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Muscle fibre composition and electromyographic features of cervical muscles following prolonged head extension in growing rats

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SUMMARY Soft tissue stretching has been proposed as one of the control factors in craniofacial morphogenesis. However, its mechanism remains unclear. The present study investigated electromyographic (EMG) activity and muscle fibre composition of cervical muscles following prolonged head extension in growing rats. Thirty-six male Wistar rats were divided into two experimental (E1, E2) and one control (C) group at 25 days of age. To induce head extension, the experimental rats were raised in cylindrical cages, which were positioned horizontally for group E1 and tilted upward at 45 degrees for group E2. At 55 days of age, EMG activity was recorded from the anterior digastricus (AD), sternohyoideus (SH), sternomastoideus (SM), longus capitis (LC), and biventer cervicis (BC) muscles in the rest position and passive head extension. EMG activity was analysed on its integrated values (IEMG), and composition of muscle fibres was evaluated by myosin ATPase reaction and fibre cross-sectional areas were calculated.

Group E1 showed a higher percentage of type I fibres and lower IEMG during passive head extension in AD. In group E2 there was a higher percentage of type I fibres, a higher IEMG at rest, and a lower IEMG during passive head extension in BC. The experimental groups demonstrated altered proportions of type IIA and IIB fibres in SM and LC. They also showed higher percentages of subtype fibres and reduced cross-section areas of type II fibres in most of the muscles investigated. These findings suggest that head extension affects fibre transition, distribution, cross-section area, and recruitment pattern in cervical muscles.

Introduction

Since Moss (1962) introduced the concept of functional matrices, much attention has been paid to the potential of soft tissues affecting facial morphology. Long-term alteration in head posture may cause muscles to exhibit different adaptive changes. Clinically, individuals with a relatively extended posture of the head tend to have facial characteristics of a skeletal open bite or Class II malocclusion (Solow and Tallgren, 1976, 1977). The hypothesis of soft tissue stretching has been proposed to explain this association between head posture and craniofacial morphology (Solow and Kreiborg, 1977). According to this hypothesis, head extension might increase tension of the ventral craniocervical soft tissues, mainly the craniocervical muscles, thus affecting the craniofacial growth pattern, but it is difficult to explain the cause–effect relationship between craniocervical muscles and craniofacial skeleton. Although Tourne (1990) suggested that altered muscular recruitment may be a plausible explanation for skeletal change, and other studies documented the relationship between muscle function and head posture (Funakoshi and Amano, 1973; Winnberg and Pancherz, 1983), our understanding is very rudimentary. In humans, head extension significantly reduces tonic activity in post-cervical muscles, while postural activity increases in sternocleidomastoideus, supra- and infra-hyoid muscles (Forsberg *et al*., 1985). However, these findings were achieved under short-term static experimental conditions. Long-term changes in the head posture may cause muscles to exhibit adaptive changes.

Postural head balance is maintained by the coordinated activities of several muscle groups of the head and neck. Head posture is also influenced by biomechanical forces arising from the vertebral column and muscles (Vidal *et al*., 1986), from inertial viscoelastic mechanisms (Keshner *et al*., 1992), and from the vestibulocollic reflex (Schor *et al*., 1988). Changes of body orientation with respect to the head evoke tonic neck and vestibulospinal reflexes, and the pattern of reflex activity depends on the direction of stimulus. Bipedal animal experiments have also shown that body posture leads to a rotation of the otic capsules, and alteration of the spine curvature and craniofacial morphology (Moss, 1961; Riesenfeld, 1966). However, the relationship of body orientation relative to head posture has not been elucidated.

The physiological properties of the cervical muscles have not been studied in conjunction with different head postures. Changes in the physiological properties should also be reflected in the fibre composition of these muscles. Based on a previous study in which growing rats were raised in restrained cages to produce head extension, growth retardation was found in both craniofacial size and craniofacial rotation, shown by smaller craniofacial dimensions in length and height, and downward rotation of the upper viscerocranium and the mandible (Gu, 1996). Using this animal model to produce a head extension, the present study was designed to explore the effects of head extension on the physiological and histochemical properties of cervical muscles, including tonic activity pattern, fibre types, and fibre cross-sectional areas. A further aim was to determine whether there is an inherent correlation between physiological properties and structural compositions of the muscles investigated, and whether body posture influences head posture. The anterior digastricus (AD), sternohyoideus (SH), sternomastoideus (SM), longus capitis (LC), and biventer cervicis (BC) were chosen to represent suprahyoid, infra-hyoid, lateral, pre-vertebral and post-vertebral muscle groups, respectively.

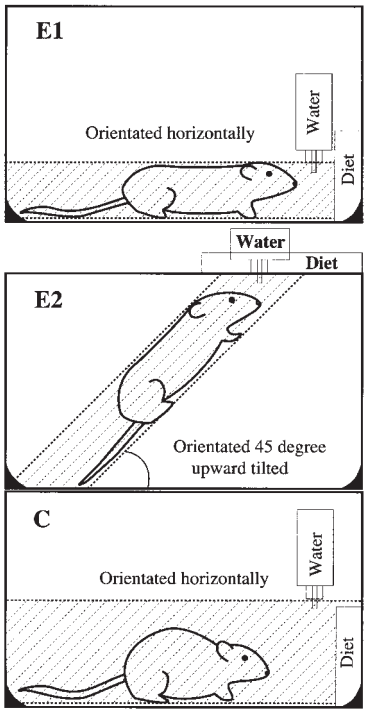

Figure 1 Cages, postures, and raising conditions. Experimental rats (E1 and E2) were raised in restrictive cylindrical cages to induce head extension, and orientated either horizontally for E1 or tilted upwards at 45 degrees for E2. Control rats (C) were raised in elliptical cages orientated horizontally. To facilitate maintenance of the animals, all cages were placed within normal breeding cages, and food and water were provided from the anterior top for E1 and C, and from above for E2.

Materials and methods

Animals

Thirty-six male Wistar rats, obtained from Kyudo Co., Kumamoto, Japan, were equally divided into two experimental (E1, E2) and one control (C) group at 25 days of age. To induce head extension, the two experimental groups were raised individually in restrictive cylindrical cages, made with overlapping wire-mesh that could expand in all dimensions once a week to allow for body growth. To observe the effects of body orientation on head posture, the cages were positioned horizontally for group E1 and tilted upward at 45 degrees for group E2. In the cages, the rats could move forward and backward for E1, and upward and downward for E2, but the cages forcefully extended their heads. As the experimental rats were kept in restrictive conditions, and their general growth might be inhibited, the rats of group C (control) were also raised in the restrictive condition. Different from the cages used for the experimental animals, those for the control animals were elliptical and horizontally orientated, so that animals could move more freely than those of E1 and E2, and did not undergo a forceful head extension (Figure 1). In a previous study to investigate the effects of head extension on the craniofacial growth in growing rats (Gu, 1996), this method had been demonstrated to produce a flattened back (reduced thoracic and lumbar curvature) and an extended head (increased craniocervical and cervicomandibular angles, and reduced posturo-mandibular angle and cervical inclination; Figure 2). Ample food and fresh water were provided every morning, and the cages were regularly cleaned. Room conditions were maintained at 24°C and 55 per cent humidity. Throughout the study, all rats were treated according to the guidelines for animal care of Kagoshima University. Approval was obtained from the institutional panel.

