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RESEARCH ARTICLE

Profiling type I collagen gene expression in growing mandibular structures

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SUMMARY

We conducted a temporal gene expression analysis with type I collagen in the coronoid process, alveolar process and mandibular angle of the rat. We observed gene expression cross-sectionally across different important physiological time points in the rat postnatal life in order to observe in which developmental stage mandibular development mainly occur. This study indicates prominent type I collagen expression at day 10 postpartum in the mandibular ramus and at day 21 in the alveolar process. These findings correspond well with previously obtained data from proliferation studies in facial bone suggesting that craniofacial growth in the rat occurs mainly between days 10 and 21.

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Introduction

Postnatal growth and development of the mandible is affected by periosteal bone remodelling and endochondral ossification. Endochondral ossification is defined as the replacement of the cartilaginous extracellular matrix by calcified bone. This type of bone formation proceeds in growth centres like the condylar cartilage (Mackie et al., 2008). However, the major part of mandibular growth occurs as a result of periosteal bone remodelling which is a combined process of bone resorption and formation executed by osteoclasts and osteoblasts (Proff and Römer, 2009; Römer et al., 2008). The postnatal growing mandible maintains its shape by periosteal bone remodelling, where bone is typically added along the posterior ramus border and resorbed along the anterior border (Enlow and Harris, 1964). This posterior drift allows a lengthening of the dental arch posteriorly. Changes in bone turnover occur primarily in response to altered mechanical loading of the bones or during healing and disease states (Parfitt, 1988; Boivin and Meunier 2002; Daegling and Hotzman, 2003). A relationship between craniofacial morphogenesis and calcium availability or masticatory function was indicated by several studies (Engström et al., 1982; Kiliaridis et al., 1992; Kiliaridis et al., 1996). Mandibular remodelling is affected by well-orchestrated appositional growth and resorptive processes and occurs in response to a functional matrix that acts upon the bone. For instance, the temporal muscle affects the coronoid process. The mandibular angle and ramus are influenced by the masseter and the medial pterygoid muscles, and the teeth are considered to provide a functional matrix for the alveolar process (Moss and Rankow, 1968; Sperber and Wald, 2001). The dentition and the functioning of the tongue and perioral muscles provide further stimuli for mandible growth. In addition, muscle dystrophy or weakness was suggested to negatively affect mandibular growth (Matsuyuki et al., 2006).

The expression profile of bone-related proteins in the growing mandible and their transcription in response to mechanical strain and physiological events is of current interest for research on skull morphogenesis. Unfortunately, the gene expression pattern of bone matrix proteins has not been sufficiently investigated, except for the condylar cartilage (Shen et al., 2005), in the growing mandible of the rat. Therefore, we aim to analyse the gene expression of type I collagen at different important physiological time points in the rat postnatal life in order to understand whether important physiological events have an influence on the transcription of type I collagen in the growing mandible. These time points coincide with the beginning of lactation (day 0), the middle phase of lactation and more mature state of total motor function (day 10 postpartum), weaning and the initiation of mastication (day 21), and sexual maturity/adulthood (day 52) (Suckow et al., 2006).

Materials and methods

Animal keeping and isolation of mandible structures

Male LEW.1 W rats (*Rattus norvegicus*) of an inbred line from Greifswald University were used as test animals. The dams

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(mothers) of male offsprings were all primiparous. The animals were kept under standardized conditions in K3 cages with a breeding diet for rats (ssniff, ssniff®RZ; Soest, Germany) and drinking water ad libitum. Each litter was kept and raised separately. The temperature was kept at 22 ± 1 °C, the relative humidity was 55–60%. A day/night light regime was imposed at 12 h respectively. Exclusively male offsprings (n=11 per time point) were used for specimen collection, since they show a larger daily weight increase. The samples were collected from animals of 4 different age groups of 11 animals each: day 0 (day of birth), day 10 (more mature state of total motor function), day 21 (end of lactation period), and day 52 (sexual maturity) (Suckow et al., 2006).

For sample collection, the animals were euthanized with ether, decapitated and the head soft tissues of the mandibular region were dissected. Subsequently, the mandibles were collected and the coronoid process, mandibular angle and the mandibular alveolar process were resected with a scalpel, rinsed with physiologic saline and placed in an Eppendorf tube. The sample was immediately deep-frozen in liquid nitrogen at −194 °C and subsequently stored at −80° until sample preparation.

Isolation of total RNA and RT-real time PCR

The tissues were frozen in liquid nitrogen and subsequently homogenized using a Mikro-Dismembrator S (B. Braun Biotech International, Germany). Subsequently, the total RNA was isolated by the RNeasy-Kit (Qiagen, Hilden, Germany) from the homogenized tissues. A 250 ng of total RNA was immediately reverse transcribed to cDNA by the QuantiTect Reverse Transcription Kit (Qiagen, Hilden Germany). The cDNA were stored at −20 °C in a freezer.

