Original Article

An electrophysiological evaluation method for the ovine facial nerve

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ABSTRACT

Introduction: Large-animal models such as sheep for facial nerve regeneration research have not yet been established because of the lack of methods for assessing the electrophysiological function of regenerated nerves. In this study, we developed a percutaneous measurement method for the evoked compound muscle action potential (CMAP) of the facial nerve in sheep.

Methods: Six 3-year-old castrated male Corriedale sheep were used in this study. Under general anesthesia, an anatomical exploration was performed to identify the course of the buccal branch of the facial nerve and its innervating muscles on one side, followed by the application of surface stimulating electrodes to the contralateral side of the face along the course of the buccal branch of the facial nerve to obtain CMAP measurements of the nasolabial levator muscle.

Results: Percutaneous CMAP measurements of the nasolabial levator muscle could be obtained in all animals by placing stimulating electrodes 1 cm apart on the line coinciding with the course of the buccal branch of the facial nerve revealed by the preceding anatomical exploration. Mean values for electrophysiological parameters were amplitude 4.7 ± 0.7 mV, duration 2.1 ± 0.6 ms, and latency 3.6 ± 0.4 ms.

Conclusion: We have established a percutaneous measurement method for CMAP of the buccal branch of the facial nerve in sheep. This method is expected to be very useful in future studies of facial nerve regeneration for long nerve defects in sheep.

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1. Introduction

Facial nerve palsy can have various causes, such as Bell’s palsy [1], auditory nerve tumors [2], trauma [3], and malignant parotid gland tumors [3], and significantly impairs the patients’ social activities and quality of life through mimetic muscle paralysis. Toward establishing new surgical treatments for facial nerve palsy, we have developed various experimental systems for facial nerve regeneration using rat models of facial nerve defects, including facial nerve regeneration in rats using a novel nerve conduit made of polylactic acid nonwoven fabric [4], facial nerve regeneration in rats using a basic fibroblast growth factor drug delivery system [5], facial nerve regeneration using dedifferentiated fat cells [6], facial nerve regeneration using adipose-derived stem cells (ADSCs) that were induced in vitro to differentiate into Schwann cell lineages [7], facial nerve reconstruction in rats using an artificial nerve conduit containing dental pulp cells extracted from incisor teeth [8], and facial nerve regeneration using an artificial nerve conduit made of a silicone tube containing a non-cultured stromal vascular fraction as the source of ADSCs [9]. We then developed a method to measure compound muscle action potential (CMAP) in these rat models of facial nerve regeneration, for which no electrophysiological evaluation method for regenerated facial nerves had been established [10]. However, the above rat models of facial nerve defect allowed for experimental nerve transplantation into defects of up to only about 7 mm in length due to the small size of the animals, and were too small to reproduce facial nerve regeneration surgery in human patients in clinical practice. For example, at least 10 cm of nerve regeneration length is required for cross-facial nerve grafting (CFNG), where an autologous nerve graft is transplanted between the facial nerve on the healthy side and the facial nerve branch on the affected side so that the
mimetic muscles on the affected side are reinnervated by neural transmission from the facial nerve on the healthy side to restore facial movement [11]. This cannot be reproduced in rat models of facial nerve palsy. Therefore, aiming to create a large-animal model of facial nerve defect, we have conducted anatomical studies of the facial [12], hypoglossal [12] and gastrocnemius [13] nerves in sheep, describing the course of the nerves in detail. Based on the facial nerve anatomy revealed through these previous studies, in the present work we established a method for measuring CMAP of the facial nerve in sheep and describe the measurement procedure.

2. Methods

2.1. Experimental design

This animal study using sheep was approved by the animal experimentation committee at IVTeC Inc (approval number: IVT19-80) and was conducted in accordance with the Act on Welfare and Management of Animals; the Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain; the Fundamental Guidelines of the Ministry of Education, Culture, Sports, Science and Technology; the Guidelines for Proper Conduct of Animal Experiments; the Guidelines on Methods of Sacrificing Animals, and other applicable regulations. A total of 6 castrated Corriedale sheep (age 3 years, body weight 30–40 kg) were used. For all electrophysiological evaluations and surgery, the sheep were intubated, and anesthesia was induced and maintained with 2–5% isoflurane under mechanical ventilation.

2.2. Identification of the course of the buccal branch of the facial nerve and detection of muscle contraction induced by electrical stimulation

The anatomical stimulation and recording sites for CMAP measurement were determined on the right (n = 3) and left (n = 3) side of the face of the 6 sheep. On the right or left side of the sheep’s face, a “modified Blair incision” was made anterior to the auricle and extended from 1.5 cm posterior to the mandibular ramus to 2 cm below the mandibular body. This flap was undermined widely to expose the platysma muscle using scissors and scalpel so that the zygomatic muscle, parotid gland, facial artery and vein, and the buccal and marginal mandibular branches of the facial nerve could be seen through the platysma fascia. An L-shaped incision was then made in the platysma overlaying the mandibular area to expose the parotid gland and the buccal branch of the facial nerve emerging from the anterior margin of the parotid gland, and the course of the buccal branch was identified, which would serve as the landmark for the application of stimulating electrodes for percutaneous CMAP measurement. The buccal branch was further traced toward the most posterior molar while dissecting surrounding tissue to confirm that it was coursing behind the zygomatic muscle into the posterior aspect of the nasolabial levator muscle. The stimulating electrodes of the evoked potential testing device (Neuropack S3, Nihon Kohden, Tokyo, Japan) were applied directly to the exposed buccal branch and a single supramaximal electric stimulus (approx. 8 mA) was applied at a frequency of 0.1 Hz to visually confirm movement of the nasolabial levator muscle upon stimulation (Fig. 1). The marginal mandibular branch of the facial nerve, which

