Biochemical adaptation in the skeletal muscle of rats depleted of creatine with the substrate analogue β -guanidinopropionic acid

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1. Rats were fed on a diet containing 1% β -guanidinopropionic acid (GPA), a creatine substrate analogue, for 6–10 weeks to deplete their muscle of creatine. This manipulation was previously shown to give a 90% decrease in [phosphocreatine] in skeletal and cardiac muscle and a 50% decrease in [ATP] in skeletal muscle only. 2. Maximal activities of creatine kinase and of representative enzymes of aerobic and anaerobic energy metabolism were measured in the superficial white, medial and deep red portions of the gastrocnemius muscle, in the soleus and plantaris muscle and in the heart. 3. Fast-twitch muscles were smaller in GPA-fed animals than in controls, but the size of the soleus muscle was unchanged. The activities of aerobic enzymes increased by 30–40% in all fast-twitch muscle regions except the superficial gastrocnemius, but were unchanged in the soleus muscle. The activities of creatine kinase and phosphofructokinase decreased by 20–50% in all skeletal-muscle regions except the deep gastrocnemius, and the activity of glycogen phosphorylase generally paralleled these changes. There were no significant changes in the activities of any of the enzymes measured in the heart. 4. The glycogen content of the gastrocnemius–plantaris complex was increased by 185% in GPA-fed rats. 5. The proportion of Type I fibres in the soleus muscle increased from 81% in control rats to 100% in GPA-fed rats, consistent with a previous report of altered isometric twitch characteristics and a decrease in the maximum velocity of shortening in this muscle [Petrofsky & Fitch (1980) *Pflugers Arch.* 384, 123–129]. 6. We conclude that fast-twitch muscles adapt by a combination of decreasing diffusion distances, increasing aerobic capacity and decreasing glycolytic potential. Slow-twitch muscles decrease glycolytic potential and become slower, thus decreasing energy demand. 7. These results suggest that persistent changes in the [phosphocreatine] and [ATP] are alone sufficient to alter the expression of enzyme proteins and proteins of the contractile apparatus, and that fibre-type-specific thresholds exist for the transformation response.

INTRODUCTION

Skeletal muscle is a very adaptable tissue which responds to an altered usage pattern by changing its structure to suit the new metabolic demands. The simplest working hypothesis is that there exists an interplay between structure and function such that signals which result from altered function feed back to modify structure. Although a number of stimuli have been identified which result in adaptive change, such as endurance training (reviewed in Holloszy & Booth, 1976), chronic stimulation (reviewed in Salmons & Henriksson, 1981), vascular insufficiency (Bylund *et al.*, 1976) and altered thyroid state (Fitts *et al.*, 1980), the nature of the intracellular signals which trigger the process of adaptation is unknown.

Using ^{31}P n.m.r., we investigated the metabolic changes which occur during isometric contraction at 4 Hz in the gastrocnemius–plantaris complex (*in vivo*) in rats which had been depleted of creatine by feeding them with a diet containing 1% of the creatine substrate analogue β -guanidinopropionic acid (GPA) for 6–10 weeks (Shoubridge & Radda, 1984). This intervention was

known to result in a 90% decrease in [phosphocreatine] and a 50% decrease in [ATP] in skeletal muscle (Fitch *et al.*, 1974). Although the phosphorylated form of the analogue (PGPA) accumulates in the muscle (Fitch *et al.*, 1975), it cannot substitute for phosphocreatine, because it is a very poor substrate for creatine kinase (Chevli & Fitch, 1979). Our results indicated that aerobic potential was enhanced in the muscles of GPA-fed animals. Using the same model, Petrofsky & Fitch (1980) showed that the isometric twitch characteristics of fast-twitch muscle (plantaris) were unaltered, whereas slow-twitch muscle (soleus) exhibited decreased twitch amplitude, increased rise and relaxation time and markedly increased isometric endurance. The maximum velocity of shortening was also decreased in the soleus muscle, but was unchanged in the plantaris muscle (Petrofsky & Fitch, 1980). Taken together these data suggested that persistent changes in [phosphocreatine] and/or [ATP] were sufficient to trigger fundamental rearrangements of the system of energy metabolism in skeletal muscle. In this study we have investigated the molecular basis of the adaptations which occur in fast- and slow-twitch skeletal muscles and in cardiac muscle in response to GPA feeding.

