Enzyme-histochemical and morphological characteristics of fast- and slow-twitch skeletal muscle after brain infarction in the rat

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Abstract

The right middle cerebral artery was permanently occluded in 12-week-old male spontaneously hypertensive rats. After the surgery the rats were subjected to repeated behavioural tests during the observation period. Fourteen weeks after surgery the fast-twitch extensor digitorum longus (EDL) and the slow-twitch soleus muscle of both sides were removed and examined with regard to muscle fibre characteristics obtained by histochemical and morphometrical methods. Comparisons were made with age-matched controls. Limb placement and the ability to traverse a beam or a rotating pole were repeatedly tested 2–13 weeks after the operation. In spite of permanent sensorimotor deficits in limb placement and when traversing a rotating pole or beam, no increase in pathological changes was noted in either EDL or soleus. The number and proportion of fibre types remained unchanged in both muscles. There was no difference in muscle fibre size in either EDL or soleus. It is concluded that brain infarction in the rat, although causing marked impairment of contralateral motor function, does not have a major influence on the muscle-fibre morphology or fibre-type composition, irrespective of muscle type.

Keywords: Cerebral infarction; Extensor digitorum longus; Skeletal muscle; Soleus; Muscle fibre type

1. Introduction

Lesions of upper motoneurones, e.g. after cerebrovascular accidents, is one of the most common causes of diminished motor performance in man. Studies of the effects of such lesions on skeletal muscle have yielded different, partly diverging results. The muscular changes reported in long-standing hemiplegia include muscle fibre atrophy affecting primarily type II fibres (Edström, 1968; Brooke and Engel, 1969; Edström, 1970; Chokroverty et al., 1976; Scelsi et al., 1984; Dietz et al., 1986; Dattola et al., 1993), or no atrophy (Landin et al., 1977; Jakobsson et al., 1991), and an increased proportion of type II (Landin et al., 1977; Jakobsson et al., 1991), or type I fibres (Scelsi et al., 1984; Dietz et al., 1986; Dattola et al., 1993). The differences between these studies can partly be ascribed to differences in the type of muscle studied (i.e. whether it has a locomotor and/or postural function), the location, severity and duration of the lesion, the degree of spasticity and the degree of disability of the subjects. These parameters will influence the activity level of the subject and, as a consequence, have an effect on the amount of muscular activity and on mechanical factors such as passive stretch, i.e. factors known to have an important influence on the muscle fibre composition and on the muscle fibre size (for Refs. see e.g. Edström and Grimby, 1986; Musacchia et al., 1988). The occurrence of altered fibre-type proportions after chronic hemiplegia has been referred to a transformation of fibre types in relation to a disuse of specific motoneurones (Jakobsson et al., 1991) or to a transynaptic degeneration of type-specific motoneurones together with a reinnervation process (Dattola et al., 1993).

In order to elucidate possible mechanisms behind the divergent observations after long-standing hemiplegia in man, a rat model was used, in which the influence of such secondary factors as stated above, can be more easily controlled for. The right middle cerebral artery (MCA) was permanently occluded in 12-week-old male spontaneously hypertensive rats (SHR). After 14 weeks the animals were killed and the fast-twitch EDL and the slow-twitch soleus...
muscles of both sides were examined with regard to histopathological, enzyme-histochemical and morphometrical characteristics. Comparisons were made with age-matched controls.

2. Material and methods

The experimental protocol was approved by the Ethics Committee for Animal Research at Lund University. Six male inbred SHR (Møllegaard Breeding Centre, Denmark), 12 weeks of age at the beginning of the experiment, were studied. All rats were subjected to a 12-h/12-h light/dark cycle and had free access to food and water.

Because the results showed no difference between the muscles from the paretic and non-paretic limb, six normal and age-matched SHR rats, not subjected to any operation, were added to the study. These rats were housed three and three in standard laboratory cages (550 × 350 × 200 mm).

2.1. Surgery

The rats were anesthetized with methohexital sodium (Brietal) 50 mg/kg i.p. and the body temperature was kept close to 37°C. After cranioectomy, the right middle cerebral artery (MCA) was occluded proximal to the striatal branches at the level of crossing the olfactory tract by means of a square knot using 10-0 monofilament nylon thread (Tamura et al., 1981; Grabowski et al., 1988). Twentyfour hours after the operation, the rats were placed in a cage, 815 mm × 610 mm × 450 mm allowing social interaction but no specific physical training.

