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Modulation of incisor eruption in rats by sympathetic efferents

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ABSTRACT

Introduction: Intact neural supply is necessary for tooth eruption. Sympathetic denervation accelerates or decelerates the eruption rate depending on the tooth condition (intact or injured). The aim of this study is to reexamine the role of the sympathetic innervation, through the observation of the effects of pre or post ganglionic chemical sympathectomy on the eruption of intact rat incisors.

Materials and methods: Different groups of rats were subjected to either ganglionic or peripheral chemical sympathectomy and the observed effects on incisor eruption were compared to those made on intact/sham groups or on rats subjected to inferior alveolar nerve (IAN) lesion.

Results: The total amount of eruption in control/naïve rats, measured over a total period of 144 h, was 3 ± 0.15 mm and decreased to 2.57 ± 0.06 mm ($n = 8$; $p < 0.01$) or 2.8 ± 0.10 mm ($n = 8$; $p < 0.05$) following treatment with guanethidine and hexamethonium, respectively. This amount decreased to 1.8 ± 0.14 mm ($p < 0.001$ vs. control, $n = 7$; or $p < 0.01$ vs. sham, $n = 5$) in rats subjected to IAN lesion.

Conclusion: Sympathectomy delayed tooth eruption. Blocking the sympathetic effectors with guanethidine exerted more potent effects than ganglionic block with hexamethonium. Intact sympathetic supply is required for tooth growth under normal conditions.

1. Introduction

Tooth eruption is a complex process where teeth move through bone to their functional positions within the oral cavity (Marks, Gorski, & Wise, 1995). Intact neural supply is essential in tooth movement through regulation of bone metabolism, bone resorption, gingiva and the periodontal ligament (Kjær, 2014). Experimental studies have shown that eruption stops when the nerve connection to the teeth is interrupted (Fujiyama et al., 2004) and that denervation can lead to dentoalveolar ankylosis with decreased width of the periodontal space. It is suggested that the Malassez epithelium may be involved in the maintenance of periodontal space and that sensory innervation might be indirectly associated with it (Fujiyama et al., 2004). Presence of intact sympathetic innervation is necessary in all tissues involved in tooth eruption (Kjær, 2014). In bone, the proximity of sympathetic nerve endings to osteoblasts provides the anatomical substrate for this osteo-neural interaction. Evidence from animal models and clinical studies suggests that a β2-adrenergic receptor mechanism underlies this interaction (Hugues et al., 2016). Chemical or surgical sympathectomy and sensory denervation can affect bone resorption (Cardinali, 2000). It seems that the sympathetic system is important for the prevention of bone loss (Kim et al., 2009) and consequently tooth eruption. Neuropeptide Y (NPY), a well-characterized transmitter in the peripheral sympathetic nervous system (SNS), is known to be present in human dental pulp and could have a modulatory role in pulpal inflammation and in reparative dentine formation (El Karim, Lamey, Linden, Awawdeh, & Lundy, 2006).

In the rat dental pulp, stimulation of sympathetic nerves causes recruitment of CD43 + PMN cells and CD43+ granulocytes. In fact, it has been shown that following sympathectomy, granulocyte presence in rat molars was impaired during orthodontic tooth movement while electrical stimulation of sympathetic nerves caused recruitment of these cells in the dental pulp. Sympathetic innervation is, therefore, important for the recruitment and migration of granulocytes during inflammatory processes in oral tissues (Csillag et al., 2004; Haug & Heyeraas, 2006).

In rats, the sympathetic innervation of teeth consists of unmyelinated axons originating in the ipsilateral superior cervical ganglion. The sympathetic nerve endings contain norepinephrine and other neuropeptides like NPY and vasoactive intestinal peptide (VIP) (Inoue, Kurosaka, & Abe, 1992; Ladizesky et al., 2001). It seems that the sympathetic system plays a role in the continuous eruption of the rat mandibular incisors through the regulation of bone formation. This regulation involves the control of bone metabolism through the

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modulation of preosteoclast differentiation and osteoclast activation (Cherruau & Saffar, 1998; Cherruau, Morvan, Schirar, & Saffar, 2003). Controversial results, however, have been reported in the old literature on the effect of sympathetic activation, as leading to an acceleration of the eruption rate (Bryer, 1957), while others reported no effect (Miller 1957; Taylor & Butcher, 1951). More recently, Ladizesky et al. (2001), Ladizesky et al. (2003) did a unilateral superior cervical ganglionectomy (SCGx) and reported varying results depending on the tooth condition (intact or shortened). Briefly, sympathetic denervation induced higher eruption rate in cut incisors. On the contrary, low or no change in the eruption rate were observed in intact incisors (Boggio, Ladizesky, Cutrera, & Cardinali, 2004). This can lead to the following two questions: first, why was the effect of sympathectomy observed only when one of the incisors is cut or injured? Second, why were there two opposite effects on the eruption of the incisors of the same animal, i.e., increased rate in the case of shortened incisors and decreased rate in the intact one? These questions deserve to be further explored.

Although the sympathetic modulation of bone metabolism and bone resorption is well established, the impact of this modulation on the growth/eruption of mandibular incisors still needs further elucidation. The aim of the present study is to quantify the role of the sympathetic nervous system in the eruption of intact (impeded) mandibular incisors and to compare the effects of chemical sympathectomy to those of inferior alveolar nerve lesion on the eruption of mandibular incisors in rats.