Abbreviations used: GPA, β -guanidinopropionic acid; PGPA, its phosphorylated form.

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MATERIALS AND METHODS

Chemicals

Enzymes, substrates and cofactors for enzyme and glycogen assays were obtained from Sigma (Poole, Dorset, U.K.) and Boehringer Mannheim (Lewes, East Sussex, U.K.). GPA was synthesized by the method of Rowley *et al.* (1971), from β -alanine and cyanamide, and was recrystallized once from hot water. All other chemicals were of analytical grade.

Animals

Male Wistar rats were used in all experiments. The animals were obtained at 3 weeks of age (50–60 g) and were given free access to ground laboratory diet or to the same diet containing 1% GPA. Experiments were performed 6–10 weeks after feeding was started, but all experiments used age-paired animals. Animals were killed by cervical dislocation. The following tissues were used in the enzyme assays: gastrocnemius muscle [whole, or divided into superficial white, medial and deep red portions as in Armstrong & Laughlin (1983)], plantaris muscle, soleus muscle and heart (left ventricle).

Enzyme assays

Fresh tissue was used in all assays. For the assays of glycogen phosphorylase, hexokinase, 6-phosphofructokinase, 2-oxoglutarate dehydrogenase, lactate dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and creatine kinase, tissues were homogenized with a Polytron (setting 5, 2×20 s) in 9 vol. of 50 mM-triethanolamine buffer, pH 7.4, containing 1 mM-EDTA, 5 mM-MgCl₂ and 20 mM-2-mercaptoethanol. The same extraction buffer was used for citrate synthase, except that 2-mercaptoethanol was omitted. All assays were done at 25 °C.

Creatine kinase, lactate dehydrogenase and citrate synthase activities were measured in the crude homogenate in the presence of 0.1% Triton X-100. Creatine kinase was assayed by the method of Oscai & Holloszy (1971). Both GPA and PGPA are competitive inhibitors of creatine kinase with respect to creatine and phosphocreatine; however, the inhibition constants are very high, in the range of 50 mM (E. A. Shoubridge, unpublished work). Since the maximal enzyme activity was assayed in a 1:20000 dilution of muscle homogenate, and the concentration of GPA plus PGPA is of the order of 30–40 μ mol/g fresh wt, the effects of this inhibition will be negligible. Lactate dehydrogenase was assayed in 50 mM-imidazole buffer, pH 7.4, containing 2.0 mM-pyruvate and 0.2 mM-NADH. Citrate synthase was assayed by the method of Morgan-Hughes *et al.* (1977). The crude homogenate was spun at 1000 g for 10 min and the supernatant used for the assay of glycogen phosphorylase (Bergmeyer, 1974) and phosphofructokinase (Opie & Newsholme, 1967). For hexokinase, oxoglutarate dehydrogenase and hydroxyacyl-CoA dehydrogenase, Triton X-100 was added to the crude homogenate to a final concentration of 0.05%. The homogenate was spun in an Eppendorff micro-centrifuge for 2×2 min and the supernatant used for enzyme assays. Hexokinase was assayed by the method of Zammit & Newsholme (1976) and hydroxyacyl-CoA dehydrogenase by the method of Bass *et al.* (1969). Oxoglutarate dehydrogenase activity was measured by a modification of the method of Cooney *et al.* (1981) in 50 mM-Mops

buffer, pH 7.4. All activities were expressed per g fresh wt.

NAD⁺-isocitrate dehydrogenase and pyruvate dehydrogenase activities were measured in isolated mitochondria. Isocitrate dehydrogenase was measured by the method of Alp *et al.* (1976). Pyruvate dehydrogenase was assayed by the coupling assay of Coore *et al.* (1971), with modification that [NAD⁺] was 10 mM. Mitochondria were preincubated for 10 min in isolation medium containing 0.1 mM-Ca²⁺ and 1.0 mM-Mg²⁺ to fully activate pyruvate dehydrogenase. Activities were expressed per mg of mitochondrial protein. Protein was measured by the method of Bradford (1976) with the Bio-Rad Laboratories kit. Bovine plasma γ -globulin was used as the standard.

Glycogen determinations

The gastrocnemius-plantaris muscle complex was freeze-clamped in rats under halothane anaesthesia (1%, in N₂O/O₂, 1:1). Glycogen was determined in a HClO₄ (8%) extract of these samples by the method of Keppler & Decker (1974).

