Induction of carbonic anhydrase III mRNA and protein by denervation of rat muscle

Nicholas D. CARTER,*§ Per J. WISTRAND,† Harvey ISENBURG,‡ Håkan ASKMARK,† Stephen JEFFERY,* David HOPKINSON‡ and Yvonne EDWARDS‡

*Department of Child Health, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE, U.K.,

†Departments of Medical Pharmacology and Neurology, Uppsala University, S-75123 Uppsala, Sweden,

and ‡MRC Human Biochemical Genetics Unit, Wolfson House, 4 Stephenson Way, London NW1 2HE, U.K.

Carbonic anhydrase III (CAIII) protein and mRNA amounts in fast- and slow-twitch rat muscles were examined after resection of the sciatic nerve. Striking changes occur in the fast-twitch anterior tibialis (AT) and extensor digitorum longus (EDL) muscles, where CAIII protein and mRNA are increased several-fold 16 days after denervation. The data suggest that these changes are regulated in part by changes in gene transcription and that they perhaps signal a fast-to-slow fibre type transition in these denervated muscles. AT and EDL show some differences in the effects of denervation, which are suggestive of variation in the timing of denervation-induced responses and/or the CAIII protein/mRNA turnover rates in the two muscles.

INTRODUCTION

Carbonic anhydrase III (CAIII) is a cytosolic M_r -29000 zinc-containing isoenzyme which is thought to facilitate the transport of CO_2 in skeletal muscle by catalysing the reversible hydration of CO_2 (for review see Tashian & Hewett-Emmett, 1984). This activity is probably involved in the maintenance of ionic balance and acid-base homeostasis within the muscle tissue (Swenson, 1984). CAIII is detected in large amounts (2% of wet wt.) in adult red slow-twitch muscles such as rat and rabbit soleus (Register *et al.*, 1978; Jeffery *et al.*, 1982; Väänänen *et al.*, 1986), but is virtually absent from adult white fast-twitch muscle, such as rodent anterior tibialis (AT) and chicken pectoralis muscle (Holmes, 1977; Jeffery *et al.*, 1982). These specific patterns of activity have been further refined to different muscle fibre types by immunohistochemical studies (Jeffery *et al.*, 1986a; Väänänen *et al.*, 1986; Wistrand *et al.*, 1987), which have shown that the concentration of CAIII is highest in type 1 fibres, variable to low in type 2A fibres and low or absent in type 2B fibres. Of the other known isoenzymes of carbonic anhydrase (see Tashian & Hewett-Emmett, 1984), CAII is present in type 1 fibres and CAI in some type 2 fibres (Jeffery *et al.*, 1986a). The function of these isoenzymes in the muscle is unknown.

The development of the specific fibre types and of some fibre-type-specific isoforms in muscle appears to depend on the innervation that the muscle fibre receives, or more specifically on the pattern of neuronal stimulation of the muscle. For example, specific neuronal activity seems to be necessary for the synthesis of adult fast and slow myosin heavy-chain isoforms (Whalen *et al.*, 1981), the adult patterns of expression of troponin (Dhoot & Perry, 1980) and tropomyosin (Roy *et al.*, 1979), and the synthesis of the fast-twitch-fibre-specific Ca^{2+} -binding protein, parvalbumin (Leberer & Pette,

1986). Denervation of the neonatal-rat soleus muscle prevents the emergence of type 1 fibres and the synthesis of slow myosin (Rubinstein & Kelly, 1978), and denervation of maturing fast-twitch muscles suppresses the increase in parvalbumin usually seen after birth (Leberer & Pette, 1986). In addition, chronic nerve stimulation induces fast-to-slow conversions of the major functional systems of the muscle fibres [for reviews see Salmons & Henriksson (1981) and Pette (1984)].

