Bull Tokyo Dent Coll (2008) 49(2): 53–58

Original Article

Relationship between Function of Masticatory Muscle in Mouse and Properties of Muscle Fibers

Shinichi Abe*, Emi Hiroki, Osamu Iwanuma, Koji Sakiyama, Yoshitaka Shirakura, Daiki Hirose, Yoshiaki Shimoo, Masashi Suzuki, Yasutoyo Ikari, Ryusuke Kikuchi, Yoshinobu Ide and Masao Yoshinari*

Department of Anatomy, Tokyo Dental College,
1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan
* *Oral Health Science Center HRC7, Tokyo Dental College,*
1-2-2 Masago, Mihama-ku, Chiba 261-8502, Japan

Received 21 December, 2007/Accepted for publication 30 January, 2008

Abstract

Mammals exhibit marked morphological differences in the muscles surrounding the jaw bone due to differences in eating habits. Furthermore, the myofiber properties of the muscles differ with function. Since the muscles in the oral region have various functions such as eating, swallowing, and speech, it is believed that the functional role of each muscle differs. Therefore, to clarify the functional role of each masticatory muscle, the myofiber properties of the adult mouse masticatory muscles were investigated at the transcriptional level. Expression of MyHC-2b with a fast contraction rate and strong force was frequently noted in the temporal and masseter muscles. This suggests that the temporal and masseter muscles are closely involved in rapid antero-posterior masticatory movement, which is characteristic in mice. Furthermore, expression of MyHC-1 with a low contraction rate and weak continuous force was frequently detected in the lateral pterygoid muscle. This suggests that, in contrast to other masticatory muscles, mouse lateral pterygoid muscle is not involved in fast masticatory movement, but is involved in functions requiring continuous force such as retention of jaw position. This study revealed that muscles with different roles function comprehensively during complicated masticatory movement.

Key words: Myofiber properties—Eating habits—Masticatory muscles—MyHC—Masticatory movement

Introduction

Recently, several studies have changes in the properties of myofibers in the masticatory and cranio-cervical muscles during the growth and development period[7,24]. Focusing on weaning in the growth and development stage, Gojo *et al.* investigated such changes in mouse masseter muscle after weaning at the protein level[11]. Furthermore, Shida *et al.* quantitatively evaluated such changes in mouse masseter muscle at the genetic level[22]. These studies found that the myofiber properties of mouse masseter muscle markedly changed after weaning, because the oral role changed from lactation to mastication. To evaluate mouse masticatory function, it is, therefore, necessary to evaluate not only the masseter muscle, but also other muscles[1,17,23]. However to the author's knowledge, no studies have investigated such changes in mouse temporal, medial or lateral pterygoid muscles.

Myosin, a protein required for muscle contraction, accounts for about half of the total protein composition of myofibrils[6,12,14–16,18]. In particular, the myosin heavy chain (MyHC), which represents the major portion of the myosin molecule, is known to best reflect muscle function[18]. MyHC consists of several isoforms and can be broadly classified into fast muscle type isoforms (MyHC-2b, MyHC-2d, MyHC-2a) and slow muscle type isoform (MyHC-1) according to contraction speed[2,4,19–21,26]. Furthermore, one study has indicated that the proportions of different MyHC isoforms characterize the properties of different muscles[13].

In this study, the function of these 4 mouse masticatory muscles was compared by investigating the expression of the genes coding the MyHC proteins (MyHC isoforms).

Materials and Methods

1. Specimens

Five male mice at 9 weeks of age were anesthetized with pentobarbital and sacrificed according to the Guidelines for Animal Experiments of Tokyo Dental College. The masseter muscle, temporal muscle, medial pterygoid muscle and lateral pterygoid muscle from all mice were used for examination at the transcriptional level.