EMG recording

At 55 days of age, the body length of the animals (tail excluded) was measured by means of a digital calliper to calculate the difference when the animals were in and out of the cages. The differences were 12–18 mm (longer in cages) for the experimental groups, and 2–5 mm for the control group. The rats were then anaesthetized by intra-peritoneal administration of alpha-chloralose (6 mg/100 g) and ethyl carbamate (Urethane, 50 mg/100 g). Tracheal cannulation was performed to prevent any influence of muscle activity from respiration during electromyographic (EMG) recording. Using 27-gauge hypodermic needles, pairs of polyurethane-coated copper wire electrodes (0.12 mm, Unique Medical Co., Fukuoka, Japan) with 1-mm bare tips were hooked into the

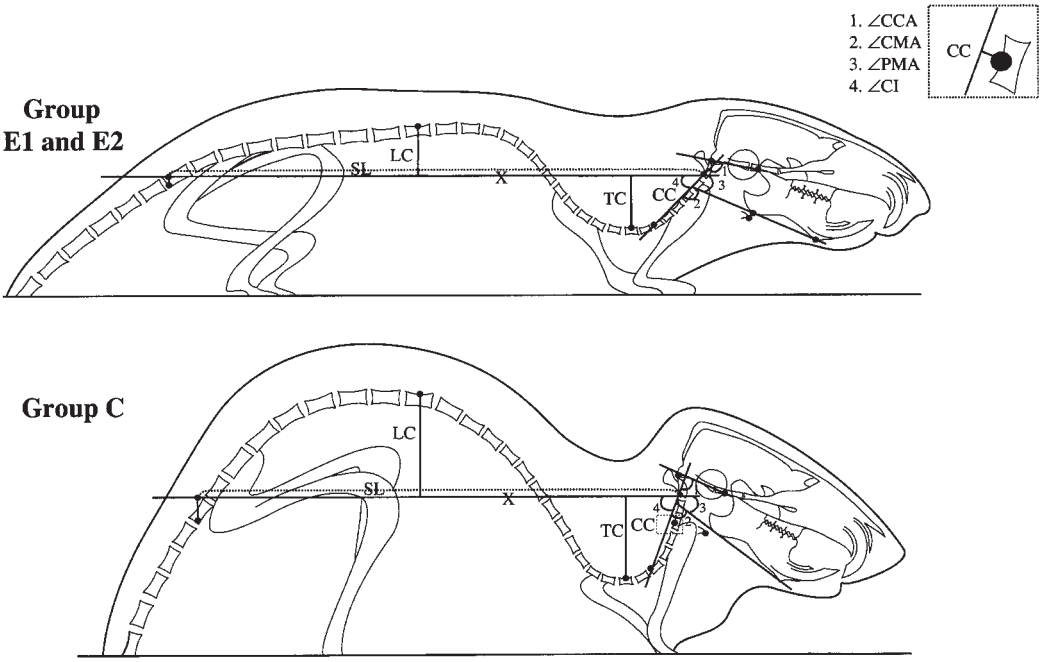

Figure 2 Posture changes of the experimental rats (E1 and E2) compared with control rats (C). The backs of animals E1 and E2 were flattened, showing the reduced thoracic and lumbar curvatures (TC and LC) and increased spinal length (SL). As a consequence, head extension was induced, and there were larger craniocervical (CCA) and cervicomandibular (CMA) angles, and a smaller posturo-mandibular (PMA) angle and cervical inclination (CI). All differences were significant ($P < 0.05$) between experimental (E1 and E2) and control (C) groups, but not between groups E1 and E2. (Reproduced with kind permission of the Japanese Orthodontic Society from Gu G M 1996 Effects of altered posture on the craniofacial morphology in growing rats: a longitudinal cephalometric analysis. Journal of Japan Orthodontic Society 55: 427–443).

exposed middle segments of AD, SH, SM, and LC, and at the C3 innervation level of BC in the left side. The paired inter-polar distances were 3 mm. The opposite ends of the electrodes were welded to a 12-pole female miniature connector, which was then sutured to the dorsal skin of rats.

The animal was fixed on a sliding plate and the maxillary incisors were connected to a force transducer (FD pickup, TB-611T, Nihon Kohden Co., Tokyo, Japan) with braided silk thread (Figure 3). The sliding plate was set in a horizontal orientation for group E1 and C, and tilted upward at 45 degrees for group E2 to provide support similar to their cage orientations. EMG recordings from the cervical muscles were first obtained with the head in the rest position. Head extension of the experimental animals was then mimicked by sliding the plate posteriorly to the distances that were equal to the differences of the total body lengths in and out of cages measured before anaesthesia. These individually passive tractions produced forces in the range of 165–200 g. For the control animals, an average of 180 g force was applied regardless of sliding distance, since the difference in body length in and out of the cage was negligible. The force signal was transformed, amplified (isometric

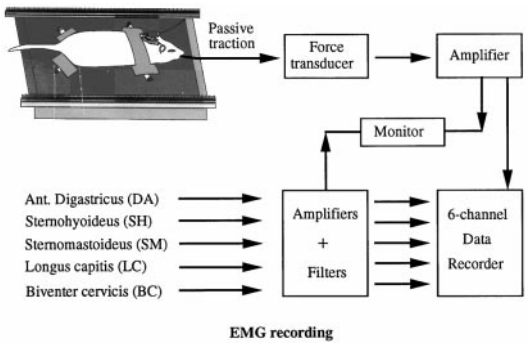

Figure 3 Diagram of EMG recording procedures.

amplifier, EF-601G, Nihon Kohden Co.), and monitored on an oscilloscope. All EMG outputs during rest and passive traction were amplified and filtered (0.03–1 KHz) by AC amplifiers (AB-621G, Nihon Kohden Co.), and stored on magnetic tape at a speed of 9.5 cm/second using a 6-channel data recorder (A-69, Sony Magnesscale Inc., Tokyo, Japan) along with the force signal. The recording time for each experimental state was at least 30 seconds and recordings were repeated three times

with a 10-minute interval. Anaesthesia was controlled at such a level that neither corneal reflex nor spontaneous eye movement occurred, and the rectal temperature was maintained at 36–38°C by a heating pad.

