Volume 219, number 1, 135–138 FEB 04895 July 1987

# Thyroid hormones regulate expression of the neural cell adhesion molecule in adult skeletal muscle

Joanne Thompson, Stephen E. Moore and Frank S. Walsh

*Institute of Neurology, Queen Square, London WC1N 3BG, England*

Received 14 May 1987

Adult rat skeletal muscle does not express detectable levels of neural cell adhesion molecule (N-CAM) and membrane activity mediated by nerve appears to repress the N-CAM gene. N-CAM expression in skeletal muscle can however be reinduced by hypothyroidism. Thyroidectomised rats re-express N-CAM mRNAs of 5.2 and 2.9 kb and Western blot analysis showed protein bands of 125 and 155 kDa. Immunofluorescence analysis also showed high levels of N-CAM in the sarcolemma. These changes in N-CAM expression could be reversed by treatment of hypothyroid rats with thyroxine. Thus thyroid hormones as well as neural influences appear to control N-CAM expression in skeletal muscle.

Neural cell adhesion molecule; Thyroid hormone; (Skeletal muscle)

## 1. INTRODUCTION

A number of studies have shown the neural cell adhesion molecule (N-CAM) to be expressed in skeletal muscle [1–9]. High levels of N-CAM are found in cell cultures of skeletal muscle [1,4,7,8] and also in embryonic muscle [1,5,7,9] correlating with the processes of myofibre formation and polyneuronal innervation. N-CAM in the muscle sarcolemma is down-regulated neonatally [1,5,7,9] and expression is confined mainly to the neuromuscular junction in adult muscle [1,5,6]. Synaptic activity has been found to regulate N-CAM expression since denervation [2,5,6], paralysis [6] or botulinum toxin treatment [2] has been found to cause a reactivation of N-CAM expression. All these changes in N-CAM expression are accompanied by qualitative and quantitative changes in individual N-CAM protein and mRNA species. The major desialo N-CAM isoform in myoblasts is a transmembrane isoform of about 140–145 kDa (N-CAM-145) while myotubes express predominantly non-transmembrane desialo isoforms of about 155 kDa (N-CAM-155) and 125 kDa (N-CAM-125) [5,8–10]. These protein changes are accompanied by changes in mRNA species. Myoblasts predominantly express a 6.7 kb species that encodes N-CAM-145 while myotubes express 5.2 and 2.9 kb species in rodents and 5.2 and 4.3 kb species in human muscle that encode N-CAM-155 and N-CAM-125 [8,10]. Alternative RNA splicing has been found to introduce a muscle-specific sequence into the extracellular domain of N-CAM-125 and N-CAM-155 but not a brain homologue of 120 kDa (N-CAM-120) showing that the maturation of skeletal muscle is accompanied by the operation of a muscle-specific splicing mechanism [11]. The plasticity of gene expression in skeletal muscle is well documented and a variety of physiological and hormonal stimuli have been found to be important controlling mechanisms [12]. To analyse the plasticity of N-CAM expression in skeletal muscle we analysed the effects of thyroid hormones, a model system that has been extensively used to study isoform transitions in myosin heavy chain (MHC) genes [13–17].

Correspondence address: F.S. Walsh, Institute of Neurology, London WC1N 3BG, England

*Published by Elsevier Science Publishers B.V. (Biomedical Division)*

## 2. MATERIALS AND METHODS

Young male rats (Sprague Dawley, 150 g) were thyroidectomised and skeletal muscle analysed at various time points up to 59 days. Treatment of hypothyroid rats with T4 was carried out by 3 intraperitoneal injections per week of 1 $\mu$g T4 in 250 $\mu$l saline.

For Northern blot analysis total RNA was isolated from leg muscle at appropriate time points, run on agarose gels, transferred to nitrocellulose and hybridised with the N-CAM probe pM1.3 [13] as described [14,15]. Immunoblot and indirect immunofluorescence analysis of N-CAM in control and 33-day hypothyroid rats and human hypothyroid myopathy samples was carried out as in [1]. The N-CAM antibody used was rabbit anti-N-CAM and the detecting antibody was fluorescein-labelled goat anti-rabbit immunoglobulin.

