REDUCTION OF MASTICATORY MOTONEURONS IN THE TRIGEMINAL MOTOR NUCLEUS OF THE TOOTHLESS (*OP/OP*) MOUSE

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ABSTRACT

In osteopetrotic (B6C3Fe-*a/a-op/op*) mice, in which no eruption of teeth ever occurs and the periodontal ligament remains less developed than in normal (B6C3Fe-*a/a*, +/?) mice. A horseradish peroxidase-wheat germ agglutinin conjugate (HRP-WGA) was injected into the masseter nerve of 3- and 5-month-old +/? and *op/op* mice. The number of larger cells labeled in the trigeminal motor nucleus (Mo5) in *op/op* mice was significantly lower by the third postnatal month than that in the Mo5 of +/? mice fed on a solid diet. This decrease in number of labeled cells was larger than that in +/? mice fed a granulated diet. These findings suggest that reduction in masticatory motoneurons in Mo5 may be due to a decrease in sensory input via the mesencephalic trigeminal nucleus (Me5) and the supratrigeminal nucleus (Su5) from periodontal mechanoreceptive afferents as a result of less-developed periodontal ligaments in *op/op* mice.

Mastication is an integrated biological activity that is carried out by the combined operation of masticatory motor apparatus, sensory input from the oro-facial sensory organs, and the central nervous system. Recently, Kubota *et al.* (5) suggested that the sensory input plays a major role in controlling formation of the masticatory system during the certain stages of growth. Moreover, Maeda *et al.* (7) reported that a liquid diet requiring no mastication and unilateral extraction of molars suppressed the development of the masticatory muscle, and they suggested that sensory input from the periodontal ligament might be involved to a great extent in controlling the development of masticatory organs in developing animals. Therefore, the present study was designed to allow more detailed analyses of the difference of the masseteric motor and sensory neurons between normal (+/?) mice and toothless (*op/op*) mice, a mutant strain mice that never developed erupted teeth.

MATERIALS AND METHODS

B6C3Fe-*a/a-op/+* male and female mice were

imported from the Jackson Laboratory (Bar Harbor, ME, U.S.A.). Newborn male mice were kept with their mothers, and infant mice were judged, by monitoring eruption of teeth on day 20 after birth, to be osteopetrotic (*op/op*) or not (+/?), and they were then separated from their mothers. The *op/op* mice were fed a granular diet, and normal animals (+/?) were fed a solid diet or granular one (CA1; Japan Clea, Tokyo, Japan). Experiments were performed during the third and fifth postnatal month. Each group of three male animals was anesthetized via intraperitoneal injection of sodium pentobarbital (0.06 mg/g body weight). The unilateral masseter nerve was surgically exposed just anterior to the temporomandibular joint after partial removal of the zygomatic arch, and 1 μ l of a solution of 5% horseradish peroxidase-wheat germ agglutinin conjugate (HRP-WGA; Toyobo, Osaka, Japan), entrapped in a 1% hypoallergenic polyacrylamide gel, was injected by means of a glass micropipette (80-100 μ m in diameter) into the nerve bundle. After 24 h, the deeply anesthetized animals were perfused through the ascending aorta with 200 ml of heparinized saline, which was followed

immediately by 300 ml of fixative (1% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4). The fixative was then flushed out with 200 ml of 10% sucrose in 0.1 M phosphate buffer, pH 7.4. Thereafter, the brainstem and trigeminal ganglion (TG) were removed, and they were stored overnight in a solution of 30% sucrose in phosphate buffer (pH 7.4) at 4°C. Transverse frozen sections of 50 μ m thickness were serially cut, and the sections were processed in accordance with the tetramethyl-benzidine (TMB) protocol of Mesulum (8). The sections were then dried on slides with chrome alum-gelatin. All treated sections were examined under a light microscope (BH2; Olympus, Tokyo, Japan), and the total number of labeled neurons was counted in the trigeminal motor nucleus (Mo5), the trigeminal mesencephalic nucleus (Me5), and TG. Moreover, labeled cells in the trigeminal motor nucleus were classified into two groups by size: those having a long-axis diameter of less than 20 μ m and those having a long-axis diameter above 20 μ m. All data were analyzed by Student's *t*-test.

Upper and lower jaws from normal (+/?) and toothless (*op/op*) mice were fixed in 10% paraformaldehyde and, after decalcification and dehydration, the specimens were embedded in paraffin. Thereafter, they were cut into serial sections at 10 μ m, and all sections were stained with hematoxylin and eosin and examined under the light microscope.

RESULTS

In normal (+/?) mice, erupted teeth and well-developed periodontal ligaments were observed in 5-month-old animals (Fig. 1A). However, in osteopetrotic (*op/op*) mice, no eruption of teeth was recognized on upper and lower jaws by the fifth postnatal month, and the formation of the tooth root was poorly recognizable. Therefore, the periodontal ligaments were hardly visible. In addition, fusion of the tooth and alveolar bone was observed near the tooth neck of every tooth (Fig. 1B). The cell bodies of the neurons innervating the masseter muscle, which were located in the Me5, TG, and Mo5 nuclei contained, the HRP-WGA conjugate that had been injected into the masseter nerve 24 h earlier. Fig. 2 shows conjugate-containing neurons at the same level in the Mo5 of 3- and 5-month-old mice. Labeled cells could be classified as larger and smaller cells. The density of distribution of which labeled cells in *op/op* mice was lower than that in

