Biochemical Characteristics of Slow Skeletal Muscle in Work-Induced Hypertrophy

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Skeletal-muscle fibres are able to respond adaptively to various functional demands by appropriate changes in protein turnover rates. These long-term modulations result either in an overall increase in fibre volume (i.e. muscle hypertrophy) or in the selective accumulation of individual subcellular components, according to the amount and type of work (Guth, 1968).

It is, on the other hand, known that there are differences at the molecular level between mammalian fast muscles and slow muscles with regard to myofibrils (see Bárány, 1967), soluble sarcoplasm (e.g. isoenzymic composition of lactate dehydrogenase; Dawson, Goodfriend & Kaplan, 1964) and the intracellular membranes (Margreth, Salviati & Mussini, 1969). These biochemical characteristics, which are ontogenically determined (Trayer & Perry, 1966; Margreth, Angelini, Valfrè & Salviati, 1970; Margreth, Salviati & Catani, 1971) and largely controlled by the specific innervation to the muscles (Mommaerts, Buller & Seraydarian, 1969), are also generally considered to be relatively stable under prevailing steady-state conditions. Partly because most previous enzymic studies have been carried mainly on mixed skeletal muscles, it is as yet unsolved question (Guth, 1968) whether these specific properties may, however, be modified during work-induced dynamic changes in normally innervated muscle, i.e. independent of effects on the frequency of nerve impulses.

Here we report results obtained from a study of the slow soleus muscle of the rat (Close, 1964; Margreth *et al.* 1970; Schiaffino, Hanzlíková & Pierobon, 1970) during compensatory hypertrophy after tenotomy of the synergistic muscles (Hamosh, Lesch, Baron & Kaufman, 1967). The hypertrophy is explained mainly by enhanced synthesis of new muscle protein, both myofibrillar and sarcoplasmic (Hamosh *et al.* 1967; Goldberg, 1968). The somewhat disproportionate accumulation of sarcoplasmic proteins in hypertrophic soleus muscle has been attributed to an additional effect on their rate of catabolism (Goldberg, 1969).

Groups of seven to ten male Wistar rats (about 200 g body wt.) were used in each experiment 5 days after tenotomy of the gastrocnemius and plantaris

muscles of the right hind leg. The soleus muscles from the operated side and the controlateral muscles (controls) were quickly excised, weighed on a precision torsion balance and pooled. The minced tissues (about 1 g) were homogenized in 9 vol. of ice-cold 0.3 M-sucrose in a flat-bottomed Perspex homogenizer operated at 1500 rev./min and then centrifuged at 650g for 10 min. Mitochondrial and microsomal fractions were sedimented from the supernatant solution by differential centrifugation at 6500g for 20 min and then 30 000g for 30 min. The soluble fraction was obtained by centrifugation at 150 000g for 60 min. Myosin was isolated from the low-speed sediment by the procedure of Szent-Györgyi (1951) with some modifications derived from Bárány (1967). After being freed from contaminating soluble proteins by washing three times with 50 mM-potassium chloride, the myofibrillar residue was extracted once with 9 vol. of 0.6 M-potassium iodide-6 mM-sodium thiosulphate. Insoluble material was removed by centrifuging at 13 000g for 20 min, and myosin was precipitated from the supernatant fraction by dilution with water (Szent-Györgyi, 1951). The precipitate was collected by centrifuging at 2500g for 10 min, dissolved in 0.5 M-potassium chloride and recentrifuged at 150 000g for 60 min. The sediment was discarded and the supernatant solution, containing purified myosin, was used for the assay of adenosine triphosphatase activity.

Myoglobin was determined by the method of Reynafarje (1963), which is based on conversion into a carbonyl dithionite-reduced derivative in phosphate-buffered medium, pH 6.6, and measurement of the extinction at 538 nm and 568 nm. Separate determinations were made in the 150 000g supernatant fraction of the tissue homogenates, containing most of the pigment, and in potassium chloride extracts. The summed values were used to calculate the total amount of myoglobin per unit fresh weight tissue. NADH-cytochrome c reductase (EC 1.6.99.-), glycerol 3-phosphate-cytochrome c reductase (EC 1.1.99.5), glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) and lactate dehydrogenase (EC 1.1.1.27) were measured spectrophotometrically in a Unicam SP. 800 recording spectrophotometer. The adenosine triphosphatase activities of myosin

and of muscle microsomal fraction were determined by measuring the liberation of P_i (Lindberg & Ernster, 1956) from ATP. Ca^{2+} uptake was determined by a Millipore filtration method (Martonosi & Feretos, 1964) and liquid-scintillation spectrometry of ^{45}Ca . The conditions for the assay of the various enzyme activities are given in the legend to Table 1. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). The overall results of the present experiments with rat soleus muscle are given in Table 1.