## 3. RESULTS

To determine whether thyroid hormones regulate muscle N-CAM expression, we made rats hypothyroid by thyroidectomy. Muscle samples were analysed at various time points after the lesion. A Northern blot analysis was first carried out to assess changes in N-CAM mRNA species. Newborn rats express mRNAs of 5.2 and 2.9 kb, whereas adult rats do not express appreciable amounts of N-CAM mRNA (fig.1). Muscle samples from hypothyroid rats were taken from 7 days and up to 59 days after the lesion and compared to control muscle samples. Control muscle samples did not express N-CAM mRNA. In contrast, hypothyroid rats expressed 5.2 and 2.9 kb mRNA transcripts at 23 days and this increased at later time points taken at 33, 38 and 59 days (fig.1). No evidence was found for the third N-CAM mRNA species of 6.7 kb that is found in myoblasts suggesting that the N-CAM mRNA that is found is predominantly expressed in myofibres. Thyroid hormone deficiency can be reversed by administration of T4 and we therefore treated rats that had been hypothyroid for 29 days with T4 for 4, 9 or 30 days (fig.1). As little as 4 days of T4 treatment was sufficient to down-regulate the 5.2 and 2.9 kb mRNAs and no transcripts were found after 9 and 30 days of T4 treatment showing that

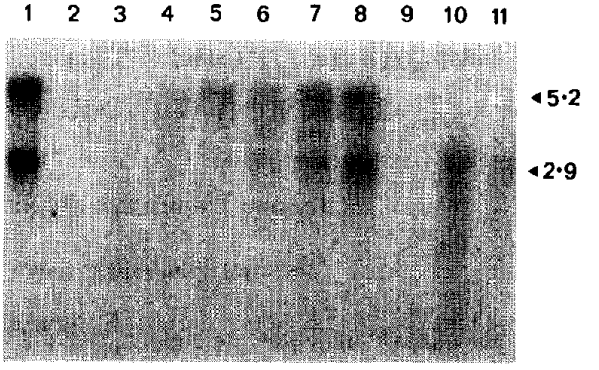


Fig.1. Northern blot analysis of control, hypothyroid and T4-treated muscle samples with N-CAM cDNA probe. Lanes: 1, newborn rat muscle; 2,3, adult rat muscle; 4–8, hypothyroid rat muscle after (4) 7 days, (5) 23 days, (6) 33 days, (7) 38 days and (8) 59 days of hypothyroidism; 9–11, samples of muscle from 29-day hypothyroid rats after 4 days (9), 9 days (10) and 30 days (11) of T4 treatment.

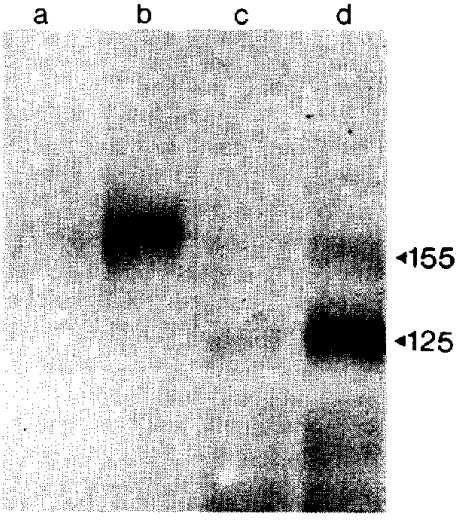


Fig.2. Immunoblot analysis of N-CAM in control and 33-day hypothyroid rats. The blot shows control (1) or hypothyroid muscle (2) and the same samples after treatment with neuraminidase to generate desialo N-CAM (track 3, control; track 4, hypothyroid muscle).

the changes in N-CAM gene expression were fully reversible. To determine whether the activation of N-CAM mRNA resulted in specific N-CAM protein expression, an immunoblot analysis was carried out. Fig.2 shows that rat muscle from control

animals has barely detectable levels of N-CAM whereas 33-day hypothyroid animals express high levels of N-CAM. Desialo N-CAM was generated by neuraminidase treatment to relate the isoforms found in hypothyroid muscle to those previously found in muscle tissue. N-CAM-125 and N-CAM-155 are the predominant desialo N-CAM band found in hypothyroid muscle consistent with the presence of 5.2 and 2.9 kb mRNA species (fig.2). Indirect immunofluorescence was used to analyse the location of N-CAM in muscle (fig.3). Control rat muscle does not express N-CAM at the sarcolemma but there is a very weak reactivity associated with satellite cells (fig.3a) [7]. Animals that were hypothyroid for 28 days showed strong N-CAM staining at the sarcolemma and in addition, there was variable staining of the myofibre cytoplasm similar to that found in other lesions of neuromuscular transmission [2,5,6] (fig.3b). To determine whether these high levels of muscle N-CAM were specific to experimental hypothyroidism, biopsies of humans with hypothyroid myopathy were examined. Control adult human muscle does not express N-CAM as was shown previously [3] (fig.3c). However, in hypothyroid myopathy there was strong staining of the muscle sarcolemma and cytoplasm (fig.3d). Thus, the observed changes in N-CAM expression in experimental hyothyroidism correlate well with changes found in human neuromuscular disease and show the generality of the observations.