2. Reverse transcription polymerase chain reaction analyses

Muscle was removed and snap-frozen in liquid nitrogen, and mRNA was extracted using the Quick Prep Micro mRNA Purification Kit (Amersham Pharmacia Biotech UK Ltd.). After establishing the optimal conditions for all primers, mRNA expression was quantified according to the standard LightCycler™ protocol. As a hot start PCR solution for the LightCycler™ (Roche Molecular Biochemicals, Mannheim, Germany), adjusted LC FastStart DNA Mastar SYBR Green I (Roche Molecular Biochemicals) was used. To the PCR mixture for demonstration runs for each diluted PCR product, or 10.2 μl of sterile water, 2 μl LC FastStart DNA Mastar SYBR Green I containing λDNA (5 pg/μl), SYBR Green I (1/60,000 dilution), and 1.6 μl $MgCl_2$ (25 mM) were added. Furthermore, after adding 0.6 μl each of Forward primer (10 pmol/μl) and Reverse primer (10 mol/μl) prepared using Oligo 5 primer design (Biogene, Ltd., Kimbolton, UK), 5 μl of each diluted PCR product was added, thus bringing the final reaction volume to 20 μl. The primers for MyHC-1, 2b, 2a and 2d were designed by selecting a unique sequence from the full DNA sequence of each isoform. The nucleotide sequence of the primers for each isoform was as follows: MyHC-1 (Forward: 5′-GAGTCCCAGGTCAACAAGC-3′, Reverse: 5′-AACCCAGAGAGGCAAGTGAC-3′, Accession: M12289); MyHC-2b (Forward: 5′-ACAGACTAAAGTGAAAGCC-3′, Reverse: 5′-CTCTCAACAGAAAGATGGAT-3′, Accession: XM_126119); MyHC-2a (Forward: 5′-CGATGATCTTGCCAGTAATG-3′, Reverse: 5′-ATAACTGAGATACCAGCG-3′, Accession: NM-144961); and MyHC-2d (Forward: 5′-GACAAACTGCAATCAAAGG-3′, Reverse: 5′-TTGGTCACTTTCCTGCACTT-3′, Accession: AJ293626). To the PCR mixture, 14.2 μl sterile water, 2 μl LC FastStart DNA Mastar SYBR Green I containing λDNA (5 pg/μl) and SYBR Green I (1/60,000 dilution), 1.6 μl $MgCl_2$ (25 mM), and 0.6 μl each of forward primer (10 pmol/μl) and reverse primer (10 pmol/μl) were added. In addition, 1 μl target DNA was added to bring the final reaction volume to 20 μl. Each PCR mixture (20 μl) prepared in the above manner was added to the glass section of each capillary. PCR was performed at 95°C for 10 min, at 95°C for 10 s, 62°C for 10 s, and 72°C for 7 s, for a total of 50 cycles. As for gene amplification, according to a melting program of 70°C for 15 s,

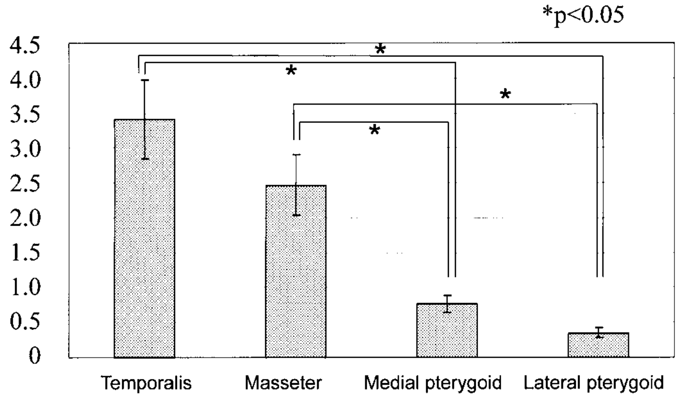

Fig. 1 Amount of MyHC-2b mRNA expression (I: S.D. mean)
Expression was most frequently observed in masseter and temporal muscles.

fluorescence was continuously monitored at a rate of 0.1 μl per second during the transition phase from 70 to 95°C. F1 (530 nm) was used as a fluorescent channel, and the gain indicated 89.9°C for MyHC-1, 89.9°C for MyHC-2b, 88.2°C for MyHC-2a, and 89.6°C for MyHC-2d. The amount of each MyHC isoform calculated by the above-mentioned methods was divided by the amount of GAPDH, which was one of the house-keeping genes, to determine the mRNA expression of each isoform. The base sequence of GAPDH was as follows: (Forward: 5′-TGAACGGGAAGCTCTCACTGG-3′, Reverse: 5′-TCCACCACCCTGTTGCTGTA-3′, Accession: NM_008084). Each PCR fragment was verified as part of a MyHC isoform with the ABI PRISM 310 Genetic Analyzer (Perkin-Elmer Japan Applied Biosystem, Tokyo, Japan).

3. Statistical comparison

Statistical comparisons were made using a one-way analysis of variance (ANOVA). Tukey's multiple comparison test was used for further comparisons between the occlusal areas ($p<0.05$), using the SPSS® software program (SPSS Japan, INC., Tokyo, Japan).

