POSTNATAL CHANGES IN THE MASSETER MUSCLE OF THE TOOTHLESS (*op/op*) MOUSE WITH LESS-DEVELOPED PERIODONTAL LIGAMENTS

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ABSTRACT

Postnatal changes in the masseter muscle were investigated in the toothless (B6C3Fe-*a/a-op/op*) mouse with less-developed periodontal ligaments. From the start to the end of the weaning period, no distinct difference in histochemical staining of succinate dehydrogenase (SDH) activity in muscle fibers was detectable between *op/op* and normal (B6C3Fe-*a/a-+/?*) mice. Thereafter, differences in the SDH activity of muscle fibers between *op/op* and *+/?* mice gradually appeared in the masseter muscles and, by the third postnatal month, severely atrophied muscle fibers were observed in the superficial region of the masseter muscle of *op/op* mice, which was composed of white and intermediate muscle fibers. However, the nature of progression and the extent of atrophy of muscle fibers in the toothless mouse differed among regions of the masseter muscle. Moreover, the decrease in diameter of muscle fibers in the *op/op* mouse was larger than that in the *+/?* mouse fed a granulated diet. These observations suggest that the inevitable decrease in the sensory input due to less-developed periodontal ligaments may be involved in the atrophy of masseter muscle fibers in the toothless mouse.

There have been many reports regarding the development and aging of various types of muscle fibers in skeletal muscles (2, 3, 6, 10, 11, 17, 21, 23). In general, histochemical and morphological properties of skeletal muscle change during development and aging to adapt to decreases or increases in activity (5, 8, 12-14, 18, 20, 21, 23). Moreover, it has been reported that efferent output from the motor neurons innervating skeletal muscles is involved in controlling histochemical and morphological properties of the muscles (2, 4, 7, 16, 19, 20). However, little attention has been paid to the effects of sensory inputs from the peripheral organs, which are related to the modulation of muscle movements, on the development and the histochemical and morphological properties of muscles. Maeda *et al.* (11, 12) previously suggested the importance of the effects of mastication on the development of the masseter muscle after the end of the weaning

period. Moreover, Maeda *et al.* (13) reported that a liquid diet requiring no mastication and unilateral extraction of molars suppressed bilaterally the development of the masseter muscle, suggesting that sensory input from the periodontal ligaments is involved in the postnatal development of the masticatory organs in developing animals. In addition, Suemune *et al.* (22) found recently that the number of masticatory motoneurons was reduced in the trigeminal motor nucleus (Mo5) in the osteopetrotic (B6C3Fe-*a/a-op/op*) mouse, in which eruption of teeth never occurs and the periodontal ligaments remains less developed than those in the normal (B6C3Fe-*a/a-+/?*) mouse. These authors have suggested that reduction in masticatory motoneurons in the Mo5 is due to a decrease in sensory input from the periodontal mechanoreceptive afferents, as a result of the less-developed periodontal ligaments in the *op/op* mouse. To analyze the

Fig. 1 Diagram of a transverse section of the mid-belly portion of the masseter muscle of the mouse. P1, P2 and P3 indicate regions examined. M, mandible; Mm, masseter muscle; Fn, facial nerve

effects of the sensory input from the peripheral nerve endings on the development and aging of the skeletal muscle, we investigated in the present study postnatal changes in the masseter muscle in the *op/op* mouse.

MATERIALS AND METHODS

B6C3Fe-*a/a*, *op/+* male and female mice were imported from the Jackson Laboratory (Bar Harbor, ME, U.S.A.). Pregnant females were kept in separate cages under alternating 12-h periods of light and dark. Newborn male mice were kept with their mothers, and infant mice were judged by monitoring eruption of incisors on day 15 after birth whether they were osteopetrotic (*op/op*) or not (*+/+*). The *op/op* mice were fed a granulated diet, and normal mice (*+/+*) were fed a solid or granulated diet (CA1; Clea Japan, Tokyo, Japan). The masseter muscles were removed under deep anesthesia on the 15th day, and first, third and fifth month after birth. Three specimens from each group of mice were cut into transverse serial sections of 10 μ m thickness in a cryostat at -20°C and rapidly air-dried. These sections were alternately stained for succinate dehydrogenase (SDH) and with hematoxylin. Histochemical staining for SDH was performed by the following procedure: air-dried sections were incubated for 20 min in the reaction mixture described by Nachlas *et al.* (15), and fixed in 10% formaldehyde. Then the sections were washed with distilled water and dried at room temperature. Both types of treated section were