Mitochondrial isolation

Mitochondria were prepared from the gastrocnemius muscles of control and GPA-fed rats by the trypsin digestion method of Davies *et al.* (1981). Each preparation was made from four muscles (GPA-fed) or three muscles (control). The final pellet was resuspended in 225 mM-mannitol/75 mM-sucrose/10 mM-Tris/HCl/500 μ M-EDTA, pH 7.4, at a protein concentration of 5–10 mg/ml. Respiratory activities were measured at 25 °C as detailed by Morgan-Hughes *et al.* (1977).

Histochemistry

Cross-sections (10 μ m) cut from whole soleus muscles at –25 °C on a cryostat microtome were stained for myofibrillar actomyosin ATPase by the method of Brooke & Kaiser (1970). The proportions of Type I and Type II fibres were determined on the entire cross-sectional area.

Presentation of data and statistics

All results are presented as means $\pm$ S.D. Statistical significance was assessed with a Student's *t* test.

RESULTS

Body and muscle weight

There was no difference between the body weights of control and GPA-fed animals. Control animals weighed 312 ± 46 g ($n = 25$) and GPA-fed animals 295 ± 27 g ($n = 26$). The relative weights of the plantaris and gastrocnemius muscles (muscle weight/body weight) were decreased by 9 and 24% respectively in GPA-fed animals, but the size of the soleus muscle was unchanged (Table 1).

Enzyme activities

Gastrocnemius muscle. In the muscle taken as a whole, the activities of glycogen phosphorylase, phosphofructokinase and lactate dehydrogenase decreased by about 30% and that of creatine kinase by 23% compared with control values. Citrate synthase activity increased by 40%. No significant changes in the activity of hexokinase were observed in any portion of the gastrocnemius muscle

Table 1. Ratios of muscle to body weight in the gastrocnemius-plantaris-soleus complexValues are means $\pm$ S.D. ($n = 5$).

Muscle... Rats	$10^4 \times$ Muscle wt./body wt.		
	Soleus	Plantaris	Gastrocnemius
Control	4.6 ± 0.5	9.5 ± 1.0	51.8 ± 3.2
GPA-fed	4.5 ± 0.3	8.6 ± 0.3	39.4 ± 0.6

in GPA-fed rats (Table 2). These results were generally similar to those obtained from the medial portion of the gastrocnemius. In this region oxoglutarate dehydrogenase and hydroxyacyl-CoA dehydrogenase activities increased by about 30% in GPA-fed rats, as did citrate synthase activity; however, the latter change was not statistically significant. In the superficial white portion of the gastrocnemius, glycogen phosphorylase activity was 12% lower, phosphofructokinase activity 40% lower and creatine kinase activity 24% lower in GPA-fed animals than in controls, but lactate dehydrogenase activity was unchanged. Although the mean activities of citrate synthase, oxoglutarate dehydrogenase and hydroxyacyl-CoA dehydrogenase were all increased by about 20% in the superficial gastrocnemius, none of these changes was statistically significant. In the deep red portion of the gastrocnemius of GPA-fed rats, both oxoglutarate dehydrogenase and hydroxyacyl-CoA dehydrogenase

activities increased by 40%, but the 16% increase in citrate synthase was not significant. There were no significant changes in the activities of creatine kinase or any of the glycolytic enzymes in this region of the gastrocnemius.

Plantaris muscle. The only significant change in the activity of hexokinase in GPA-fed animals occurred in the plantaris muscle, where the activity increased by 29%. Glycogen phosphorylase, phosphofructokinase and creatine kinase activities all decreased by 30–40% in GPA-fed animals, whereas citrate synthase activity increased by 40% and oxoglutarate dehydrogenase activity increased by 31% (Table 2).

Soleus muscle. The changes in the activities of the glycolytic enzymes and creatine kinase were similar to those seen in fast-twitch muscles from GPA-fed rats. Glycogen phosphorylase decreased by 60%, and phosphofructokinase and creatine kinase by 34%. There were no significant changes in the activities of aerobic enzymes (Table 2).

Heart. No significant changes were observed in the activities of any of the enzymes that were measured in the heart (Table 2).