The cDNA was 10 fold diluted with water and 1 μl of diluted cDNA per 25 μl of PCR reaction volume were taken. In addition, each reaction contained 20 μM primers (see Table 1) and 12.5 μl of 2× Mastermix (QuantiTect SybrGreen PCR Kit, Qiagen). Real time PCR was performed in triplets for each cDNA on Abi7000 (Applied Biosystems). The real time amplifications included an initial step of 15 min at 95 °C (polymerase heat activation), followed by 45 cycles at 95 °C for 30 s (denaturation), 65 °C for 30 s (annealing/elongation) and 72 °C for 30 s (data collection). This technique allows the identification of the cycling point where the PCR product is detectable by means of fluorescence emission (threshold cycle or Ct value). A melting curve was performed to check the specifity of the amplicons (Fig. 1). The relative expression of mRNA was calculated by the delta Ct method, where delta CT is the value obtained by subtracting the Ct value of ß-actin from the Ct value of the target mRNA, as described elsewhere (Nishimura et al., 2009). Furthermore, the relative amount of target mRNA relative to ß-actin is expressed as $2^{-(\text{delta Ct})}$.

Statistical analysis

Statistical evaluation was performed using the SPSS program (version 15.0 for Windows). The gene expression is given as copy of type I collagen/copy ß-actin mRNA ± SD. Gene expression was compared among the groups (time points: 0, 10, 21 and 52 d) for significance with the Kruskal–Wallis test. If there was a total significance level of $p \leq 0.05$, pairwise comparisons were made using the Mann–Whitney U test, and the nominal p values are given. All tests were two-sided. Statistical significance was established at $p \leq 0.05$ (*) or $p \leq 0.01$ (**).

Table 1
Primers used in this present study with their accession number and sequence.

Name	Sequence	T_M	Acession
Col1a1-fwd	5′-CTG GAA ACA TCG GCC ACA CA-3′	65 °C	NM_053304
Col1a1-rev	5′-CAC CCC ACC CCT TCA CAG AG-3′	65 °C	
ß-Actin-fwd	5′-CTC ATG CCA TCC TGC GTC TG-3′	65 °C	NM_031144.2
ß-Actin-rev	5-GGC AGT GGC CAT CTC TTG CT-3′	65 °C	

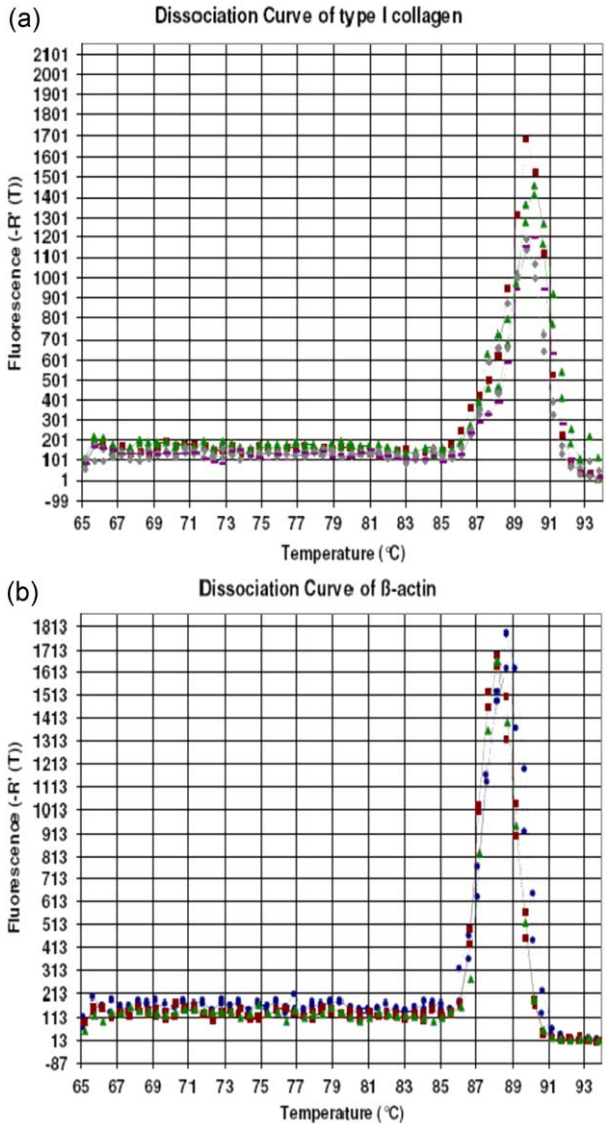

Fig. 1. Product specificity of the amplicons was examined using the melting curve. The melting curves of type I collagen (a) and ß-actin (b) exhibit high primer specificity.

Results

Day 0 (neonatal/begin of lactation)

(a) Coronoid process: a gene expression of 28.9 ± 9.38 copies of type I collagen/copy ß-actin mRNA was measured at postpartum in the coronoid process. Gene expression was statistically significant lower than in comparison to day 10 (p=0.01), and was not statistically different (p=0.83) from that at day 52 day postpartum (Fig. 2). However, the change of gene expression of type I collagen between postpartum and day 21 was found to be statistically significant (p=0.02).

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