EMG analysis

EMG recordings were sampled for 30 seconds using the Bio-Information Multi-Task Analysing System (BIMUTAS, Kissei Co., Matsumoto, Japan; PC 9801 DA, NEC Co., Tokyo, Japan) with a sampling frequency of 2 KHz. The 30-second sampling was divided into three consecutive 10-second segments. Integrated EMG activities (IEMG) of the first 2.56 seconds from each 10-second segment were calculated for both experimental conditions (Figure 4). For normalization, IEMG during passive head extension was expressed as a proportion of the corresponding IEMG at rest. As no significant difference was found among the values from the three consecutive 10-second segments, all values ($3 \times 3 = 9$) in each animal from each experimental condition were averaged for statistical analysis.

Muscle histochemistry

After EMG recording, the rats were immediately killed and the same cervical muscles on the right side were dissected. All muscles were transected at the same levels as the electrodes were inserted on the left side and their entire cranial portions were removed for histological analysis (Figure 5). These muscle specimens were orientated on OCT compound (Tissue-Tek, Miles Inc., Elkhart, USA) and frozen in liquid nitrogen. Using

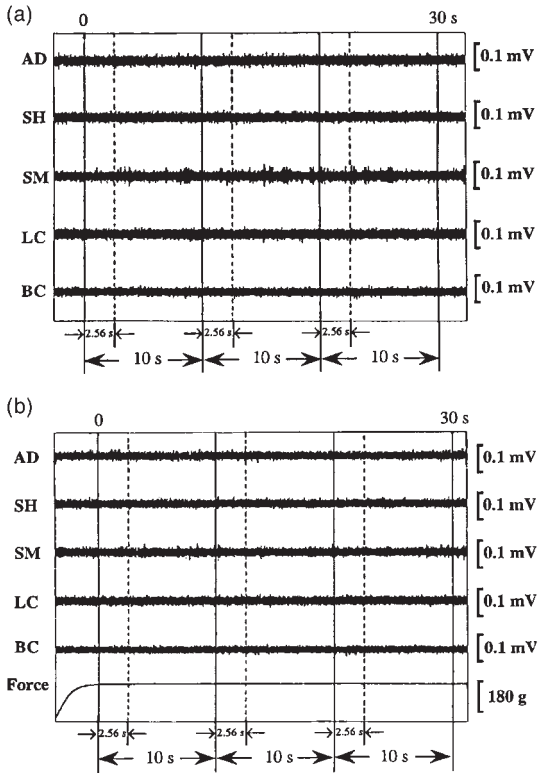

Figure 4 Raw EMG tracing of rat C-2 to show the method of EMG analysis. (a) Rest position and (b) passive traction. The 30-second EMGs were divided into three 10-second segments, and the first 2.56 seconds from each were sampled for integrated EMG activity (IEMG). AD, anterior digastricus; SH, sternohyoideus; SM, sternomastoideus; LC, longus capitus; BC, biventer cervicis. Vertical scale bars indicate EMG voltage and force level. EMG recordings mimicking head extension were performed by sliding the plate posteriorly with 165–200 g passive traction forces.

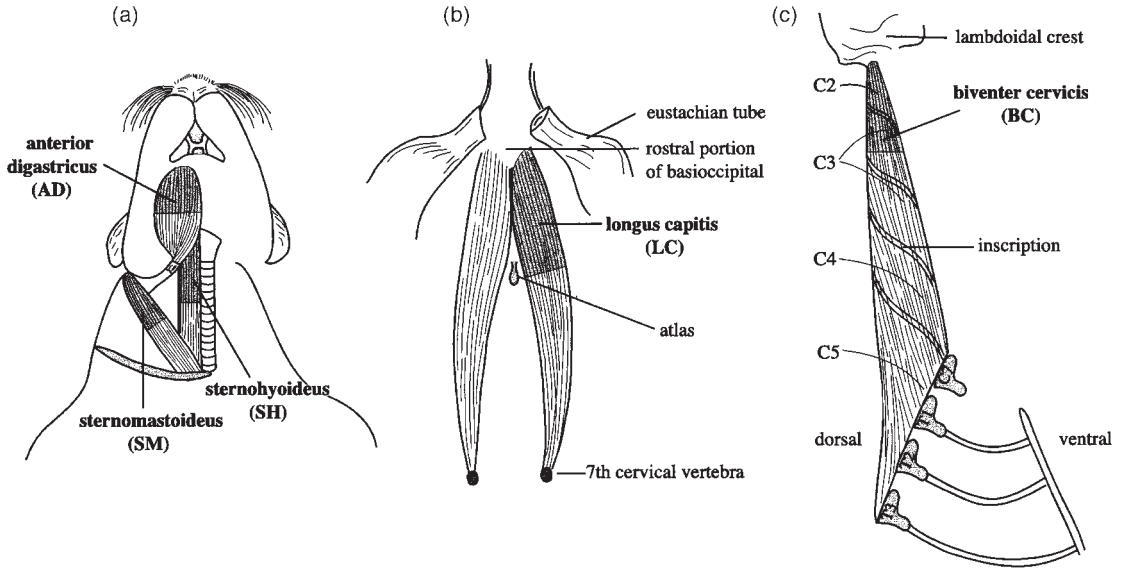

Figure 5 Location and approach to obtaining muscle specimens. (a) AD, SH, and SM, and (b) LC were transected at the mid-belly using a ventral approach; and (c) BC was cut at the level of the third cervical nerve using a dorsal approach. All cranial portions of the five muscles were taken for histological analysis.

a cryostat (2800 E, Hitachi Kenki Fine Tech, Co., Ltd, Tokyo, Japan), serial cross-sections of 8 μm thickness were obtained at –20°C.

Histochemical evaluation of muscle fibre types was based upon the pH liability of myosin adenosine triphosphatase (mATPase) activity. Following a modified method of Brooke and Kaiser (1970), the acid pre-incubations (pH 4.15, 4.25, 4.4, 4.5, and 4.6) were performed for 5 minutes in a solution of barbital acetate and 0.1 M HCl, whereas the alkaline pre-incubation (pH 10.7 and 10.8) was performed for 15 minutes in a solution of 0.1 M barbital sodium and 0.18 M $CaCl_2$. Both acid and alkaline pre-incubated sections were washed for 30 seconds in 0.1 M barbital sodium and 0.18 M $CaCl_2$ (pH 9.6) and incubated for 45 minutes in 0.1 M barbital sodium, 0.18 M $CaCl_2$, and ATP disodium salt (pH 9.6). These procedures were performed at room temperature, 19–21°C.