Two features of CAIII expression suggest that the CAIII gene may be under the positive control of slow-muscle-fibre-type neuron activity. One is the marked relationship between fibre type and CAIII protein distribution in adult muscle, and the other is the pattern of the distribution of CAIII during development. For example in humans, where all adult muscles are a mixture of type 1 and type 2 fibres, CAIII protein is detectable in fetal muscle at 10 weeks gestation, but remains in low amount until about 25 weeks gestation, when there is a steep rise to give 50% of the adult value at birth (Jeffery *et al.*, 1980; Lloyd *et al.*, 1986). Similarly in the rat soleus, the amount of CAIII 3 weeks after birth is about 25% of the adult value, and rises rapidly to the adult value during the next 10 weeks (Jeffery *et al.*, 1988). Thus in both species the major increase in CAIII amount occurs at a stage in development when fetal polyneuronal innervation has regressed and the specific fibre/nerve impulse patterns are being established. We have investigated these interrelationships further by examining the changes which occur, after nerve resection, to rat CAIII and CAII proteins and to CAIII mRNA contents in slow- and fast-twitch muscles.

MATERIALS AND METHODS

Animal procedures and tissue preparation

Male or female adult Sprague-Dawley rats, weighing 250–350 g, were anaesthetized with pentobarbitol (50 mg/

Abbreviations used: CAIII, carbonic anhydrase III; AT, anterior tibialis; EDL, extensor digitorum longus; poly(A)⁺ RNA, polyadenylated RNA.

§ To whom reprint requests should be addressed.

kg) intraperitoneally. Then 10 mm of the right sciatic nerve was resected about 20 mm proximal to the hind-leg knee joint on one side; the contra-lateral hind leg was used as control. After various time intervals, up to 71 days, the rats were killed. The tibialis anterior (AT), extensor digitorum longus (EDL) and soleus muscles were dissected from both hind legs. For determination of CAIII and CAII proteins by radioimmunoassay, the muscles were homogenized (1:20, w/v) in 10 mM-phosphate buffer, pH 7.4, containing 0.5% Triton X-100. The homogenate was centrifuged at 1000 *g* for 10 min and the supernatant stored at -70°C until analysed. For determination of total protein the muscles were snap-frozen in isopentane and stored at -70°C until used. Homogenates for SDS/polyacrylamide-gel electrophoresis were prepared in distilled water (1:1, w/v) with an Ultra-Turrax blender and centrifuged at 10000 *g* for 10 min to obtain clear supernatants.

SDS/polyacrylamide-gel electrophoresis

Samples of aqueous muscle supernatants were analysed by SDS/polyacrylamide-gel electrophoresis (24 mA, 4 h) in the Tris/HCl, Tris/glycine buffer system (Laemmli, 1970) and 12%-acrylamide running gels with 5% stacking gels. Protein polypeptides were detected with Coomassie Brilliant Blue. A mixture of proteins of known molecular size was used to calibrate each gel. Before electrophoresis, the samples were denatured at 100°C for 2 min in an equal volume of a solution containing 2.5% (v/v) SDS, 6 mM- β -mercaptoethanol, 40 mM-Tris/HCl (pH 8.3) and 20% (v/v) glycerol.

Quantitative determination of CA proteins

Immunoassays of amounts of CAIII and CAII protein in the muscle supernatants (see above), usually diluted 1:100–1:1000 in water for CAIII and 1:10 for CAII, were carried out with rat CAIII- and CAII-specific antibodies, essentially as described previously (Shiels *et al.*, 1984). The sensitivity of this method is 0.2 ng of enzyme protein/ml of tissue fluid, and the precision is 5% in duplicate determinations for both enzymes. Haemoglobin was determined in the supernatants by a cyanmethaemoglobin method (Zade-Oppen, 1960) to allow correction for blood contamination. It is known that red and white muscles differ with respect to density of capillary network (higher in red fibres), and denervation increases muscle blood flow (see Hudlicka, 1973). Protein was determined by standard procedures.

