. AChE activity was measured, as previously described (Gisiger and Stephens, 1988), by the spectrophotometric method of Ellman et al. (1961) in the presence of 10^{-5} M of the non-specific cholinesterase inhibitor tetraisopropylpyrophosphoramide (iso-OMPA) (Sigma). Citrate synthase (EC 4.1.3.7) activity was assayed according to Srere (1969). Protein concentration of the muscle extracts was determined using the method of Lowry et al. (1951).

Sedimentation analyses of AChE molecular forms were performed by ultracentrifugation of 50- or 100-μl aliquots of the muscle extracts, loaded on 5–20% sucrose gradients. The gradients were made up in a buffer identical to that used for homogenization except for these modifications: EDTA and aprotinin were omitted, while 50 nM $MgCl_2$ was added. The muscle extract samples were centrifuged at 4°C for 21 hr at 40,000 rpm in a Beckman SW 41 rotor. Approximately 45 fractions were collected from the gradients and assayed for AChE activity as described above. The molecular forms of AChE were identified according to the nomenclature of Bon et al. (1979) on the basis of their apparent sedimentation coefficients, as determined by comparison with 3 standards (Gisiger and Stephens, 1983).

The extraction methods and the homogenization buffer used in this study were those found to be optimal during our preliminary assays. As indicated previously (Gisiger and Stephens, 1984), the criteria for optimal quality of extraction were 2-fold: highest amounts of heavy molecular forms and the best yield of overall AChE activity. These criteria take into account that some degree of proteolysis during preparation of the AChE samples can never be completely excluded.

Analysis of the data. In order to adequately evaluate the significance of AChE variations induced by training, the muscle content in AChE molecular forms was expressed as absolute activity per muscle rather than in relative percentage of total activity. The activity per muscle of the molecular forms was computed from the total AChE activity per muscle, as determined from an aliquot of the total extract and from the proportions of the forms obtained from the sedimentation pattern. In order to accurately measure the proportions of the 3 major forms, A_{12}, G_4, and G_1, we simultaneously estimated the contribution to the sedimentation profile of the 2 minor forms, A_8 and G_2. Indeed, separate analyses of the asymmetric and globular forms, performed in mouse (Gisiger and Stephens, 1983) as well as rat (Gisiger, unpublished results), have shown that both fast and slow muscles always contain significant amounts of A_8 and G_2, even when the presence of these forms is completely masked by dominant peaks of G_4 and G_1. By contrast, the contribution of A_4 never exceeds 5% of the muscle AChE content and therefore was neglected. The activity of a form was determined by summing the enzymatic activities of its peak. In the regions where the peaks overlapped, the activities of the fractions were corrected by taking into account that the shape of the peaks is Gaussian. This adjustment yielded proportions of A_8 and G_2 that were in good agreement with those measured after complete separation of these minor forms (Gisiger and Stephens, 1983; V. Gisiger, unpublished observations). Calculations of baseline as well as proportions of the 5 AChE molecular forms were performed using a microcomputer program previously developed (Gisiger and Stephens, 1988) from an algorithm adapted from that proposed by Dreyfus et al. (1984). The gradients illustrated in the figures were drawn by a plotter using SAS procedures.

Statistical analysis was performed between group means using 2-tailed Student's *t*-test. The level of significance was selected at the 0.05 level of confidence.

Results

The effect of the prolonged increase in neuromuscular activity on AChE and its molecular forms has been studied in 5 different rat hindlimb muscles. They included 4 predominantly fast muscles, i.e., GAST, PL, TA, and EDL (Buchthal and Schmalbruch, 1980; Armstrong and Phelps, 1984), as well as the slow-twitch SOL (Luff, 1981). On a functional basis, these 5 muscles belong to 2 antagonist groups: in the running exercise, GAST, PL, and SOL act as ankle extensors while TA and EDL operate as ankle flexors (Rasmussen et al., 1978; Gruner et al., 1980). In addition, all 5 muscles are easy to remove in their entirety. This property, which allows precise determination of AChE activity per muscle, made it possible to estimate the absolute amounts of the AChE molecular forms present in each muscle.