mounted with glycerin. All treated sections were examined under a light microscope (BH2; Olympus, Tokyo, Japan). The smallest diameter of each muscle fiber was measured by the methods of Brooke and Engel (1) and Wakisaka *et al.* (24). As shown in Fig. 1, measurements of muscle fibers were carried out in three different regions of each hematoxylin-stained section in the mid-belly region of the masseter muscle. The number of muscle spindles in the masseter muscle of each mouse in examined groups was counted by using all transverse serial sections. All data were analyzed by Student's *t*-test.

RESULTS

Fig. 2 shows postnatal changes in the SDH activity in a superficial portion (P1) of the masseter muscle in *op/op* and *+/+* mice. In 15-day-old mice, no distinct differences were detectable in the SDH activity between *op/op* and *+/+* animals (Fig. 2, A and D). One month after birth, postnatal month, slightly atrophied muscle fibers were observed in the region of the masseter muscle in the *op/op* mouse, as compared with fibers in the normal mouse (Fig. 2, B and E). By the third postnatal month, almost all of the muscle fibers in this region atrophied in the masseter muscle of the *op/op* mouse. The most severely atrophied fibers were detected in the lateral component, and classification of fibers on the basis of SDH activity was not possible (Fig. 2F), though two types of fibers, namely, white and intermediate muscle fibers, were detected in this region in the normal mouse (Fig. 2C). In a deeper region (P3), no distinct differences in terms of SDH activities of muscle fibers were detectable between *+/+* and *op/op* mice until the end of the first postnatal month (Fig. 3, A, B, D and E). After three months, atrophied fibers were observed in the masseter muscle of *op/op* mouse (Fig. 3F), although the extent of atrophy of muscle fibers in the P3 region appeared slightly lower than that in the P1 region. A distinct difference in SDH activity between masseter muscles of 3-month-old *op/op* and *+/+* mice was observed in the intrafusal muscle fibers (Fig. 3, C and F), and the number of muscle spindles was slightly but not significantly reduced (Table 1). By contrast, in the P2 region under the facial nerve, we could not detect any differences in SDH activities among muscle fibers even when 3-month-old *op/op* mice were compared with normal mice (Fig. 4). Fig. 5 shows differences in the muscle diameter between masseter muscles

Fig. 2 Postnatal changes in the SDH activity in the P1 region of the masseter muscle. A, B and C, +/?. D, E and F, *op/op*; A and D, 15-day-old mice; B and E, 1-month-old mice; C and F, 3-month-old mice. Arrowhead indicates severely atrophied region. +/? and *op/op* mice were fed a solid and granulated diet, respectively. $\times 255$

of 5-month-old +/? and *op/op* mice fed a granulated diet. Fiber diameters were significantly decreased in the P1 ($P < 0.001$) and P3 ($P < 0.05$) regions, when compared with those in normal mice, though there was no significant difference in the body weight between +/? and *op/op* mice (Table 1 and Fig. 5). In the P2 region, we could not detect any

significant difference in fiber diameters.

DISCUSSION

Sensory input from the oro-facial sensory organs plays a major role in controlling formation of the masticatory system during a certain stage of growth

Fig. 3 Postnatal changes in the SDH activity in the P3 region of the masseter muscle. A, B and C, +/?. D, E and F, *op/op*; A and D, 15-day-old mice; B and E, 1-month-old mice; C and F, 3-month-old mice. Arrowheads indicate muscle spindles. +/? and *op/op* mice were fed a solid and granulated diet, respectively. $\times 255$

(9). Maeda *et al.* (13) reported that a liquid diet requiring no mastication and unilateral extraction of molars bilaterally suppressed the development of the masticatory muscle, and they suggested that sensory input from the periodontal ligaments might be involved in controlling the development of masticatory organs in developing animals. Sue-

mune *et al.* (22) suggested that an inevitable decrease in sensory input to Mo5 motoneurons, via trigeminal mesencephalic nucleus (Me5) and supra-trigeminal nucleus (Su5), from periodontal sensory endings, as a result of the less-developed periodontal ligaments in *op/op* mice, might cause the significant reduction in the number of masticatory moto-

Table 1 Differences in the Number of Muscle Spindles in the Masseter Muscle between 3- and 5-Month-Old Normal (+/?) and Toothless (*op/op*) Mice

Animals	Body weight (g)	Number of muscle spindles
+/?		
3-month-old	30.9 ± 2.5	54 ± 7
<i>op/op</i>		
3-month-old	27.8 ± 2.3	46 ± 3
5-month-old	32.7 ± 1.0	48 ± 5

Each value is the mean ± SD from 3 animals.