RNA preparation and analysis

Individual flash-frozen muscles were powdered and homogenized in ice-cold 4 M-LiCl/8 M-urea. After standing for 48 h at 4°C , total RNA was prepared by centrifugation and phenol extraction. Poly(A)⁺ RNA was purified by a passage through oligo(dT)-cellulose, and yields were assessed spectrophotometrically at 260 nm. Variable amounts (0.5–2.0 μg) of poly(A)⁺ RNA were denatured in 1 M-glyoxal and were either applied directly to a nylon membrane (Pall Biodyne; Pall Corp., Glen Cove, NY, U.S.A.) with a slot-blot apparatus, or were electrophoresed in phosphate buffer in a 1.1% agarose gel before Northern blotting (Thomas, 1980). Filters were baked for 1 h at 80°C and hybridized to ^{32}P -labelled DNA probes according to the manufacturers' instructions. After hybridization, membranes were washed in $0.2 \times \text{SSC}$ ($\text{SSC} = 0.15 \text{ M-NaCl}/15 \text{ mM-}$

sodium citrate, pH 7.0)/0.1% SDS at 50°C and exposed to X-ray film for 24 h at -70°C . Insert DNA from the recombinant plasmid pCA15 was used as a probe (Lloyd *et al.*, 1985). The insert DNA is complementary to a full-length 1.7 kb human CAIII mRNA (Lloyd *et al.*, 1986) and was prepared by *EcoRI* digestion and recovery by electroelution from agarose gels. Identical membrane filters were also screened with the 1.0 kb insert cDNA complementary to human skeletal-muscle α actin, pGF3 (Gillespie *et al.*, 1984). The intensity of the signal on the slot-blot autoradiographs was assessed by densitometric scanning with a Joyce-Loebl Chromoscan 3 densitometer.

RESULTS

CAIII protein

Cleared supernatants were prepared from AT, EDL and soleus muscles 16 days post-denervation. This method extracts greater than 90% of soluble protein (Jeffery *et al.*, 1982). CAIII in control and denervated muscles were analysed by SDS/polyacrylamide-gel electrophoresis, and the polypeptide patterns obtained are shown in Fig. 1. The band of M_r 29000, previously defined as CAIII by using a specific antibody (Lloyd *et al.*, 1985), is strikingly increased in the denervated AT and EDL samples compared with the controls, but is slightly less prominent in the soleus muscle. At least five other polypeptides can be seen to be increased in the denervated AT and EDL compared with the controls, including the M_r -17000 polypeptide, previously identified as myoglobin (Askmark *et al.*, 1984), and several other

Fig. 1. Polypeptide profiles of aqueous extracts of rat muscles 16 days after resection of the sciatic nerve (dn) and in contralateral control muscles (C)

Abbreviation: SOL, soleus.

Table 1. Amounts of CAIII protein determined by radioimmunoassay in the hind-leg muscles of individual rats at different times after denervation

Small amounts of CAII were also detected by radioimmunoassay in these muscles, but these probably originate from contaminating blood (see the Results section). The amounts of CAII were not influenced by denervation. Abbreviations: C, control; D, denervated.

		Time after denervation (days)...		CAIII (μg/mg of soluble protein)											
		2		4		8		16			32		71		
Muscle	Rat no...	1	2	3	4	5	6	7	8	9	10	11	12	13	
Soleus	C	51.7	49.8	37.1	27.7	54.6	37.6	58.0	—	—	35.6	46.1	177.1	136.0	
	D	46.7	30.9	41.0	28.9	56.0	39.5	79.8	—	—	64.6	42.9	49.0	47.7	
EDL	C	2.9	3.7	2.4	1.8	2.9	2.3	5.5	4.9	2.8	2.9	5.8	6.2	8.1	
	D	4.0	5.2	4.3	2.4	9.7	7.3	38.6	33.1	32.6	51.9	46.3	35.9	102.1	
AT	C	2.5	6.8	2.5	2.6	3.9	6.9	9.4	—	—	12.3	18.2	17.5	13.4	
	D	3.1	6.4	4.0	3.2	13.7	18.6	53.9	—	—	83.2	89.6	98.6	65.4	

components are less prominent after denervation. The soleus polypeptide pattern is relatively unchanged overall by denervation.