Specific enzyme activities and respiratory rates in isolated mitochondria.

Specific enzyme activities (activity per mg of mitochondrial protein) were measured in mitochondria isolated from the gastrocnemius muscles of control and

Table 2. Maximal enzyme activities in the gastrocnemius, plantaris, soleus and heart muscles of control rats and of rats fed on a diet containing 1% GPA for 6–10 weeks

Enzyme activities were measured as outlined in the Materials and methods section. Values are means $\pm$ S.D. for the numbers of determinations shown in parentheses. Abbreviations: C, control; A, GPA-fed; n.d., not determined. Statistical significance of differences between control and GPA-fed animals: ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$.

Muscle...		Muscle enzyme activities ($\mu\text{mol}/\text{min}$ per g fresh wt., at 25 °C)						
		Gastrocnemius				Plantaris	Soleus	Heart
		Whole	Superficial	Medial	Red			
Hexokinase	C	n.d.	0.48 ± 0.08 (7)	0.69 ± 0.10 (4)	0.98 ± 0.20 (6)	1.05 ± 0.08 (4) ^a	1.34 ± 0.17 (4)	6.10 ± 0.12 (4)
	A	n.d.	0.53 ± 0.08 (8)	0.74 ± 0.09 (5)	1.18 ± 0.20 (7)	1.35 ± 0.15 (4)	1.38 ± 0.22 (4)	5.83 ± 0.69 (4)
6-Phosphofructokinase	C	52.4 ± 7.8 (5) ^c	61.6 ± 12.9 (5) ^b	58.5 ± 17.7 (4) ^a	35.4 ± 9.3 (5)	49.4 ± 11.6 (5) ^b	10.2 ± 1.3 (5) ^c	9.2 ± 1.8 (5)
	A	34.2 ± 1.9 (5)	40.5 ± 3.0 (5)	29.0 ± 3.6 (4)	25.5 ± 4.0 (5)	30.1 ± 2.2 (5)	6.7 ± 0.5 (5)	8.4 ± 1.4 (5)
Phosphorylase	C	38.8 ± 3.7 (5) ^b	42.4 ± 3.7 (5) ^a	38.9 ± 3.9 (5) ^b	24.9 ± 5.1 (5)	36.7 ± 3.5 (5) ^c	5.9 ± 1.5 (5) ^b	7.1 ± 0.9 (5)
	A	27.5 ± 3.5 (5)	37.2 ± 3.0 (5)	27.2 ± 4.7 (5)	22.3 ± 3.1 (5)	26.4 ± 1.3 (5)	2.4 ± 1.6 (5)	7.1 ± 0.9 (5)
Lactate dehydrogenase	C	521 ± 45 (5) ^b	519 ± 59 (5)	476 ± 78 (5)	404 ± 67 (5)	472 ± 139 (5)	145 ± 33 (5)	385 ± 51 (5)
	A	375 ± 75 (5)	492 ± 34 (5)	370 ± 69 (5)	330 ± 53 (5)	410 ± 43 (5)	134 ± 14 (5)	354 ± 52 (5)
Citrate synthase	C	15.0 ± 1.7 (4) ^b	12.7 ± 2.1 (5)	16.4 ± 3.2 (4)	28.0 ± 4.0 (5)	20.0 ± 2.9 (5) ^b	19.6 ± 3.1 (5)	111.0 ± 12.9 (5)
	A	21.2 ± 2.2 (4)	15.9 ± 2.4 (5)	20.7 ± 3.2 (5)	32.4 ± 4.7 (5)	28.1 ± 3.4 (5)	19.1 ± 1.7 (5)	98.7 ± 20.3 (5)
2-Oxoglutarate dehydrogenase	C	n.d.	0.66 ± 0.16 (6)	0.93 ± 0.10 (4) ^a	1.43 ± 0.24 (7) ^c	1.41 ± 0.09 (4) ^a	1.24 ± 0.10 (4)	5.88 ± 1.01 (4)
	A	n.d.	0.79 ± 0.16 (8)	1.20 ± 0.13 (5)	1.98 ± 0.17 (8)	1.84 ± 0.28 (4)	1.30 ± 0.09 (4)	6.21 ± 0.92 (4)
3-Hydroxyacyl-CoA dehydrogenase	C	n.d.	2.42 ± 0.51 (4)	3.38 ± 0.35 (4) ^b	5.32 ± 0.37 (4) ^a	n.d.	n.d.	20.0 ± 1.9 (5)
	A	n.d.	2.75 ± 0.58 (5)	4.26 ± 0.34 (5)	7.55 ± 1.29 (5)	n.d.	n.d.	20.2 ± 8.0 (5)
Creatine kinase	C	773 ± 123 (5) ^a	904 ± 106 (5) ^b	748 ± 73 (4) ^b	606 ± 122 (5)	731 ± 52 (5) ^c	260 ± 75 (5)	340 ± 44 (5)
	A	593 ± 105 (5)	690 ± 31 (5)	601 ± 49 (5)	548 ± 151 (5)	487 ± 64 (5)	171 ± 26 (5)	321 ± 18 (5)