After 14 weeks the rats were killed by an overdose of methohexital and EDL and m. soleus were carefully dissected free from surrounding tissue and clamped at a standardized length with the ankle and knee joints fixed at angles of approximately 90°. The muscles were then weighed, frozen in freon chilled with liquid nitrogen and stored at –80°C until processed further.

2.2. Behavioural testing

The functional outcome was repeatedly tested during three postoperative months using tests that a normal rat has no difficulty with. In the limb placing test, modified as earlier described (Ohlsson and Johansson, 1995) after De Ryck et al. (1989), the maximum score (= normal performance) for each side was 16 points. Coordination and integration of motor movement were evaluated by the ability to traverse a beam or a rotating pole (Ohlsson and Johansson, 1995). The beam was 1,750 mm long and 19 mm wide and placed 700 mm above the floor. A wall was alternately placed 13 mm to the left or the right of the beam (rats are more inclined to traverse the beam when a wall is placed next to the beam). The performance was graded from 0 to 6; 0 = the rat falls down; 1 = is unable to traverse the beam but remains sitting across the beam; 2 = falls down while walking; 3 = can traverse the beam but the affected hindlimb does not aid in forward locomotion; 4 = traverses the beam with no more than 50% foot slips; 5 = crosses the beam with a few foot slips; 6 = crosses the beam with no foot slips. The pole, 45 mm in diameter and 1,500 mm in length, rotated alternately to the left or right with three turns per minute. The same scoring was used as for beam-walking except that score 3 = the rat jumps with both hindlimbs together apparently supporting the weak hindlimb with the opposite strong limb, and score 4 = the affected hindlimb is used less than 50% of the steps.

2.3. Determination of infarct volume

The brains were frozen in isopentane chilled to −40°C and stored at −80°C until 20 µm thick coronal sections were cut in a cryostat at −20°C. At 300 µm intervals, sections were taken for determination of infarct volume, a total of 15 sections from each rat. Since all that remains of the infarct 14 weeks after an MCA occlusion is a cystic cavity, the remaining area of the right hemisphere was determined and subtracted from the contralateral hemispheric area and the infarct volume calculated in % of contralateral hemispheric volume (Ohlsson and Johansson, 1995). The infarct volume, or rather the total tissue loss, will at this late stage of the infarct include some secondary atrophy particular of the thalamus. We therefore additionally calculated the cortical tissue in % of contralateral cortex. The image analyzing system consisted of a video camera (Dage MTI, MI, USA), a light box (Imaging Research Inc., Ont., Canada) and a Macintosh computer as earlier described (Ohlsson and Johansson, 1995; Grabowski et al., 1995).

2.4. Histological technique

The muscle was cut at its greatest girth (EDL), or at the motor point (soleus), perpendicular to its longitudinal axis into serial 10 µm thick cross-sections in a cryostat (−20°C). The muscles were stained with haematoxylin-eosin (Dubowitz, 1985) and modified trichrome (Engel and Cunningham, 1963), and for succinate dehydrogenase (SDH) (Nachlas et al., 1957) and myofibrillar ATPase (mATPase) (Padykula and Herman, 1955) after acid (pH 4.35 and 4.5) preincubations (Brooke and Kaiser, 1969, 1970). Soleus was also stained for mATPase after 55 min of formaldehyde fixation at 4°C (Hayashi and Freiman, 1966). The fibres were classified into types I and II, and the type II fibres were further subdivided into types IIA, IIB and IIC according to their ATPase staining characteristics (Brooke and Kaiser, 1970). In soleus, some of the type I fibres had staining characteristics intermediate to those of typical type I and type IIC fibres, and these fibres were denoted IC (see Ansvèd and Larsson, 1989). A direct
correlation between the myosin heavy chain (MHC) composition and the histochemical staining for mATPase has been found in single fibres of rabbit soleus muscle (Staron and Pette, 1986). Intermediate fibres, corresponding to the types IIC and IC in the present study, were characterized by the coexistence of fast and slow MHCs in varying proportions (Staron and Pette, 1986).

