EFFECT OF THYROIDECTOMY ON THE DISTRIBUTION OF THE FAST AND SLOW FORMS OF TROPONIN I IN RAT SOLEUS MUSCLE

Gurtej K. DHOOT and S. Victor PERRY
Departments of Immunology and Biochemistry, University of Birmingham, PO Box 363, Birmingham B15 2TT, England

Received 4 September 1981

1. Introduction

The effects of thyroid hormones on the properties of muscle in both experimental and clinical situations are well-documented [1–9]. These studies indicate that the speeds of contraction and relaxation in skeletal muscle are related to the thyroid hormone status and that the changes in physiological properties of the muscles observed when this status is altered are associated with appropriate changes in the proportion of type I and type II fibres [6,7,9,10]. The modifications that occur in the myosin light chain composition after thyroidectomy correlate well with the changes in fibre types and physiological properties [8,10]. As similar changes in myosin light chain composition can be induced by cross innervation and by chronic electrical stimulation, some workers have concluded that the thyroid effect on skeletal muscle may be neurally mediated [8]. On the other hand, the results in [11] suggest that thyroid hormones may have a direct effect on gene expression at least so far as mitochondrial enzymes are concerned.

Many of the studies on thyroid effects have been carried out on rat soleus muscle without making allowance for the pronounced changes in the proportion of type I and type II fibres which in the normal animal take place for at least up to 24 weeks after birth [12,13]. We have, therefore, re-examined the effect of thyroidectomy on fibre type changes in the rat soleus using the immunoperoxidase procedure with antibodies to the fast and slow forms of troponin I to identify cell types and paying particular attention to the continuing maturation process that occurs in this muscle. Our studies suggest that thyroid hormones can directly affect expression of the genes controlling the synthesis of the components of the troponin complex in the absence of active nerve.

Address correspondence to S. V. P.

2. Materials and methods

Ten 12-week-old (310–320 g) thyroidectomized Wistar strain rats purchased from Charles River (UK) Ltd, Manston Road, Margate, Kent, were weight-matched with a control group of 16 rats of the same strain purchased from the same source. Three to four days after removal of the thyroid, under pentobarbitone anaesthesia and taking sterile precautions, the sciatic nerve in both control and thyroidectomized rats was cut in the proximal part of the thigh of the right leg and reflected to prevent reinnervation of soleus and other lower leg muscles. Thyroidectomized rats failed to grow further and in most cases lost weight. Pronounced atrophy also occurred in the lower right leg muscles of the denervated control animals (table 1). At different time intervals after denervation, the rats were again anaesthetized, weighed and checked for reinnervation by visual examination of the whole muscle and sections made from it. In some animals the possibility of reinnervation was checked by staining for nerve fibres by the acetylcholine esterase and silver method as in [13]. Soleus muscle from both the control and denervated legs were removed and weighed at the intervals indicated in section 3.

Frozen sections (6 μm thick) across the whole of the middle of the soleus muscle were cut and stained for myosin ATPase after acid or alkaline preincubation and for fast and slow forms of troponin I by the immunoperoxidase technique as in [14]. The proportion of cells staining for fast troponin I were counted

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

Table 1
Effect of denervation and thyroidectomy on cells staining for fast troponin I in rat soleus muscle

	No. of animals	Age at examination (weeks)	Age at denervation (weeks)	Time thyroidectomized (weeks)	Cells staining[a] for fast troponin I (% of total)	Animals (g)	Soleus (mg)
Controls							
Nerve intact	6	12	–	–	31.0 ± 1.6	315 ± 5	157 ± 5
	4	24.5	–	–	1.9 ± 0.7	448 ± 9	193 ± 9
Denervated	4	24.5	12.5		74.4 ± 2.8	448 ± 9	31 ± 2
	2	32.5	24.5	–	43.6	535	34
Thyroidectomized							
Nerve intact	2	20	–	8	0.15	295	108
	4	24	–	12	0.08 ± 0.04	278 ± 5	109 ± 4
	2	32	–	8	0.05	306	130
Denervated	2	20.5	12.5	8	11.8	295	44
	4	24.5	12.5	12	13.6 ± 0.15	278 ± 5	43 ± 2
	2	32.5	24.5	20	10.4	306	46

[a] Cells staining for fast troponin I include both type II and intermediate cells. Figures are average values and standard errors are indicated when cells in sections from ⩾3 different muscles were counted

as in [13]. For each muscle at least 2500 cells were counted.