Table 3. Enzyme activities in mitochondria isolated from the gastrocnemius muscles of control and GPA-fed rats:

Values are means $\pm$ S.D. ($n = 5$). ^aControl and GPA-fed significantly different ($P < 0.01$).

Enzyme	Activity (nmol/min per mg of protein at 25 °C)	
	Control	GPA-fed
Pyruvate dehydrogenase	102 $\pm$ 15	95 $\pm$ 11
2-Oxoglutarate dehydrogenase	166 $\pm$ 19	173 $\pm$ 21
NAD ⁺ -isocitrate dehydrogenase	134 $\pm$ 17	113 $\pm$ 16
Hydroxyacyl-CoA dehydrogenase	634 $\pm$ 22	620 $\pm$ 27
Citrate synthase	2053 $\pm$ 113	1829 $\pm$ 93 ^a
Creatine kinase	2483 $\pm$ 233	2473 $\pm$ 155

GPA-fed rats to test whether the increase in mitochondrial enzyme activity seen in fast-twitch muscles was associated with a qualitative change in the nature of the mitochondria (Table 3). The only significant difference in the six enzymes measured was a small relative decrease (11%) in the activity of citrate synthase in mitochondria isolated from GPA-fed animals. We also measured respiratory rates and respiratory control ratios with a variety of substrates (Table 4). No differences were observed in the State-3 (ADP-stimulated) rate of O₂ consumption with any of the substrates tested, nor in the degree of coupling.

Glycogen

Glycogen content was measured in the gastrocnemius-plantaris complex of control and GPA-fed rats. It increased by 185% in the muscles of GPA-fed rats, from 41.4 $\pm$ 4.8 ($n = 11$) to 76.0 $\pm$ 14.6 ($n = 10$) μ mol/g wet wt., expressed as glucosyl units ($P < 0.001$).

Histochemistry

The proportion of Type I fibres was assessed in soleus muscles on the entire cross-sectional area. Control muscles were 81 $\pm$ 5% Type I, whereas soleus muscles from GPA-fed rats were 100% Type I ($n = 5$).

DISCUSSION

The experimental diet has a negligibly small effect on growth rate. Although the mean body weight of GPA-fed

rats was less than that of controls after 6–10 weeks of GPA feeding, the difference was not significant. Similar results have been obtained with rats of both sexes and of different strains (Shields & Whitehair, 1973; Fitch *et al.*, 1978; Petrofsky & Fitch, 1980). There are, however, changes in the relative weights of the muscles in the gastrocnemius-plantaris-soleus complex in GPA-fed animals. The difference between fast and slow muscle that we observed confirms a previous report (Fitch *et al.*, 1978), although a much larger decrease in the weight of the plantaris (29%) was observed in the latter study. The largest change in relative muscle size occurred in the muscle with the greatest proportion of Type IIb fibres (gastrocnemius: 62% Type IIb; Armstrong & Laughlin, 1983). This is consistent with previously reported measurements of muscle fibre cross-sectional areas in GPA-fed rats (Shields *et al.*, 1975), in which no changes were observed in the mean cross-sectional areas of fibres from a Type-I-fibre-rich area, whereas a 2-fold decrease was measured in Type IIb fibres from the superficial white region of the gastrocnemius muscle.

