Journal of Neurology, Neurosurgery, and Psychiatry 1987;50:439–442

Short report

Cell adhesion molecule N-CAM is expressed by denervated myofibres in Werdnig-Hoffman and Kugelberg-Welander type spinal muscular atrophies

FRANK S WALSH,* STEPHEN E MOORE,* BRIAN D LAKE†

From the Institute of Neurology, Queen Square, and the Hospital for Sick Children,† Great Ormond Street, London UK*

SUMMARY Immunocytochemical analysis utilising antibody to neural cell adhesion molecule (N-CAM) was carried out on skeletal muscle biopsies from patients with childhood spinal muscular atrophy. Children with both Werdnig-Hoffmann and Kugelberg-Welander disease showed positive N-CAM reactivity. There were however differences in the N-CAM expression profiles in these two sets of patients. All myofibres were positive for N-CAM in the Werdnig-Hoffmann patients. This included both the normal sized fibres and the atrophic fibres. In contrast only the atrophic fibres were positive in the Kugelberg-Welander patients. No reactivity was found associated with the large hypertrophic fibres. It is likely that in the Werdnig-Hoffmann patients the positive N-CAM reactivity reflects unstable innervation of myofibres that had been previously innervated. A similar mechanism may operate in the Kugelberg-Welander patients, but the innervation of the hypertrophic fibres is more stable as they are able to repress N-CAM expression. These results contrast with a lack of N-CAM expression found previously on muscle biopsies from adults with denervation disease.

Neural cell adhesion molecule (N-CAM) is believed to be involved in controlling cell-cell interactions in a variety of tissue systems.[1] N-CAM is specific gene product of skeletal muscle cells that is expressed by myoblasts and myotubes in cell culture and in developing muscle but not in innervated adult myofibres.[2-4] From the onset of synaptogenesis during muscle development, N-CAM expression becomes increasingly restricted. In adult myofibres N-CAM expression is retained only at the neuromuscular junction.[3 5 6] This apparent correlation between N-CAM expression and synaptogenesis is consistent with the results of experimental muscle denervation[4-6] and toxin induced paralysis[4 5] experiments. After denervation N-CAM is rapidly re-expressed at the sarcolemma and upon reinnervation it is again repressed. N-CAM therefore appears to be a good correlate to innervation status of the myofibre. In addition, anti-N-CAM appears to block the initial stages of recognition between spinal cord neurites and myotubes in vitro,[2] also indicating a possible role in nerve-muscle interactions.

We have recently examined a series of muscle biopsy specimens from adult patients with chronic denervating diseases, and in these no N-CAM reactivity was found associated with atrophic fibres.[7] One possible explanation for this observation is that the animal experiments consisted of acute denervations of only a few weeks duration, while the biopsy samples studied were all from chronic denervations. We have now analysed N-CAM expression in a series of biopsies from children with spinal muscular atrophy of the acute Werdnig-Hoffmann and milder Kugelberg-Welander types and compare the results with those of the adult series.

Address for reprint requests: Dr FS Walsh, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

Received 4 February 1986 and in revised form 9 July 1986.
Accepted 10 July 1986

Patients and methods

The muscle biopsy samples used in the present study were obtained from children presenting at the Hospital for Sick Children, Great Ormond Street, London. Frozen cryostat sections (8 μm) were cut and placed on coverslips for indirect immunofluorescence analysis. All techniques for indirect immunofluorescence and haematoxylin and eosin (H/E) staining analysis of biopsy samples have been described previously.[8] The antibody used in the present study was rabbit anti-D2-CAM[9] that reacts with N-CAM and was generously donated by Dr E Bock, Protein Laboratory, University of Copenhagen. The detecting antibody was fluorescein labelled sheep anti-rabbit immunoglobulin. For fibre type analysis, sections were also reacted with monoclonal antibody (McAb) 29.1D12[10] which reacts with adult fast myosin heavy chain and this antibody was detected with rhodamine labelled sheep anti-mouse immunoglobulin. Standard fibre typing using an ATPase method with and without acid preincubation was compared with the results of McAb 29.1D12.

Results

Eleven cases of childhood spinal muscular atrophy were analysed for expression of N-CAM in skeletal muscle. The figure shows a typical example of a muscle biopsy from a patient with Werdnig-Hoffmann disease (a, b) and another with Kugelberg-Welander disease (c, d) stained with H/E and anti-N-CAM. The biopsy (fig a, b) from a 24 day old patient with Werdnig-Hoffmann disease exhibits normal and slightly hypertrophic muscle fibres (18–28 μm) interspersed with numerous very small myofibres (3–4 μm). N-CAM staining of this specimen (fig b) shows that all fibres express N-CAM to some degree, and the more atrophied fibres have a higher level of N-CAM expression. Fibre typing analysis showed that the normal sized fibres were type 1, while the smaller fibres were type I or II.