3. Results and discussion

Over the period studied which, in most cases, was from age 12–24 weeks, the proportion of cells staining for fast troponin I in normal rat soleus muscle changed from 31.0–2.1% (fig.1a,b; table 1). If the animals were thyroidectomized at 12 weeks, even fewer cells (i.e., <1 in 2000) staining for fast troponin I could be detected at 24 weeks (fig.2a). Thus, although suppression of synthesis of troponin I is a feature of maturation of normal rat soleus, in the absence of the thyroid gland the process was speeded up to the extent that suppression of synthesis of fast troponin I was virtually complete 8 weeks after thyroidectomy. Over the same period of thyroidectomy very little change in the proportions of fibre types could be observed in the extensor digitorum longus or tibialis anterior muscles from the same animal.

Denervation of the immature soleus muscle of the rat causes a stimulation of the synthesis of fast troponin components in most cells [13]. If denervation was carried out at 12 weeks (table 1), at 24 weeks the muscle showed a great reduction in weight (table 1) and ~74% of the cells stained for fast troponin I (fig.1c). If denervation was carried out 3–4 days after thyroidectomy at 12 weeks, the proportion of cells containing fast troponin I at 24 weeks was 13.6%, significantly higher than the proportion of such cells present in normal muscle and in soleus muscle from thyroidectomized animals with the nerve intact (fig.2). Although the cells had undergone considerable atrophy after denervation in the absence of the thyroid gland, a large fraction of the cells, i.e., ~60% of the total and which in the absence of the nerve would have expressed the gene controlling the synthesis of fast troponin I, had not done so. These cells still contained the slow form of troponin I. This suggests that thyroid hormones or possibly imbalance of other hormones produced by thyroidectomy can directly influence the synthesis of the fast and slow forms of the

Fig.1. Cross sections of rat soleus muscle stained with antibodies to fast troponin I by immunoperoxidase [1] before and after denervation (bar = 300 μm): (a) muscle from 12.5-week-old rat; (b) muscle from 24.5-week-old rat; (c) muscle from 24.5-week-old rat, denervated at 12.5 weeks.