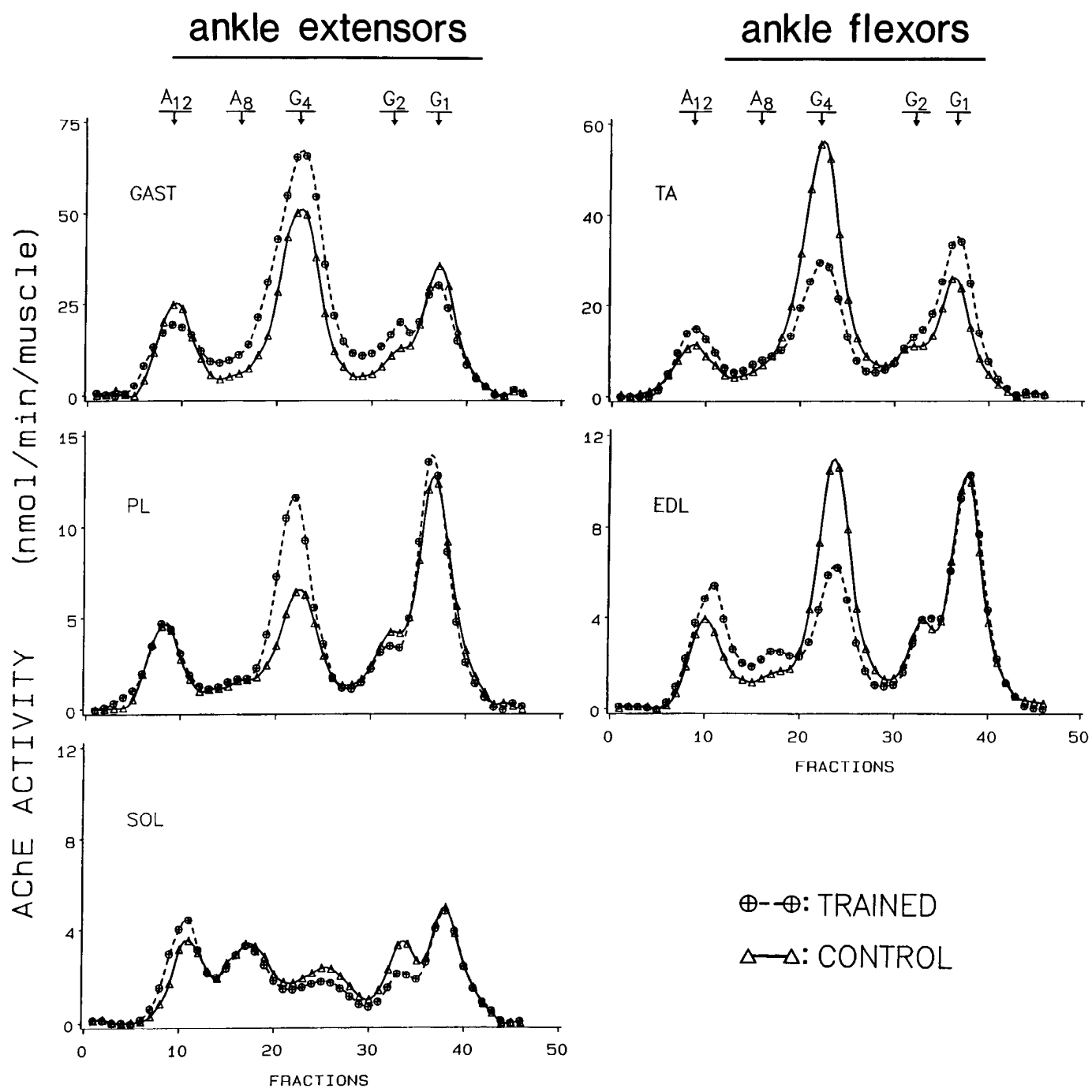


Figure 1. Effect of endurance running training on the content in AChE molecular forms of antagonist muscles of the rat hindlimb. Shown are actual AChE distributions that are representative of the average content in AChE molecular forms observed in 8–12 samples of each control and trained muscle (see Tables 2, 3). The distribution of the molecular forms expressed as activity per muscle was computed from the overall AChE activity per muscle and the sedimentation profile. The AChE distribution of SOL is represented at the same scale as that of EDL, which exhibits similar amounts of A_{12}. Extractions, sedimentation analyses, and measurements of AChE activity were performed as indicated in Materials and Methods. The AChE molecular forms were identified according to Bon et al. (1979) on the basis of their apparent sedimentation coefficients. *GAST,* Gastrocnemius; *PL,* plantaris; *SOL,* soleus; *TA,* tibialis anterior; *EDL,* extensor digitorum longus.

Control muscles

AChE analysis of muscles taken from cage-confined rats (Fig. 1; Table 3) clearly confirmed the characteristic divergence in muscle AChE content according to the contractile properties which has been described in several species (Bacou et al., 1982; Gisiger and Stephens, 1982, 1982–1983, 1983; Groswald and Dettbarn, 1983; Lomo et al., 1985). SOL exhibited the specific molecular form distribution that differentiates the mammalian slow-twitch muscle from its fast-twitch counterparts, i.e., low G_4 and increased A_8 proportion relative to A_{12}.

The AChE profiles obtained from several samples of a given rat muscle varied markedly from each other (Fig. 2, left). This fluctuation sharply contrasted with the remarkable reproduc-

Table 1. Citrate synthase activity of control and trained muscles

	GAST	PL	SOL	TA	EDL
Control	199.46	157.44	182.64	213.61	165.98
	±22.76	±29.80	±18.64	±17.02	±19.05
	(10)	(5)	(7)	(6)	(8)
Trained	274.41*	216.99*	252.52*	232.43	224.12*
	±34.25	±7.28	±46.29	±32.42	±48.35
	(7)	(6)	(10)	(12)	(7)

Activities are given in nmol/min × mg^{-1} protein, together with the number of muscles examined (n). Values are $\bar{x}$ ± SD; asterisks denote statistically significant differences between group means ($p < 0.02$, 2-tailed).

ibility of the AChE patterns exhibited by the muscles of the 129/ReJ mice strain (Gisiger and Stephens, 1983). The extent of the differences is illustrated in Figure 2, left. In addition to the average profile, Figure 2 shows the most as well as the least active gradients that have been observed for each muscle. The other samples produced intermediate AChE patterns ranging from one extreme to the other. For the sake of the clarity of the presentation, the profiles with highest, mean, and lowest activities were arbitrarily designated as "high," "average," and "low," respectively (Fig. 2). Several facts make it unlikely that the variations of the AChE profiles resulted from degradation of AChE molecules during the experimental process. First, in most cases, the variations selectively affected G_4 (Fig. 2, left) and this occurred without concomitant changes in proportions of G_1, as would be expected from artifactual alterations of the forms (proteolysis, etc.). As shown in Figure 2, left, these variations included significant differences in absolute amounts of G_4. Consequently, the variations of G_4 in a given muscle were generally paralleled by corresponding changes in both total AChE activity per muscle (Fig. 2, left) and overall specific activity. Second, whatever the G_4 level, the distributions as well as the activities of AChE molecular forms exhibited by a pair of contralateral muscles taken from the same rat were very similar (see, for example, *GAST*, Fig. 3). Third, a close examination of all AChE analyses of control muscles revealed a general trend. Indeed, we found a definite tendency for all the 4 fast muscles taken from the same rat to share the same type of G_4 level. For example, among the cases illustrated in Figure 2, left, GAST, PL, and TA exhibiting the lowest G_4 content originated from a single rat. The same was true in the case of TA and EDL with average G_4 as well as for GAST, TA, and EDL with the highest G_4 levels. Taken together, these facts suggest that the diversity in AChE profiles observed among samples of a given fast muscle corresponds to genuine differences. SOL also exhibited variations in its AChE profile (Fig. 2, left). However, they were limited and much less specific, affecting several AChE molecular forms.

General effects of training

Following the 12-week running training program, the body weight of the trained rats was very similar to that of the controls, i.e., 295 gm ± 10 vs 289 gm ± 13 ($\bar{X}$ ± SD). The trained muscles showed no signs of hypertrophy and, accordingly, the protein concentration of the extracts obtained from control and trained muscles was not significantly different, except in the case of TA, which exhibited a slight increase (12%) (Table 2). The extent of the muscles' adaptation to enhanced activation was evaluated by analyzing the activity of the mitochondrial enzyme citrate synthase (Table 1). Our running training program raised the activity of this enzyme in all 5 muscles examined. In GAST, PL, EDL, and SOL, the increases were of similar magnitude (35–38%; $p < 0.02$) while in TA, the change was limited to 9% (not significant). Such increases in citrate synthase activity were similar to what is usually reported for the type of training used in this study (Baldwin et al., 1972; Holloszy and Booth, 1976; Jasmin et al., 1988).

