Morphogenesis of the juxtaoral organ in humans

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Abstract

The juxtaoral organ was studied using light microscopy in 55 human embryos and 90 fetuses at different stages of development. The juxtaoral organ arises from the epithelium at the bottom of the transverse opening of the primitive mouth during O’Rahilly stage 16 and becomes detached from the epithelium after O’Rahilly stage 18. The juxtaoral organ is innervated by the buccal nerve from O’Rahilly stage 20 onward, and its connective tissue capsule is clearly visible after week 11 of development. This study enabled us to describe three main periods of juxtaoral organ development: (1) the period of epithelial condensation and invagination, at O’Rahilly stages 16–17; (2) the period during which the juxtaoral organ becomes detached from the oral epithelium and is innervated, at O’Rahilly stages 18–23; and (3) the period during which the connective tissue capsule is formed, after week 11 of development. We also analysed the juxtaoral organ of five additional fetuses by immunohistochemistry with anti-NF-200 to verify their innervation. The results show that the juxtaoral organ may have a function in the mechanical activity of the region.

Key words development; embryology; human; juxtaoral organ.

Introduction

When studying the development of the salivary glands, Chievitz (1885) observed a thin epithelial cord in human embryos near the parotid duct. This structure has been given various names (see Discussion), including the Chievitz organ. In subsequent studies, Ramsay (1935) demonstrated that this organ is a constant feature in human embryos and fetuses and concluded that there was no evidence that the juxtaoral organ has an endocrinal or exocrine function, as this is a transient structure that disappears before birth.

After embryological (Zenker & Halzl, 1953), histochemical (Zenker et al. 1961) and ultrastructural studies (Mayr & Salzer, 1967; Mayr, 1979) on Chievitz’s organ, it was designated the ‘juxtaoral organ’, the name by which it is known today (Salzer & Zenker, 1962).

The anatomical location is quite specific: deep to the medial pterygoid muscle at the level of the pterygomandibular raphe and in close relation to fibres of the buccal nerve (Kramer & Zenker, 1974). Histologically, the juxtaoral organ was reported (Zenker & Salzer, 1961; Salzer & Zenker, 1962) to comprise three layers or strata that surround the epithelium: the ‘stratum fibrosum internum’ formed by diverse layers of loose connective tissue; the ‘stratum fibrosum externum’ made up of a dense connective tissue that surrounds the organ; and the nervous fibres that form the ‘stratum nervosum’. The presence of vascularization has not been reported by any authors.

The function of the juxtaoral organ remains controversial. The activity and distribution of some enzymes in the organ were studied and compared with results obtained in oral mucosa, parotid gland and minor salivary glands of the oral region (Müller & Zenker, 1981). These studies suggested that the juxtaoral organ is a receptor that may have a neuroendocrine function. Other authors have proposed a possible role for the juxtaoral organ as a mechanoreceptor (Jeanneret-Gris, 1980; Mandl et al. 1993).
Some authors have claimed that the juxtaoral organ can be mistaken for an oral carcinoma (Lutman, 1974; Danforth & Baughman, 1979; Mikó & Molnár, 1981; Geist et al. 1984). It is therefore important to be aware of its existence in order to prevent diagnostic errors (Eversole & Leider, 1978; Jensen et al. 1979; Tschen & Fechner, 1979; Sciubba & Sachs, 1980). On the other hand, cases of hyperplasia and tumours of the juxtaoral organ have also been reported (Leibl et al. 1976; Soucy et al. 1990; Vadmal et al. 1998; Bénateau et al. 2003; Ide et al. 2003).

The aim of this study is to contribute to the knowledge of the juxtaoral organ by providing a terminology and at the same time analysing its vascularization and innervation in human specimens from weeks 7 to 17 of development.

Materials and methods

Developmental study

Fifty-five embryos and 90 human fetuses from the collection of the Embryology Institute of the Universidad Complutense de Madrid were studied (Fig. 1). In the embryos, crown–rump length (CRL) ranged from 9 to 31 mm (O’Rahilly stages 16–23). In the fetuses, CRL ranged from 35 to 150 mm (weeks 9–17 of development). The parameters used to determine gestational age were CRL, weight and cranial perimeter (O’Rahilly & Müller, 1996). All specimens were obtained from ectopic pregnancies or spontaneous abortions, and no part of the material gave indications of possible malformation. Approval for the study was granted by the Ethical Committee of the Faculty of Medicine of the University Complutense of Madrid.

