THE EFFECT OF TOOTH EXTRACTION ON PERIODONTAL LIGAMENT MECHANORECEPTORS REPRESENTED IN THE MESENCEPHALIC NUCLEUS OF THE CAT

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(Accepted 19 June 1989)

Summary—When a force is applied to a tooth, mechanoreceptors in the periodontal ligament are stimulated. When teeth are extracted the remnants of the periodontal ligament break down and disappear, but it is not known what happens to the mechanoreceptor neurones that innervated it. The present study seeks to determine the effect of tooth extraction on the population of periodontal ligament mechanoreceptor neurones represented in the mesencephalic nucleus of the fifth cranial nerve. The incisor and canine teeth were extracted from adult cats; terminal experiments were performed between 7.5 months and 2 yr later. Recordings were made in the mesencephalic nucleus with microelectrodes, and neurones were identified in the inferior alveolar nerve that previously innervated the periodontal ligament of one of the extracted mandibular teeth. The majority of these neurones responded only to electrical stimuli applied to the edentulous ridge of the mandible in the area where the incisor or canine teeth had previously been. It was not possible to stimulate them mechanically, despite the use of large forces. A small number had reinnervated new soft-tissue sites. They could be mechanically stimulated and were found adjacent to the area in which the mandibular incisor and canine teeth had been. Thus the population of periodontal ligament mechanoreceptor neurones represented in the mesencephalic nucleus do not all degenerate after tooth extraction. As the majority of those still present do not appear to reinnervate new tissues in which they can be mechanically stimulated, it is unlikely that they have any functional role after tooth loss.

INTRODUCTION

Extraction of the teeth destroys the terminals of pulpal and periodontal ligament neurones, but the subsequent fate of the axons or the cell bodies is not known. The neurones may survive and reinnervate different tissues, such as the oral mucosa, the periodontium of the edentulous mandible, or even the underlying bone that fills the space previously occupied by the teeth. Alternatively, they may degenerate completely. In a study in man (Heasman, 1984), a post-mortem comparison was made between the number of fibres in the inferior alveolar nerve in dentate and edentulous subjects. The nerves in the edentulous subjects contained approx. 20% fewer myelinated fibres than those of dentate subjects. This rather small difference suggests that many fibres which originally innervated the teeth and periodontal ligaments were still present in the inferior alveolar nerve.

After tooth loss, the remnants of the periodontal ligament break down and disappear (reviewed in Simpson, 1968). Information from a large number of previous studies (reviewed in Dubner, Sessle and Storey, 1978; Linden, 1989) provides a basis from which to investigate the fate of periodontal afferents after tooth extraction. A large number of periodontal mechanoreceptor afferents have their cell bodies in the trigeminal ganglion; in addition, there are many such afferents with cell bodies in the mesencephalic nucleus of the fifth nerve (Linden, 1978). This nucleus also contains the cell bodies of jaw-elevator muscle spindles (Cody, Lee and Taylor, 1972) and Type P mechanoreceptors (Linden, 1978). The only mesencephalic nucleus neurones with axons in the inferior alveolar nerve are those which innervate periodontal mechanoreceptors, and the sites of the receptors have been located in the periodontal ligament by Linden and Scott (1989). It follows, therefore, that a study involving recording from the mesencephalic nucleus and stimulation of the inferior alveolar nerve could be expected to provide information on the post-extraction fate of the population of periodontal mechanoreceptor afferents that have their cell bodies in the mesencephalic nucleus.

We have now planned experiments in which, after removal of a number of cat teeth, an adequate time was allowed for full healing and possible rearrangement of the innervation to take place. Recordings would then be made from cells in the mesencephalic nucleus in response to electrical stimulation of the inferior alveolar nerve. Those which also responded to mechanical stimulation of the remaining teeth would be given no further attention. The others which did not respond to tooth stimulation would be presumed to be neurones which had previously innervated the periodontal ligament of the extracted teeth. These would be subjected to further study by mechanical and electrical stimulation of tissues either adjacent to or in the edentulous area.

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The questions addressed were as follows:

1. Are the peripheral processes of mesencephalic nucleus neurones, which normally innervate the periodontal ligament, still present in the inferior alveolar nerve after tooth extraction but not effective as periodontal mechanoreceptors? The finding of a central response to electrical stimulation of their axons in the inferior alveolar nerve, but a lack of response to mechanical stimulation of the remaining teeth would answer this question.