Fibre types were distinguished on photographs (×50) of sections pre-incubated at pH 4.25, 4.5, and 10.7. The number of each fibre type was counted on the photographs of sections pre-incubated at pH 4.5, where all fibre types could be recognized. Their numbers were expressed as percentage ratios of type I (slow twitch) and IIA (fast twitch fatigue resistant) to type IIB (fast twitch fatigable) fibres (I + IIA)/IIB, as well as ratios of type IIA/IIB.

Photographs (×100) of sections pre-incubated at pH 4.5 were scanned (Epson GT-6000, Epson Co., Tokyo, Japan) and transferred to a personal computer (Power Macintosh 7100/80AV, Apple Co., San Francisco, USA). Cross-sectional areas of each fibre type were measured using imaging software (NIH image 1.44, Symantec Co., Bethesda, USA). Counting of fibres and measurement of fibre area were performed repeatedly on two slides (pH 4.5) per muscle. Their values were averaged for statistical analysis.

Statistics

A univariate analysis of variance (ANOVA) was performed to examine the differences in body weight, IEMG, percentages and ratios of fibre type, and fibre cross-section areas among the three groups. For multiple comparisons between the groups, a Chi-square (for percentage of fibre type) or a Student–Newman–Keuls (SNK, for other parameters) test was chosen. The statistical significance was set at $P \leq 0.05$ for both Chi-square and SNK tests. A Pearson's correlation analysis was used to assess the association between IEMGs and fibre types.

Results

Body weight increased constantly without significant group differences throughout the experimental period (Figure 6).

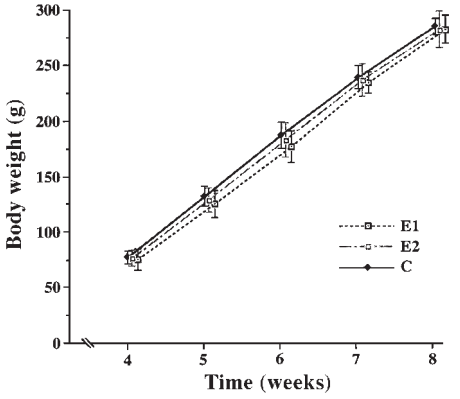

Figure 6 Mean body weights of the experimental and control groups (12 rats per group) from 22 to 55 days. The vertical bars at each point indicate standard deviation. There were no significant differences among the three groups (ANOVA).

Integrated EMG activity

The level of the rest IEMG in BC was significantly higher in group E2 than in groups E1 and C, while other muscles showed no differences (Figure 7a). The proportional IEMG of passive traction in DA was lower in group E1 than in groups E2 and C, and those of SM and BC were lower in group E2 than in groups E1 and C (Figure 7b).

Identification of muscle fibre types

Myosin mATPase reactions of cervical muscle fibres are illustrated in Figure 8 and identification of each fibre

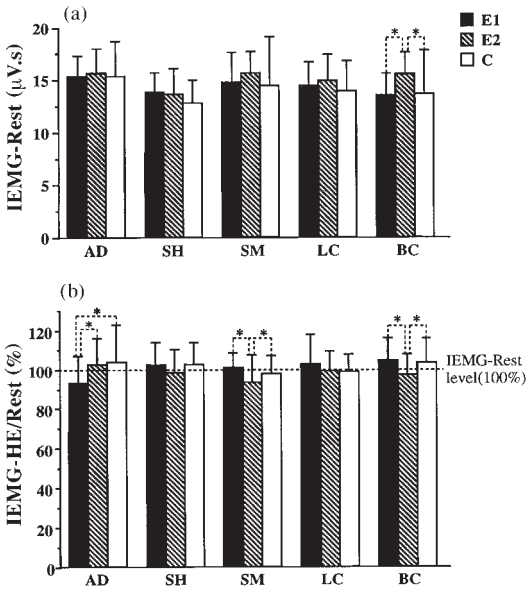

Figure 7 EMG activity of cervical muscles. (a) Integrated EMG at rest (IEMG-Rest). (b) Normalized integrated EMG activity at head extension (IEMG-HE/IEMG-Rest × 100 per cent). The vertical bars indicate standard deviation and asterisks indicate significant differences (*$P < 0.05$) between each group (SNK test).

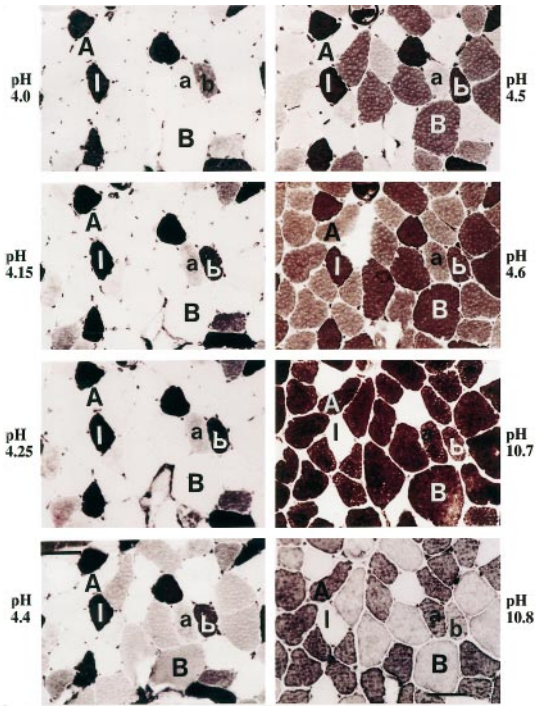

Figure 8 Myosin ATPase reaction in serial cross-sections of longus capitus of rat E2-6, based on a broad acid pre-incubation at pH 4.0, 4.15, 4.25, 4.4, 4.5, and 4.6, and alkaline pre-incubation at pH 10.7 and 10.8 for identifying fibre types. Bar equals 50 μm. Type I fibres (I) constantly remained reactive from 4.0 to 4.6. Type IIA (a) and type IIB (b) fibres were inhibited until pH 4.6 and 4.5, respectively, and were stained moderately for both at pH 10.7 and strongly for IIA, but weakly for IIB at pH 10.8. Note that the reaction for subtype IICA (a) fibres remained weak and for IICB (b) fibres strong in the range of pH 4.0–4.6 in the acid media, as did type I fibres. Type IICA showed the stain intensity between type IIA and IIB, and type IICB showed the stain intensity between type I and IIB at pH 4.5. Again, both type IICA and IICB fibres showed the same stain intensity as type IIA and IIB, respectively at pH 10.7 and 10.8.