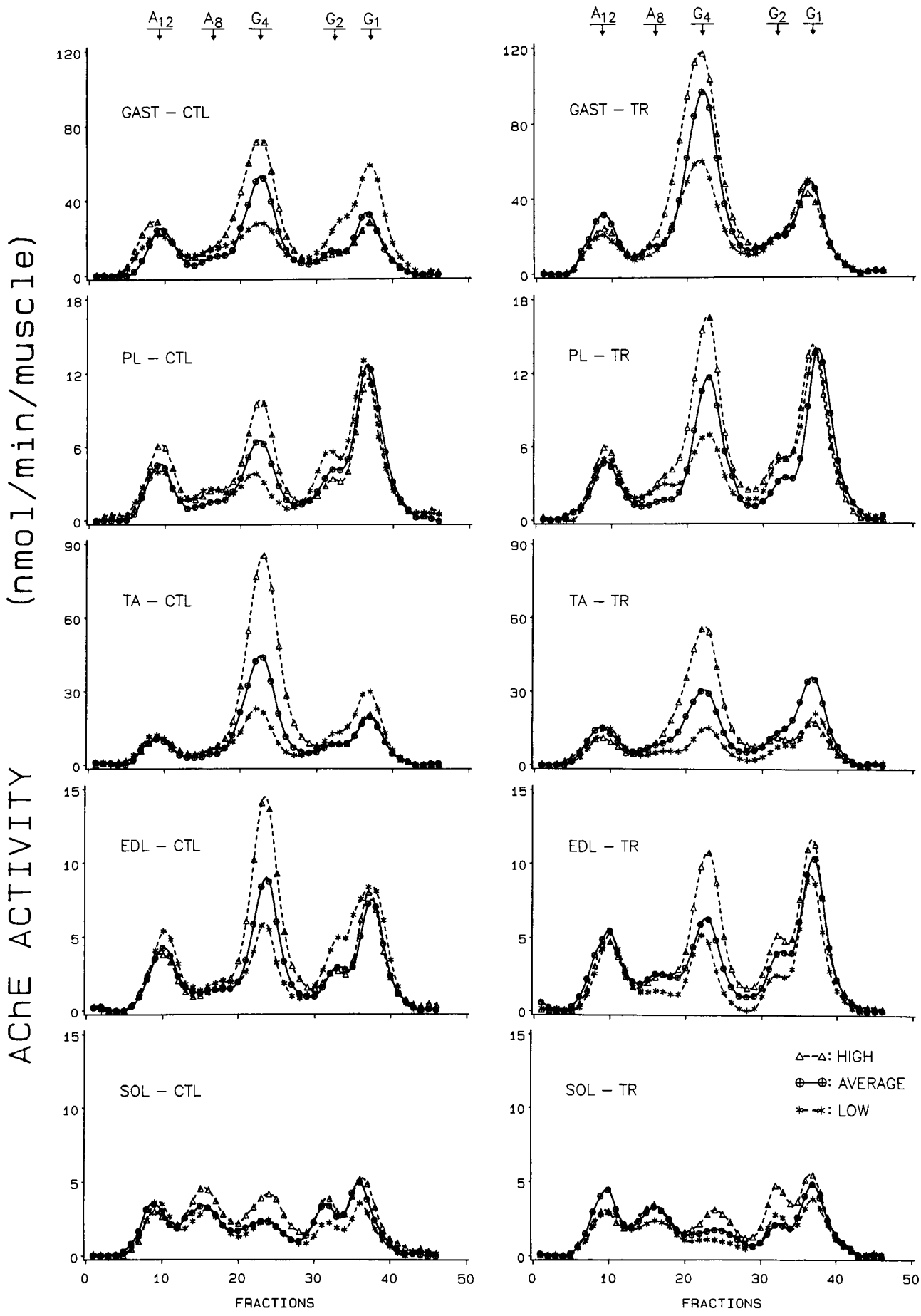
Effect of training on AChE

In contrast to its uniform effect on citrate synthase, training affected AChE in a very discriminate way according to the twitch properties as well as the function of the muscles. Both the content (activity per muscle) and concentration (specific activity) of AChE were increased in the ankle extensors, GAST and PL (18–28%), but reduced in the ankle flexors, TA and EDL (9–18%) (Table 2). The slow-twitch SOL exhibited a decrease in AChE activity despite the fact that it is also an ankle extensor (Table 2). These changes in total AChE activity were the consequence of significant alterations of the muscle content in AChE molecular forms. Remarkably, however, the impact of training was quite diverse according to the type of molecular forms as well as the properties and function of the muscles.

Asymmetric forms. As a result of the training, A_{12} and A_8 were generally elevated in the fast muscles, independent of whether the overall muscle AChE activity was increased or reduced by the running program (Figs. 1, 4; Table 3). However, the increases, which were highly variable (2–33%), were significant only for A_{12} in 2 muscles: PL (33%; $p < 0.02$) and TA (22%; $p < 0.01$). In trained SOL, A_{12} was also slightly elevated, but the main change involved A_8, which was decreased by about 20% ($p < 0.01$).

Globular forms. The most distinctive effect of the training program was seen on G_4, in terms not only of its extent, but also of its direction (Fig. 1). Indeed, as was the case for overall AChE activity, prolonged running training had an opposite effect on the tetramer level in the 2 antagonist groups of fast muscles.

Training increased dramatically the proportions of G_4 in both ankle extensors GAST and PL. These changes in tetramer percentage translated into highly significant increases in the amounts of G_4 per muscle as well as per mg of protein. In trained GAST, the activity per muscle and the specific activity of G_4 were increased by 53% ($p < 0.0002$) and 51% ($p < 0.0005$), respectively, whereas in trained PL, the 2 parameters were elevated by 52% ($p < 0.003$) and 56% ($p < 0.0006$), respectively, as compared to controls (Fig. 4; Table 3). In contrast, training markedly reduced G_4 in the ankle flexors, TA and EDL (Fig. 1). The decreases also involved important amounts of tetramer; actually, they nearly mirrored the G_4 increases observed in the ankle extensors. Thus, in TA, the G_4 level per muscle and specific activity were reduced by 36% ($p < 0.03$) and 44% ($p <$


A12
A8
G4
G2
G1
GAST – CTL
GAST – TR
PL – CTL
PL – TR
TA – CTL
TA – TR
EDL – CTL
EDL – TR
SOL – CTL
SOL – TR
ACHE ACTIVITY (nmol/min/muscle)
FRACTIONS
△–△: HIGH
⊕–⊕: AVERAGE
✳–✳: LOW