2. What tissues are innervated by these erstwhile periodontal mechanoreceptor neurones which survive after tooth extraction? Stimulation of various tissues and sites in or adjacent to the extraction area would answer this.

These questions are of interest because there is considerable evidence that periodontal mechanoreceptors provide the afferent input to reflexes involving the masticatory muscles and salivary secretion (reviewed in Linden, 1989). However, these reflexes have also been observed in edentulous subjects wearing full dentures (Matthews and Yemm, 1970; Bellwood and Heath, 1987).

MATERIALS AND METHODS

Anaesthesia was induced in 10 adult cats, weighing 1.7-3.6 kg, with ketamine hydrochloride (22 mg kg\(^{-1}\) injected intramuscularly). The left mandibular and maxillary canine and incisor teeth were extracted, antibiotics were administered intramuscularly (Amoxicillin Trihydrate 7.5 mg kg\(^{-1}\), Beecham, Brentford, England) and the animals were allowed to recover. The teeth were removed using a watchspring scaler and dental extraction forceps. When a tooth fractured, the remaining part of the root was removed with the watchspring scaler. It was not necessary in any of the experiments to remove bone surgically to extract the teeth. The maxillary teeth were removed to prevent trauma to the lower edentulous ridge. The incisor teeth were extracted to allow a substantial segment of the mandibular bone to be edentulous. It was decided that a minimum period of at least 6 months should be allowed for sufficient healing of the tooth socket. In the first instance, terminal experiments were performed at 7.5 months (2 animals) and 8.5 months (2 animals) after the extractions. These were followed by further experiments at 1 yr (2 animals), 18 months (2 animals) and 2 yr (2 animals) after the extractions.

In the terminal experiments, anaesthesia was induced in the 10 adult cats, now weighing 2.0-4.0 kg, with ketamine hydrochloride (22 mg kg\(^{-1}\), injected intramuscularly) and maintained with \(\alpha\)-chloralose (initial loading dose 50 mg kg\(^{-1}\), continuous infusion 4.6-23 mg h\(^{-1}\), intravenously). The animals were artificially ventilated through a tracheostomy tube with 8.5 months (2 animals) after the extractions. These experiments were performed with glass-insulated, gold and platinum-black coated tungsten microelectrodes (tip length 15-20 \(\mu\)m, impedance 50 \(\Omega\) to 1 \(\Omega\) at 1 kHz; Merrill and Ainsworth, 1972), directed caudally at a 30° angle to the vertical into the left mesencephalic nucleus. The whole of the accessible region of the nucleus was explored at 200 \(\mu\)m intervals (A4-P4, 2.3 mm lateral to the midline; Berman, 1968), while giving square-wave pulses (5-8 V, 0.1 ms duration, 1 Hz) to the inferior alveolar nerve through the stimulating electrodes. This stimulus was always sufficient to produce a substantial jaw-opening reflex. Output from the microelectrodes was amplified through an a.c. preamplifier and amplifier, and recorded on FM tape for subsequent analysis. The data were displayed on oscilloscope screens and by a high-speed signal store on a BBC microcomputer.

When a neurone was found in the mesencephalic nucleus which responded to electrical stimulation of the inferior alveolar nerve, mechanical stimuli were applied to the remaining teeth. If they did respond to forces applied to the remaining teeth, they were assumed to be intact periodontal ligament mechanoreceptors and not studied further. If a neurone did not respond to the remaining mandibular teeth, then mechanical and electrical stimuli were applied to the following sites: the oral mucous membrane, the skin overlying the mandible and the edentulous ridge in which the left mandibular incisor and canine teeth had previously been. Mechanical stimulation was applied with a fine camel-hair brush, tungsten wire, a hand-held glass rod and a pair of insulated college tweezers. This allowed a large range of forces to be applied. Electrical stimulation of these areas was also performed with an isolated constant-current stimulator. A range of stimuli up to 10 mA were applied through either a concentric bipolar needle electrode or bipolar electrodes set approx. 2-3 mm apart.