CAIII and CAII proteins in muscle supernatants were determined quantitatively by radioimmunoassay at intervals of 2–71 days after denervation.

The results for CAIII (Table 1 and Fig. 2) are in broad agreement with the SDS/polyacrylamide-gel-electrophoresis analysis and confirm that a marked increase in CAIII protein relative both to the total soluble-protein concentration and to the tissue wet weight occurs after denervation of the AT and EDL, but not the soleus, muscle. There is some variation in CAIII protein in the contralateral control muscles, with a suggestion of an upward trend after an extended period of time, which is particularly noticeable in soleus and to a lesser degree in the AT muscle. This could be due to rat-to-rat individual variation, an age effect or a response to some humoral stimulus similar to the cross-over effect described by Staron *et al.* (1987) in electro-stimulated

muscle. Thus, in order to take account of this variation in the control material, the amounts of CAIII in the denervated muscles are expressed relative to the control values in the same-day contralateral muscle (Fig. 2). When the values for each time point are plotted, the greatest increase of CAIII is seen in the EDL. The maximum is an 8-fold rise at 16 days after denervation if the CAIII contents are expressed relative to other soluble proteins, and a 13-fold change at 32 days post-denervation if expressed relative to tissue wet weight (Fig. 2). There is a 5–6-fold increase in CAIII protein in the AT muscle, irrespective of the method used to express the data, and in soleus the CAIII contents show scarcely any change in the denervated muscle.

The amounts of CAII ranged between 0.5 and 1.1 $\mu\text{g}/\text{g}$ wet wt., with no significant difference between the muscle before and after denervation. These values correspond closely to those, 0.17–0.9 $\mu\text{g}/\text{g}$ wet wt., which can be calculated to originate from the blood content of the muscles, which was found to vary between 0.02 and 0.1% of whole blood. In these calculations the whole blood was taken to contain 850 μg of CAII/ml (Lönnerholm *et al.*, 1986).

Total RNA

Muscles from 14 animals, 16 days after sciatic-nerve resection, were used for RNA preparation. The individual muscles varied in weight, but in general the AT muscles were the largest and the soleus and EDL the smallest (controls, average weight, AT 639.7 mg, EDL 152.4 mg, soleus 135.2 mg). After denervation there was a marked loss of muscle weight, easily seen after 1 week, and by 16 days the weight loss was about 64%, 61% and 54% for the AT, soleus and EDL respectively (Table 2). In general it appears that the yields of total RNA per mg of tissue were higher by 1.5–3-fold in the denervated samples than in the corresponding controls. Although this may indicate a real increase in translational capacities, it is more likely to be due to the loss of muscle weight, since the average total yields of RNA in each denervated and control muscle pair are very similar (Table 2). This would imply that the weight loss is largely attributable to wasting of the contractile protein mass, and takes place without depletion of the total RNA pool.

Fig. 2. Changes in the amounts of CAIII protein in rat muscle after sciatic-nerve resection assessed by radioimmunoassay

CAIII protein is expressed per mg wet wt. of tissue (—) or per μg of soluble protein (—) and plotted as a ratio of the denervated/control muscles. Each point represents the average results for two animals, except at 16 days, where data were collected from three animals for EDL and from a single animal for AT and soleus.

Table 2. Effects of 16 days of denervation on muscle weight, total RNA and poly(A)⁺ RNA concentrations

Data are means $\pm$ S.D. ($n = 14$). Abbreviations: C, control; D, denervated.