The figure (c, d) shows micrographs from a 9 year old patient with Kugelberg-Welander disease. Here two size populations of myofibres are apparent; the larger hypertrophic fibres (55–110 μm diameter) are N-CAM negative while the atrophied fibres (4–16 μm diameter) are N-CAM positive with some variation in staining intensity. Fibre type analysis of this section shows that the atrophied fibres are all type II and such fibre type grouping indicates that they have been denervated and reinnervated by collateral sprouting

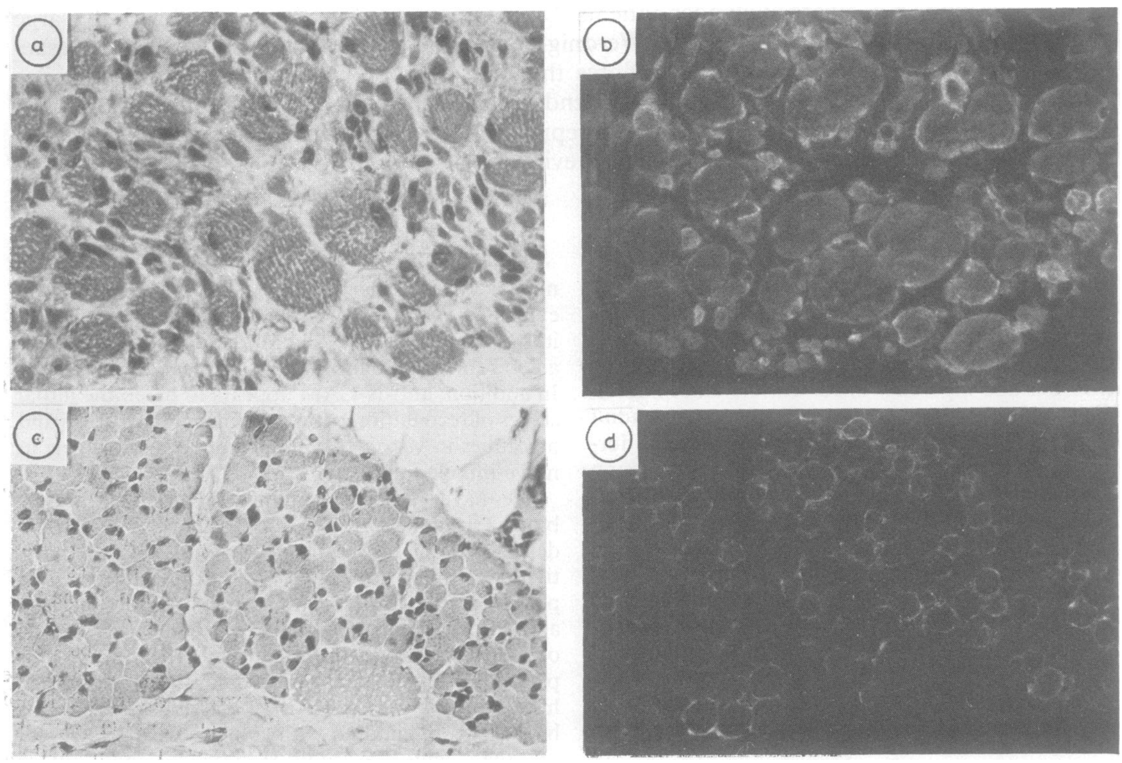

Fig *Indirect immunofluorescence staining of muscle biopsy samples from a case of Werdnig-Hoffmann disease (a, b) and Kugelberg-Welander syndrome (c, d). Sections were stained with H/E (a, c) and anti-N-CAM (b, d).*

of an adjacent motor neuron. All of the biopsies tested in the present study showed denervated fibres and these were positive for N-CAM reactivity, suggesting that N-CAM expression is a general response to denervation in these patients.

Discussion

Experimental denervation of skeletal muscle causes a number of structural and functional changes to occur. At the sarcolemma there is a reexpression of a variety of molecules, such as the nicotine acetylcholine receptor,[11] apamin binding sites[12] and N-CAM.[4-6]

We have studied N-CAM expression in 11 juvenile spinal muscular atrophy biopsies. The two cases illustrated in the figure are typical of the results found. All fibres in the Werdnig-Hoffmann biopsies are positive for N-CAM expression. Thus fibres which are of normal size are positive as well as atrophic fibres. In contrast, the older patients with Kugelberg-Welander disease present a different picture. Here only the small atrophic fibres are positive, while the large hypertrophic fibres are negative for N-CAM. These data suggest that there are differences in N-CAM expression in these two diseases that may possibly reflect a different pathogenesis. In the Werdnig-Hoffmann patients the atrophic fibres are clearly denervated, but it is not possible to state categorically whether they were innervated and were then denervated or whether they were never innervated. The observation that the normal sized fibres are also positive for N-CAM may suggest that these fibres have an unstable innervation, and these may with time become atrophic. If this is the case, then it may be more likely that the atrophic fibres were once innervated, but in an unstable manner. The mixed fibre types of the small fibres adds weight to the proposal that they were once innervated. This model would predict that there is an ongoing process of denervation in these patients, and that with time, all fibres would become denervated. The factors controlling this lack of stability of innervation are unknown at present. The Kugelberg-Welander patients behave in a different manner with respect to N-CAM expression. Here there are two populations of myofibres. The innervated hypertrophic fibres are N-CAM negative, while the atrophic fibres are N-CAM positive. It is clear in these patients that all myofibres were innervated at some point and a large number of these have become denervated. The difference between these patients and the Werdnig-Hoffmann patients is that the large fibres are N-CAM negative. This shows that these fibres are fully innervated and can repress N-CAM expression in a similar manner to normal myofibres. If the innervation of these hypertrophic fibres is unstable, then it is clearly over a much longer timescale than in the Werdnig-Hoffmann patients. Whether the difference in N-CAM expression in these two sets of patients reflects a different pathogenetic mechanism is not known but merits further study.

