Distribution patterns in glycoconjugate expression during the development of the rat palate

ALBRECHT R. ZSCHÄBITZ 1, HANS K. BIESALSKI 2, VOLKER KRAHN 1, HANS J. GABIUS 3, HARALD WEISER 4, ALEXANDER KHAW 1, CHRISTIAN HEMMES 3 and ECKART STOFFT 1

1 Institute of Anatomy and Cell Biology, Johannes Gutenberg-University of Mainz, Saarstr. 19-21, 55099 Mainz, Germany; 2 Department of Biological Chemistry and Nutrition, University of Stuttgart-Hohenheim, Germany; 3 Institute for Pharmaceutical Chemistry, Ludwig Maximilians-University of Munich, Germany; and 4 Research Department, Hoffmann-La Roche Ltd., Basle, Switzerland

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Summary
The distribution of complex carbohydrate structures during the embryonic development of the rat palate was analysed by examining lectin-binding patterns in serial paraffin and cryostat sections. With few exceptions, the binding patterns showed a general increase in lectin receptors in the more developed stages of palatogenesis. High mannose oligosaccharides were especially amplified during development. Terminal fucose molecules were not expressed. In contrast, terminal sialic acid molecules were ubiquitously distributed in epithelial and mesenchymal tissues. Non-sialylated terminal N-acetylglucosamine was specifically restricted to evolving bone matrix. Before palatal fusion, quantitative but not qualitative differences were detected between oral, nasal, and medial-edge epithelial surfaces. The only exception was LCA, which specifically marked epithelial cells at the tip of palatal shelves. A very selective affinity for Jacalin was demonstrated in the oral epithelium of the palate after day 16, suggesting the presence of sialylated terminal galactose-(β-1,3)-N-acetylgalactosamine. PNA specifically marked the basal lamina of the oral side of palatal processes. The binding patterns of DBA, GSL I A, SBA, and VVA indicated that the epithelium of the tongue is characterized by terminal α- and β-galactose residues, whereas palatine cells possess only molecules with β-anomery. During palatogenesis, glycosaminoglycans patterns were significantly modified. Our data suggest that alteration of complex carbohydrate structures may play a central role in modulating cell-cell and cell-matrix interactions. The significance of these findings, however, remains to be elucidated.

Introduction
In numerous morphological and biochemical studies, the development of secondary palatal shelves has been analysed in order to understand how cells and extracellular matrix components interact during ontogenesis. It has been shown that numerous mechanisms can influence differentiation of embryonic orofacial tissue. The distribution of extracellular matrix proteins has been analysed in some detail (Morris-Wiman & Brinkley, 1992), with an emphasis on hormone- and growth factor-induced regulation (Sharpe et al., 1993). However, our knowledge concerning craniofacial biology has been complicated by the variety of interacting factors and by rudimentary comprehension of the mechanisms involved in normal palatogenesis (Slavkin, 1984).

Knudsen et al. (1985) have demonstrated histochemically that during development of palatal processes, the concentration and distribution of acetylated and sulphated glycosaminoglycans show characteristic modifications. Proteoglycan synthesis and accumulation has been found to change significantly in the palate just prior to reorientation (Pratt et al., 1973). Therefore, it has been suggested that accumulation of these molecules, exerting an intrinsic tissue pressure, provides the impetus for shelf orientation. There is, however, no certainty about the importance of proteoglycans during craniofacial development (Perris et al., 1991). Far less is known about the role of oligosaccharide side-chains during palatogenesis. It has been shown that controlled modification of these carbohydrate structures, situated at cell surfaces or as components of the extracellular matrix, is an important regulatory mechanism during organ development and growth (Sénéchal et al., 1983). Modified sugar molecules are involved in intercellular recognition and adhesion, and equally in receptor and transport activities. In other
words, the arrangement of sugar monomers in glyco-proteins or glycolipids conveys biological information (Gabius, 1991).

A logical first step in establishing the role of glyco-conjugates in the developing palate is to determine their structural composition at various phases of development. Lectins were utilized to detect minor differences in carbohydrate structures histochemically. This specific class of molecules are sugar-binding receptors different from enzymes and antibodies (Damjanov, 1987). They are classified on the basis of their preferential binding to D-pyranose monosaccharides. However, while some lectins bind to sugar molecules irrespective of adjacent structures, others react only if additional conditions are fulfilled, e.g., position within the carbohydrate chain (terminal/internal component), anomic specificity (α- /β-forms), extended binding sites (di- or tri-saccharides), and linear or branched sugar structures. Lectins with wide specificities usually detect more sites than those with narrower binding requirements. Staining with a battery of lectins characterized by the same nominal carbohydrate affinity, but with different binding profiles, is, therefore, of potential value for determining the distribution of complex oligosaccharide structures in situ (Walker, 1989).