Muscle		Av. weight (mg)	Av. weight loss (%)	Total RNA		Poly(A) ⁺ RNA	
				($\mu\text{g}/\text{mg}$ wet wt.)	($\mu\text{g}/\text{whole}$ muscle)	(ng/mg wet wt.)	($\mu\text{g}/\text{whole}$ muscle)
Soleus	C	135.2 $\pm$ 20.6	61.2	1.78 $\pm$ 0.6	241	64.4 $\pm$ 39.9	8.7
	D	52.2 $\pm$ 13.0		4.05 $\pm$ 3.1	211	68.5 $\pm$ 48.8	3.6
EDL	C	152.4 $\pm$ 14.7	53.9	1.63 $\pm$ 0.8	248	34.8 $\pm$ 15.0	5.3
	D	70.3 $\pm$ 7.9		2.60 $\pm$ 1.9	183	194.2 $\pm$ 92.4	13.7
AT	C	639.7 $\pm$ 66.8	63.7	0.80 $\pm$ 0.4	511	29.6 $\pm$ 11.7	18.9
	D	232.8 $\pm$ 29.5		2.48 $\pm$ 1.8	577	94.4 $\pm$ 65.8	22.0

The results of the estimates of the poly(A)⁺ RNA fraction are rather heterogeneous (Table 2), and it is difficult to decide how much they are affected by the weight loss induced by denervation and whether the apparent increase in poly(A)⁺ RNA concentration in the denervated EDL and the decrease in the denervated soleus are significant.

CAIII mRNA

Estimates of the CAIII mRNA concentrations in the three sets of muscle were obtained from slot-blot assays and Northern-blot analysis. The slot-blot assays (Fig. 3a) were carried out on pooled muscle samples for eight animals (Expt. 1) or six animals (Expt. 2), and the Northern analysis (Fig. 3b) was carried out on individual muscles dissected from three separate animals.

The estimates of mRNA per mg of muscle tissue derived from slot-blot assay (Table 3) revealed markedly lower contents of CAIII mRNA in the denervated red slow-twitch soleus muscles compared with the contralateral controls [denervated (D)/control (C) ratio 0.3]. In contrast, the white fast-twitch EDL and AT muscles showed higher contents of CAIII mRNA in the denervated specimens compared with the controls. In Expt. 1 the change is more marked in the EDL muscles (D/C ratio 6.2) than in the AT muscles (D/C 4.2), and Expt. 2 the effect of denervation is more pronounced in AT (D/C 13.9) than in EDL (D/C 2.0).

These results are in keeping with the radio-immunoassay estimates of the CAIII-protein contents in these three types of muscle in the control and denervated specimens (Fig. 2), except that the effect of denervation on CAIII protein was most marked in the EDL muscle, whereas for the mRNA estimates the change is most pronounced in the AT muscle, when the results of both experiments are combined as an average.

Differences in the response to denervation between slow-twitch (soleus) and fast-twitch (EDL and AT) muscles are also apparent when the CAIII mRNA contents are expressed as a proportion of the poly(A)⁺ mRNA [CAIII/ μg of poly(A)⁺ RNA; Table 3]. There is a marked decline in the relative concentration of the CAIII mRNA associated with denervation of the red slow-twitch SOL muscles (D/C 0.3) compared with white fast-twitch AT muscles (D/C 2.8). These results were confirmed by Northern-blot analysis (Fig. 3b) of the mRNA prepared from individual muscle biopsies. The consistent finding associated with denervation was a relative decrease in the CAIII mRNA in the slow-twitch soleus muscles and a marked increase in the fast-twitch AT muscles. The results obtained from the EDL muscles were more heterogeneous, however, both on slot-blot assay (Table 3) and on Northern analysis (Fig. 3b). An elevated CAIII mRNA signal was found after

Fig. 3. Amounts of CAIII mRNA in poly(A)⁺ RNA derived from control (c) and denervated (d) rat muscle

(a) Slot-blot assay of 2.0, 1.0 and 0.5 μg of poly(A)⁺ RNA. The results from Expt. 1 are shown in full, and for comparison the results for AT of Expt. 2 are included. (b) Northern-blot analysis of 2 μg of poly(A)⁺ RNA from muscles of three individual rats. ³²P-labelled insert DNA pCA15 was used as probe. Abbreviation: SOL, soleus.