The observation of N-CAM expression in denervated fibres of juvenile spinal muscular atrophy biopsies correlates in a general manner with in vivo animal denervation experiments[4-6] but contrasts with previous negative findings in adult denervated biopsies. However, this apparent anomaly may be due to differences in duration or severity of denervation or simply shows that there is a different pathogenetic mechanism operating in these different groups of patients, and that comparison with animal models may not be appropriate in all cases. In addition, the re-expression of at least one of the nerve regulated membrane molecules, the nicotinic acetylcholine receptor, is for relatively short periods of time only. Thus six weeks after denervation of rats, Ringel *et al*[13] could not find detectable levels of nicotinic receptors assessed by α-bungarotoxin immunohistochemistry. Whether there are other differences between childhood and adult samples from denervated muscle remains to be determined. Fidzianska[14] has also pointed out a number of major differences in certain cell structures including the sarcolemma, at the light and electron microscope level in muscle biopsies from patients with Werdnig-Hoffmann disease and amyotrophic lateral sclerosis. However, it is not known whether the present data on N-CAM represents molecular correlates of these changes. An additional correlate of denervation in Werdnig-Hoffmann muscle is that there is no evidence of satellite cell activation[15] in contrast to experimental denervation studies.[16] As such, it is likely that the N-CAM that is found on the biopsy samples is synthesised by denervated fibres themselves, and that satellite cells do not contribute to the high levels found.

References

1 Edelman GM. Modulation of cell adhesion during induction, histogenesis and perinatal development of the nervous system. *Ann Rev Neurosci* 1984;7:339–77.
2 Rutishauser U, Grumet M, Edelman GM. Neural cell adhesion molecule mediates initial interactions between spinal cord neurons and muscle cells in culture. *J Cell Biol* 1983;**97**:145–52.
3 Moore SE, Walsh FS. Specific regulation of N-CAM/D2-CAM cell adhesion molecule during skeletal muscle development. *EMBO J* 1985;**4**:623–30.
4 Moore SE, Walsh FS. Nerve dependent regulation of N-CAM expression in skeletal muscle. *Neurosci* 1986;**18**:499–505.
5 Covault J, Sanes JR. Neural cell adhesion molecule (N-CAM) accumulates in denervated and paralysed skele-

tal muscle. *Proc Natl Acad Sci USA* 1985;**82**:4544–8.

6 Rieger F, Grumet M, Edelman GM. N-CAM at the vertebrate neuromuscular junction. *J Cell Biol* 1985;**101**:285–93.

7 Walsh FS, Moore SE. Expression of cell adhesion molecule N-CAM in diseases of adult human skeletal muscle. *Neurisci Letts* 1985;**59**:73–8.

8 Hurko O, Walsh FS. Human fetal muscle-specific antigen is restricted to regenerating myofibres in diseased adult muscle. *Neurology* 1983;**33**:737–43.

9 Ibsen S, Berezin V, Norgaard-Pederson B, Bock E. Enzyme-linked immunoadsorbent assay of the D2-glycoprotein. *J Neurochem* 1983;**41**:356–62.

10 Moore SE, Hurko O, Walsh FS. Immunocytochemical analysis of fibre type differentiation in developing skeletal muscle. *J Neuroimmunol* 1984;**7**:137–49.

11 Fambrough DM. Control of acetylcholine receptors in skeletal muscle. Physiol Rev 1979;**59**:165–227.

12 Schmid-Antomarchi H, Renaud J-F, Romey G, Hugues M, Schmid A, Lazdunski M. The all-or-none role of innervation in expression of apamin receptor and of apamin-sensitive Ca^{++}—activated K^{+} channel in mammalian skeletal muscle. *Proc Natl Acad Sci USA* 1985;**82**:2188–91.

13 Ringel SP, Bender AN, Engel WK. Extrajunctional acetylcholine receptors. *Arch Neurol* 1976;**33**:751–8.

14 Fidzianska A. Morphological differences between the atrophied small muscle fibres in amyotrophic lateral sclerosis and Werdnig-Hoffmann disease. *Acta Neuropathol* 1976;**34**:321–7.

15 Saito Y. Muscle fibre type differentiation and satellite cell population in Werdnig-Hoffmann disease. *J Neurol Sci* 1985;**68**:75–87.

16 Murray MA, Robbins N. Cell proliferation in denervated muscle; identity and origin of dividing cells. *Neuroscience* 1982;**7**:1823–33.