Early life undernutrition in rats

2. Some contractile properties of skeletal muscles from adult animals

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(*Received 7 September 1981 – Accepted 26 November 1981*)

1. The contractile properties of soleus and extensor digitorum longus muscles from animals at 12 months of age whose mothers had been undernourished during pregnancy and lactation (PU rats) have been compared with those of muscles from age-matched controls.

2. Body-weight and muscle wet weight of PU rats was significantly reduced. Muscle:body-weight values were, however, no different from controls.

3. No significant alterations in whole muscle speeds of contraction or relaxation could be detected when compared with those of age-matched controls.

4. Twitch and tetanic forces of both SOL and EDL were greater per unit weight of muscle in PU rats. Apart from SOL twitch these differences were significant. The tetanus:twitch values were, however, not different.

It is well documented that the histology, biochemistry and physiology of skeletal muscle alters during development (Close, 1972). The muscle fibres of rats are immature at birth and all have 'slow'-twitch characteristics (Close, 1972). Their differentiation into recognizable 'fast' and 'slow'-twitch fibres, typical of adult mammalian skeletal muscles, is critically dependent on an intact nervous system (Shafiq *et al.* 1972). Intense growth activity is seen in the peripheral nervous system during this differentiation. Multiple synapses are made with muscle fibres which then are believed, in the rat, to compete for a permanent synaptic connection (Brown *et al.* 1976; Riley, 1977) during the first 2 weeks of life. It seems likely that it is this permanent contact, possibly via an effect due to the resulting normal activity induced in muscle fibres, which affects the differentiation of 'fast' and 'slow'-twitch fibres. Certainly cross-innervation of adult 'slow'- and 'fast'-twitch muscle with 'fast' and 'slow' nerves respectively does significantly alter their properties (Buller *et al.* 1960; Close, 1965) and denervation causes a slowing of contraction time of 'fast'-twitch muscles (Eccles *et al.* 1962). Hence the reduction in central nervous tissue resulting from early undernutrition (Dobbing & Smart 1974; Thomas *et al.* 1979), particularly if similar changes also occur in the peripheral system (Sourander *et al.* 1974), could affect the differentiation of skeletal muscle fibre types.

Intensive exercise or partial removal of a muscle results in the appearance of a preponderance of large-diameter fibres (Goldspink, 1962) containing an increased myofibril content (Goldspink, 1965). Restriction of an animal's food supply has been shown to result in a preponderance of small-diameter fibres (Goldspink, 1965). Such changes in muscle have been related to changes in performance, with exercise-hypertrophied fibres being

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stronger than small-diameter fibres (Goldspink, 1965), but these changes are fully reversible (Goldspink & Ward, 1979).

Hence skeletal muscle adapts rapidly to a wide variety of influences. It is perhaps not surprising, therefore, that in mature animals (*a*) undernutrition should bring about changes in muscle physiology and histochemistry and (*b*) these changes are fully reversible (Goldspink & Ward, 1979; Bedi *et al.* 1982). The fact that it has been shown that muscle from rats which had been undernourished during the gestation and suckling period undergoes irreversible histochemical and histological changes (Haltia *et al.* 1978; Howells *et al.* 1978; Bedi *et al.* 1982) poses the question as to whether, and to what extent, the physiological properties of these muscles may also be permanently altered. As far as can be ascertained no equivalent study has been reported.

'Slow'-twitch soleus (SOL) and 'fast'-twitch extensor digitorum longus (EDL) muscles of previously-undernourished (PU) rats have been examined in vivo to determine twitch tension, contraction time, half-relaxation time and maximal tetanic tension. The results are discussed with relation to findings concerning the histology and histochemistry of muscle from PU rats (Bedi *et al.* 1982).

METHODS

Two groups of adult male rats were used. One consisted of seven offspring (PU rats) of mothers given a restricted diet during pregnancy and lactation as described by Bedi *et al.* (1982) and the other was composed of six age-matched controls whose mothers were fed *ad lib.* during this period. After weaning, all litters were fed an unrestricted diet. Final experiments were done between 12 and 15 months after birth.