Table 2 Differences in Diameter of Extrafusil Muscle Fibers in the P1, P2 and P3 Regions of the Masseter Muscle between 5-Month-Old Normal (+/?) and Toothless (*op/op*) Mice

Animals	Diameter (μm)		
	P1	P2	P3
+/?	48.6 ± 11.8 (232)	50.8 ± 13.1 (227)	47.2 ± 11.9 (244)
<i>op/op</i>	31.6 ± 11.2** (305)	48.0 ± 13.8 ^{NS} (235)	43.5 ± 11.2* (274)

Each value is the mean ± SD from 3 animals. The number of muscle fibers measured is indicated in parentheses. * $P < 0.05$ and ** $P < 0.001$ vs +/?, NS, not significant

Fig. 4 SDH activity in the P2 region of the masseter muscle of 3-month-old mice. A, +/? mouse; B, *op/op* mouse; I, intermediate muscle fiber; W, white muscle fiber. +/? and *op/op* mice were fed a solid and granulated diet, respectively. × 120

Fig. 5 Differences in diameters of muscle fibers between 3-month-old +/? and *op/op* mice in the P1, P2 and P3 regions. ★, $P < 0.05$ and ★★, $P < 0.001$ vs +/?; NS, not significant. Animals in both groups were fed a granulated diet. Each value is the mean from 3 animals. Solid bars, +/? mice; checkered bars, *op/op* mice

neurons in Mo5. In the present study, we observed rapid progression of atrophy of extrafusal muscle fibers in the masseter muscle after weaning, and distinct atrophy appeared in some superficial regions of the masseter muscle, which were composed of white and intermediate muscle fibers. The degree of atrophy in the regions of the muscle in *op/op* mice was higher than that in mice fed a fine-grained diet (12, 13). On the other hand, the degree of atrophy in a deeper region of the masseter muscle, which was mainly composed of red and intermediate fibers, was almost the same as that in mice fed a fine-grained diet, or in mice whose unilateral molars were extracted (12, 13). In addition, there is a critical difference in findings between mice fed a fine-grained diet (12) and *op/op* mice fed granulated diet in the present study; in mice fed a fine-grained diet, white and red, but not intermediate, fibers were atrophied, whereas in *op/op* mice, all types of muscle fibers were atrophied. Niederle and Mayr (16) showed that all types of muscle fibers were atrophied by denervation, though the course of denervation atrophy was different among types of fibers. Thus, the manner of muscle atrophy in the masseter muscle of *op/op* mice was similar to that in the denervated muscle, rather than to that in the masseter muscle of mice fed a fine-grained diet. Therefore, our present observations do not contradict our previous findings that the number of motor neurons innervating the masseter muscle is reduced in *op/op* mice with less-developed periodontal ligaments (22). Moreover, the present results support our previous suggestion that sensory input from the periodontal ligaments is involved to a great extent in controlling the development of masticatory organs in developing animals (13). In the present study, however, we do not know the reason why the degree of fiber atrophy differed between regions in the masseter muscles. Mechanical load applied to the masseter muscle may differ between the *op/op* and *+/?* mice, if there is a morphological change in maxillo-facial bones in the former.

In intrafusal muscle fibers, we observed a distinct decrease in SDH activity in *op/op* mice. Maeda *et al.* (13) previously suggested that decreases in the SDH activity and the diameter of intrafusal muscle fibers in mice fed a liquid diet might be caused secondarily by the low tension and limited contraction that were consequence of the atrophied extrafusal muscle fibers. Suemune *et al.* (22) could not detect any significant reduction in the number of smaller neurons in Mo5, which innervate muscle

spindles in the masseter muscle, although there was a slight decrease in the number; they also found that the number of Me5 neurons innervating muscle spindles in the masseter muscle was slightly but not significantly reduced in 5-month-old *op/op* mice. Accordingly, it is unclear why intrafusal muscle fibers atrophy in *op/op* mice with less-developed periodontal ligaments. This remains to be further elucidated.

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