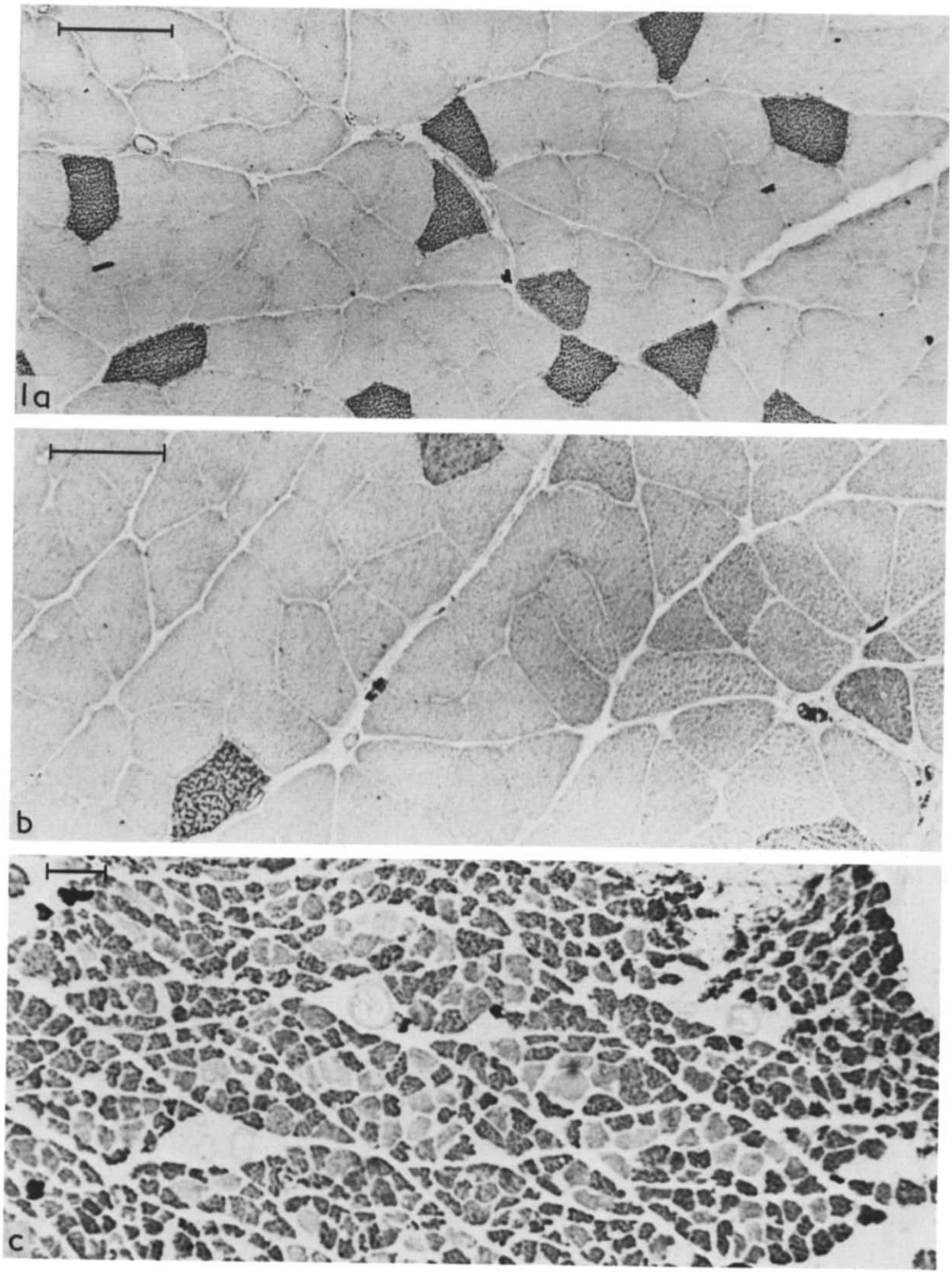

Fig.1

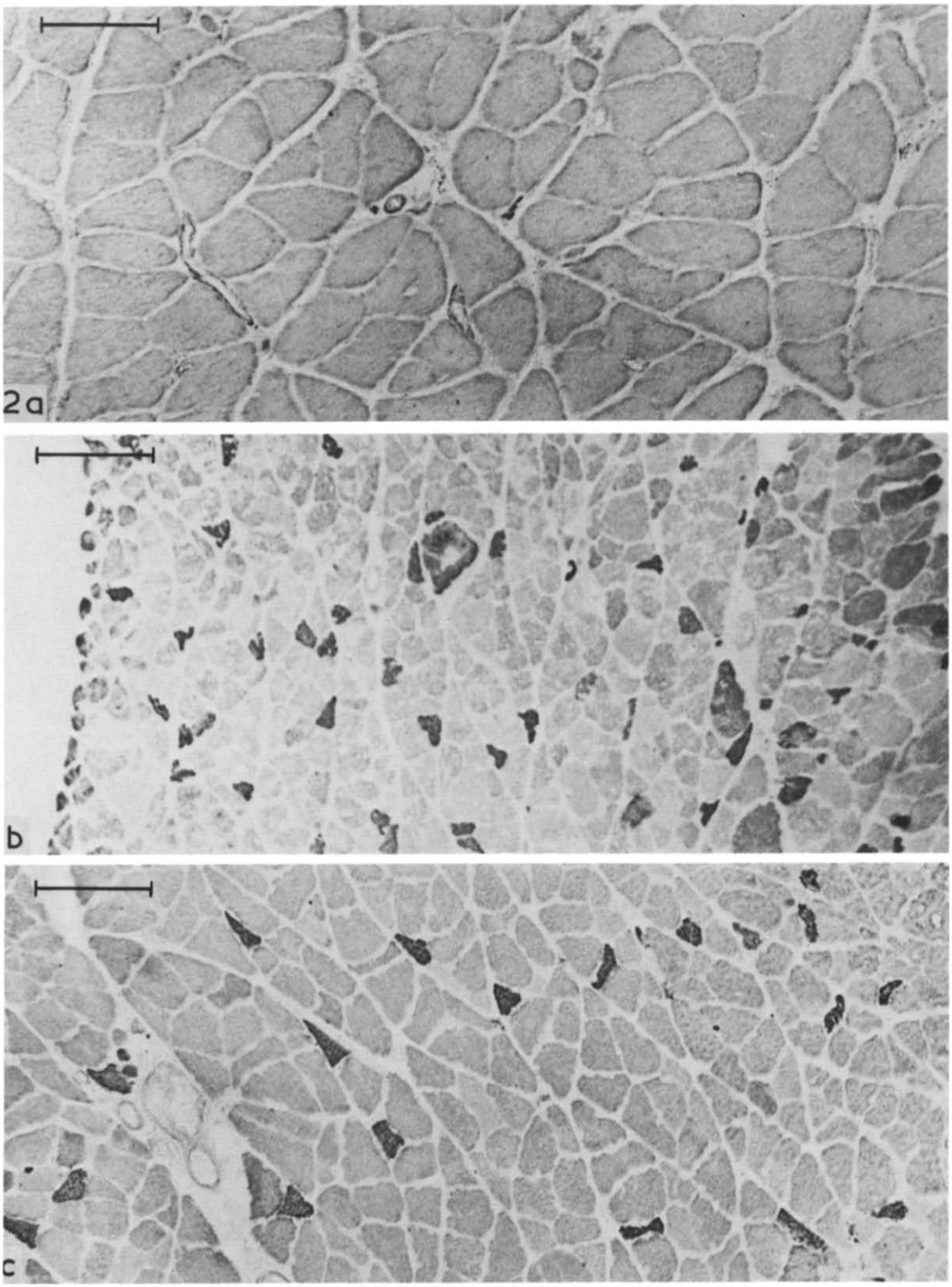

Fig.2

troponin components by a mechanism that does not involve the nerve. This effect is much more marked in soleus muscle compared to fast muscles such as extensor digitorum longus and tibialis anterior and may be due to the greater density of T3 receptors in soleus muscle as suggested in [15].

References

[1] Lambert, E. H., Underhahl, L. O., Beckett, S. and Mederloss, L. O. (1951) J. Clin. Endocrinol. 11, 1186–1205.
[2] Wilson, J. and Walton, J. M. (1959) J. Neurol. Neurosurg. Psychiat. 22, 320–324.
[3] Takamori, M., Gutmann, L. and Sharne, S. R. (1971) Arch. Neurol. 25, 535–546.
[4] Gold, H. K., Span, S. F. and Braunwald, E. (1970) J. Clin. Inv. 49, 849–854.
[5] Grossie, J. (1978) Am. J. Physiol. 234, C90–C95.
[6] Iauzzo, D., Patel, P., Chen, V., O'Brien, P. and Williams, C. (1977) Nature 270, 74–76.
[7] McKeran, R. O., Sharin, G., Andrews, M., Ward, P. and Mair, W. G. P. (1975) J. Clin. Pathol. 28, 659–663.
[8] Johnson, M. A., Mastalgia, F. L., Montgomery, A., Pope, B. and Weeds, A. G. (1980) in: Plasticity of Muscle (Pette, D. ed) pp. 607–615, Academic Press, New York.