Results

The expression of MyHC-2b mRNA was frequently noted in the masseter and temporal muscles, and the amount was higher in the temporal muscle than in the masseter muscle. Furthermore, the expression was slight in the medial and lateral pterygoid muscles (Fig. 1).

Expression of MyHC-2d mRNA was found in the masseter, temporal, and lateral pterygoid muscles, and the highest amount was observed in the medial pterygoid muscle (Fig. 2).

Almost no expression of MyHC-2a mRNA was detected in the masseter or temporal muscles. However, slight expression was detected in the medial pterygoid muscle. The lateral pterygoid muscle showed a higher expression than the medial pterygoid muscle (Fig. 3).

Almost no expression of MyHC-1 mRNA was detected in the masseter, temporal or medial pterygoid muscles, whereas the lateral pterygoid muscle exhibited frequent expression of MyHC-1 mRNA (Fig. 4).

Discussion

Among the fast-type isoforms of the muscle contractive protein myosin, MyHC-2b has been reported to show the fastest contraction speed, whereas MyHC-2a shows the slowest contraction speed[3,5,10]. Adult mouse masseter

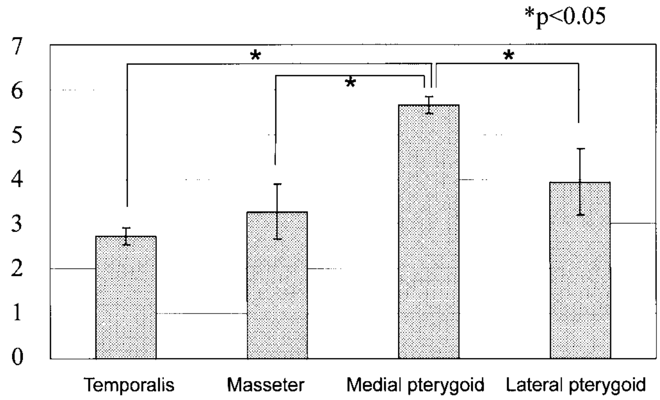

Fig. 2 Amount of MyHC-2d mRNA expression (I: S.D. mean)
Expression was most frequently observed in the medial pterygoid muscle.

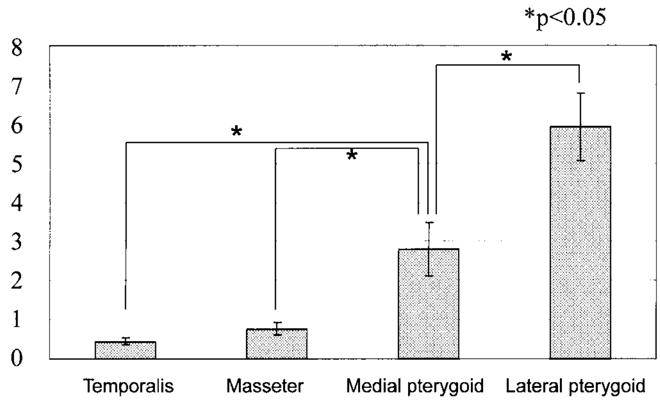

Fig. 3 Amount of MyHC-2a mRNA expression (I: S.D. mean)
Expression was only slightly detected in medial and lateral pterygoid muscles.

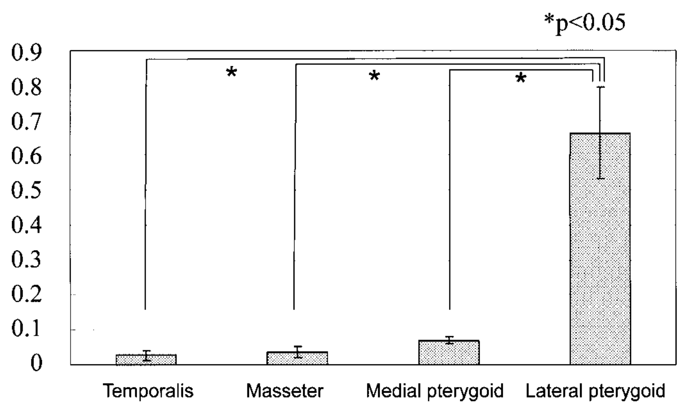

Fig. 4 Amount of MyHC-1 mRNA expression (I: S.D. mean)
Expression was most frequently observed in lateral pterygoid muscle.