All specimens were fixed in 10% formalin and embedded in paraffin for processing. Sections were 7–25 µm thick, depending on specimen size. Sections were stained with haematoxylin–eosin, azan and Masson’s trichromic dye (McManus & Mowry, 1968). The study was carried out using a Nikon Eclipse E400 microscope and a Nikon DXM 1200 digital camera coupled to a Pentium IV PC.

Immunohistochemical (IHC) procedure

Samples to confirm the innervation of the juxtaoral organ were obtained by the bilateral dissection of five human fetuses (three females, two males). These were aged 28, 32, 34 and 35 weeks, and one had reached full term. These specimens were additional to those used in the developmental study and did not present any malformation.

After removing the skin from the cheek region, the parotid gland was removed and the masseter muscle detached. The interpterygoid space was entered by going around the anterior margin of the ramus of the mandible. The medial pterygoid muscle was dissected and the whole specimen including the buccal fat pad was removed. The samples obtained were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). After fixation, the samples were dehydrated in a graded series of ethanol and finally embedded in paraffin, and 5-µm serial sections were prepared.

For the immunofluorescence reaction, the following procedure was followed: sections were incubated for...
1 h at room temperature in 10% normal goat serum (NGS) (Sigma, USA) and 0.2% Triton X-100 (Merck, Germany) diluted in phosphate-buffered saline (PBS). They were incubated in monoclonal mouse antibody directed against neurofilament 200 kDa (NF-200; clone NE-14, Sigma, USA) in a 1 : 150 dilution at 4 °C for 48 h and then washed in PBS (3 × 15 min). This was followed by incubation at 4 °C for 24 h with the immunoglobulin fraction from goat anti-mouse serum conjugated to fluorescein isothiocyanate (FITC) (Chemicon, Temecula, CA, USA) diluted 1 : 100. The sections were washed in PBS (3 × 15 min) and mounted without dehydration in Vectashield mounting medium (Vector, Burlingame, CA, USA). All antibody dilutions were carried out in 0.2% Triton X-100, 1% NGS in 0.1 M PBS at pH 7.4. Controls were treated in the same way as above, except that incubation in the primary antibody solution was eliminated to demonstrate that the secondary antibodies reacted only with their respective primary antibody.

Sections were studied using a Zeiss Axioplan Z Imaging microscope equipped with a Metamorph digital imaging acquisition system (version 4.5) (Universal Imaging Corp.).

Some sections not used for immunohistochemistry were processed with haematoxylin–eosin and azan (McManus & Mowry, 1968).

Results

The juxtaoral organ was bilateral in all analysed specimens.

O’Rahilly stages 16–17 (8–14 mm CRL)

Embryos at O’Rahilly stage 16 revealed an epithelial thickening in the proximity of the mandibular nerve at the bottom of the transverse opening of the primitive mouth. This epithelial condensation corresponds to the prospective region of the juxtaoral organ (Fig. 2). During O’Rahilly stage 17, the condensed epithelium invaginates into the neighbouring mesenchyme in relation to the buccal nerve (Fig. 3).

O’Rahilly stages 18–19 (14–18 mm CRL)

During these stages, it was found that the anlage of the juxtaoral organ is not connected to the oral epithelium. At stage 19, the juxtaoral organ presents a lumen and is medial to the blastema of the temporal muscle (Fig. 4). During these stages, an epithelial invagination appears ventrally to the juxtaoral organ anlage but independent of it; this invagination, surrounded by condensed mesenchyme and lateral to the ramus of the mandible, corresponds to the anlage of the parotid gland (Fig. 5).

O’Rahilly stages 20–23 (18–31 mm CRL)

During these stages, the juxtaoral organ is present as an epithelial cord with a lumen. There is a clear difference
in the locations of the juxtaoral organ and the parotid gland; the former is located medial to the medial pterygoid muscle, and the parotid gland is lateral to the masseter muscle. Moreover, during this stage the juxtaoral organ is innervated by the buccal nerve (Fig. 6a,b).