Table 3. Amounts of CAIII mRNA in rat muscles 16 days after denervation

Abbreviations: IPA, integrated peak area for CAIII mRNA; C, control; D, denervated.

Muscle	Expt. 1		Expt. 2		Average D/C	Expt. 1		Expt. 2		Average D/C
	IPA/10 mg of muscle	D/C	IPA/10 mg of muscle	D/C		IPA/ μ g of poly(A) ⁺ RNA	D/C	IPA/ μ g of poly(A) ⁺ RNA	D/C	
Soleus	C	35.1	0.15	32.6	0.3	54.5	0.1	50.6	0.5	0.3
	D	5.3		15.5		7.7		22.6		
EDL	C	0.6	6.2	5.0	4.1	1.8	1.1	14.4	0.4	0.7
	D	3.7		9.9		1.9		5.1		
AT	C	1.7	4.2	1.6	9.1	5.7	1.3	5.4	4.4	2.8
	D	7.2		22.2		7.6		23.5		

Table 4. Amounts of actin mRNA in rat muscles 16 days after denervation

Abbreviations: IPA, integrated peak area for actin mRNA; C, control; D, denervated.

Muscle	Expt. 1		Expt. 2		Average D/C	Expt. 1		Expt. 2		Average D/C
	IPA/10 mg of muscle	D/C	IPA/10 mg of muscle	D/C		IPA/ μ g of poly(A) ⁺ RNA	D/C	IPA/ μ g of poly(A) ⁺ RNA	D/C	
Soleus	C	22.2	0.05	34.7	0.09	34.5	0.05	53.9	0.12	0.09
	D	1.1		4.6		1.6		6.7		
EDL	C	11.7	0.32	11.1	0.47	33.5	0.05	32.0	0.11	0.08
	D	3.7		7.0		1.6		3.6		
AT	C	5.5	1.16	14.7	1.53	18.7	0.36	49.6	0.60	0.48
	D	6.4		28.0		6.8		29.7		

denervation in some animals (e.g. track 1, Fig. 3*b*, and Expt. 1, Table 3), but there was no change, or even a decreased signal, in others (e.g. tracks 2 and 3, Fig. 3*b*, and Expt. 2, Table 3).

Actin mRNA

Estimates of actin mRNA concentrations were also made on the control and denervated muscle samples by Northern analysis and slot-blot assay. In each case the actin mRNA concentration relative to the total poly(A)⁺ RNA content decreased markedly on denervation (Table 4). This effect was least pronounced in the fast-twitch AT muscle. A similar pattern of response was observed if the actin mRNA concentration was expressed relative to the wet weight of muscle tissue, and indeed on this estimation the AT actin mRNA increased above control values on denervation.

DISCUSSION

We have examined the possibility that the expression of the muscle-specific carbonic anhydrase (CAIII) gene is influenced by neuronal activity. CAIII protein and mRNA contents in fast- and slow-twitch rat muscles have been examined after resection of the sciatic nerve, and marked changes have been observed. The most striking alterations occur in the fast-twitch AT and EDL muscles, which show significant elevation of CAIII protein after denervation, and the effects are greater than can be attributed to the consequences of muscle atrophy after denervation. Furthermore, since the CAIII protein

and the specific CAIII mRNA are both increased several-fold 16 days after denervation, it seems likely that the increases are regulated, at least in part, by changes in gene transcription rather than by increased translatability of the mRNA. This appears to involve the 'switching on' of CAIII expression in the normally non-expressing type-2 fast-twitch muscle fibres, and agrees with immunohistochemical studies which have demonstrated significant amounts of CAIII protein in type-2 fibres after denervation (Wistrand *et al.*, 1987). Although increased transcription seems to be the most likely interpretation, the possibility that the CAIII mRNA is more stable in the denervated muscles than in the controls cannot be excluded.