The myosin adenosine triphosphatase values are of especial interest, since they were previously correlated by Bárány (1967) with the intrinsic speed

of shortening in the same muscle. There is a tendency in hypertrophic soleus muscle towards greater emphasis of the slow-type catalytic properties of myosin. This might perhaps be attributed to accumulation, at the early stages of hypertrophy, of foetal-type myosin, which shares most of the properties of slow-type myosin but in an exaggerated form (Trayer, Harris & Perry, 1968). Lesch, Parmley, Hamosch, Kaufman & Sonnenblick (1968) have reported a decrease of the intrinsic speed of shortening in rat soleus muscle undergoing compensatory hypertrophy.

The Ca^{2+} -uptake activity of skeletal-muscle sarcoplasmic reticulum is generally found to be

Table 1. *Work-induced changes in rat soleus muscle*

	Control muscle				Hypertrophic muscle			
	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Average muscle wt. (mg)	61	91	92	88	102	119	116	121
Myosin ATPase (μ mol of P_i formed/min per mg of protein)								
EDTA-stimulated	—	0.58	0.66	0.84	—	0.49	0.35	0.61
Ca^{2+} -stimulated	—	0.38	—	—	—	0.32	—	—
Microsomal Mg^{2+} -stimulated ATPase (μ mol of P_i formed/min per mg of protein)								
No addition	0.58	1.22	—	0.61	0.65	1.00	—	0.76
EGTA (1 mM) added	0.70	1.08	—	0.41	0.65	0.96	—	0.67
Ca^{2+} uptake (μ mol/mg of protein)								
Initial rate (1 min)	—	0.11	—	0.10	—	0.07	—	0.10
Maximal capacity (10 min)	—	0.52	—	0.43	—	0.48	—	0.44
NADH-cytochrome <i>c</i> reductase (μ mol of cytochrome <i>c</i> reduced/min per mg of protein)	—	0.092	—	0.065	—	0.089	—	0.087
Lactate dehydrogenase (μ mol of NADH oxidized/min per mg of protein)								
With 0.33 mM-pyruvate (<i>A</i>)	—	1.91	4.16	3.22	—	1.63	2.78	2.90
With 10 mM-pyruvate (<i>B</i>)	—	1.12	1.33	1.61	—	0.84	0.85	1.21
Isoenzyme ratio (<i>A/B</i>)	—	1.7	3.1	2.0	—	1.9	3.3	2.4
Myoglobin (mg/g fresh tissue wt.)	—	—	2.82	2.86	—	—	2.48	2.26

matched to the rate of ATP hydrolysis by myosin (Margreth *et al.* 1969), and is accordingly low in rat soleus muscle. Likewise the Mg^{2+} -stimulated adenosine triphosphatase activity of the microsomal fraction from soleus muscle exhibits a low degree of sensitivity to added ethanedioxybis(ethylamine)-*NNN'*-tetra-acetate, in agreement with earlier studies on slow muscles from other mammalian species (Sreter & Gergely, 1954; Mommaerts *et al.* 1969). Neither of these properties nor the rotenone-insensitive NADH-cytochrome *c* reductase activity (Margreth *et al.* 1971) of the corresponding fraction from hypertrophic muscle reveal substantial changes (Table 1).

Reis & Wooten (1970) have established a quantitative relationship between myoglobin concentration and duration of contraction in cat skeletal muscles. In the rat slow soleus muscle, myoglobin concentration (Table 1) remains high during hypertrophy by comparison with published values of 1.3–1.6 mg/g of muscle in predominantly fast leg muscles in the same species (Reynafarje, 1963). Likewise the isoenzyme ratios of lactate dehydrogenase are at least as high in hypertrophic soleus muscle as in the control muscle (Table 1), i.e. there is an unvaried predominance of the H form of enzyme, which is inhibited by high concentrations of pyruvate (Margreth *et al.* 1970). In conformity with these several results, as well as with the knowledge that the enzymes of the glycerol 3-phosphate cycle (Estabrook & Sacktor, 1958) are relatively inactive in slow muscles (see Pette, 1966; Margreth *et al.* 1969), additional experiments showed that the specific activities of mitochondrial glycerol 3-phosphate-cytochrome *c* reductase (Ringler & Singer, 1959) and of soluble glycerol 3-phosphate dehydrogenase (Brosemer, 1965) were very low in both control and hypertrophic soleus muscles. The respective activities were, in the order given, 5 nmol and 4 nmol of cytochrome *c* reduced/min per mg of mitochondrial protein at 30°C, and 63 nmol and 43 nmol of NADH oxidized/min per mg of soluble protein at 25°C.

Thus the present results indicate that the molecular composition of both myofibrillar and sarcoplasmic components is remarkably uniform throughout compensatory hypertrophy of the rat soleus muscle. These findings imply a very rigid homeostatic regulation, by neurogenic and possibly intrinsic factors, over the biochemical characteristics of slow muscle, i.e. akin to irreversible differentiation of tissues. The validity and more general applicability of these concepts must, how-

ever, await further work with different experimental models and different muscles.

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