type in Table 1. Type I fibres showed strong (in the range of pH 4.0–4.6) reaction for acid-stable mATPase, and a weak (pH 10.7) or negative (pH 10.8) reaction for akaline-stable mATPase, whereas type II fibres showed a reciprocal relationship. Type IIA fibres were stained moderately (pH 4.6) after acid pre-incubation, and moderately (pH 10.7) or strongly (pH 10.8) after alkaline pre-incubation. Type IIB fibres were stained strongly (pH 4.5–4.6) after acid pre-incubation, and moderately (pH 10.7) or weakly (pH 10.8) after alkaline pre-incubation. The myofibres with positive ATPase reactions after both acid and alkaline pre-incubation were subdivided into two subtypes on the basis of pH sensitivity. The myofibres that stained weakly (in the range of 4.0–4.5) or moderately (pH 4.6) for ATPase after acid pre-incubation, and moderately (pH 10.7) or strongly (pH 10.8) for ATPase after alkaline pre-incubation were termed type IICA. The myofibres that stained moderately (pH 4.0) or strongly (in the range of 4.15–4.4) for ATPase after acid pre-incubation, and moderately (pH 10.7) or weakly (pH 10.8) for ATPase after alkaline pre-incubation were named type IICB. Type IICA and IICB could be clearly distinguished from type I fibres with the mATPase reaction after pre-incubation at pH 10.7, showing almost the same reaction of type IICA as type IIA and of type IICB as type IIB at pH 10.8. On the other hand, type IICA and IICB could also be distinguished from type II fibres after pre-incubation in the range of pH 4.0–4.4, showing positive mATPase reaction. They had a continuum of mATPase reaction from acid to alkaline media.

Distribution and cross-sectional area of muscle fibres

Percentages of each fibre type in the three groups are shown in Table 2. In AD, the percentage of type I fibres was significantly higher in group E1, but lower in group E2 than in group C. In SH, group E2 showed a lower

Table 1 Identification of each fibre type based on the staining intensity of the mATPase activity.

Type/pH	4.0	4.15	4.25	4.4	4.5	4.6	10.7	10.8
I	+++	+++	+++	+++	+++	+++	+	–
IIA	–	–	–	–	–	++	++	+++
IIB	–	–	–	–	+++	+++	++	+
IICA	–	+	+	+	+	++	++	+++
IICB	+	+++	+++	+++	++	++	++	+

+++, stained strongly; ++, stained moderately; +, stained weakly; –, unstained.

+++ , the similar stain intensity between type IIB and IICB.

+++ , the similar stain intensity between type IIA and IICA.

+++ , the similar stain intensity between type I and IICB; between type I and IICA.

Table 2 Percentages of fibre types of cervical muscles in the 55-day-old rats.

Muscle	E1	E2	C	*F* value	Chi-square test		
	n = 12	*n* = 12	*n* = 12		E1:C	E2:C	E1:E2
AD							
I	13.55 ± 3.18	9.31 ± 1.95	11.81 ± 2.32	5.97**	*	*	*
IIA	33.71 ± 6.27	33.68 ± 3.39	33.28 ± 4.94	0.02^{NS}	–	–	–
IIB	49.69 ± 6.62	53.28 ± 5.43	53.58 ± 5.36	1.28^{NS}	–	–	–
IICA	2.11 ± 1.29	2.42 ± 1.27	0.76 ± 0.94	4.94*	*	*	NS
IICB	0.94 ± 0.62	1.31 ± 0.77	0.58 ± 0.47	2.87^{NS}	–	–	–
SH							
I	4.58 ± 1.79	3.00 ± 2.21	6.86 ± 3.60	5.75**	NS	*	NS
IIA	21.99 ± 3.56	27.54 ± 3.90	23.58 ± 3.50	6.68**	NS	*	*
IIB	68.72 ± 4.26	62.80 ± 4.85	67.38 ± 4.29	5.24*	NS	*	*
IICA	4.22 ± 2.25	5.34 ± 3.15	1.91 ± 1.49	5.43**	*	*	NS
IICB	0.48 ± 0.53	1.32 ± 1.63	0.27 ± 0.40	3.07^{NS}	–	–	–
SM							
I	15.02 ± 3.61	16.11 ± 2.69	15.04 ± 4.42	0.32^{NS}	–	–	–
IIA	35.35 ± 4.69	31.45 ± 4.14	44.28 ± 4.35	23.18***	*	*	*
IIB	45.90 ± 6.94	47.69 ± 4.75	39.17 ± 6.34	5.67**	*	*	NS
IICA	2.31 ± 1.14	2.15 ± 1.40	0.84 ± 0.92	4.80*	*	*	NS
IICB	1.42 ± 1.44	2.61 ± 1.55	0.68 ± 0.49	6.14**	NS	*	*
LC							
I	18.62 ± 2.77	18.25 ± 4.40	21.04 ± 5.07	1.30^{NS}	–	–	–
IIA	37.84 ± 4.56	32.16 ± 6.92	47.40 ± 3.13	21.23***	*	*	*
IIB	35.64 ± 5.43	38.44 ± 3.93	28.63 ± 6.12	9.24***	*	*	NS
IICA	7.18 ± 4.25	9.21 ± 3.87	2.46 ± 1.67	9.22***	*	*	NS
IICB	0.72 ± 0.70	1.94 ± 1.21	0.46 ± 0.52	8.44***	NS	*	*
BC							
I	17.00 ± 2.70	19.41 ± 3.33	15.87 ± 2.62	4.28*	NS	*	NS
IIA	24.19 ± 4.89	22.21 ± 3.52	24.71 ± 3.18	1.23^{NS}	–	–	–
IIB	55.61 ± 5.60	55.71 ± 4.83	57.80 ± 4.71	0.66^{NS}	–	–	–
IICA	1.35 ± 1.66	1.04 ± 1.00	0.58 ± 0.58	1.19^{NS}	–	–	–
IICB	1.85 ± 1.96	1.63 ± 1.36	1.04 ± 1.45	0.75^{NS}	–	–	–

Values are means ± SD; AD, anterior digastricus; SH, sternohyoideus; SM, sternomastoideus; LC, longus capitis; BC, biventer cervicis.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant.

percentage of type IIB, but higher type IIA fibres than both groups E1 and C. Group E2 also showed a lower percentage of type I fibres than group C. In SM and LC, the percentage of type IIA fibres exhibited a significant decrease with a corresponding increase of type IIB fibres in both groups E1 and E2 compared with group C. In BC, group E2 had a higher percentage of type I fibres than group C. All muscles in the experimental groups, except for BC, had significantly elevated subtype IICA fibres. In addition, group E2 showed higher percentages of subtype IICB fibres in SM and LC. Figure 9 illustrates a sample of the distribution of the fibre types in the five muscles from an experimental animal.