## 4. DISCUSSION

A number of studies have analysed N-CAM expression in developing and adult skeletal muscle and in adult muscle subjected to a variety of classic lesions of neuromuscular transmission [1–9]. A

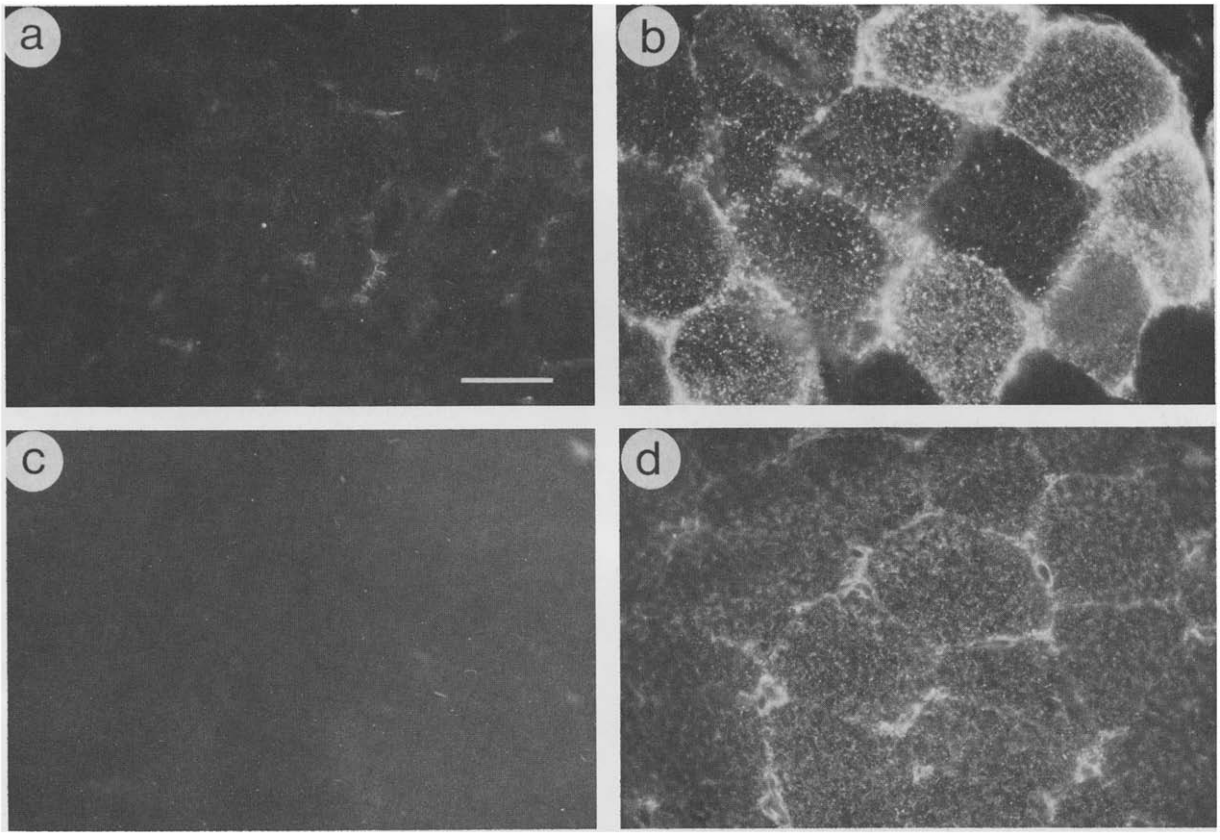


Fig.3. Indirect immunofluorescence analysis of N-CAM in control rat (a), 33-day hypothyroid rats (b), control human (c) and human hypothyroid myopathy (d) samples. Scale bar represents 100 $\mu$m.