2.5. Morphometrical technique

The total number of fibres and the number of different fibre types and subtypes were counted from magnified (×100–150) photomicrographs of whole muscle cross-sections. The cross-sectional areas and the ‘lesser diameters’ of muscle fibres were measured directly from the microscope via a CCD-camera (Hamamatsu C3077, Hamamatsu Photonics KK, Japan) connected to an image-analysis processor (Vidas, Kontron Bildanalyse, GmbH, Munich, FRG). Measurements comprised 200 fibres of type I (soleus) or IIB (EDL) and 50 each of the other fibre types. If the total number of fibres of the respective type was smaller than these numbers, then all the fibres of that specific type were measured.

2.6. Statistics

Means and standard deviations were calculated from individual values by standard procedures. Statistical intergroup comparisons were made by means of the Mann-Whitney U-test and corrected for multiple comparisons according to Bonferoni-Holmes. Differences were considered significant at \( p < 0.05 \).

3. Results

3.1. Behavioural tests and infarct volume

Sensorimotor deficits remained until the end of the experiment as summarized in Table 1. MCA occluded rats had large infarcts extending over somatosensory and parietal regions as well as part of the caudate-putamen (Fig. 1). The total tissue loss was 28 ± 13% of the contralateral hemisphere and the relative cortical tissue loss was 39 ± 8% of contralateral cortex.

| Table 1 | Behavioural scores 2 and 13 weeks after the occlusion of the middle cerebral artery |
|---------|---------------------------------|-----------------------------|
|         | 2 Weeks            | 13 Weeks          | Normal score |
| Limb placement | non-paretic side | 15.8 ± 0.4 | 16 ± 0 | 16 ± 0 |
|          | paretic side       | 8.2 ± 4.4         | 9.8 ± 4.6 | 16 ± 0 |
| Pole    | non-rotating      | 2.8 ± 2.2         | 3.3 ± 2.4 | 6 ± 0 |
|          | rotating to the left | 3.0 ± 2.1 | 3.5 ± 2.9 | 6 ± 0 |
|          | rotating to the right | 2.5 ± 1.9 | 2.3 ± 2.5 | 6 ± 0 |
| Traversing a beam |                  | 3.7 ± 2.2 | 3.7 ± 2.1 | 6 ± 0 |

3.2. Body and muscle weight

The body weight of MCA occluded rats increased from 253 ± 10 g to 328 ± 42 g at the end of the experiment. The body weight did not differ between the MCA occluded rats and the controls, whereas the muscle weight of both soleus and EDL was significantly smaller in the controls (Table 2). The muscle weight did not differ between the paretic and non-paretic side of the MCA occluded animals (Table 2).

3.3. Histopathology and enzyme-histochemistry

In both EDL and soleus of MCA occluded rats, there was a sporadic occurrence of angulated atrophic fibres and centrally placed nuclei, but no difference was observed between the paretic and non-paretic side or between the MCA occluded rats and the controls. Similarly, there was no statistically significant difference regarding the number and proportion of fibre types and the total number of
Body weight, muscle weight and numbers and proportions of different muscle fibre types in the soleus and extensor digitorum longus muscles of MCA occluded and control rats