The ratios of type (I+IIA)/IIB were significantly lower in SH of both experimental groups and in AD of group E2. The ratio of type IIA/IIB was significantly high in SH of group E2, but low in SM and LC of both experimental groups (Table 3).

Cross-sectional areas of type IIB fibres were the largest of the three fibre types, whereas type I and type IIA fibres were similar (Table 4). In AD, the areas of type I fibres were smaller in group E2, and type IIA fibres were larger in groups E1 and E2 than in group C. In SH, type I fibres were larger in group E2 than in group C. In SM, type IIA and IIB fibres were smaller in groups E1 and E2 than in group C. In LC, group E1 showed the smallest areas of all three fibre types, and group E2 showed larger type I and IIA fibres, but smaller type IIB fibres compared with group C. In BC, the areas of type I and IIA fibres were smaller in both experimental groups than in group C.

Correlation between IEMGs and fibre types

IEMG correlated positively with the percentage of type I fibres in AD and with type IIA fibres in BC of group E1, positively with the percentage of type IIA fibres in SH and BC of group E2, and positively with the percentage of type I fibres, but negatively with type IIB fibres in LC of group C (Table 5).

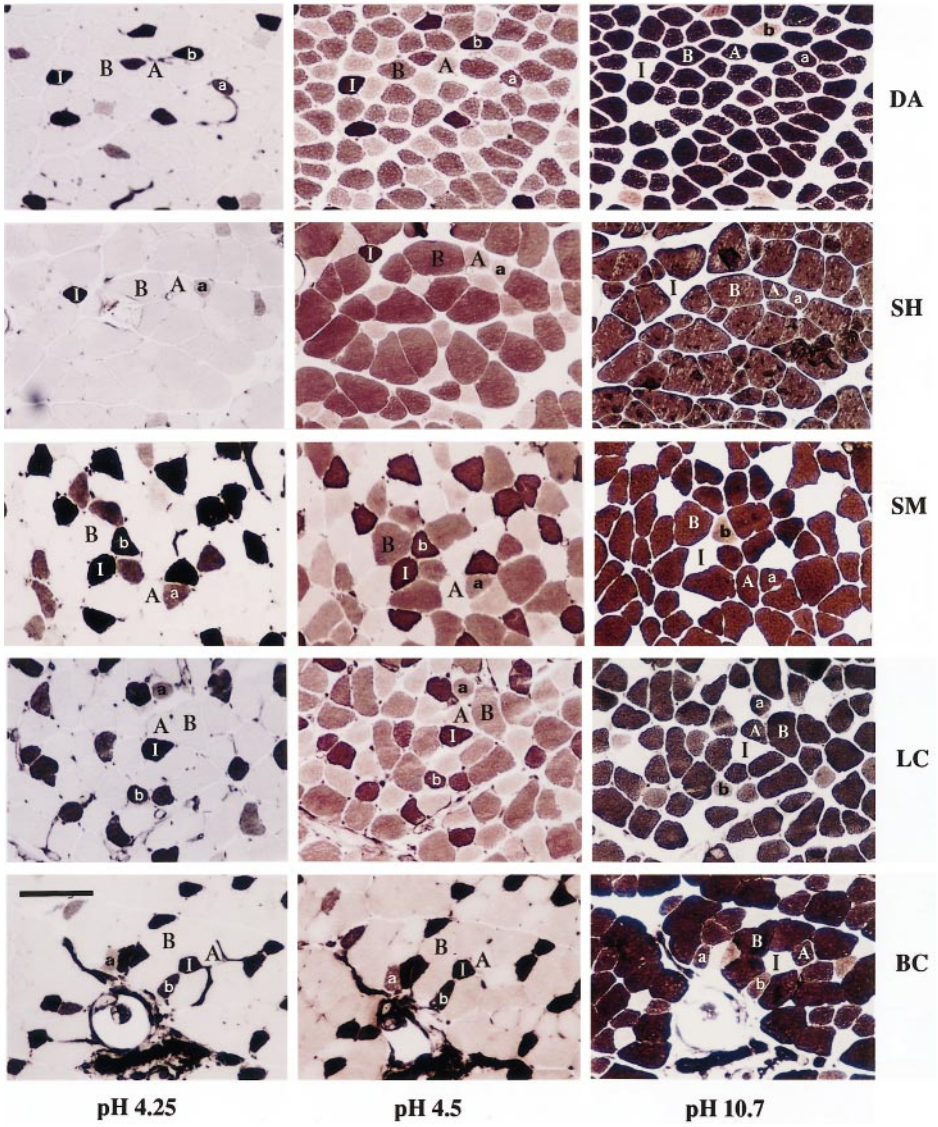

Figure 9 Distribution of fibre types of five cervical muscles in the experimental rat E1-3. Note the inter-myofibrillar pattern of staining reaction is apparent in the transitional fibres (I, type I; A, type IIA; B, type IIB; a, type IICA; b, type IICB) of each muscle following both acid (pH 4.25, 4.5) and alkaline (pH 10.7) pre-incubations. Bar equals 25 μm. See Figure 4 for muscle abbreviations.

Discussion

Altered EMG activity of cervical muscles

In the present study, one of the aims was to observe the EMG activity of cervical muscles in relation to long-term head extension with different body orientations. However, there was less significance than expected. Group E2 had higher activity of BC in the rest position (Figure 7a), which might indicate that a tilted upward body posture with a lower centre of body gravity has changed the equilibrium of the muscle kinetic system. Responding to such a body posture, head extension might evoke a tonic reflex of cervical extensor muscles because BC, the major post-cervical muscle, is known to support and elevate the head (Richmond and Abrahams, 1975) and to be active tonically (Richmond and Vidal, 1988). Also such a body posture requires a physiological adjustment of vision and head position, so that the centre of gravity of the head is moved forwards more or less relative to the occipital condyle. In all primates, as the centre of head gravity is moved forward, the rotational moment at the occipital condyle becomes longer, increasing the work of the post-cervical muscles to maintain a constant head posture (Schultz, 1942; Vig *et al*., 1983).

On the other hand, lower EMG levels of AD in group E1, and SM and BC in group E2 during passive head extension (Figure 7b), might indicate that the daily

Table 3 Ratio of type (I + IIA)/IIB and type IIA/IIB fibres of cervical muscles in the 55-day-old rats (%).