The changes in enzyme activities that occur during GPA feeding are specific to different muscles and fibre types. The activities of both creatine kinase and phosphofructokinase in GPA-fed rats changed in parallel, decreasing by 20–50% in all regions examined except the deep red portion of the gastrocnemius. The activity of glycogen phosphorylase generally paralleled these changes, except that in the superficial white gastrocnemius there was only a small (12%) decrease in its activity. These data suggest that the activities of creatine kinase and key glycogenolytic enzymes are co-ordinately regulated in response to GPA feeding. This contrasts with previous observations in dystrophic and denervated avian skeletal muscle, in which the activities of three glycolytic enzymes (aldolase, enolase and glyceraldehyde-3-phosphate dehydrogenase) were shown to change independently of creatine kinase (Shackelford & Leberherz, 1981; Petell & Leberherz, 1985). Type I and IIa fibres do not respond to the depletion of the phospho-creatine pool in the same fashion in fast and slow muscles. The activities of creatine kinase and the glycolytic enzymes are unchanged in the deep red gastrocnemius (30% Type I, 62% Type IIa), whereas dramatic changes are observed in the soleus muscle, which becomes 100% Type I.

The activities of oxoglutarate dehydrogenase and hydroxyacyl-CoA dehydrogenase increased by 30–40%

Table 4. Oxygen consumption and respiratory control ratios in mitochondria isolated from the gastrocnemius muscles of control and GPA-fed rats

Values are means $\pm$ S.D. ($n = 5$).

Substrates	Rats...	Respiratory rate (ng-atoms of O/min per mg of protein) (at 25 °C)		Respiratory control ratio	
		Control	GPA-fed	Control	GPA-fed
5 mM-Pyruvate + 2.5 mM-L-malate		223 $\pm$ 36	231 $\pm$ 47	8.8 $\pm$ 3.3	8.4 $\pm$ 1.8
10 mM-DL-Oxoglutarate + 2.5 mM-L-malate		275 $\pm$ 49	251 $\pm$ 43	10.6 $\pm$ 3.6	12.0 $\pm$ 5.6
40 μ M-DL-Palmitoylcarnitine + 2.5 mM-L-malate		105 $\pm$ 34	104 $\pm$ 51	8.5 $\pm$ 4.0	7.7 $\pm$ 4.5
10 mM-Succinate		294 $\pm$ 78	281 $\pm$ 50	3.6 $\pm$ 1.1	3.9 $\pm$ 1.1
2 mM-Ascorbate + 50 μ M-tetramethyl- <i>p</i> -phenylenediamine		518 $\pm$ 51	517 $\pm$ 114	1.7 $\pm$ 0.2	1.7 $\pm$ 0.4

in all fast muscle regions of GPA-fed animals except the superficial white gastrocnemius. The magnitude of the increase in the activities of these enzymes in the different regions of the gastrocnemius muscle correlated with the relative proportion of Type I fibres. Citrate synthase activity generally paralleled these changes, although the increased activity was not always significantly different from controls. These changes are consistent with a 30–40% increase in mitochondrial content. Qualitatively, mitochondria isolated from the gastrocnemius muscles of control and GPA-fed rats were virtually identical with respect to specific enzyme activity, respiratory rate and degree of coupling. The small relative decrease in the activity of citrate synthase in mitochondria from GPA-fed rats (11%), although consistent with the results on whole tissue homogenates (citrate synthase compared with oxoglutarate dehydrogenase and hydroxyacyl-CoA dehydrogenase activity), is unlikely to be physiologically important. Rather, it may reflect active mitochondrial biosynthesis and turnover. The increase in aerobic potential in fast-twitch muscles might involve an increase in size or number of mitochondria or an elaboration of the mitochondrial reticulum. It may also partly reflect decreased fibre size in the absence of mitochondrial proliferation. Our experiments do not allow us to discriminate between these possibilities.

The different responses of different muscle and fibre types to GPA feeding occur in the face of very similar alterations in the intracellular milieu. [Phosphocreatine] has been reported to decrease by about 90% and [ATP] by 50% in the gastrocnemius muscle (Fitch *et al.*, 1974), gastrocnemius–plantaris complex (Shoubridge & Radda, 1984), plantaris and soleus muscle (Fitch *et al.*, 1978) and tibialis anterior muscle (Fitch *et al.*, 1975) after at least 6 weeks of GPA feeding. Shields *et al.* (1975) reported that total creatine concentrations decreased by 87% in both the superficial white and deep red portions of the gastrocnemius muscle. Concomitant with the depletion of creatine, GPA accumulates. The mean total GPA pool (GPA plus PGPA) was 32–38 $\mu\text{mol/g}$ fresh wt. in all of the above studies. Thus the extent of creatine depletion and GPA accumulation in skeletal muscle appears to be independent of muscle and fibre type. There are, however, some significant differences in the heart. We have recently shown that while [phosphocreatine] is decreased by 90%, [ATP] is unchanged, and the accumulation of total analogue (GPA plus PGPA) is only 38% of that found in the gastrocnemius–plantaris complex of the same animals (Shoubridge *et al.* 1985).