muscle has been shown to consist of only fast-type muscle fibers[7,24]. Gojo *et al.* (2002) studied functional change in this type of masseter muscle in detail by observing MyHC-2a/MyHC-2b composition, and reported that its properties greatly changed during the peri-weaning period[11]. In addition, Usami *et al.* (2003) found no such weaning-associated characteristic changed in limb muscle[25]. They showed that MyHC-2b, which is thought to have a fast contraction speed and a high contraction force, was predominantly expressed during the weaning period, suggesting this to be the result of a shift to a chewing motion, thereby leading to further development of the function of the masseter muscle. There have been several reports on the function of MyHC-2b. In a study on adult rat extensor digitorum longus (EDL), which, similar to mouse masseter muscle, is classified as a fast-type muscle requiring a very high contraction force, EDL was shown to be composed solely of MyHC-2b. In addition, it has become clear that, when the function of EDL is reduced by experimental denervation, MyHC-2a expression occurs during reduction of MyHC-2b[9]. This demonstrates that EDL require a high contraction force which ultimately acquires the MyHC-2b isoform, while a decreased function eliminates the need for a high contraction force, thus leading to expression of MyHC-2a.

Our results showed that, among mouse masticatory muscles, the temporal muscle showed the fastest contraction rate and strongest force, followed by masseter muscle. Furthermore, although the lateral pterygoid muscle showed only a weak force, expression of MyHC-1 with continuous force was frequently observed in this muscle; therefore, it is suggested that, during jaw movement, this muscle plays a role in adjusting load applied to the temporomandibular joint by other masticatory muscles.

One report has compared the myofiber properties of these 4 masticatory muscles in human[8]. Although differences were observed in masticatory functioning between human and mouse masticatory muscle, certain similarities have also been noted. For example, during masticatory movement, the temporal muscle showed a stronger force than the masseter muscle, and jaw function was adjusted by the lateral pterygoid muscle with a weak force.

Muscles with different roles are, therefore, believed to function comprehensively during complicated masticatory movement.

Acknowledgements

This study was supported by a grant-in-aid for scientific research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (No. 19592931: Shinichi Abe), by a grant from the Foundation of the Japan Medical Association' by a grant from Oral Health Science Center HRC7 (Shinichi Abe and Masao Yoshinari), Tokyo Dental College' and by a "High-Tech Research Center" Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) of Japan, 2006–2011.

References

1) Abe S, Maejima M, Watanabe H, Shibahara T, Agematsu H, Doi T, Sakiyama K, Usami A, Gojo K, Hashimoto M, Yoshinari M, Ide Y (2002) Muscle-fiber characteristics in the adult mouse tongue muscles. Anat Sci Int 77:145–148.
2) Allen DL, Leinwand LA (2002) Intracellular calcium and myosin isoform transitions. Calcineurin and calcium-calmodulin kinase pathways regulate preferential activation of the 2a myosin heavy chain promoter. J Biol Chem 277:45323–45330.
3) Bottinelli R, Schiaffino S, Reggiani C (1991) Force-velocity relations and myosin heavy chain isoform compositions of skinned fibers from rat skeletal muscle. J Physiol 437: 655–672.
4) Brueckner JK, Itkis O, Porter PD (1996) Spatial and temporal patterns of myosin heavy chain expression in developing rat extraocular muscle. J Muscle Res Cell Motil 17: 297–312.
5) Davoli R, Fontanesi L, Cagnezzo M, Scotti E,

Buttazzoni L, Yarle M, Russo V (2003) Identification of SNPs, mapping and analysis of allele frequencies in two candidate genes for meat production traits: the porcine myosin heavy chain 2B (MYH4) and the skeletal muscle myosin regulatory light chain 2 (HUMMLC2B). Anim Genet 34:221–225.

6) Doi T, Abe S, Ide Y (2003) Masticatory function and properties of masseter muscle fibers in microphthalmia (*mi/mi*) mice during postnatal development. Ann Anat 185:435–440.

7) Eason JM, Schwartz GA, Pavlath GK, English AW (2000) Sexually dimorphic expression of myosin heavy chains in the adult mouse masseter. J Appl Physiol 89:251–258.

8) Eriksson PO, Thornell LE (1983) Histochemical and morphological muscle-fibre characteristics of the human masseter, the medial pterygoid and the temporal muscles. Arch Oral Biol 28:781–795.