An attempt was made to locate the terminal part of three neurones in separate experiments while recording in the mesencephalic nucleus. The mucoperiostium overlying the edentulous ridge was reflected in the area in which the canine and incisor teeth had previously been. It was possible to apply mechanical and electrical stimulation to the periostium itself and directly onto the bone of the edentulous ridge. After this procedure the bone of the edentulous ridge was gently pared away using a dental stone under saline irrigation. This allowed the tissue in the centre part of the edentulous ridge to be mechanically and electrically stimulated. The procedure was not done in every cat because it necessitated the termination of the experiment because of damage to the peripheral site. After all of the experiments in which the bone of the edentulous ridge was not pared away, radiographs were taken of both sides of the mandible. These were examined to determine if the edentulous ridge area had healed satisfactorily after tooth extraction.
RESULTS

In each of the 10 cats, neurones were found in the mesencephalic nucleus which responded to electrical stimulation of the inferior alveolar nerve, but not to mechanical stimulation of the remaining teeth. Twenty-three such neurones were found in all; each animal had between 1 and 4 neurones with this type of response. As in previous mesencephalic nucleus studies (Corbin and Harrison, 1940; Linden, 1978), they were situated in the caudal part of the nucleus. The neurones were found at each of the different recovery periods after tooth extraction.

Of the 23 neurones, 20 did not respond to mechanical stimulation of any of the tissues supplied by the inferior alveolar nerve, i.e. the mucoperiosteum of the edentulous ridge, the lingual and buccal oral mucous membrane and the hairy and non-hairy skin overlying the mandible. Despite high-intensity mechanical stimuli (i.e. forces considered as noxious), it was not possible to elicit a response from any of these 20 neurones. They could only be stimulated electrically by placing the bipolar electrodes 2-3 mm apart, one pole on either side (i.e. buccal and lingual) of the edentulous ridge.

An example of one such neurone is shown in Fig. 1. The electrodes were moved along the edentulous ridge to find the most distal position and the lowest threshold current at which the neurones could be stimulated. It was often found that current of a similar magnitude excited the neurones from the most proximal part of the edentulous ridge (i.e. the area adjacent to the first premolar tooth) to a position more distal (i.e. towards the midline). In all cases there was a position in which any further distal movement of the stimulating electrodes did not elicit a response. The most distal position at which a response was elicited was assumed to be the site at which the terminal part of the neurone was situated. The position of the bipolar electrodes for 18 of the 20 neurones in the edentulous ridge suggested that the terminal part was in the area where the canine tooth root had previously been. The terminal position of the remaining 2 neurones appeared to be in the area where the incisor teeth had been. The most distal position of the bipolar electrodes necessary to stimulate the majority of neurones in the edentulous ridge is shown in Fig. 2.

In the three experiments in which bone was removed from the edentulous ridge in an attempt to locate the precise position of the terminal part of the neurone, it was not possible at any stage to stimulate mechanically the neurones by probing either the periosteum or the mandibular bone itself. However, they could still be stimulated electrically with a stimulus applied across the edentulous ridge.

Of the 23 neurones found, only three responded to mechanical stimulation of the soft tissues as well as to electrical stimulation of the inferior alveolar nerve. The first such receptor was found in an experiment performed 8.5 months after tooth-extraction. It responded to mechanical stimulation of the hairy skin of the lip in an area adjacent to where the canine tooth had been. Figure 3 shows the discharge of this neurone to mechanical stimulation. The receptor was of a very low threshold; it responded to very gentle stroking of the down hairs alone with a fine camel-hair brush; there appeared to be no directional sensitivity. In the centre of the receptive field a thicker hair was present which resembled a tylotrich hair. The neurone responded to displacement of this hair; however, this may have been because its displacement was mechanically transmitted to the down-hair follicles which then stimulated the receptor. It was not slowly adapting and there was no resting discharge. The response resembled that of the Type D hair follicle units described by Brown and Iggo (1967).

The second receptor was located in the non-keratinized oral mucosa at the junction with the mucoperiosteum covering the edentulous ridge,
adjacent to where the canine tooth had previously been. It was found in an experiment performed 18 months after tooth extraction. There was no resting discharge and the receptor adapted out when a force was applied. It responded to stretching of the oral mucous membrane, probing with the glass rod and pinpoint stimulation with the tungsten wire.

The third receptor, found in an experiment performed 2 yr after tooth extraction, was in the non-hairy pigmented skin of the lip in an area adjacent to where the 2nd incisor tooth had been. Its response was similar to the neurone in the non-keratinized oral mucous membrane. None of the receptors could be adequately classified using the criteria for cutaneous receptors proposed by Horch, Tuckett and Burgess (1977). The position of the three mechanoreceptors in the soft tissues is shown in Fig. 4.

Radiographs showed no evidence of any tooth fragments in the area where the canine or incisor tooth had been extracted. The sockets had healed well and the whole area in which the teeth were once present appeared to have been replaced with new bone. From the radiographic examination there appeared to be nothing abnormal about the extraction sites in any of the animals.