The aim of this morphological study was to characterize the topographical arrangement of glycoconjugates during development of secondary palatal shelves by using a panel of exogenous lectins. The results were compared with those revealed by other histochemical methods. Special emphasis was placed on extracellular matrix structures. Because palatal closure in rats occurs on day 16 of gestation, the results of developmental day 15 to 17 is reviewed extensively in this paper. The observations of the present study indicate that a regionally specific and time-related arrangement of specific oligosaccharides occurs during shelf outgrowth, re-orientation and fusion.

Materials and methods

Animals, insemination and experimental conditions

Mature Sprague–Dawley rats, aged 12 weeks, were obtained from the Biological-Medical Institute (Füllingsdorf, Switzerland). The animals were kept at room temperature (25°C) under pathogen-free conditions for two weeks prior to insemination. They were exposed to a 12 h light (from 6 a.m. to 6 p.m.) and a dark cycle. The presence of a vaginal plug immediately after the males was regarded as evidence of successful mating and was considered as day 0. Pregnant rats were killed under mild ether anaesthesia on day 13, 15–17, 20 and 21 of gestation and fetuses were excised from uteri. A total of 94 palates was considered for examination in the study.

Tissue processing

Individual heads were either snap frozen in liquid nitrogen and then stored at −70°C or fixed by immersion in either Bouin's fluid or phosphate-buffered 4% formaldehyde and embedded in paraffin wax. Heads collected on day 20 and 21 were demineralized in EDTA (pH 7.4) at 4°C for 21 days (Frank et al., 1992). Serial frontal cryosections, 7 μm thick, were cut in a Reichert cryostat at −20°C, fixed in acetone and stored at −70°C. Prior to further procedure, paraffin sections (cut at 6 μm) were deparaffinized with xylene, rinsed in two changes of absolute ethanol and gradually rehydrated.

Lectin histochemistry

After quenching the endogenous peroxidase activity with 0.3% H2O2 in methanol for 30 min, sections were pre-incubated with 0.1% BSA in PBS to minimize non-specific protein–protein interactions and washed three times in PBS. Subsequently, specimens were incubated with biotinylated markers (Camon, Wiesbaden, Germany; Table 1) in a humidified chamber for 45 min at room temperature and rinsed three times in PBS. Following this, the samples were incubated for 60 min with an avidin–biotin–peroxidase reagent (ABC-Vectorstain, Wiesbaden) and again washed in PBS. The peroxidase-binding sites were visualized with a fresh solution of diaminobenzidine-tetrahydrochloride and H2O2 for 5 min (Zschaibitz et al., 1992). Some sections were counterstained with Haematoxylin. Finally, the samples were dehydrated, cleared and mounted.

For further analyses of the binding sites, serial sections were treated with the following enzymes prior to lectin staining: (a) Vibrio cholerae neuraminidase (Boehringer Mannheim, Germany; 1 U ml⁻¹) for 10 h at 37°C to eliminate terminal sialic acids (Stoward et al., 1980); (b) diastase (Sigma, Deisenhofen, Germany; 1 mg ml⁻¹) for 30 min at 37°C to differentiate glycogen from other polysaccharides (Hennigar et al., 1986).

The intensity of staining was graded subjectively and scored as negative = −, faintly visible = (+), weak = +, medium = + +, and strong = ++ +.

Controls. Non-specific negative controls were incubations without added lectins or avidin. In order to investigate possible non-specific protein–protein interactions, biotinylated carrier protein BSA was applied. The specificity of lectin staining was ascertained by incubation of the lectin with the corresponding inhibiting sugars (0.2 M/l; Table 1).

Histochemical staining procedures

Sections were stained with Haematoxylin–Eosin, Azan periodic acid–Schiff (PAS), Alcian blue at pH 1 or pH 2.5, Alcian blue pH 2.5–PAS, and Alcian blue at various critical electrolyte concentrations (Cook, 1990). The distribution of hyaluronic acid was analysed according to Girard et al. (1986).

Results

As a rule, cryostat sections revealed weaker lectin-marking than did paraffin sections. With GSL I, and PNA, positive reactions were obtained only in fixed specimens. The binding results were not much affected by the two different fixatives, even though Bouin-fixed tissue tended to demonstrate clearer results (Tables 2 and 3). Non-specific negative controls revealed no staining at all. After incubation with their reciprocal inhibiting carbohydrates, no lectin-binding reactions were detected, with the exceptions of DSL and PHA-E, where a faint staining was consistently observed.