Weeks 9–10 of development

The juxtaoral organ is situated medial to the insertion of the temporal muscle and the masseter and mandible, and lateral to the buccinator muscle. During this developmental period, some branches of the buccal nerve innervate the wall of the oral vestibule in the cheek (Fig. 7).

Weeks 11–12 of development

During this period of development, the juxtaoral organ is surrounded by the condensed connective tissue that constitutes the capsule. Some branches from the buccal nerve and artery cross the capsule and spread in the epithelial tissue of the juxtaoral organ (Fig. 8). This innervation was found in all specimens studied although the buccal nerve showed different distribution patterns. After this developmental period, we also observed that

Fig. 4 Human embryo ESC-14 (17 mm CRL; O’Rahilly stage 19). Frontal section. Azan staining. Arrow indicates juxtaoral organ. B, buccal nerve; T, temporal muscle; C, Meckel’s cartilage; L, lingual nerve; D, inferior alveolar nerve. Bar, 100 µm.

Fig. 5 Human embryo ESC-14 (17 mm CRL; O’Rahilly stage 19). Frontal section. Azan staining. P, parotid gland; C, Meckel’s cartilage; D, inferior alveolar nerve; J, mandible. Arrows indicate condensed mesenchyme. Bar, 200 µm.

Fig. 6 (a) Human embryo P1 (29 mm CRL; O’Rahilly stage 23). Frontal section. HE staining. Y, juxtaoral organ; B, buccal nerve; MA, masseter muscle; DP, parotid duct; J, mandible; C, Meckel’s cartilage; L, lingual nerve; PM, medial pterygoid muscle; D, inferior alveolar nerve. Bar, 500 µm. (b) Enlargement of Fig. 5(a). Arrow indicates branch of buccal nerve for the juxtaoral organ. Bar, 50 µm.
the epithelial tissue presents exophytic formations made up of a layer of flat epithelial cells that surround other larger cells with clearer cytoplasm (Fig. 9).

**Weeks 13–17 of development**

During this developmental period there was a morphological change in the juxtaoral organ. The epithelial structure revealed epithelial outgrowths without apparent lumens. The vascularization and innervation were noteworthy (Fig. 10).

The results are summarized in Fig. 11.

**Immunohistochemistry**

Figures 12 and 13 depict consecutive sections of the same specimen. Figure 12 shows the juxtaoral organ formed by the epithelium and numerous outgrowths surrounded by connective tissue capsule. The capsule is in close proximity to a thick nerve. Figure 13 shows positive anti-NF-200 labelling corresponding to the thick nerve seen in Fig. 12. Figure 13 also shows other nerve fibres not visible in the connective tissue capsule in Fig. 12 that display positive anti-NF-200 labeling.

**Discussion**

The juxtaoral organ was discovered by Chievitz (1885) when studying development of the salivary glands. He considered it to be a rudimentary epithelial structure. This structure subsequently received various names, including the orbital inclusion (Schulte, 1913), Chievitz organ (Broman, 1916) and buccopharyngeal tract (Brachet, 1919). Zenker (1953) designated it the buccotemporal
organ because of its topographical location in mammals. The name ‘juxtaoral organ’ was proposed by Salzer & Zenker (1962).

The presence of the juxtaoral organ has been reported in a wide range of species such as fish, amphibians, reptiles, birds and mammals (Zenker & Halzl, 1953; Boyd & Hughes, 1960; Grüneberg, 1971; Jeanneret-Gris, 1980).

The juxtaoral organ was present bilaterally in all the specimens studied here and was found to have an increasingly complex morphology during development. Initially, only the invaginated epithelium is present. The connective tissue capsule appears from week 11 of development onward. After week 13 of development, numerous epithelial outgrowths were observed. After

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**Fig. 10** Human fetus Te-1 (96 mm CRL; week 13 of development). Transverse section. HE staining. Epithelium of the juxtaoral organ (E). Epithelial outgrowths (arrowheads). B, buccal nerve; BU, buccinator muscle. Connective tissue capsule (arrows). Nerve (*). Bar, 200 µm.