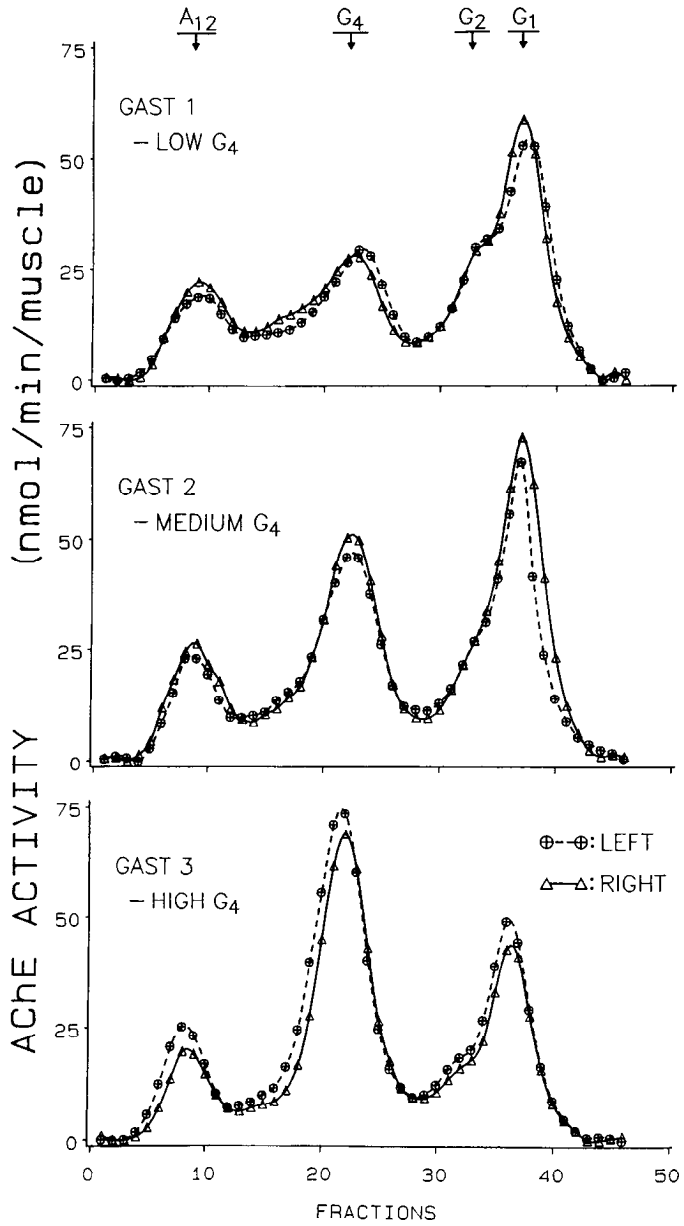


Figure 3. Comparison of the content in AChE molecular forms in pairs of contralateral GAST taken from 3 control rats. Shown are examples of GAST exhibiting low, medium, and high G_4 amounts. Otherwise as in Figure 1.

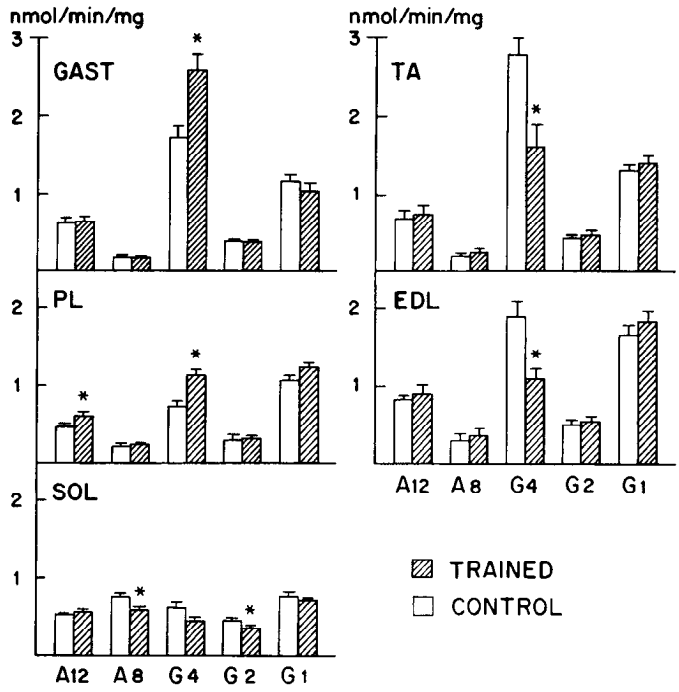


Figure 4. Specific activities of the AChE molecular forms in control and trained muscles. The columns represent means ± SEM of the specific activity of the molecular forms measured in 8–12 samples of each control and trained muscle. The specific activity of each form was computed from the total AChE specific activity and the relative proportion of the form (see Materials and Methods). *Asterisks* indicate statistically significant differences between means ($p < 0.05$, 2-tailed).

0.02), respectively, while in EDL, the 2 parameters decreased by 39% ($p < 0.02$) and 43% ($p < 0.005$) (Fig. 4; Table 3).

The two other globular forms exhibited only small changes in the 4 fast muscles (Fig. 4; Table 3): after training, G_1 varied between 88 and 120% of the control values and G_2 between 97 and 122%. These variations were generally nonsignificant and they showed no relationship with the particular functional role of the muscles.

In the slow-twitch SOL, the effect exerted by the training program on its globular forms clearly departed from that observed in the fast muscles. Indeed, the trained SOL exhibited small reductions of both G_4 and G_2, while G_1 was almost unaffected (Figs. 1, 4; Table 3).

Another important observation was the marked variability in AChE content exhibited by the individual samples of each trained muscle (Fig. 2). Actually, it resembled that displayed by the control muscles. In particular, the variations also affected mainly G_4 of the fast muscles, and here, too, there was a definite tendency for all the 4 fast muscles originating from the same rat to share a common G_4 type (for instance, high, average, or low). Thus, the high-G_4 trained GAST and EDL, in Figure 2, right, originated from one rat and the low-G_4 trained GAST and PL from another. Most important, however, this variability did not hinder the impact of training on the AChE content. In the trained SOL, as for the control SOL, the variability in the AChE content was not limited to G_4 but involved several molecular forms.

Sciatic nerve

A previous study has already shown that running training does not change the AChE specific activity of the sciatic nerve trunk (Jasmin et al., 1987). However, a possible effect on the indi-

Figure 2. Variability of the content in AChE molecular forms among individual samples of control (*CTL*) and trained (*TR*) muscles. In addition to the average AChE content, illustrated by a representative actual AChE distribution, the figure shows the sedimentation profiles exhibiting the lowest and the highest amount of G_4 observed among the 8–12 samples of each control and trained muscle that were examined. Otherwise as in Figure 1.

Table 2. AChE activity (per muscle and per mg protein) and protein concentration of the extracts of control and trained muscles

	GAST	PL	SOL	TA	EDL
AChE per muscle (nmol/min)					
Control	801.83 ±99.03	136.32 ±15.93	87.97 ±11.93	517.92 ±123.74	129.91 ±12.45
Trained	968.29* ±143.06	173.73* ±21.52	79.55 ±17.48	476.19 ±82.9	123.18 ±31.21
AChE per mg protein (nmol/min × mg $^{-1}$)					
Control	4.03 ±0.42	2.70 ±0.37	3.09 ±0.43	5.42 ±1.36	5.17 ±0.73
Trained	4.77* ±0.61	3.46* ±0.38	2.64* ±0.49	4.44* ±0.84	4.74 ±0.82
Protein concentration (mg/ml of extract)					
Control	19.46 ±2.37	20.41 ±2.79	11.40 ±0.86	19.20 ±1.29	10.13 ±1.01
Trained	20.36 ±2.32	20.09 ±1.60	12.05 ±1.09	21.56* ±1.91	10.30 ±1.91
Number of muscles					
Control	12	12	9	12	8
Trained	12	11	10	12	9

Values are $\bar{x} \pm$ SD; asterisks denote statistically significant differences between means ($p < 0.05$, 2-tailed). The muscles were homogenized in 2.5 ml (PL, SOL, and EDL), 5 ml (TA), or 10 ml (GAST) of buffer (see Methods).