<table>
<thead>
<tr>
<th>Animal weight (g)</th>
<th>Muscle weight (mg)</th>
<th>Type I Number</th>
<th>%</th>
<th>Type IC Number</th>
<th>%</th>
<th>Type IIC Number</th>
<th>%</th>
<th>Type IIA Number</th>
<th>%</th>
<th>Type IIB Number</th>
<th>%</th>
<th>Total fibre number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paretic side</td>
<td>328 ± 42</td>
<td>142 ± 21</td>
<td>*</td>
<td>2321 ± 337</td>
<td>87 ± 5</td>
<td>11 ± 10</td>
<td>0 ± 0</td>
<td>54 ± 51</td>
<td>2 ± 2</td>
<td>294 ± 107</td>
<td>11 ± 4</td>
<td>2680 ± 323</td>
</tr>
<tr>
<td>Non-paretic side</td>
<td>141 ± 21</td>
<td>2300 ± 396</td>
<td>*</td>
<td>86 ± 4</td>
<td>36 ± 22</td>
<td>1 ± 1</td>
<td>83 ± 71</td>
<td>3 ± 3</td>
<td>241 ± 123</td>
<td>9 ± 5</td>
<td>2660 ± 365</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>346 ± 20</td>
<td>2493 ± 190</td>
<td>91 ± 2</td>
<td>16 ± 12</td>
<td>1 ± 0</td>
<td>50 ± 24</td>
<td>2 ± 1</td>
<td>186 ± 69</td>
<td>7 ± 2</td>
<td>2744 ± 192</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-paretic side</td>
<td>183 ± 26</td>
<td>102 ± 33</td>
<td>*</td>
<td>4 ± 1</td>
<td>649 ± 248</td>
<td>23 ± 6</td>
<td>2010 ± 397</td>
<td>73 ± 6</td>
<td>2760 ± 560</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>189 ± 15</td>
<td>92 ± 34</td>
<td>*</td>
<td>3 ± 1</td>
<td>684 ± 206</td>
<td>25 ± 6</td>
<td>1981 ± 426</td>
<td>72 ± 7</td>
<td>2757 ± 494</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paretic side</td>
<td>124 ± 13</td>
<td>78 ± 27</td>
<td>*</td>
<td>3 ± 1</td>
<td>595 ± 156</td>
<td>24 ± 4</td>
<td>1825 ± 506</td>
<td>73 ± 4</td>
<td>2498 ± 623</td>
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</tr>
</tbody>
</table>

* p < 0.05 in comparison with controls, * * p < 0.01 in comparison with controls.

Size of muscle fibres in the soleus and extensor digitorum longus muscles of MCA occluded and control rats

<table>
<thead>
<tr>
<th>Type I</th>
<th>Diameter (µm)</th>
<th>Type IC</th>
<th>Diameter (µm)</th>
<th>Type IIC</th>
<th>Diameter (µm)</th>
<th>Type IIA</th>
<th>Diameter (µm)</th>
<th>Type IIB</th>
<th>Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soleus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paretic side</td>
<td>1747 ± 425</td>
<td>41.9 ± 5.0</td>
<td>1787 ± 642</td>
<td>39.2 ± 3.9</td>
<td>1688 ± 486</td>
<td>39.5 ± 5.5</td>
<td>1932 ± 355</td>
<td>43.7 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Non-paretic side</td>
<td>1612 ± 69</td>
<td>40.3 ± 1.1</td>
<td>1786 ± 475</td>
<td>41.0 ± 5.1</td>
<td>1347 ± 377</td>
<td>36.2 ± 8.2</td>
<td>1860 ± 254</td>
<td>43.6 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1689 ± 197</td>
<td>41.3 ± 2.5</td>
<td>1579 ± 280</td>
<td>40.2 ± 4.6</td>
<td>1333 ± 255</td>
<td>39.6 ± 7.8</td>
<td>1758 ± 108</td>
<td>41.8 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>EDL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paretic side</td>
<td>609 ± 87</td>
<td>25.0 ± 1.6</td>
<td>570 ± 142</td>
<td>27.3 ± 2.4</td>
<td>841 ± 164</td>
<td>27.3 ± 2.4</td>
<td>1867 ± 360</td>
<td>41.9 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Non-paretic side</td>
<td>729 ± 171</td>
<td>26.8 ± 3.2</td>
<td>762 ± 104</td>
<td>26.7 ± 1.9</td>
<td>1981 ± 197</td>
<td>43.7 ± 2.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>549 ± 87</td>
<td>21.8 ± 2.1</td>
<td>821 ± 102</td>
<td>27.4 ± 2.1</td>
<td>1754 ± 290</td>
<td>40.7 ± 3.6</td>
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</tr>
</tbody>
</table>

* p < 0.05 in comparison with controls.
4. Discussion

Previous studies have revealed a high correlation between the degree of brain damage after right MCA occlusion and deficits in contralateral sensorimotor performance in the rat 3 weeks after surgery, although this correlation is weakened with time (Grabowski et al., 1988; Grabowski et al., 1991). Functional testing by means of a paw-reaching test, is highly impaired contralateral to the lesion even 3 months after surgery and the rats predominantly remove pellets with the ipsilateral intact paw (Grabowski et al., 1993). Significant lasting deficits were evident also in the tests used in the present study (Table 1). The housing conditions have been shown to influence functional outcome after focal brain infarcts. Rats in a larger cage improve more than rats in standard laboratory cages but not to the same extent as rats housed in an enriched environment (Ohlsson and Johansson, 1995; Grabowski et al., 1995; Johansson and Ohlsson, 1996). The lower muscular weight of the control animals in the present study is most likely related to relative inactivity since they were housed in smaller cages than were the experimental animals. The MCA occlusion had no primary effect on muscle weight since there was no difference between the paretic and non-paretic side.