[9] Johnson, M. A., Mastalgia, F. L. and Montgomery, A. (1980) FEBS Lett. 110, 230–235.
[10] Nicol, C. J. M. and Brucc, D. S. (1981) Pflugers Arch. 390, 73–79.
[11] Winder, W., Fitts, R., Holloszy, J., Kaiser, K. and Brooke, M. (1980) in: Plasticity of Muscle (Pette, D. ed) pp. 581–592, Academic Press, New York.
[12] Kugelberg, E. (1976) J. Neurol. Sci. 27, 269–289.
[13] Dhoot, G. K. and Perry, S. V. (1981) submitted.
[14] Dhoot, G. K., Gell, P. G. H. and Perry, S. V. (1978) Exp. Cell Res. 117, 357–370.
[15] Janssen, J. W., Van Hardeveld, C. and Kassenaar, A. A. H. (1979) Acta Endocrinol. 90, 81–89.

Fig.2. Cross sections of thyroidectomized control and denervated rat soleus muscle stained with antibodies to fast troponin I as described in legend to fig.1 (bar = 300 μm): (a) muscle from 24.5-week-old rat, thyroidectomized at 12 weeks; (b) muscle from 24.5-week-old rat, thyroidectomized at 12 weeks, denervated at 12.5 weeks; (c) muscle from 32-week-old rat, thyroidectomized at 12 weeks, denervated at 24 weeks.