Animals were anaesthetized with Inactin, 100 mg/kg. Two muscles were investigated, SOL, a characteristically 'slow'-twitch muscle and EDL, a 'fast'-twitch muscle. The animal was laid on its back for investigating EDL or its front for SOL. The leg was opened with a longitudinal incision and the required muscle dissected free. The distal tendon was cut and attached with inelastic thread to an isometric force transducer (Ether Ltd). A thick thread was passed through the knee and tied firmly to an immovable bar. The ankle was clamped to stabilize the preparation. The skin surrounding the incision was drawn up to a circular bar by means of stitches to form a pool through which paraffin oil was passed at 37°. This served to prevent dessication or cooling of the surface of the muscle being investigated. The sciatic nerve was dissected and sectioned. The distal end was attached to silver wires through which stimulating pulses were passed. Supramaximal voltages as 0·2 ms square waves were applied from a Devices isolated stimulator. Either single pulses or trains could be produced using a Devices Gated pulse generator and Digitimer. The operating procedure typically took 30 min and the animal was then left for a further 30 min before stimulation.

Under isometric recording conditions contraction time, half-relaxation time, twitch tension and maximal tetanic tension of muscles from control and experimental animals were measured. Permanent records of muscle responses were made using a Grass polygraph via a 7Pl amplifier. Values of rise times, half-relaxation times and twitch tensions were determined using a 5000-series Tektronix storage oscilloscope. Rise and fall times are expressed in ms and total muscle tension in g and unit muscle tension as g/wet weight of muscle.

At the end of the experiment animals were killed with an overdose of Inactin and SOL and EDL muscles removed, blotted dry and weighed. The properties of muscles are given as means (± 1 SE). The significance of differences between groups was evaluated using an unpaired Student's *t* test.

Table 1. *Comparison of body and muscle weights of well fed control (C) and previously-undernourished (PU) rats*

(Values are means with 1 standard error. Body-weights were of live animals; no. of animals in parentheses)

	C (6)		PU (7)		Statistical significance of difference: *P*
	Mean	SE	Mean	SE	
Body-wt (g)	511·7	16·4	364·9	15·6	< 0·02
Muscle wet wt (mg): EDL	250·3	10·9	187·3	5·9	< 0·02
:SOL	205·8	10·9	157·1	12·8	< 0·02
Muscle wt: body-wt ($\times 10^{-3}$): EDL	0·484	0·015	0·505	0·011	0·29
:SOL	0·387	0·019	0·409	0·018	0·4

EDL, extensor digitorum longus; SOL, soleus.

Table 2. *Comparison of isometric contractions in normal well-fed control (C) and previously-undernourshed (PU) rat muscles*

(Values are means with 1 standard error)

	C		PU	
	Mean	SE	Mean	SE
EDL				
Time to peak (ms)	19·5	0·4	20·7	0·5*
Half-relaxation time (ms)	15·8	1·7	14·6	0·8
Twitch tension (g)	68·9	6·1	61·1	4·5
Maximum tetanic tension (g)	338·4	43·5	330·0	11·4
SOL				
Time to peak (ms)	37·9	1·3	39·0	1·6
Half-relaxation time (ms)	44·0	3·9	44·7	3·7
Twitch tension (g)	38·3	3·8	33·8	6·1
Maximum tetanic tension (g)	165·4	16·7	149·4	10·7

EDL, extensor digitorum longus; SOL, soleus.

* The only measurement to show a change approaching statistical significance ($P = 0·07$).

RESULTS

The mean body-weight of the seven PU rats used in this study was 30·6% less than that of the six aged-matched controls (Table 1). This deficit in absolute weight was statistically significant. SOL and EDL muscles from PU rats were also lighter than those of controls. The mean deficit was 23·7% for SOL and 25·2% for EDL. Again the differences were statistically significant (Table 1). Nevertheless, body-weight: muscle weight was not significantly altered for SOL or EDL from PU rats and normal rats (Table 1).