Table 3. Activity per muscle of the AChE molecular forms of control and trained muscles

	GAST	PL	SOL	TA	EDL
A_{12}					
CTL	123.14 ±22.58	21.70 ±6.18	14.63 ±1.82	64.63 ±13.56	20.76 ±2.51
TR	128.39 ±25.83	29.03* ±8.75	16.57 ±2.94	79.33* ±15.11	23.52 ±6.15
A_8					
CTL	30.98 ±8.90	9.73 ±3.52	21.20 ±3.34	20.46 ±6.40	7.72 ±2.01
TR	31.84 ±5.85	10.97 ±3.17	17.68 ±4.79	25.97 ±7.68	9.00 ±3.44
G_4					
CTL	340.97 ±84.52	37.04 ±12.92	17.56 ±6.21	268.30 ±130.77	47.53 ±14.62
TR	523.36* ±119.45	56.19* ±14.54	12.99 ±6.45	171.85* ±93.36	29.07* ±13.52
G_2					
CTL	74.32 ±25.99	14.07 ±4.48	12.89 ±2.50	41.39 ±7.92	12.59 ±4.25
TR	72.74 ±11.81	15.76 ±2.53	10.61 ±3.36	50.85* ±12.13	14.63 ±6.44
G_1					
CTL	232.18 ±68.20	53.84 ±9.76	21.67 ±4.81	123.00 ±36.90	41.72 ±11.36
TR	211.79 ±68.36	61.71 ±8.61	21.43 ±3.43	148.09 ±40.06	47.21 ±11.31

Data are expressed in nmol/min/muscle. Values are $\bar{x} \pm$ SD of 8–12 muscles (see Table 2). Asterisks denote significant differences ($p < 0.05$, 2-tailed). CTL, Control muscles; TR, trained muscles.

Table 4. Specific activity of total AChE and of the individual molecular forms of the sciatic nerves of control and trained rats

	Total	A_{12}	G_4	G_2	G_1
Control	235.03	6.64	134.52	27.05	66.74
	±47.88	±1.47	±25.99	±6.90	±21.54
Trained	243.19	6.42	138.56	27.57	70.20
	±37.22	±2.81	±21.47	±4.70	±18.22

Data are expressed in nmol/hr × mg^{-1} protein. Values are $\bar{x}$ ± SD of 15 nerves.

vidual molecular forms could not be excluded. The present study provided the opportunity to examine this point. Our analyses confirmed that the AChE specific activity of the rat sciatic nerve is not significantly changed after running training (Table 4). In addition, sedimentation analysis demonstrated that the exercise program did not affect the AChE molecular forms, either. As shown in Figure 5 and Table 4, the specific activities of the AChE molecular forms were almost identical in sciatic nerves taken from trained and control rats.

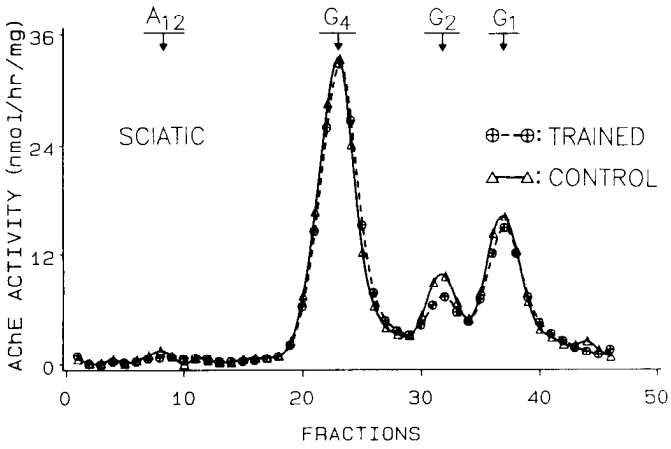


Figure 5. Comparison of the distribution of AChE molecular forms in sciatic nerves of control and trained rats. The sedimentation profiles are actual ones that were closest to the average values observed in 15 sciatic nerves of control and trained rats. The distribution of the molecular forms, expressed as specific activity, was computed from the total AChE specific activity and the sedimentation profile. The forms were identified according to Bon et al. (1979) as indicated in Materials and Methods.

Discussion

The running training program used in this study had a very diversified and complex impact on the muscle AChE content: (1) exercise preferentially affected the pool of G_4 characterizing fast muscles; (2) the G_4 changes, although prominent, were opposite in antagonist fast muscles; (3) the fast muscles displayed, in addition, a moderate increase in asymmetric forms, independent of their particular function and the type of G_4 changes they showed; (4) the slow-twitch SOL exhibited a small, but significant, decrease of AChE which affected all the molecular forms, except A_{12}. Furthermore, this study yielded additional information about muscle AChE of cage-confined rats.

Control muscles

One striking result was the observation that the content in AChE molecular forms exhibited by a given rat muscle varied over a much larger range than previously observed in the normal 129/ReJ mice strain (Gisiger and Stephens, 1983). The control experiments we performed yielded 3 facts that may throw some light on the origin of this variation: (1) the variability affected mainly G_4 so that the AChE content of the various samples of a given muscle could be distinguished by its level of tetramer (for instance, high, medium, or low); (2) a similar type of G_4 content was observed among the muscles taken from the same rat; (3) the AChE content of contralateral muscles, as established by separate homogenizations and sedimentation analyses, was virtually identical. These results make it unlikely that the AChE variability has an artifactual origin. The fact that the type of G_4 content was a property specific to the whole rat and not to individual muscles raises a definite possibility that this variability was caused by a genetic heterogeneity of the rat strain. Actually, it is likely that breeding of the peculiar 129/ReJ mice strain, which does not show such extensive AChE variations (Gisiger and Stephens, 1983), is much more tightly controlled than that of the more common Sprague-Dawley rat strain used in this study. This view is further strengthened by the observation that Sprague-Dawley rats exhibit significantly larger individual variations of AChE activities than other, more inbred rat strains (Edwards and Brimijoin, 1982). Alternatively, because of its analogy with the changes induced by running, the G_4 variability might also be related to individual differences in the level of natural cage activity, which are actually observed among these laboratory animals. Remarkably, however, this variability did not mask the effect of training (Figs. 2, 4; Table 3).