Although the overall conclusion is straightforward, detailed examination of the data reveals complexities owing to differences in the response to denervation shown by the two fast-twitch muscles, AT and EDL. The protein and mRNA data for these two muscles are not in close agreement when compared 16 days after denervation. In particular, the amount of CAIII mRNA in EDL is relatively low compared with the protein content, whereas in the AT the mRNA content is relatively high. This may reflect differences in the timing of denervation-induced responses in AT and EDL, and/or variations in the CAIII protein/mRNA turnover times in the two muscles. In addition the CAIII mRNA apparently decreases relative to other mRNAs in the EDL mRNA pool after denervation, whereas in the AT there is a 3-fold increase in the proportion of CAIII mRNA. This implies that the relative rates of synthesis

of other member mRNAs in the muscle mRNA pools are changing and that the patterns of change are different in the two muscles. This is further evidenced by the greater loss of actin mRNA in EDL (92 % of control) compared with AT (52 % of control).

It is not clear why the AT and EDL muscles should respond differently to denervation. There may be some effect of fibre composition. Both muscles comprise predominantly type 2 fibres, but there are some type 1 fibres in the AT and a higher proportion of type 2A (intermediate) than 2B (fast) in AT compared with the EDL (Jeffery *et al.*, 1986a; Wistrand *et al.*, 1987). It is noteworthy that a similar variation in the extent of myoglobin induction among denervated muscles of similar fibre composition has also been reported (Askmark *et al.*, 1984).

If the differentiation of fibre-type-specific metabolism and biochemistry is dependent on a particular pattern of nerve activity, then it might be supposed that, in the absence of neuronal stimulation, mature fibres would gradually lose their fibre-specific features and take on the characteristics of an undifferentiated immature muscle fibre. However, the induction of expression of CAIII in EDL and AT to values close to those found in the normal adult soleus muscle suggests that the change perhaps reflects a fast-to-slow transition. This agrees with various other reports in the literature; for example, myoglobin, a protein usually confined to type 1 fibres, is increased 3-fold in denervated EDL and AT (Askmark *et al.*, 1984), to values higher than those found in fetal tissue (Weller *et al.*, 1986) and similar to those of adult soleus. In addition, the synthesis of parvalbumin, characteristic of type 2 fibres, is decreased after denervation to a value characteristic of slow-twitch muscle (Leberer & Pette, 1986).

There is some evidence that denervation may lead to the reverse, slow-to-fast, transition in soleus. For example, 30 days after cord section in the rat there is histochemical evidence of an increased proportion of fast fibres in the soleus, and this normally slow muscle acquires myosin light chains of the fast-muscle type (Rubinstein & Kelly, 1978), although it is not clear whether these are the adult fast isoforms. However, the rapid appearance of fast-fibre-specific proteins is not a general feature. The low contents of parvalbumin in fetal and adult soleus are unaffected by denervation (Leberer & Pette, 1986). Furthermore, the denervated soleus is apparently slow to lose proteins characteristic of type 1 fibres, since little change is seen in the CAIII or myoglobin protein contents during the 32 days after denervation (Askmark *et al.*, 1984; the present work). Eventual loss of CAIII protein from denervated soleus is presumably heralded by the decrease of CAIII mRNA to 30 % of control values.

How neural impulses are translated into gene activity within the muscle fibre is unknown. The molecular signal for switching on muscle CAIII synthesis may be a growth factor secreted by the nerve ending, or could relate directly to a neurotransmitter. Alternatively the situation may be the result of hormonal control and similar to that found in hypophysectomized female rats, where the absence of growth hormone appears to switch on the

synthesis of CAIII protein in the liver (Jeffery *et al.*, 1986b).

This work is supported by the Swedish Medical Research Council, grants 2874 (for P. J. W.) and 6755 (for N. D. C.). We thank Mrs. Laine Bennick-Björkman and Mrs. Gun Jahnke for skilful technical assistance and Mrs. D. Trinder for typing the manuscript.