general picture has emerged showing that N-CAM is present in embryonic muscle but not adult muscle and that denervation or paralysis reinduces N-CAM expression implying that muscle activity appears to control N-CAM levels. These data are consistent with a role for N-CAM controlling the innervation potential of myofibres and suggest that N-CAM down-regulation may be a functional signal in the shift from polyneuronal innervation to monosynaptic input. However, there is only a very general association between the timing of withdrawal of polyneuronal innervation and N-CAM levels and other parallels such as a shift from a hypothyroid state at birth to a neonatal euthyroid state [16] can also be made. In addition, the neonatal down-regulation of N-CAM mediated by activity is difficult to reconcile with studies on chick embryos that show a synchronous down-regulation of N-CAM in primary and secondary myofibres that are innervated at different times and in human muscle diseases such as myotonic dystrophy, where there are high levels of sarcolemmal N-CAM and no evidence of impairment of neuromuscular transmission (Walsh, unpublished). These studies suggest that innervation status may not be the sole controlling element in N-CAM expression in muscle and that other influences such as hormones, growth factors or other unidentified factors may be important. Thyroid hormones have been shown to regulate MHC genes in highly specific ways in skeletal and cardiac muscle [16–20]. Hypothyroidism reinduces embryonic MHC in soleus muscle and neonatal MHC in masseter muscle [19,20] showing that gene products that are not normally expressed in adult muscle can be reinduced by hypothyroidism. N-CAM is one additional muscle gene product whose expression is controlled in part by hormonal status and it seems likely that a complex interplay of hormonal and neural influences determines final N-CAM levels.

## ACKNOWLEDGEMENTS

This work was supported by the Muscular Dystrophy Group of Great Britain and the Wellcome Trust. F.S.W. is a Wellcome Trust Senior Lecturer.

## REFERENCES

[1] Moore, S.E. and Walsh, F.S. (1985) EMBO J. 4, 623–630.
[2] Moore, S.E. and Walsh, F.S. (1986) Neuroscience 18, 499–505.
[3] Walsh, F.S. and Moore, S.E. (1985) Neurosci. Lett. 59, 73–78.
[4] Grumet, M., Rutishauser, U. and Edelman, G.M. (1982) Nature 295, 693–695.
[5] Rieger, F., Grumet, M. and Edelman, G.M. (1985) J. Cell Biol. 101, 285–293.
[6] Covault, J. and Sanes, J.R. (1985) Proc. Natl. Acad. Sci. USA 82, 4544–4548.
[7] Covault, J. and Sanes, J.R. (1986) J. Cell Biol. 102, 716–730.
[8] Covault, J., Merlie, J.P., Goridis, C. and Sanes, J.R. (1986) J. Cell Biol. 102, 731–739.
[9] Tosney, K.W., Watanabe, L., Landmesser, L. and Rutishauser, U. (1986) Dev. Biol. 114, 437–452.
[10] Moore, S.E., Thompson, J., Kirkness, V., Dickson, J.G. and Walsh, F.S. (1987) J. Cell Biol., in press.
[11] Dickson, J.G., Gower, H.J., Barton, H., Prentice, H.M., Moore, S.E., Cox, R.D., Quinn, C.A., Putt, W. and Walsh, F.S. (1987) submitted.
[12] Goridis, C., Hirn, M., Santoni, M.-J., Gennarini, G., Deagostini-Bazin, H., Jordan, B.R., Keifer, M. and Steinmetz, M. (1985) EMBO J. 4, 631–635.
[13] Dickson, J.G., Prentice, H.M., Kenimer, J.G. and Walsh, F.S. (1986) J. Neurochem. 46, 787–794.
[14] Dickson, G., Prentice, H., Julien, J.-P., Ferrari, G., Leon, A. and Walsh, F.S. (1986) EMBO J. 5, 3449–3453.
[15] Strohman, R.C. and Wolf, S. (1985) Gene Expression in Muscle, Plenum, New York.
[16] Gambke, B., Lyons, G.E., Haselgrove, J., Kelly, A.M. and Rubinstein, N.A. (1983) FEBS Lett. 156, 335–339.
[17] Butler-Browne, G.S., Herlicoviez, D. and Whalen, R.G. (1984) FEBS Lett. 166, 71–75.
[18] Whalen, R.G., Toutant, M., Butler-Browne, G.S. and Watkins, S.C. (1985) J. Cell Biol. 101, 603–609.
[19] Mahdavi, V., Strehler, E.E., Periasamy, M., Wieczorek, D., Izumo, S., Grund, S., Strehler, M.-A. and Nadal-Ginard, B. (1986) in: Molecular Biology of Muscle Development (Emerson, C. et al. eds) pp.345–361, A.R. Liss, New York.
[20] Izumo, S., Nadal-Ginard, B. and Mahdavi, V. (1986) Science 231, 597–600.