Trained muscles

The most significant finding of this study is the preferential and highly differentiated effect exerted by running training on the pool of G_4 characterizing fast muscles which resulted in marked opposite G_4 changes in antagonist muscles, i.e., increase in the ankle extensors (GAST and PL) vs decrease in the ankle flexors (TA and EDL). The relationship between the G_4 elevation displayed by GAST and PL and the dynamic role they play during running appears straightforward. In opposition, the cause of the G_4 reduction in TA and EDL is not as clear. The fact that the ankle flexors exhibited an increased level of citrate synthase indicates that they were significantly solicited during running training. Electromyographic data support this conclusion (Gruner et al., 1980; Roy et al., 1985). Consequently, the G_4 decrease in TA and EDL cannot be ascribed to a reduction of neuromuscular activity. Nor could any consistent causal link be established with the various functional alterations exhibited by these muscles during running: muscle-loading characteristics, speed of shortening, duration, frequency and amplitude of EMG, etc. (see, for instance, Gruner et al., 1980; Gardiner et al., 1982b, 1986; Roy et al., 1985). Hence, the G_4 decrease seems to be linked to the antagonist role played by the ankle flexors during running.

What this antagonist activity consists of has been investigated mainly in the cat by detailed cinematographic, ergodynamic, and EMG studies (Goslow et al., 1973; Grillner, 1975; Rasmussen et al., 1978). The less extensive analyses performed in the rat indicate that biomechanics of locomotion is basically the same in both quadrupeds (Gruner et al., 1980; Gardiner et al., 1986). Activation of the ankle extensors GAST and PL begins when the hindlimb is extended toward the ground and continues

throughout the phase during which the limb pushes the animal forward. The contraction of GAST and PL during this "stance phase" (Goslow et al., 1973) generates a significant part of the propulsive force. The ankle flexors, in turn, are activated throughout the "swing phase" (Goslow et al., 1973) during which the hindlimb is recovered and protracted forward, leading into the next stride. The biomechanical action of the ankle flexors is more complex than that of the extensors. First, TA and EDL slow the extension movement of the ankle and change its direction to one of flexion by which the foot clears the terrain. They then continue to contract in order to hold the foot in flexion as the limb accelerates while being brought forward in a more rostral position relative to the moving body. As a consequence, the contraction of the ankle flexors must overcome the inertia forces generated by deceleration during the transition from extension to flexion and, most important, by acceleration during the protraction of the hindlimb. More specifically, during the swing phase, the flexors develop a force counteracting the torque generated at the ankle joint by the limb speed changes (Grillner, 1975). In addition, ankle flexors are again shortly activated, simultaneously with the extensors, as the limb is extended toward the ground. It has been proposed that this isometric contraction of the flexors contributes to the precise placing of the foot on the ground by balancing the contraction of the extensors (Engberg, 1964; Grillner, 1975). Thus, whereas the role of ankle extensors, namely the generation of the propulsive force, is indeed clearly dynamic, the activity of the ankle flexors, which is directed mainly against inertia forces, entails a predominant tonic component during running. Taking into account the biomechanical data, the results presented here support the conclusion that the G_4 content of a fast muscle is high or low according to whether the actual activity it is used to perform is predominantly dynamic or tonic.

It is important to stress that both the inertia to be overcome in order to maintain the foot flexed against acceleration during the swing phase and the isometric activation of the flexors during placement of the foot significantly increase with the speed of the animal's motion (Engberg, 1964; Grillner, 1975; Rasmussen et al., 1978). This might explain why Fernandez and Donoso (1988) observed a G_4 elevation in TA as a result of their mild walking (8.5 m/min without inclination) treadmill exercise program, instead of the reduction reported here. At such low speed, the tonic component of the TA activity is considerably reduced in favor of a preponderant dynamic type of action. In this view, the G_4 increase in TA reported by these authors is consistent with the relationship between G_4 content and type of muscle activity proposed here. The type of activity, dynamic or tonic, as the cause of the G_4 changes, is obviously a complex factor. Nonetheless, it constitutes one of the fundamental components of the muscle's dynamics (for instance, Grillner, 1975; Rasmussen et al., 1978). Interestingly, the body of converging evidence that triggered this study (see introductory remarks) already pointed toward the type of activity, taken as an entity, as the specific factor regulating the muscle G_4 content. One of the aims of our ongoing studies is to refine this relationship.

The effect of training on the AChE asymmetric forms clearly differed from that on G_4. First, the running program resulted generally in an elevation of A_{12} and A_8 in the trained fast muscles: interestingly, the increase was statistically significant in 2 antagonist muscles (PL and TA) exhibiting opposing G_4 modifications. Second, the changes in asymmetric forms were much more limited than the ones displayed by G_4. This indicates that the asymmetric forms are much less sensitive to the activity level than G_4. The moderate increase we observed appears as a corrective adjustment of endplate AChE to a chronic and substantial increase of synaptic activation.

In contrast to the fast muscles, the slow-twitch SOL reacted to our running training program with a very limited AChE reduction which was distributed over several molecular forms. However, its citrate synthase activity was increased to the same extent as in trained fast muscles. This oxidative adaptation, together with electromyographic data (Roy et al., 1985), attests that SOL was significantly activated by our training program. Nevertheless, there are indications that, during running, the weight-bearing role of the SOL still remains prominent (Rasmussen et al., 1978; Gardiner et al., 1982b). This would be consistent with the marginal decrease of the already small pool of G_4 found in SOL.

Remarkably, the sciatic nerve showed no modification of its AChE content following training. Nonetheless, taking into account the matching of the G_4 content of the motor nerves with that of their muscles (Gisiger and Stephens, 1982, 1982–1983, 1984; Bacou et al., 1985), together with the mirrored AChE changes exhibited by the trained antagonist muscles, the absence of an effect of training on sciatic nerve AChE was not unanticipated. Indeed, if, as one would expect, training induced in the motor nerves opposite G_4 changes parallel to those in their respective muscles (increase in extensors versus decrease in flexors), then these mirrored modifications are likely to mutually offset one another when AChE is measured at the level of the sciatic nerve trunk.

Functional impact of the pool of G_4 molecules characterizing fast muscles

Especially important is the strong confirmation brought by this report that, in innervated mature fast muscles, their characteristic G_4 pool is subject to a specific regulation, completely distinct from that controlling the asymmetric forms (Massoulié et al., 1980; Gisiger and Stephens, 1983). In particular, the present study produced additional evidence that the size of the G_4 pool characterizing fast muscles is independent of both the oxidative capacity and the content in AChE asymmetric forms of these muscles (Fernandez and Donoso, 1988; Gisiger and Stephens, 1988). This fact strengthens the conclusion that, whereas the oxidative enzymes and the asymmetric forms implement their functional role at the level of each individual fiber, the G_4 pool, in turn, fulfills a specific global need of the entire muscle (or of an anatomical subunit if the muscle is subdivided into heads; Gisiger and Stephens, 1988). Such a view is consistent with the observation that the G_4-rich environment that the G_4 pool characteristic of fast muscles provides at the motor zone to each endplate is collectively contributed by neighboring fibers (Gisiger and Stephens, 1988). This led to the proposal that the functional role of the G_4 pool is fulfilled by the part of it that is placed outside the muscle fibers, as both membrane-bound and secreted molecules.