There are clearly different strategies of adaptation to the depletion of the total creatine pool in different muscles and different fibre types, and different costs in terms of physiological performance. The loss of 90% of the phosphocreatine pool drastically decreases the total amount of energy buffer, the rate at which it can be delivered (the flux from phosphocreatine to ATP is decreased from 15.7 to 1.3 $\text{mm} \cdot \text{s}^{-1}$; Shoubridge & Radda, 1984) and the magnitude of the diffusion gradients for phosphocreatine in the cell. One would predict that these alterations would have their greatest impact on large fast-twitch fibres with little aerobic potential, i.e. Type IIb fibres (see, e.g., Mainwood & Rakusan, 1982; Meyer *et al.*, 1984). The fibres in the superficial gastrocnemius muscle (predominately Type IIb) respond primarily by decreasing diffusion distances, thus enhancing O_2 delivery, and by decreasing anaerobic potential. The

fibres in the medial gastrocnemius and plantaris, both of which are composed of a greater proportion of Type IIa fibres, decrease diffusion distances rather less than the superficial gastrocnemius, and decrease glycolytic potential but increase aerobic potential by 20–40%. In the deep red area of the gastrocnemius, which contains only 8% Type IIb fibres (Armstrong & Laughlin, 1983), the major adaptation would appear to be increased aerobic potential, and no change in fibre size (Shields *et al.*, 1975). Fast-twitch muscles from GPA-fed rats have nearly twice the glycogen content of control muscles. A 30–40% increase in glycogen content has been reported in muscle from the whole hind limb of GPA-fed mice (Annesley & Walker, 1980). In total global ischaemia the rate of glycogenolysis in the muscles from GPA-fed mice was found to be faster than in controls (Annesley & Walker, 1980), suggesting altered glycogenolytic regulation and/or increased glycogen availability in response to GPA feeding.

We can conclude from the above considerations that the adaptation in fast-twitch muscle involves a fundamental rearrangement of aerobic/anaerobic potential to permit rapid oxidation of glycogen. The isometric and isotonic contractile characteristics of fast-twitch muscles (plantaris) are unaltered by GPA feeding (Petrofsky & Fitch, 1980). Thus, on a per-gram basis, fast-twitch muscles conserve their power output characteristics. The cost of the adaptation, however, can be measured in terms of the decrease in absolute force production, which is a direct function of the decrease in muscle cross-sectional area.

We have examined the functional consequences of the biochemical adaptation in the gastrocnemius–plantaris complex during isometric contraction at 4 Hz by using ^{31}P n.m.r. (Shoubridge & Radda, 1984). Maximal changes in [phosphocreatine], [ATP] and intracellular pH (pH_i) were observed after 4 min of supramaximal stimulation. In control muscles [phosphocreatine] decreased by 34 mM, [ATP] by 4.9 mM and pH_i by 0.79 unit, whereas $[\text{P}_i]$ increased by 43.9 mM. In the muscle from GPA-fed rats [phosphocreatine] decreased by 2.1 mM, [PGPA] by 6.5 mM, [ATP] by 2.5 mM and pH_i by 0.46 unit, whereas $[\text{P}_i]$ increased by 13.5 mM. (All values are measured in terms of intracellular water, assuming it is 0.67 times fresh wt.) If one assumes that there is no difference in the rate of net acid extrusion in control and GPA-fed animals and that buffering capacity is constant (at 50 mmol/l per pH unit), one can estimate [intracellular lactate] from the above data. This estimate is 29.7 mM in GPA-fed rats and 71.7 mM in control animals. Because there was no difference in the physiological performance of the muscles, we can calculate that the muscles of GPA-fed animals obtained a minimum of 60% more of their ATP requirements aerobically as compared with control animals.

