Lectin-binding Glycoconjugates in the Tongues of the Rat and Guinea Pig as Revealed by Lectin-Gold-Silver Methods

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Summary: Lectin-gold-silver (LT-G-S) procedures using two lectins (RCA-I and Con A) were applied to appropriately prepared paraffin sections of tongues in the rat and guinea pig. In the lingual mucous membranes of the rat and guinea pig, positive RCA-I-G-S and Con A-G-S reactions were obtained in the intercellular spaces and the cytoplasm of epithelial cells of the basal and intermediate layers respectively. Likewise, the LT (RCA-I and Con A)-G-S techniques gave rise to varying intensities of positive reactions in the serous and mucous gland acini, nerve and muscle fiber bundles and connective tissue elements. The results obtained in the present study indicate that in the rat and guinea pig tongues the histologic structures mentioned contain varying amounts of β-D-galactose and α-D-mannose or α-D-glucose residues of glycoconjugates, and that such histochemical properties of some of the lingual structures can be correlated with their possible histophysiological functions.

Morphological and histological studies have so far been made on various histological structures of the tongue in a number of mammalian species (Schumacher, 1927; Bloom & Fawcett, 1975; Iwasaki et al., 1987; Shimamura, 1987; Iino & Kobayashi, 1988; Kobayashi & Iwasaki, 1989, Ohshima et al., 1990; Kobayashi, 1990; Fujita & Fujita, 1992). To the best of the present authors' knowledge, however, few investigations have hitherto been performed into the histochemistry of lectin-binding glycoconjugates involved in mammalian tongues.

In the present study, therefore, attempts have been made to visualize histochemical properties of lectin-binding glycoconjugates contained in the tongues of a couple of mammalian species, so as to elucidate the histophysiological functions of a variety of histologic structures in these digestive organs.

Materials and Methods

Ten adult Sprague-Dawley rats and five adult Hartley guinea pigs of both sexes were used in the present study. Tongues were removed from the donor animals sacrificed by exsanguination following Nembutal anesthesia. The organs were immediately dissected into two pieces of the same size and these were fixed in ethanol containing 1% acetic acid at 4°C for 12–24 hrs. The tissue pieces were then dehydrated in 100% ethanol, cleared in benzene and embedded in paraffin wax, according to usual techniques. Sections were cut at a thickness of 4 to 6 μm and mounted on glass slides without any adhesives. The tissue sections were then dewaxed in xylene, hydrated in an ethanol series of descending concentrations and subjected to the following histological and histochemical staining techniques with or without combined control procedures.

1. Histological staining: hematoxylin-eosin (HE)
2. Histochemical stainings: The lectin-gold-silver (LT-G-S) staining procedures previously reported (Murata & Yamada, 1985; Fujimori et al., 1988) were followed.
3. Control procedures for the LT-G-S stainings:
   a) To substantiate the binding specificities of the both lectins for their specified saccharide residues the LT-G solution was replaced by that containing an appropriate monosaccharide: 0.1M of...
Results

I. Results obtained with hematoxylin-eosin (HE)-stained sections

In the rat, the mucous membrane of the lingual dorsal surface was thrown into papillae, which could be grouped into filiform (Fig. 1), fungiform, circumvallate and foliate ones. Such mucous membrane was found to be covered by stratified squamous epithelium, and this epithelium consisted of basal, intermediate and horny layers (Fig. 1). In the epithelium lining the filiform papillae, the superficial cells of the horny layer were keratinized in varying degrees with each papilla (Fig. 1). In the cores of each type of the lingual papillae and the subpapillary lamina propria, fibrous connective tissues of different densities were visualized (Fig. 1).

In the root of the rat tongue, at least two types of lingual glands were detected, one type was serous, and the other being mucous (Fig. 2).

The major portions of the rat tongue surrounded by the lingual mucous membrane were occupied by bulks of stratified muscle fiber bundles (Fig. 3), and bundles of myelinated and non-myelinated nerve fibers were seen to be interposed between the bulks of the muscle fiber bundles (Fig. 3).