The highly differentiated response exhibited by the G_4 pool to changes in muscle activity, together with the independence of the G_4 and A_{12} regulations, supports the proposal that G_4 fulfills a specific and essential function, definitely distinct from that of A_{12} (Gisiger and Stephens, 1983, 1988). This conclusion is strengthened by evidence indicating that the 2 AChE endproducts G_4 and A_{12} are assembled along 2 separate biosynthetic

pathways (Toutant and Massoulié, 1987) and occupy distinct intramuscular localizations. Thus, whereas the bulk of G_4 molecules is concentrated around the endplates (Gisiger and Stephens, 1988), no less than 70% of all the asymmetric forms present in rodent muscle are clustered within the neuromuscular junctions (Dreyfus et al., 1983; Fernandez et al., 1984). This marked distribution divergence makes the likelihood remote that the G_4 pool specific of fast muscles may significantly contribute to the preservation of synaptic transmission efficacy achieved by the asymmetric forms; nor would one expect that activity-induced G_4 changes, of the amplitude reported here, would notably affect synaptic transmission. In contrast, the cojunctional compartment of G_4, in particular the membrane-bound and secreted G_4 molecules concentrated around the endplates, occupy a perfect strategic position in order to very effectively remove ACh molecules diffusing away from the postsynaptic membrane (Gisiger and Stephens, 1982–1983). The available data suggest that, whereas only little of the transmitter released by a single quantal event is able to leave the synaptic cleft (still up to 20%, according to Wathey et al., 1979; see also Rotundo and Fambrough, 1986), the amount of ACh escaping AChE molecules immobilized on junctional structures is actually far from negligible during muscle activation. Indeed, *in vivo* muscle stimulation is delivered in trains of rapidly succeeding impulses and an effective stimulus corresponds to a pulse of 10^{-4} M ACh over a background level of about 10^{-8} M (for review, see Changeux, 1981). Training appears to significantly modify the concentration of G_4 molecules around the endplates and, consequently, the hydrolysis rate of ACh evading the junction-bound AChE.

Accordingly, the G_4 alterations are expected to affect the extent of ACh accumulation around the endplates, and therefore the background level of ACh within the junctions. Interestingly, there is evidence that even slight increases in the synaptic background concentration of transmitter significantly depress 2 important synaptic properties, i.e., the excitability of the motor endplate (Changeux, 1981) as well as the adaptation to activity mediated by activation of the nicotinic receptors (Gisiger and Stephens, 1982–1983; Gisiger, 1989). Altogether, these facts raise a definite possibility that, while controlling the synaptic background concentration of ACh, the G_4 pool characterizing fast muscles acts as a modulating factor in these regulatory mechanisms.

References

Armstrong, R. B., and R. O. Phelps (1984) Muscle fiber type composition of the rat hindlimb. Am. J. Anat. *171:* 259–272.

Bacou, F., P. Vigneron, and J. Massoulié (1982) Acetylcholinesterase forms in fast and slow rabbit muscle. Nature *296:* 661–664.

Bacou, F., P. Vigneron, and J. Y. Couraud (1985) Retrograde effect of muscle on forms of acetylcholinesterase in peripheral nerves. J. Neurochem. *45:* 1178–1185.

Baldwin, K. M., G. H. Klinkerfuss, R. L. Terjung, P. A. Mole, and J. O. Holloszy (1972) Respiratory capacity of white, red, and intermediate muscle: Adaptive response to exercise. Am. J. Physiol. *222:* 373–378.

Bon, S., M. Vigny, and J. Massoulié (1979) Asymmetric and globular forms of acetylcholinesterase in mammals and birds. Proc. Natl. Acad. Sci. USA *76:* 2546–2550.

Brimijoin, S. (1983) Molecular forms of acetylcholinesterase in brain, nerve and muscle: Nature, localization and dynamics. Prog. Neurobiol. *21:* 291–322.

Brzin, M., J. Sketelj, V. M. Tennyson, T. Kiauta, and M. Budininkas-Schoenebeck (1981) Activity, molecular forms, and cytochemistry of cholinesterases in developing rat diaphragm. Muscle Nerve *4:* 505–513.

Buchthal, F., and H. Schmalbruch (1980) Motor unit of mammalian muscle. Physiol. Rev. *60:* 90–142.

Butler, I. J., D. B. Drachman, and A. M. Goldberg (1978) The effect of disuse on cholinergic enzymes. J. Physiol. (Lond.) *274:* 593–600.

Changeux, J. P. (1981) The acetylcholine receptor: An "allosteric" membrane protein. Harvey Lect. *75:* 85–254.

Crockett, J. L., V. R. Edgerton, S. R. Max, and R. J. Barnard (1976) The neuromuscular junction in response to endurance training. Exp. Neurol. *51:* 207–215.

Dreyfus, P. A., F. Rieger, and M. Pinçon-Raymond (1983) Acetylcholinesterase of mammalian neuromuscular junctions: Presence of tailed asymmetric acetylcholinesterase in synaptic basal lamina and sarcolemma. Proc. Natl. Acad. Sci. USA *80:* 6698–6702.

Dreyfus, P. A., A. Friboulet, L. H. Tran, and F. Rieger (1984) Polymorphism of acetylcholinesterase and identification of new molecular forms after sedimentation analysis. Biol. Cell *51:* 35–41.

Edwards, J. A., and S. Brimijoin (1982) Divergent regulation of acetylcholinesterase and butyrylcholinesterase in tissues of the rat. J. Neurochem. *38:* 1393–1403.

Ellman, G. L., K. D. Courtney, V. Andres, and R. M. Featherstone (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. *7:* 88–95.

Engberg, I. (1964) Reflexes to foot muscles in the cat. Acta Physiol. Scand. *62* (suppl. 235).

Fernandez, H. L., and J. A. Donoso (1988) Exercise selectively increases G_4 AChE activity in fast-twitch muscle. J. Appl. Physiol. *65:* 2245–2252.

Fernandez, H. L., N. C. Inestrosa, and J. R. Stiles (1984) Subcellular localization of acetylcholinesterase molecular forms in endplate regions of adult mammalian skeletal muscle. Neurochem. Res. *9:* 1211–1230.

Gardiner, P. F., M. Lapointe, and D. Gravel (1982a) Exercise effects on recovery of muscle acetylcholinesterase from reduced neuromuscular activity. Muscle Nerve *5:* 363–368.

Gardiner, K. R., P. F. Gardiner, and V. R. Edgerton (1982b) Guinea pig soleus and gastrocnemius electromyograms at varying speeds, grades, and loads. J. Appl. Physiol. *52:* 451–457.

Gardiner, P., R. Michel, C. Browman, and E. Noble (1986) Increased EMG of rat plantaris during locomotion following surgical removal of its synergists. Brain Res. *380:* 114–121.

Gisiger, V. (1989) Role of hyperpolarization generated by the Na^+-K^+ pump in the trans-synaptic induction of RNA synthesis in sympathetic neurons. Gif Lectures in Neurobiology. J. Physiol. (Paris) *83:* 148–163.