**DISCUSSION**

After tooth extraction, 23 neurones were found in the mesencephalic nucleus which responded to electrical stimulation of the inferior alveolar nerve, but not to mechanical stimulation of the remaining teeth. The majority of these failed to respond to any form of stimulation of the edentulous area other than high-intensity electrical stimulation across the ridge. Only 3 of the 23 neurones could be stimulated by low-intensity stimulation of the hairy skin or mucous membrane. Neurones in the inferior alveolar nerve that respond to either electrical stimulation alone or to mechanical and electrical stimulation of soft tissues have not been observed in previous studies on the mesencephalic nucleus (Linden, 1978; Linden and Scott, 1989). Therefore, our first conclusion is that some periodontal mechanoreceptor neurones survive tooth extraction. It seems likely that many do not survive as in a previous study (Linden and Scott, 1989) on 13 cats in which all of the mandibular teeth were present. 51 neurones were recorded in the mesencephalic nucleus which responded to forces applied to the mandibular canine tooth. In our present study on 10 cats, only 23 neurones were found which would have originally innervated the periodontal ligaments of the extracted teeth.

It is possible that in extracting the teeth, neurones which innervated the periodontal ligament were damaged and subsequently degenerated. There is no information from previous studies on the cell numbers in the mesencephalic nucleus after tooth extraction or damage to the inferior alveolar nerve. However, trigeminal nerve damage is followed by degeneration of some of the cell bodies in the mesencephalic nucleus (Imamoto, 1972). In contrast, studies on peripheral nerves supplying other areas have shown that neurones do survive axotomy for long periods even if complete regeneration is not possible (Davis et al., 1978). Horch and Lisney (1981) established that the number of axons in nerves proximal to transection lesions is similar to normal. It is known that cell death is more likely when an injury to the neurone is close to the cell body than when an injury is a considerable distance from it (reviewed in Lieberman, 1974). In our study any damage to the neurones innervating the periodontal ligament would have been likely to have occurred at their terminal parts, but this did not lead to all of them degenerating subsequently.

The majority of the neurones that we found appeared to innervate the mandibular edentulous ridge. This is consistent with results of a histological study in the rat in which neurones were found in the mandibular bone 10 months after tooth extraction (Hansen, 1980). It is possible that our neurones still had receptive endings, but because they were now surrounded by mineralized bone the adequate stimulus needed to elicit a discharge of impulses could not be applied. Alternatively the receptor endings could have degenerated when the remnants of the periodontal ligament disappeared—this requires further study.

![Fig. 3. A trace to show action potentials recorded from a neurone that responded to mechanical stimulation of the hairy skin of the lip in an experiment performed 8.5 months after tooth extraction. The mechanoreceptor responded only when the force was initially applied and removed as indicated by the arrows.](image)

![Fig. 4. Diagram to show the position of 3 receptors recorded in the mesencephalic nucleus that responded to mechanical stimulation. (A) a receptor in the hairy skin of the lip in an experiment performed 8.5 months after tooth extraction; (B) a receptor located at the junction between non-keratinized oral mucosa and the mucoperiosteum of the edentulous ridge in an experiment performed 18 months after tooth extraction; (C) a receptor on the non-hairy pigmented skin of the lower lip in an experiment performed 2 yr after tooth extraction.](image)
The 3 neurones in soft tissues were all in areas adjacent to where the extracted teeth had previously been. The most likely explanation for the apparent reinnervation of new sites by the eristwhile periodontal afferents is that extraction of the teeth damaged neurones innervating these sites. The periodontal mechanoreceptor neurones bereft of receptor terminals may have entered and regenerated along the severed endoneurial tubes of the axons which originally innervated the soft tissues.

Our results suggest that most periodontal mechanoreceptor neurones with their cell bodies in the mesencephalic nucleus no longer have a functional role after tooth extraction, because although they can survive they cannot subsequently be mechanically stimulated. Therefore, our findings do not provide an explanation for the observation that masticatory and salivary reflexes can be elicited by contact between artificial teeth in edentulous subjects, although these reflexes are known to involve periodontal afferents in the intact dentition. Our study demonstrates that whatever may be the receptor mechanisms and central connections in the reflexes in edentulous subjects, they are unlikely to involve the periodontal mechanoreceptor neurones with their cell bodies in the mesencephalic nucleus. It is possible that periodontal mechanoreceptor neurones with their cell bodies in the trigeminal ganglion or a separate group of receptors are involved.

Acknowledgement—This work was supported by a grant from the Medical Research Council.

REFERENCES