Muscle	E1	E2	C	*F* value	SNK test		
	n = 12	*n* = 12	*n* = 12		E1:C	E2:C	E1:E2
AD							
(I+IIA)/IIB	26.91 ± 5.52	18.84 ± 5.26	24.03 ± 4.26	5.73**	NS	*	*
IIA/IIB	0.71 ± 0.23	0.65 ± 0.13	0.64 ± 0.17	0.43^{NS}	–	–	–
SH							
(I+IIA)/IIB	5.69 ± 2.58	3.99 ± 2.36	9.01 ± 5.53	4.93**	*	*	NS
IIA/IIB	0.33 ± 0.07	0.45 ± 0.09	0.36 ± 0.07	6.85**	NS	*	*
SM							
(I+IIA)/IIB	21.63 ± 7.83	22.36 ± 4.43	20.77 ± 7.24	0.15^{NS}	–	–	–
IIA/IIB	0.82 ± 0.23	0.68 ± 0.15	1.17 ± 0.28	13.49***	*	*	NS
LC							
(I+IIA)/IIB	34.47 ± 7.90	28.30 ± 6.40	36.66 ± 11.03	2.70^{NS}	–	–	–
IIA/IIB	1.11 ± 0.27	0.89 ± 0.23	1.83 ± 0.74	11.38***	*	*	NS
BC							
(I+IIA)/IIB	23.40 ± 5.40	25.36 ± 5.85	20.17 ± 6.09	2.26^{NS}	_	_	_
IIA/IIB	0.45 ± 0.14	0.41 ± 0.09	0.44 ± 0.09	0.39^{NS}	–	–	–

Values are means ± SD; AD, anterior digastricus; SH, sternohyoideus; SM, sternomastoideus; LC, longus capitis; BC, biventer cervicis.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant.

Table 4 Cross-sectional areas of each fibre type of cervical muscles in the 55-day-old rats (mm^2).

Muscle	E1	E2	C	*F* value	SNK test		
	n = 12	*n* = 12	*n* = 12		E1:C	E2:C	E1:E2
AD							
Type I	123.47 ± 32.90	110.76 ± 27.47	120.39 ± 25.78	4.97**	NS	*	*
Type IIA	126.51 ± 36.63	124.82 ± 32.96	116.93 ± 34.40	5.62**	*	*	NS
Type IIB	173.69 ± 47.25	176.24 ± 51.64	170.93 ± 51.14	0.95^{NS}	–	–	–
SH							
Type I	88.83 ± 22.87	97.65 ± 29.49	83.58 ± 17.71	4.38*	NS	*	NS
Type IIA	94.64 ± 23.91	92.28 ± 31.62	94.65 ± 28.76	0.36^{NS}	–	–	–
Type IIB	309.93 ± 159.46	336.94 ± 164.36	316.90 ± 154.50	2.53^{NS}	–	–	–
SM							
Type I	153.57 ± 53.97	164.73 ± 42.18	158.66 ± 42.08	1.79^{NS}	–	–	–
Type IIA	162.76 ± 54.54	168.20 ± 48.80	177.44 ± 50.10	5.50**	*	*	NS
Type IIB	294.23 ± 113.90	311.12 ± 128.83	338.79 ± 121.46	7.54***	*	*	NS
LC							
Type I	112.88 ± 31.00	142.49 ± 52.23	129.55 ± 43.72	21.99***	*	*	*
Type IIA	158.46 ± 45.51	181.89 ± 64.05	169.83 ± 56.18	12.42***	*	*	*
Type IIB	265.04 ± 87.43	284,92 ± 98.50	302.05 ± 99.01	6.66**	*	*	NS
BC							
Type I	134.97 ± 45.71	132.16 ± 48.79	154.39 ± 43.64	7.90**	*	*	NS
Type IIA	164.48 ± 55.84	164.22 ± 57.27	178.24 ± 48.79	3.30*	*	*	NS
Type IIB	332.21 ± 112.27	352.22 ± 117.37	352.49 ± 142.06	2.38^{NS}	_	_	_

Values are means ± SD; AD, anterior digastricus; SH, sternohyoideus; SM, sternomastoideus; LC, longus capitis; BC, biventer cervicis.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant.

posture in cages has made these muscles physiologically adapted. Consequently, their EMG reactions were lower when a passive forward traction was exerted. SM and BC are important muscles participating in head-neck movements, as well as maintaining head-neck posture. In both rats and cats, SM is the chief muscle connecting the skull and the shoulder girdle, and BC is the major connection between the skull and the vertebral column (Richmond and Vidal, 1988). When these muscles are provoked by a prolonged altered posture, the loading and stretching eventually results in their functional adaptation. SM has been reported to be

Table 5 Correlation coefficients between integrated activity (IEMG) of cervical muscles and the percentage of muscle fibre types in the 55-day-old rats (n = 36).

EMG	Fibre type								
	Type I			Type IIA			Type IIB		
	E1	E2	C	E1	E2	C	E1	E2	C
IEMG-Rest									
AD	0.48*	–0.32	0.33	0.21	–0.37	0.09	0.13	–0.32	0.01
SH	–0.37	0.09	0.19	–0.25	0.56**	–0.16	0.06	0.39	0.22
SM	–0.20	–0.36	0.14	–0.39	–0.27	0.03	–0.16	–0.39	–0.36
LC	0.11	0.04	0.58**	0.16	–0.12	0.14	0.00	–0.36	–0.56**
BC	0.18	–0.20	0.24	0.55**	0.43**	0.09	–0.09	–0.11	0.25

IEMG-Rest: IEMG at rest position; AD, anterior digastricus; SH, sternohyoideus; SM, sternomastoideus; LC, longus capitis; BC, biventer cervicis.
*$P < 0.05$; **$P < 0.01$.

recruited strongly during forceful head movement (Richmond *et al*., 1992), and BC is most active during head extension (Baker and Wickland, 1988; Roucoux *et al*., 1989).

Transformation of muscle fibre types

Table 2 shows that the cervical muscles in the control rats had a much higher percentage of type II than type I fibres. It also shows that type IIB fibres were predominant in AD (53.6 per cent), SH (67.4 per cent), and BC (57.8 per cent), whereas type IIA fibres were relatively frequent in SM (44.3 per cent) and LC (47.4 per cent). In experimental rats, however, the distribution of the three fibre types changed. The percentage of type I fibres increased or decreased in AD, SH, and BC, and an inverse relationship between type IIA and IIB fibres was observed in SM and LC; furthermore, all five cervical muscles showed high subpopulation of type II fibres. The continuum of staining intensities from acid to alkaline pre-incubations supports an assumption that these subtype fibres are transitional fibres between type IIA and IIB fibres, or between type I and II fibres. It is the transition that results in the redistribution of type I, IIA, and IIB fibres.