**Fig. 11** Summary of the results.

**Fig. 12** Human fetus (week 28 of development). HE staining. E, epithelium of the juxtaoral organ. Nerve (arrow). Connective tissue capsule (*). Bar, 100 µm.

**Fig. 13** Human fetus (week 28 of development). Immunofluorescence. Nerve fibres labelled with anti-NF-200 innervate the juxtaoral organ (arrows). Bar, 50 µm.

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week 28 of development, IHC and histological staining of the specimens showed the capsule to be made up of condensed connective tissue with abundant innervation and vascularization. These morphological data suggest that this is not an involuted structure.

There is considerable disparity of opinion regarding the onset of the juxtaoral organ anlage. Zenker (1982) reported its appearance in embryos of 7.5–12 mm CRL, whereas Kleiss & Kleiss (1985) observed it in an embryo of 19.5 mm CRL but at a more advanced period of development, suggesting that its development commences even earlier. However, Sanz et al. (1998) described its origin in specimens at O’Rahilly stage 23.

Ramsay (1935) and Zenker (1982) pointed out that the anlage of the juxtaoral organ is close to that of the parotid gland in the transverse opening of the primitive mouth but their onset does not take place at the same time. This coincidence in place but not in time has led some authors to confuse the anlage of the juxtaoral organ with that of the parotid gland. Our studies revealed an invagination of the juxtaoral organ during O’Rahilly stage 17, and the mesenchyme of the region did not appear to be condensed. The anlage of the parotid gland appeared during O’Rahilly stage 18–19 (Mérida Velasco et al. 1991), and the neighboring mesenchyme showed evident condensation (García et al. 1991). The importance of the mesenchyme in salivary gland development was previously reported. The mesenchymal condensation is thought to prepare the way for migration of the glands (García et al. 1991). Since the first studies by Grobstein (1953), the salivary glands have been considered an example of epithelial–mesenchymal interaction. Hence, the model of glandular branches or outgrowths is due to mesenchymal induction, while the salivary gland type is determined before the epithelium–mesenchymal interaction can take place (Denny et al. 1997). These epithelial–mesenchymal interactions do not seem to be necessary at the onset of development of the juxtaoral organ, because the mesenchyme does not appear condensed. However, from week 13 of development onward epithelial outgrowths appear in the juxtaoral organ, possibly as a consequence of interaction with the surrounding connective tissue capsule.

In accordance with previous descriptions by Kleiss & Kleiss (1985), we found that the juxtaoral organ became detached from the oral epithelium during O’Rahilly stages 18 and 19. However, Bujard (1911) reported that this occurred in a specimen with a CRL of 35 mm, while Ramsay (1935) described the detachment in a 10-week-old fetus. Boyd & Hughes (1960) reported that this detachment happened in specimens with 43-mm CRL.

The relationship between the juxtaoral organ and the buccal nerve was briefly mentioned by Brachet (1919). Ramsay (1935) reported that the buccal nerve emitted branches to the juxtaoral organ in a 32-mm CRL specimen. Boyd & Hughes (1960) observed innervation at 30 mm CRL, when the nerve fibres of the buccal nerve had not yet reached the oral epithelium. Our results show that the prospective region of origin of the juxtaoral organ is in relation to the mandibular nerve. Subsequently, during O’Rahilly stage 17 and after its invagination, the juxtaoral organ is in relation to the buccal nerve. We observed innervation of the juxtaoral organ during O’Rahilly stage 20.

Our group had already studied the course of the buccal nerve and its distribution pattern in the same human fetuses as those used in this study (Mérida Velasco et al. 2001). We investigated the different nerve patterns in relation to the innervation of the juxtaoral organ and verified that the innervation does not vary with different distribution patterns of the buccal nerve. On the other hand, aplasias of the buccal nerve have been described in humans, and innervation of the juxtaoral organ proceeded from the maxillary nerve in these cases (Kaspar & Leibl, 1965).