As expected, control EDL muscles had a faster contraction time, half-relaxation time and produced greater twitch and tetanic tensions than control SOL muscles. All differences were statistically significant. Maximal tetanic tension was obtained by applying stimuli at 100 Hz to EDL and 40 Hz to SOL. The results obtained from muscles from normal and PU rats is summarized in Table 2. Comparison with their controls showed that SOL from PU rats was not significantly changed in any factor measured. A similar comparison for EDL from PU rats also showed no significant change, although a slowing of the twitch approached

Table 3. *Twitch and tetanic tension (g/g wet weight of muscle) from normal well-fed control (C) and previously-undernourished (PU) rats*

(Values are means with 1 standard error. Maximum tetanic frequency for EDL was 100 Hz and for SOL was 40 Hz).

Group	C		PU		Statistical significance of difference: P
	Mean	SE	Mean	SE	
EDL					
Twitch tension (g/g wet wt)	273·3	19·1	323·5	15·9	0·06
Tetanic tension (g/g wet wt)	1378·8	103·9	1634·5	64·2	0·05
SOL					
Twitch tension (g/g wet wt)	185·3	15·5	207·8	22·0	0·4
Tetanic tension (g/g wet wt)	792·4	60·9	970·7	63·6	0·06

EDL, extensor digitorum longus; SOL, soleus.

significance at $P < 0·05$. In view of the observed decrease in SOL and EDL weights from PU rats, the results for muscle tensions have been expressed as force output per unit weight of muscle in Table 3. Here it can be seen that there was an increased twitch and tetanic force output per unit wet weight of muscle. Apart from the SOL twitch tension, the increased force output from muscles from PU rats approached significance at $P < 0·05$. Tetanus: twitch values were little changed in SOL muscles from PU rats, 4·5 *v.* 4·4 in control muscles. With EDL the corresponding value was 5·4 in PU rats and 4·9 for control muscles.

DISCUSSION

Although the group of PU rats used in this physiological investigation were older than those used for the histological examination reported in the previous paper (Bedi *et al.* 1982) similar deficits in total body-weight and wet weights of SOL and EDL were found with respect to control animals. However, in both groups of rats muscle weight: body-weight was no different from that for control animals.

The values obtained for normal EDL and SOL twitch and tetanic tensions, and for speeds of contraction were in general agreement to those obtained by other workers (Close, 1969; Barany & Close, 1971; Gutmann *et al.* 1974). The speed of contraction of a whole muscle reflects the relative proportions of fibre types present (Goldspink, 1977). These may be 'fast'-twitch (type II) fibres or 'slow'-twitch (type I) fibres (see Bedi *et al.* 1982). EDL has a major proportion of type II fibres and is fast contracting and fast relaxing. SOL, which has a major proportion of type I fibres, is slow to develop tension and to relax. Any change in the proportions of 'fast' and 'slow' fibres present might be detectable as an alteration in speed of contraction and relaxation of whole muscle. The present results indicate that contractions of EDL from PU rats were slower than those of EDL from normal rats. However, the differences were small (approximately 7%) and only statistically significant at $P < 0·07$. They are consistent with findings reported by Bedi *et al.* (1982) that EDL of PU rats showed a tendency to decreased proportions of white fibres. No alteration of fibre types was found in SOL from these rats, which correlates with the insignificant change (approximately 3% increase) in contraction time found.