When focusing on the results of pH 4.25, 4.5, and 10.8 (Figure 8), it can be seen that type IICA fibres showed a similar mATPase reaction to type IIA fibres at 10.8, and an intermediate reaction between type IIA and IIB fibres at 4.5, and between type I and II at 4.25. The pH sensitivity of mATPase activity in type IICA is comparable to type IIX fibres in rats (a low reaction at pH 4.3, high at 10.4, and intermediate at 4.6), a large sublet of type II fibres, which is co-expressed with IIA and IIB myosin heavy chain (Gorza, 1990), and also comparable to type IIAB fibres (Ingjer, 1979; Staron *et al*., 1983). Therefore, it is considered that type IICA fibres are an ongoing transformation between type IIA and IIB fibres, which are probably variable hybrids of IIA/IIB. This can be supported by the fact that the population of type IICA significantly increased in most muscles studied and that a shift of fibre distribution occurred, especially the ratio of type IIA and IIB. For instance, the transformation from type IIA to type IICA or IICB, or both, leads to an increased proportion of type IIB fibres in SM and LC. These phenomena are in agreement with the results induced by physical training in human muscles (Jansson *et al*., 1978, 1990; Müntener, 1982; Schantz *et al*., 1982).

On the other hand, type IICB fibres can be considered to be the transitional fibres between types I and II based on their similar mATPase reaction type I fibres at pH 4.25, type IIB fibres at 10.7, and intermediate reaction between type I and II at 4.5. The pH sensitivity of mATPase activity of IICB fibres is comparable to type IIC fibres (Brooke and Kaiser, 1970), which are a primitive form of type II myofibres, and are capable of differentiating into type IIA or IIB fibres (Brooke *et al*., 1971), or with type II intermediate fibres (Kugelberg, 1976; Suzuki and Cassens, 1980), which have a continuous scale of intermediate reactions between types I and II in the process of transformation of type II myofibre units to type I myofibre units. Its population was fewer than type IICA in most of the muscles investigated, except in the SM and LC of group E2. Fibre transition occurs more easily between the fast fibre types (IIA and IIB) than between slow and fast fibre types (I and II). The high population of type IICB in the SM and the LC in group E2 might indicate that this specific posture has demanded a predominant content of slow high-oxidative fibres and these type IICB fibres are in the process of transition from type II to type I. Distinction between type IICA and IICB combining immunological methods is required because

a precise identification of each fibre type could not be obtained without the immunological demonstration of distinct myosin heavy chain isoform expression according to Gorza (1990). However, whether it is type IICA or IICB, they are all variable IIA/IIB or I/IIA hybrids signifying a progressing fast→slow or slow→fast transition process.

Transitional fibres have been demonstrated in electrically stimulated mammalian muscles (Pette and Vrbova, 1992) or cross-innervated muscles of rats (Münterner *et al*., 1987), in the masseter muscle of miniature swine (Anapol and Herring, 2000), and in the masseter muscle of rats following occlusal interference (Nishide *et al*., 2001). In humans, prolonged and intense endurance training can induce a conversion from type II to type I (Gollnick *et al*., 1972), or from type IIB to type IIA (Anderson and Henriksson, 1977; Green *et al*., 1979), or a high percentage of type IIC fibres (Jansson and Kaijser, 1977). The results of the present study suggest that the muscle fibre transformations did not change the proportion of slow to fast twitch fibres, but did change the ratio within fast twitch fibres (IIA/IIB) or the ratio of (I + IIA)/IIB.

Cross-sectional area of muscle fibres

Although muscle fibre area does not directly indicate force, cross-sectional areas have been used to evaluate muscle strength (Roy *et al*., 1984), as well as to provide an estimation of maximum force-developing capacity for predicting the behaviour of musculo-skeletal systems (Pellionisz and Peterson, 1988). In the present study, it was found that the cross-sectional areas of most muscles generally decreased in all three fibre types with head extension; however, the individual muscle showed different changes (Table 4). AD, SH, and BC did not show any alteration of cross-sectional areas in type IIB fibres, which are the largest of the three fibre types. The muscle with the most obvious changes of fibre area was LC, which is stronger, and the least change was SH, which is the weakest. The fibre areas of SM and BC, which are long and parallel-fibred muscles, generally decreased. In contrast, irregular alterations of fibre area were found in AD and LC, which are short and oblique-fibred muscles. These results suggest that the mean cross-sectional areas of muscle fibres depend on their structure, including the size, length, strength, and orientation, and on the contraction properties, as well as on the response of individual muscles to head extension.

EMG activity and fibre composition

EMG activity has been reported to relate to the composition of fibre types in the region sampled by the electrode (Bigland and Lippold, 1954; Buchthal and Schmalbruch, 1980). The current data show that the level of tonic activity of LC in the control rats correlated positively with type I fibres and negatively with type IIB fibres, and that the high activity of BC in group E2 correlated positively with its type IIA fibres (Table 5). These findings are consistent with the association of positive correlation of EMG activity with the percentage of slow-twitch fibres, and negative correlation with the ratio of type IIB/(I + IIA) fibres in jaw muscles of cats (Gorniak, 1986) and with the association of tonic activity showing a predominant content of slow, oxidative fibres in BC of cats (Richmond and Abrahams, 1975).

Correlation of EMG activity with regional distribution of fibre types suggests that a cervical muscle showing greater activity is likely to contain a higher percentage of type I or type IIA fibres, as well as a higher ratio of type (I + IIA)/IIB or IIA/IIB than a muscle showing lower activity, because motor units of type I are recruited initially, followed by type IIA, fast high-oxidative fibres. These two classes of fibres bear most of the load during normal, submaximal activity (Walmsley *et al*., 1978; Armstrong, 1980). In this study, however, some muscles with high percentages of type I and type IIA fibres in the experimental groups did not show expected high EMG activity. The ongoing transition fibre type IICA, which occupied a significantly high subpopulation, is functionally relevant to causal correlations because the motor units of type IICA fibres are composed of average, rather than more oxidative fibres; they have slightly lower tension in terms of ATP used and shortening speed, and therefore are likely to have a different activity pattern. On the other hand, besides the relative content of fast and slow fibres, substantial variations are present in many important features of muscle organization for each functionally related cervical muscle, including their origin and insertion, and the role and mechanics of their actions in response to head extension and body posture.

Conclusions

1. Head extension causes modifications of cervical muscles. These include the adaptation of the physiological properties, the shift of fibre type distribution, and the reduction of cross-section areas of type II fibres.
2. Transitional fibres play an important role in the functional and structural heterogeneity of cervical muscles.
3. Tilted upward body orientation influences the extent and direction of head extension, and enhances the tonic activity of cervical dorsal muscles (BC) in association with its predominant oxidative fibres.

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