Fig. 1 Erupted tooth in a normal (B6C3Fe, +/?) 5-month-old mouse (A) and an impacted tooth in the upper jaw of an osteopetrotic (B6C3Fe, *op/op*) 5-month-old mouse (B). Arrow and arrowhead indicate the periodontal ligament and the tooth neck, respectively. DE, decalcified enamel. Bar, 100 μ m

+/? mice. Table 1 shows numbers of labeled cells in the Me5, TG, and Mo5 of normal (+/?) mice fed a solid diet or granular one, and in toothless (*op/op*) mice fed a granular diet. In specimens prepared from animals at 3 months after birth the total number of labeled cells in the Mo5 was significantly lower in *op/op* mice than in the normal (+/?) mice fed a solid diet. This significant decrease was due to a lower number of larger, rather than smaller labeled cells. A granular diet caused no significant decrease in the number of labeled cells in the Mo5 of +/?

Fig. 2 Labeled cells in the trigeminal motor nucleus of 3-month-old (A, B) and 5-month-old (C, D) mice. A, C, +/+; B, D, *op/op*. Solid and open arrows indicate larger and smaller cells, respectively. Bar, 100 μ m

mice, though a decreasing tendency in terms of the total number of labeled cells was observed in the Mo5. The number of labeled cells in the Me5 of *op/op* mice was also slightly but not significantly reduced, and no difference was seen in the TG.

DISCUSSION

It is well known that the size distributions of cell bodies in the Mo5 and of myelinated fibers are bimodal. Most of the smaller cell bodies of the Mo5 that innervate the masseter and temporal muscles measured 8–14 μ m in diameter, and the larger ones measured 28–42 μ m in diameter. The larger cells had features typical of α motor neurons that inner-

vate extrafusal muscle fibers, whereas the smaller ones had those of γ motor neurons that innervate the intrafusal muscle fibers (6). In the present study, we observed a significant decrease only in the number of larger cells of the Mo5 in *op/op* mice with less-developed periodontal ligaments. It was reported that the mesencephalic periodontal afferents project mainly to the supratrigeminal nucleus (Su5) in the cat and rat (2, 10), and the Su5 neurons serve as interneurons of the disynaptic path between Me5 neurons and larger Mo5 motoneurons (10), as shown in Fig. 3. Moreover, it has been reported from electrophysiological studies that these Su5 neurons serve as interneurons that are involved in masticatory reflexes (1, 3), and

Table 1 Numbers of Me5, TG and Mo5 Cells Labeled by Application of Horseradish Peroxidase-Wheat Germ Agglutinin Conjugate to the Masseter Nerve of Normal (B6C3Fe-+/?) and Osteopetrotic (B6C3Fe-op/op) Mice

Animals (Diet)	Age (Month)	Me5	TG	Mo5 Total	(Larger > 20 μ m)	(Smaller < 20 μ m)
+/?						
(solid)	3	544 $\pm$ 51	212 $\pm$ 30	529 $\pm$ 16	(363 $\pm$ 16)	166 $\pm$ 15)
	5	534 $\pm$ 11	191 $\pm$ 70	505 $\pm$ 62	(318 $\pm$ 58)	187 $\pm$ 22)
+/?						
(granular)	3	554 $\pm$ 36	200 $\pm$ 23	468 $\pm$ 9	(300 $\pm$ 9)	167 $\pm$ 8)
	5	556 $\pm$ 23	186 $\pm$ 13	497 $\pm$ 7	(342 $\pm$ 4)	155 $\pm$ 10)
op/op						
(granular)	3	480 $\pm$ 28	230 $\pm$ 45	376 $\pm$ 33*	(204 $\pm$ 23*)	172 $\pm$ 12)
	5	413 $\pm$ 97	204 $\pm$ 26	386 $\pm$ 22*	(205 $\pm$ 2*)	181 $\pm$ 23)

Each value was obtained from 3 animals. Me5, trigeminal mesencephalic nucleus; TG, trigeminal ganglion; Mo5, trigeminal motor nucleus. * $P < 0.05$ vs +/? (solid diet)

Fig. 3 Schema of projections from the periodontal ligament, muscle spindle, and extrafusal muscle fiber of the masseter muscle. Mo5, trigeminal motor nucleus; Me5, trigeminal mesencephalic nucleus; Su5, supratrigeminal nucleus; Ia, group Ia sensory neurons; II, group II sensory neurons; α , α motor neuron; γ , γ motor neuron; Ms, muscle spindle in the masseter muscle; Emf, extrafusal muscle fiber in the masseter muscle. Solid and open squares in the Mo5 indicate larger and smaller trigeminal motor neurons, respectively.

intracellular recording studies have revealed that the Su5 contains not only inhibitory neurons but also facilitatory ones to the jaw-closing motoneurons (1, 3, 4, 9, 11). On the other hand, Me5 neurons and the smaller Mo5 neurons innervating muscle spindles (Fig. 3) showed no significant decrease in number in the present study. These results lead us to conclude that the inevitable decrease in the sensory input to Mo5 motoneurons, via Me5 and Su5, from periodontal sensory endings, due to the less developed periodontal liga-

ment in *op/op* mice, may cause the significant reduction in masticatory motoneurons in the Mo5.

However it is still unclear why, in *op/op* mice, Me5 and Mo5 neurons both of which innervate the muscle spindles and play important roles in modulating movement of the jaw, did not decrease in number. We shall continue to examine this issue in detail.

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