Gisiger, V., and H. R. Stephens (1982) Acetylcholinesterase content in both motor nerve and muscle is correlated with twitch properties. Neurosci. Lett. *31:* 301–305.

Gisiger, V., and H. R. Stephens (1982–1983) Correlation between the acetylcholinesterase content in motor nerves and their muscles. Gif Lectures in Neurobiology. J. Physiol. (Paris) *78:* 720–728.

Gisiger, V., and H. R. Stephens (1983) Asymmetric and globular forms of AChE in slow and fast muscles of 129/ReJ normal and dystrophic mice. J. Neurochem. *41:* 919–929.

Gisiger, V., and H. R. Stephens (1984) Decreased G_4 (10S) acetylcholinesterase content in motor nerves to fast muscles of dystrophic 129/ReJ mice: Lack of a specific compartment of nerve acetylcholinesterase? J. Neurochem. *43:* 174–183.

Gisiger, V., and H. R. Stephens (1988) Localization of the pool of G_4 acetylcholinesterase characterizing fast muscles and its alteration in murine muscular dystrophy. J. Neurosci. Res. *19:* 62–78.

Goslow, G. E., Jr., R. M. Reinking, and D. G. Stuart (1973) The cat step cycle: Hind limb joint angles and muscle lengths during unrestrained locomotion. J. Morph. *141:* 1–41.

Granbacher, N. (1971) Relation between the size of muscle fibers, motor endplates and nerve fibers during hypertrophy and atrophy. Z. Anat. Entwickl.-Gesch. *135:* 76–87.

Grillner, S. (1975) Locomotion in vertebrates: Central mechanisms and reflex interaction. Physiol. Rev. *55:* 247–304.

Groswald, D. E., and W. D. Dettbarn (1983) Characterization of acetylcholinesterase molecular forms in slow and fast muscle of rat. Neurochem. Res. *8:* 983–995.

Gruner, J. A., J. Altman, and N. Spivack (1980) Effects of arrested cerebellar development on locomotion in the rat: Cinematographic and electromyographic analysis. Exp. Brain Res. *40:* 361–373.

Gupta, R. C., K. E. Misulis, and W. D. Dettbarn (1985) Changes in

the cholinergic system of rat sciatic nerve and skeletal muscle following suspension-induced disuse. Exp. Neurol. *89:* 622–633.

Guth, L., W. C. Brown, and J. D. Ziemnowicz (1966) Changes in cholinesterase activity of rat muscle during growth and hypertrophy. Am. J. Physiol. *211:* 1113–1116.

Holloszy, J. O., and F. W. Booth (1976) Biochemical adaptations to endurance exercise in muscle. Annu. Rev. Physiol. *38:* 273–291.

Jasmin, B. J., P.-A. Lavoie, and P. F. Gardiner (1987) Fast axonal transport of acetylcholinesterase in rat sciatic motoneurons is enhanced following prolonged daily running, but not following swimming. Neurosci. Lett. *78:* 156–160.

Jasmin, B. J., P.-A. Lavoie, and P. F. Gardiner (1988) Fast axonal transport of labeled proteins in motoneurons of exercise-trained rats. Am. J. Physiol. *255:* C731–C736.

Jasmin, B. J., V. Gisiger, and P. F. Gardiner (1989) Opposite effects of exercise training on the pool of G_4 acetylcholinesterase in antagonist fast muscles. In *Proc. of the XXXIst Congress of the Int. Union of Physiol. Sciences,* Helsinki, Finland, July 1989, p. 296.

Lomo, T., J. Massoulié, and M. Vigny (1985) Stimulation of denervated rat soleus muscle with fast and slow activity patterns induces different expression of acetylcholinesterase molecular forms. J. Neurosci. *5:* 1180–1187.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193:* 265–275.

Luff, A. R. (1981) Dynamic properties of the inferior rectus, extensor digitorum longus, diaphragm and soleus muscles of the mouse. J. Physiol. (Lond.) *313:* 161–171.

Massoulié, J., and S. Bon (1982) The molecular forms of cholinesterase and acetylcholinesterase in vertebrates. Annu. Rev. Neurosci. *5:* 57–106.

Massoulié, J., S. Bon, and M. Vigny (1980) The polymorphism of cholinesterase in vertebrates. Neurochem. Int. *2:* 161–184.

Müntener, M., and W. Zenker (1986) Fiber type and non-endplate acetylcholinesterase in normal and experimentally altered muscles. Anat. Embryol. *173:* 377–383.

Rasmussen, S., A. K. Chan, and G. E. Goslow, Jr. (1978) The cat step cycle: Electromyographic patterns for hindlimb muscles during posture and unrestrained locomotion. J. Morph. *155:* 253–269.

Rieger, F., M. L. Shelanski, and R. L. Sidman (1983) The multiple molecular forms of acetylcholinesterase in "motor end-plate disease" in the mouse (*med* and *med* allelic forms): Sensitivity of the 10 S form to partial or total loss of muscle activity. Exp. Neurol. *79:* 299–315.

Rotundo, R. L., and D. M. Fambrough (1986) Function and molecular structure of acetylcholinesterase. In *Myology: Basic and Clinical,* A. G. Engel and B. Q. Banker, eds., pp. 791–808, McGraw-Hill, New York.

Roy, R. R., W. K. Hirota, M. Kuehl, and V. R. Edgerton (1985) Recruitment patterns in the rat hindlimb muscle during swimming. Brain Res. *337:* 175–178.

Skau, K. A. (1983) The acetylcholinesterase abnormality in dystrophic mice is a reflection of a maturational defect. Brain Res. *276:* 192–194.

Skau, K. A., and S. Brimijoin (1981) Abnormal distribution of skeletal muscle acetylcholinesterase molecular forms in dystrophic mice. Exp. Neurol. *74:* 111–121.

Snyder, D. H., D. H. Rifenberick, and S. R. Max (1973) Effects of neuromuscular activity on choline acetyltransferase and acetylcholinesterase. Exp. Neurol. *40:* 36–42.

Srere, P. A. (1969) Citrate synthase. In *Methods in Enzymology,* J. M. Lowenstein, ed., Vol. 13, pp. 3–11, Academic, New York.

Toutant, J.-P., and J. Massoulié (1987) Acetylcholinesterase. In *Mammalian Ectoenzymes,* A. J. Kenny and A. J. Turner, eds., pp. 289–328, Elsevier, Amsterdam.

Wathey, J. C., M. M. Nass, and H. A. Lester (1979) Numerical reconstruction of the quantal event at nicotinic synapses. Biophys. J. *27:* 145–164.

Yeakley, J. M., J. L. Janavs, and C. G. Reiness (1987) Muscle activity pattern regulates postnatal development of acetylcholinesterase molecular forms in normal mice and mice with motor endplate disease. J. Neurosci. *7:* 4084–4094.