2. Materials and methods

2.1. Animals

Experiments were performed on adult Sprague-Dawley female rats (250–300 g) with completely developed mandibular incisors. Rats were kept in groups of 3 to 5 in plastic cages under standard colony conditions (12 h dark/light cycle, temperature 22 ± 2 °C) with free access to food and water. All procedures were carried out with strict adherence to the guidelines for pain experimentation in animals (Swiss Academy of Medical Sciences, Swiss Academy of Sciences (1997)) and approved by the Institutional Animal Care Committee. Experiments were conducted between 8 AM and 10 PM.

2.2. Experimental groups

Rats divided into 5 groups based on the different experimental procedures used, as summarized in Table 1.

2.3. Experimental procedures

All experimental surgical procedures were performed under deep general anesthesia achieved by injection of atropine (Atropine sulfate, Laboratoire Aguettant; dilution 1:10 in saline, 0.05 mg/kg) and chlorpromazine (Largactil®, 8 mg/kg) intra-peritoneally (i.p.), followed 10 min later by intraperitoneal (*i.p.*) injection of ketamine (Ketalar®, 50 mg/kg).

Table 1
Summary of the procedures used in the different experimental groups.

Group	n	Procedure
I	7	Control, no procedure
II	8	Block of sympathetic efferent/effector synapses with guanethidine
III	8	Block at ganglionic level of sympathetic nerves with hexamethonium
IV	7	Left inferior alveolar nerve exposed and cut
V	5	Sham group, exposed but intact, left inferior alveolar nerve

2.3.1. Chemical block of the sympathetic efferents

The chemical block was done using two different drugs. Guanethidine was used to obtain a post ganglionic block, while hexamethonium was used for a pre-ganglionic effect. The determination of the drug concentrations was based on previous work from our group about the optimal dosage without overt side effects (Saadé et al., 2008; Safieh-Garabedian et al., 2002). The rate of eruption of mandibular incisors in all groups was measured before and after the administration of drugs.

a) Guanethidine: 0.2 ml of guanethidine (1-[2-Guanethinoethl] octahydroazocine) monosulfate (1:1), cat. no. G-8520, Sigma, St. Louis, MO, USA (30 mg/kg) was daily administered subcutaneously for a period of one week. This treatment regimen was used in group II to provoke a prolonged chemical denervation of sympathetic fibers at the post ganglionic level (Coderre, Abbott, & Melzack, 1984).
b) Hexamethonium: 0.2 ml of hexamethonium Chloride (Hexane-1, bis [trimethylammoniumchloride]), cat. No. H-2138, Sigma, St. Louis, MO, USA (10 mg/kg) was administered subcutaneously daily for a period of one week for rats in group III. This treatment has been shown to block sympathetic fibers at the preganglionic level (Hong & Henry, 1992; Li, Gong, Sun, Zhou, & Kong, 2015).

2.3.2. Interruption of the inferior alveolar nerve (IAN)

The procedure used for exposing the left IAN was performed following the method previously described by Kassab, Hage, Jabbur, and Chidiac, 2013. Briefly, this consisted of exposing the bony canal of the IAN, through blunt separation of the fascicles of the masseter muscle and carefully removing the bone cover to expose the nerve (Fig. 1). After exposing the left IAN, the nerve was gently removed from its bony canal and cut leaving the two ends of the nerve separated at a distance of about 2 mm. Care was taken to keep intact the vascular supply running with the nerve.

2.3.3. Measurement of tooth eruption

The measurement method adopted was based on calculating the distance between two fixed reference points; the first localized on the tooth itself, at the junction of its buccal and distal surfaces (clear differentiation in enamel's color). This landmark would allow having a stable and reproducible guide on all animals. A groove was produced at this point, using a metallic disc (HI-FLEX, EDENTA AG, Hauptstrasse 7, CH-9434 AU/SG Switzerland) mounted on a straight hand piece. The second reference point was a tattoo (Drawing Ink, Rotring, Sanford GmbH D-22510 Hamburg, Germany) placed on the distal margin of the attached gingiva.

The distance between the tattoo on the gingiva and the groove on the tooth was labeled (a), while the distance between the tattoo and the incisal edge of the tooth corresponded to (b) (Fig. 2). A digital caliper (Electronic Digital Caliper, accuracy 0.01 mm; Starret 799A-6/150) was used to make the measurements. Each measurement was repeated three

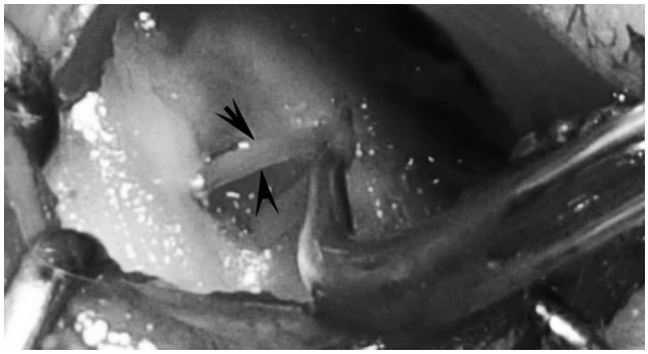

Fig 1. Photograph showing the isolated alveolar nerve elevated from its bony canal by the hook of a glass rode.

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