Fig. 1. Localization of the facial nerve and mimetic muscles in sheep as determined by facial anatomical exploration. When electric stimulation was applied via the stimulating electrodes applied directly to the buccal branch of the facial nerve at the site where it emerged from the anterior margin of the parotid gland and coursed toward the nasolabial levator muscle, the movement of the exposed nasolabial levator muscle upon stimulation was visually confirmed. *: buccal branch; ■: marginal mandibular branch of facial nerve; ●: parotid gland; ▲: nasolabial levator muscle; ★: zygomatic muscle.

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was exposed along with the buccal branch, was also electrically stimulated with the same settings to determine whether the nasolabial levator muscle was also innervated by the marginal mandibular branch (Fig. 1).

2.3. Percutaneous CMAP measurement

The side of the face contralateral to the one used for the above anatomical exploration was used to percutaneously apply electrical stimulation to the buccal branch of the facial nerve and measure CMAP of the mimetic muscles (nasolabial levator muscle) with the evoked potential testing device (Neuropack S3, Nihon Kohden). Two surface stimulating electrodes were placed 1 cm apart on the line along the course of the buccal branch of the facial nerve as revealed in the preceding anatomical exploration. The recording microelectrode (NM-450C, Nihon Kohden) was inserted to a depth of about 1 cm at the mid-point on the line connecting the nostril and the angle of the mouth, where the nasolabial levator muscle is located (Fig. 2A). A single supramaximal stimulus (approx. 8 mA) was applied at a frequency of 0.1 Hz. Ten CMAP measurements were obtained from each animal, from which amplitude, latency, and duration were determined and average values were calculated for each parameter. The method for calculating amplitude, latency, and duration was described in our previous report on measuring CMAP in rats [10]. Briefly, amplitude is the differential voltage between the baseline (zero) and positive peak potentials. Latency is the time interval between the beginning of stimulus artifacts and when the CMAP trace rises over the zero line. Duration is the time interval between the zero-crossing points of the rising and falling trace (Fig. 2B).

3. Results

The anatomical exploration performed prior to CMAP measurement revealed that the course of the buccal branch of the facial nerve was beneath the line connecting (a) the mid-point of the line connecting the outer edge of the orbit and the mandibular angle and (b) the most posterior molar. The point (a) coincided with the connecting the outer edge of the orbit and the mandibular angle (Fig. 2B).

The side of the face contralateral to the one used for the above anatomical exploration was used to percutaneously apply electrical stimulation to the buccal branch of the facial nerve and measure CMAP of the mimetic muscles (nasolabial levator muscle) with the evoked potential testing device (Neuropack S3, Nihon Kohden). Two surface stimulating electrodes were placed 1 cm apart on the line along the course of the buccal branch of the facial nerve as revealed in the preceding anatomical exploration. The recording microelectrode (NM-450C, Nihon Kohden) was inserted to a depth of about 1 cm at the mid-point on the line connecting the nostril and the angle of the mouth, where the nasolabial levator muscle is located (Fig. 2A). A single supramaximal stimulus (approx. 8 mA) was applied at a frequency of 0.1 Hz. Ten CMAP measurements were obtained from each animal, from which amplitude, latency, and duration were determined and average values were calculated for each parameter. The method for calculating amplitude, latency, and duration was described in our previous report on measuring CMAP in rats [10]. Briefly, amplitude is the differential voltage between the baseline (zero) and positive peak potentials. Latency is the time interval between the beginning of stimulus artifacts and when the CMAP trace rises over the zero line. Duration is the time interval between the zero-crossing points of the rising and falling trace (Fig. 2B).

3. Results

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Fig. 2. Sites of percutaneous measurement of CMAP of the buccal branch of the facial nerve in sheep and CMAP wave patterns. A. Stimulating electrodes were applied to two sites on the line connecting (a) the midpoint of the line connecting the outer edge of the orbit and the mandibular angle and (b) the most posterior molar. The recording microelectrode was inserted to a depth of about 1 cm at (c) the midpoint on the line connecting the nostril and the angle of the mouth, where the nasolabial levator muscle is located. CMAP, compound muscle action potential.
5. Conclusions

We have established a percutaneous measurement method for CMAP of the buccal branch of the facial nerve in sheep. This method is expected to be very useful in future studies of facial nerve regeneration for long nerve defects in sheep.

Declaration of competing interest

The authors declare no conflict of interest.

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Table 1

| Animal no. | No. 1 | No. 2 | No. 3 | No. 4 | No. 5 | No. 6 | Ave. | SD |
|------------|------|------|------|------|------|------|------|----|
| Amplitude (mV) | 3.7  | 5.3  | 4.7  | 4.0  | 5.1  | 5.4  | 4.7  | 0.7|
| Duration (ms)  | 1.2  | 2.4  | 2.0  | 2.8  | 2.5  | 1.5  | 2.1  | 0.6|
| Latency (ms)   | 4.1  | 3.5  | 3.8  | 4.0  | 3.2  | 3.1  | 3.6  | 0.4|

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