9) Erzen I, Primc M, Janmot C, Cvetko E, Sketelj J, d'Albis A (1999) Myosin heavy chain profiles in regenerated fast and slow muscles innervated by the same motor nerve become nearly identical. Histochem J 31:277–283.

10) Fenk R, Ak M, Kobbe G, Steidl U, Arnord C, Korthals M, Hunerliturkoglu A, Rohr U, Kliszewski S, Bernhardt A, Haas R, Kronenwett R (2004) Levels of minimal residual disease detected by quantitative molecular monitoring herald relapse in patients with multiple myeloma. Haematologica 89:557–566.

11) Gojo S, Abe S, Ide Y (2002) Characteristics of myofibers in the masseter muscle of mice during postnatal growth. Anat Histol Embryol 31:1–9.

12) Honda A, Abe S, Hiroki E, Honda H, Iwanuma O, Yanagisawa N, Ide Y (2007) Activation of caspase 3, 9, 12 and Bax in masseter muscle of mdx mice during necrosis. J Muscle Res Cell Motil 29:243–247.

13) Hori A, Ishihara A, Kobayashi S, Ibata Y (1998) Immunohistochemical classification of skeletal muscle fibers. Acta Histochem Cytochem 31:375–384.

14) Kurokawa K, Abe S, Sakiyama K, Takeda T, Ide Y, Ishigami K (2007) Effects of stretching stimulation with different rates on the expression of MyHC mRNA in mouse cultured myoblasts. Biomed Res 28:25–31.

15) Lee W, Abe S, Kim H, Usami A, Honda A, Sakiyama K, Ide Y (2006) Characteristics of muscle fibers reconstituted in the regeneration process of masseter muscle in an mdx mouse model of muscular dystrophy. J Muscle Res Cell Motil 27:235–240.

16) Machino M, Maeda N, Masuda T, Kumegawa M (1981) Postnatal differentiation of masticatory organs in developing mice. Study on muscle fiber differentiation of M. masseter superficialis. Bull Josai Dent Univ 10:111–116.

17) Okubo K, Abe S, Usami A, Agematsu H, Nakamura H, Hashimoto M, Ide Y (2006) Changes in muscle-fiber properties of the murine digastric muscle before and after weaning. Zool Sci 23:1079–1084.

18) Pette D, Sarton RS (1990) Cellular and molecular diversities of mammalian skeletal muscle fibers. Rev Physiol Biochem Pharmacol 116: 1–76.

19) Sartorius CA, Lu B, Acakpo-Satchivi L, Jacobsen RP, Byrnes C, Leinwand LA (1998) Myosin heavy chain 2a and 2d are functionally distinct in mouse. J Cell Biol 141:943–953.

20) Schiaffino S, Gorza L, Sartore S, Saggin L, Ausoni S, Vianello M, Gundersen K, Lomo T (1989) Three myosin heavy chain isoforms in type 2 skeletal muscle fibers. J Muscle Res Cell Motil 10:197–205.

21) Schiaffino S, Reggiani C (1996) Molecular diversity of myofibrillar proteins: Gene regulation and functional significance. Physiol Rev 76:371–425.

22) Shida T, Abe S, Sakiyama K, Agematsu H, Mitarashi S, Tamatsu Y, Ide Y (2005) Superficial and deep layer muscle-fiber properties of the mouse masseter before and after weaning. Arch Oral Biol 50:65–71.

23) Suzuki K, Abe S, Kim H, Usami A, Iwanuma O, Okubo H, Ide Y (2007) Changes in the muscle fibre properties of the mouse temporal muscle after weaning. Anat Histol Embryol 36:103–106.

24) Tuxen A, Kirkeby S (1990) An animal model for human masseter muscle: Histochemical characterization of mouse, rat, rabbit, cat, dog, pig, and cow masseter muscle. J Oral Maxillofac Surg 48:1063–1067.

25) Usami A, Abe S, Ide Y (2003) Myosin heavy chain isoforms of the murine masseter muscle during pre- and post-natal development. Anat Histol Embryol 32:244–248.

26) Wakisaka H, Hijikata T, Yohro T (1993) Muscle fiber-type analysis aided by a personal computer. Biomed Res 14:353–361.

Reprint requests to:
Dr. Shinichi Abe
Department of Anatomy,
Tokyo Dental College,
1-2-2 Masago, Mihama-ku,
Chiba 261-8502, Japan
Tel: +81-43-270-3571
Fax: +81-43-277-4010
E-mail: abe567jp@yahoo.co.jp