In the present study, morphological and enzyme-histochemical characteristics of fast- and slow-twitch hindlimb muscles were evaluated 14 weeks after surgery. In spite of the marked contralateral motor dysfunction, no significant differences were noted in morphological, morphometrical or enzyme-histochemical muscle characteristics between the paretic and non-paretic side, or between MCA occluded and control rats irrespective of muscle type. This contrasts to the findings in longstanding hemiplegia in man, where muscle fibre atrophy and alterations in the proportion of fibre types have been reported (for references, see Jakobsson et al., 1991; Dattola et al., 1993). The increase in the percentage of type II muscle fibres of the tibialis anterior of patients with severe chronic hemiparesis was ascribed to a disuse of high-threshold motor units, leading to a slow-to-fast muscle fibre transition (Jakobsson et al., 1991). Morphological changes and atrophy of muscle fibres, primarily affecting type II fibres, have also been reported in patients with upper motoneurone lesions (e.g. Edström, 1968; Edström, 1970; Scelsi et al., 1984; Dietz et al., 1986) and was suggested to result from a combination of disuse, loss of central trophic influence and transsynaptic degeneration (Chokroverty et al., 1976). There is no unequivocal anatomical evidence for claiming that acute transsynaptic degeneration occurs in spinal motoneurones of mammals after interruption of corticospinal projections (see Cowan, 1970). However, electromyographic (EMG) studies in patients with upper motoneurone lesions, but without signs of peripheral nerve injuries, have revealed denervation activity in limb muscles starting 2–3 weeks after the lesion followed by a decrease in such activity in the course of weeks or months, having diminished considerably or disappeared within 6 months (Spaans and Wilt's, 1982; Benecke et al., 1983; Brown and Snow, 1990). Such denervation activity was most frequently observed in distal muscles and affected muscles of both upper and lower extremities, the former to a somewhat larger degree (Benecke et al., 1983; Brown and Snow, 1990; Dattola et al., 1993). The decrease in denervation activity occurred in parallel with the development of spasticity and increasing voluntary activation, suggesting the occurrence of a transsynaptic degeneration of alpha-motoneurones with a subsequent regeneration and reinnervation (Benecke et al., 1983). In the present study, no morphological or morphometrical signs of a denervation–reinnervation process was observed despite a significant impairment of motor function of the hindlimb contralateral to the lesion. This suggests that the origin of the motor impairment is all within the central nervous system and that the motor unit is left intact. The results further suggest that the different and often divergent results from human studies on the long-term effects of upper motor lesions on the muscle is related to secondary factors. These include eg. differences in the severity and duration of the lesion, the age at onset, the type of muscle studied, i.e. factors affecting the degree of residual muscle activity and passive stretch.

It is probable that lesions of upper motoneurones have somewhat different effects in different species due to differences in the organization and plasticity of the central and peripheral nervous system. This must be borne in mind when comparisons are made between results from animal models such as the present one and human studies. In the present study, transsynaptic denervation may have been compensated for by regeneration of denervated α-motoneurones which would prevent long-standing morphological changes in the muscle fibres. It is also plausible
that the morphological changes in skeletal muscles reported after lesions of the upper motor neurones in man but not seen in the present study is attributable to a diminished plasticity with age. The rats used in this study were considered young adult (approximately 26 weeks of age) whereas the patients of those human studies reporting morphological muscle changes were generally middle-aged or old. To further elucidate the possible occurrence of a transsynaptic degeneration causing morphological changes in the muscle, examination at shorter time intervals after the surgery should be performed. Such a study should also include older rats in order to establish a possible effect of age.

However, it is clear from the present results that although the presence of a transsynaptic degeneration cannot be completely ruled out in the rat, it has no significant long-standing effect on fibre morphology or fibre-type proportion.

It is concluded that brain infarction in the rat does not have a long-term effect on fibre-type composition or morphology of muscle fibres of either fast- or slow-twitch hind-limb muscles, despite a significant impairment of motor function.

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References