REFERENCES

- Askmark, H., Coulson, M. & Roxin, L.-E. (1984) *Muscle Nerve* **7**, 656–661
- Dhoot, G. K. & Perry, S. V. (1980) *Exp. Cell Res.* **127**, 75–87
- Gillespie, G., Lloyd, J., Hopkinson, D. & Edwards, Y. (1984) *J. Muscle Res. Cell Motil.* **5**, 457–464
- Holmes, R. S. (1977) *Comp. Biochem. Physiol. B* **57**, 117–120
- Hudlicka, O. (1973) *Muscle Blood Flow: Its Relation to Muscle Metabolism and Functions*, pp. 1–211, Swets and Zeitlinger, Amsterdam
- Jeffery, S., Edwards, Y. H. & Carter, N. D. (1980) *Biochem. Genet.* **18**, 843–849
- Jeffery, D., Edwards, Y. H., Jackson, M. J., Jeffery, S. & Carter, N. D. (1982) *Comp. Biochem. Physiol. B* **73**, 971–975
- Jeffery, S., Carter, N. D. & Smith, A. (1986a) *J. Histochem. Cytochem.* **34**, 513–516
- Jeffery, S., Wilson, C., Mode, A., Gustafsson, J. A. & Carter, N. (1986b) *J. Endocrinol.* **110**, 123–126
- Jeffery, S., Merry, B., Holehan, A. & Carter, N. D. (1988) *Biochem. J.* **250**, 303–305
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
- Leberer, E. & Pette, D. (1986) *Biochem. J.* **235**, 67–73
- Lloyd, J., Isenberg, H., Hopkinson, D. & Edwards, Y. H. (1985) *Ann. Hum. Genet.* **49**, 241–245
- Lloyd, J., McMillan, S., Hopkinson, D. & Edwards, Y. H. (1986) *Gene* **41**, 233–239
- Lönnnerholm, G., Wistrand, P. J. & Barany, E. (1986) *Acta Physiol. Scand.* **126**, 51–60
- Pette, D. (1984) *Med. Sci. Sports* **16**, 517–528
- Register, A. M., Koester, M. K. & Noltmann, E. A. (1978) *J. Biol. Chem.* **253**, 4143–4152
- Roy, R. K., Sreter, F. & Sankar, S. (1979) *Dev. Biol.* **69**, 15–30
- Rubinstein, N. A. & Kelly, A. M. (1978) *Dev. Biol.* **62**, 473–485
- Salmons, S. & Henriksson, J. (1981) *Muscle Nerve* **4**, 94–105
- Shiels, A., Jeffery, S., Wilson, C. & Carter, N. (1984) *Biochem. J.* **218**, 281–284
- Staron, R. S., Gohlsch, B. & Pette, D. (1987) *Pflugers Arch.* **408**, 444–450
- Swenson, E. R. (1984) *Ann. N. Y. Acad. Sci.* **429**, 547–561
- Tashian, R. E. & Hewett-Emmett, D. (eds.) (1984) *Ann. N. Y. Acad. Sci.* **429** (several chapters)
- Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5201–5205
- Väänänen, H. K., Takala, T. & Morris, D. C. (1986) *Histochemistry* **86**, 175–179
- Weller, P. A., Price, M., Isenberg, H., Edwards, Y. H. & Jefferys, A. (1986) *Mol. Cell. Biol.* **6**, 4539–4547
- Whalen, R. G., Sell, S. M., Butter-Browns, G., Schwartz, K., Bouveret, P. & Pinset-Harstrom, I. (1981) *Nature (London)* **292**, 805–809
- Wistrand, P. J., Carter, N. D. & Askmark, H. (1987) *Comp. Biochem. Physiol. A* **86**, 177–184
- Zade-Oppen, M. (1960) *Acta Soc. Med. Ups.* **65**, 249–257