The findings that both SOL and EDL from PU rats developed greater tension per unit weight than their controls is interesting. Generally, 'fast'-twitch fibres develop more tension than 'slow'-twitch fibres, although the former will fatigue more rapidly. Hence, the histological results (Bedi *et al.* 1982) showing that (1) EDL and PU rats had a reduced

proportion of white fibres and (2) there was no such change in SOL, when compared to normal controls, cannot explain these changes. Their suggestion that an alteration in myofibril content may occur in PU muscle fibres could however contribute to an increase in force per unit weight. However, concomitant biochemical and membrane changes may alter the ability of the muscle to develop tension. For example the timing of the phases of excitation contraction coupling, which include the conduction velocity of the action potential, release of calcium ions from the sarcoplasmic reticulum, initiation of the contraction process (Sandow, 1966; Mulieri, 1972) may influence the tension generated by the whole muscle. It has been suggested that an alteration of the latency of release of Ca^{2+} would alter twitch tension (Hoyle, 1980). Certainly a reduction in the amount of released Ca^{2+} may reduce twitch tension (Sandow *et al.* 1975).

It may be concluded from this relatively limited study of muscle from PU rats that minor differences in muscle contractile properties may persist in relatively old animals. Even at 12 months of age animals from undernourished mothers are stronger per unit weight of muscle.

J. L. S. and K. S. B. were financed from grants to Professor John Dobbing from the Medical Research Council and the National Fund for Research into Crippling Diseases.

REFERENCES

Barany, M. & Close, R. I. (1971). *J. Physiol., Lond.* **213**, 455.
Bedi, K. S., Birzgalis, A. R., Mahon, M., Smart, J. L. & Wareham, A. C. (1982). *Br. J. Nutr.* **47**, 417.
Brown, M. C., Jansen, J. K. S. & Van Essen, D. (1976). *J. Physiol., Lond.* **261**, 387.
Buller, A. J., Eccles, J. C. & Eccles, R. M. (1960). *J. Physiol., Lond.* **150**, 417.
Close, R. (1965). *Nature, Lond.* **206**, 831.
Close, R. (1969). *J. Physiol., Lond.* **204**, 331.
Close, R. (1972). *Physiol. Rev.* **52**, 129.
Dobbing, J. & Smart, J. L. (1974). *Br. Med. Bull.* **30**, 164.
Eccles, J. C., Eccles, R. M. & Kozak, W. (1962). *J. Physiol., Lond.* **163**, 324.
Goldspink, D. F. (1977). *J. Physiol., Lond.* **264**, 283.
Goldspink, G. (1962). *Comp. Bioch. Physiol.* **7**, 157.
Goldspink, G. (1962). *Am. J. Physiol.* **209**, 100.
Goldspink, G. & Ward, P. S. (1979). *J. Physiol., Lond.* **296**, 453.
Gutmann, E., Melichna, J. & Syrovy, I. (1974). *Physiol. Bohemoslav.* **23**, 19.
Haltia, M., Berlin, O., Schucht, H. & Sourander, P. (1978). *J. Neurol. Sci.* **36**, 25.
Howells, K. F., Matthews, D. R. & Jordan, T. C. (1978). *Res. Exp. Med.* **173**, 35.
Hoyle, G. (1980). *Comp. Biochem. Physiol.* **66A**, 57.
Mulieri, L. A. (1972). *J. Physiol., Lond.* **223**, 333.
Riley, D. A. (1977). *Brain Res.* **134**, 279.
Sandow, A. (1966). *M.C.V. Quarterly* **2**, 82.
Sandow, A., Pagala, M. K. D. & Sphicas, E. (1975). *Biochim. Biophys. Acta* **404**, 157.
Shafiq, S. A., Asiedu, S. A. & Milhorat, A. T. (1972). *Exp. Neurol.* **35**, 529.
Sourander, P., Sima, A. & Haltia, M. (1974). *Early Malnutrition and Neonatal Development* (*Symposium of Swedish Nutritional Foundation XII.* p. 39. Uppsala: Almqvist & Wiksell.
Thomas, Y. M., Bedi, K. S., Davies, C. A. & Dobbing, J. (1979). *Early Human Develop.* **3**, 109.

Printed in Great Britain