Innervation of the juxtaoral organ by buccal nerve branches was confirmed by immunohistochemistry with anti-NF-200 (200 kDa) in five additional human fetuses. This antibody has been found to be a good marker of nerve fibres (Triviño et al. 2002). Immunohistochemical studies with glial fibrillary acidic protein (GFAP) and neuron-specific enolase (NSE), two non-selective markers of nerve fibres, have reported contradictory results. Thus, Soucy et al. (1990) found positive immunostaining for GFAP and NSE but Mandl et al. (1993) and Vadmal et al. (1998) reported negative results for the same markers.

As mentioned earlier, Zenker’s group structured the organization of the juxtaoral organ into three layers or strata that surround the epithelium: the ‘stratum fibrosum internum’, the ‘stratum fibrosum externum’ and the ‘stratum nervosum’. Although this structural organization has been adopted by other authors (Leibl et al. 1976; Jeanneret-Gris, 1980; Mikó & Molnár, 1981), our opinion is that it may lead to confusion, because the innervation of the juxtaoral organ does not constitute an independent, differentiated layer, as the nerve
fibres are distributed throughout the connective tissue capsule, and because this structural organization makes no reference to the vascularization of the juxtaoral organ. We propose a structural organization of the juxtaoral organ in two parts, the epithelium and the surrounding connective tissue capsule, with the latter containing both the vessels and the nerves.

The function of the juxtaoral organ is controversial. It has been described as a rudimentary salivary gland (Broman, 1916) and an internal secretory organ (Schulte, 1913). Other researchers have described it as an epithelial structure without any functional importance (Brachet, 1919; Ramsay, 1935; Boyd & Hughes, 1960; Sanz et al. 1998). Studies performed by Zenker's group suggested that the juxtaoral organ is a receptor with a possible neuroendocrine function (Zenker et al. 1961; Zenker & Salzer, 1961; Salzer & Zenker, 1962; Mayr & Salzer, 1967; Müller & Zenker, 1981). In experimental rat models, the morphology of the juxtaoral organ was considered to be subordinated to the hypophysis (Salzer & Zenker, 1968; Fasching et al. 1974). Other authors considered it to be a mechanoreceptor structure related to mastication (Jeanneret-Gris, 1980; Mandl et al. 1993; D’Andrea et al. 1999). Malinovsky (1990) proposed the term ‘sensory nerve formation’ for other peripheral sensory structures with a multimodal mechanosensory function that have a similar morphology to that of the juxtaoral organ.

Our group previously classified the development of the temporomandibular joint into three different stages (Mérida Velasco et al. 1999). The blastematic stage (weeks 7–8 of development) was characterized by buccal movements and by innervation of the juxtaoral organ (O’Rahilly stage 20). During the cavitation stage of the temporomandibular joint (weeks 9–11 of development), we observed innervation of the wall of the temporomandibular joint into three different stages (weeks 7–8 of development) was characterized by buccal movements and by innervation of the juxtaoral organ. During the maturation stage (after week 12 of development), the juxtaoral organ showed numerous outgrowths with a marked increase in innervation and vascularization. These data suggest that the juxtaoral organ may have a function in the mechanical activity of the region; the cheek is known to be important for both mastication and swallowing (Williams, 1998).

The exophytic formations found after weeks 11–12 of development, made up of a layer of flat epithelial cells surrounding other larger cells with a clearer cytoplasm, can be observed in illustrations published by other authors (Ramsay, 1935; Danforth & Baughman, 1979; Tschen & Fechner, 1979; Klacsman & Taxy, 1980; Mikó & Molnár, 1981; Müller & Zenker, 1981; Geist et al. 1984). These formations are similar to the epithelial remnants of the dental lamina or Malassez’s epithelial remnants (Hodson, 1962; Eversole & Leider, 1978; Wysocki & Wright, 1981) and have been interpreted as nodular hyperplasias of the juxtaoral organ (Leibl et al. 1976). For Jeanneret-Gris (1980), these formations can change in response to pressure, suggesting a possible mechanoreceptor function.

The clinical importance of the juxtaoral organ has been emphasized by some authors (D’Andrea et al. 1999; Pantanowitz & Balogh, 2003). Characteristics of the differential diagnosis between perineural invasion by carcinoma and the juxtaoral organ have recently been described (Pantanowitz & Balogh, 2003).

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