The adaptations in slow-twitch muscle are different from those in fast-twitch muscle. The isometric twitch characteristics of the soleus muscle are altered, resulting in decreased twitch amplitude and increased rise and relaxation times, and the maximum velocity of shortening is decreased (Petrofsky & Fitch, 1980). Thus the soleus muscle becomes slower (decreased power-generating capabilities) and endurance time is consequently much increased. The histological evidence (100% Type I fibres) suggests that the molecular basis for this alteration is, in part, a shift in the composition of the myosin isoforms.

It probably also involves changes in the nature of the Ca^{2+} -binding proteins of the sarcoplasmic reticulum, although these have not been examined. Fibre diameter and aerobic potential are unchanged in soleus muscle, although there is a very significant decrease in anaerobic potential. The cost of the adaptation in slow muscle can be measured in terms of a decrease in power output.

There are some similarities in the metabolic adaptations that occur in response to GPA feeding and those that occur in response to endurance exercise training. The adaptive change that results from endurance training is a function of the intensity of the training programme (Holloszy & Booth, 1976). Green *et al.* (1983) subjected rats to a vigorous 15-week endurance-training programme and measured the activity patterns of several representative enzymes in energy metabolism in the superficial white and deep red portion of the vastus lateralis muscle. In terms of fibre-type distribution (based on myofibrillar ATPase staining), these muscle regions correspond roughly to the superficial white gastrocnemius and the plantaris muscle used in this study (Armstrong & Laughlin, 1983). The activities of citrate synthase and hydroxyacyl-CoA dehydrogenase increased by about 65% in the deep vastus in response to training, whereas the activities of glycogen phosphorylase, lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase decreased by 30–40%. The change in the activity of hexokinase was transitory but its activity was increased by about 35% after 15 weeks of training. These results are very similar to those that we report here for the plantaris muscle of GPA-fed rats. The effects of training and GPA feeding are, however, markedly different in superficial white muscle (Type IIb fibres). Training induced 3–4-fold increases in the activities of mitochondrial enzymes, but the activities of glycolytic enzymes were unchanged (Green *et al.*, 1983). GPA feeding resulted in small, but statistically insignificant, increases in the activities of mitochondrial enzymes and decreases in the activities of glycogen phosphorylase and phosphofructokinase. Neither GPA feeding nor endurance training (Holloszy & Booth, 1976) induce adaptive changes in the enzymic machinery of the heart.

The nature of the intracellular signals involved in mediating the feedback process in which muscle structure adjusts to the demands of an altered usage pattern remain unknown. Changes in cellular energy and redox state have been suggested to be the trigger for the adaptive increase in aerobic potential that occurs in the muscles of patients with peripheral vascular insufficiency (Bylund *et al.*, 1976; Bylund-Fellenius *et al.*, 1981). Our data suggest that persistent changes in the concentrations of high-energy phosphate metabolites (phosphocreatine and ATP) are alone sufficient to alter the expression of both enzyme proteins and the proteins of the contractile apparatus, and thus lend support to the above suggestion.

Many studies have attempted to mimic the adaptive response to training by chronic nerve stimulation (reviewed by Salmons & Henriksson, 1981). The pattern of chronic stimulation (slow or fast motor neurone) has been shown to be relatively unimportant in eliciting transformation, only the total stimulation time (Hudlicka *et al.*, 1982; Eerbeek *et al.*, 1984), indicating that the integral of some intracellular signal over time is important in the adaptive process. The nature and extent of the transformation that we have observed in the muscle of GPA-fed rats is different in slow and fast muscles,

although the chemical stimulus is virtually the same. This suggests that the decoding mechanism which interprets these intracellular signals is muscle- and fibre-type specific and that different thresholds exist in different muscles. It is well known that equivalent stimulation of a slow and a fast muscle results in much more dramatic changes in phosphocreatine, ATP, pH_i and lactate etc. in fast muscles than in slow muscles (Hintz *et al.*, 1982). We suggest that the changes in [phosphocreatine] and [ATP] that occur in the soleus muscle of GPA-fed animals are inappropriately large, i.e. supraphysiological, and as such have triggered a more extensive transformation in slow muscle than in fast muscle. This hypothesis predicts that one ought to be able to titrate the transformation response by manipulating the steady-state concentrations of phosphocreatine and ATP.

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