In the guinea pig, the lingual mucous membrane was more or less comparable in figures of papillae, their cores and subpapillary connective tissue to that of the rat (Fig. 4), however, the lingual papillae of different types were apparently larger in size than corresponding those noted in the rat (Fig. 4).

In the root of the guinea pig tongue, two types of lingual glands, serous and mucous, were demonstrated and some of these glandular acini were surrounded by bundles of stratified muscle fibers (Fig. 5).

As in the rat tongue, the major portions of the guinea pig tongue surrounded by the lingual mucous membrane consisted of bulks of stratified muscle fiber bundles (Fig. 6), and bundles of myelinated and non-myelinated nerve fibers were embedded in the bulks of the muscle fiber bundles (Fig. 6).

II. Results obtained with lectin-gold silver (LT-G-S)-stained sections.

(A) Results obtained in the rat tongue.

In the lingual mucous membrane of the male rat, the RCA-I-G-S procedure resulted in a characteristic staining pattern; vividly positive reactions were distinguished in intensity in particular at the apex of filiform papillae (Fig. 7). In contrast, the RCA-I-G-S reaction was exceedingly feeble in intensity or negative in the intercellular spaces of the keratinized horny layer of the epithelium (Fig. 7). The RCA-I-G-S procedure gave rise to moderate or rather weak positive reactions in the connective tissues of the lingual papillae and subpapillary lamina propria (Fig. 7).

In the root of the male rat tongue, unusually strong positive RCA-I-G-S reactions were detected in the acinar and ductal cells of the serous glands (Fig. 8). In addition, vivid positive reactions were noted in small numbers of thin crescent-shaped cells abutting upon acinar and ductal cells of the mucous glands, which exhibited moderate, weak, doubtful or negative reactions (Fig. 8). In the serous and mucous glands, further, the connective tissue elements such as the capsules, septa and septula were found to show moderate or weak positive RCA-I-G-S reactions (Fig. 8).

In the male rat tongue, the RCA-I-G-S procedure resulted in rather strong positive reactions of the nerve fiber bundles, moderate positive reactions of the interfibrillar spaces of the stratified muscle fibers, moderate or weak positive reactions of the muscle fibers and weak positive reactions of the connective tissues surrounding the nerve and muscle fiber bundles (Fig. 9).

In the lingual mucous membrane of the male rat, the Con A-G-S technique gave rise to peculiar staining pattern; strong and moderate positive reactions were detected in the cytoplasm of cells of the basal and intermediate layers of the stratified epithelium respectively, and cells of the horny layers of the epithelium showed varying intensities of positive reactions (strong, moderate, weak or doubtful ones) (Fig. 10). However, the intercellular spaces of all the epithelial layers showed negative reactions (Fig. 10). In addition, the connective tissues of the papillary cores and subpapillary lamina propria exhibited rather weak positive Con A-G-S reactions (Fig. 10).

In the root of the male rat tongue, the acinar and ductal cells of the serous glands, together with striated muscle fibers surrounding the glands were seen to show strong positive reactions (Fig. 11), whereas the acinar and ductal cells of the mucous glands exhibited doubtful or negative reactions (Fig. 11).

In the male rat tongue stained with the Con A-G-S procedure, strong or moderate positive reactions of the nerve fiber bundles and of the stratified muscle fibers were demonstrated, however, the connective tissue elements surrounding the nerve and muscle fiber bundles revealed weak positive, doubt-
ful or negative reactions (Fig. 12).

In the tongue of female rats, nearly all the histologic structures such as the mucous epithelium (Fig. 13), connective tissue elements (Figs. 13 & 14), serous and mucous gland cells (Fig. 14), striated muscle (Fig. 14) and nerve fiber bundles were found to exhibit RCA-I-G-S reactions which were nearly comparable in intensity to those in the male rat tongues.

Likewise, the Con A-G-S reactions of the epithelial (Figs. 15 & 16), connective (Fig. 15), muscular (Fig. 16) and nervous tissue elements in the female rat tongues were not significantly different in intensity from those in the tongues of male animals.

In the lingual tissues of the rat subjected to the control procedures such as RCA-I-G β-D-gal-S (Fig. 17) and Con A-G α-MM-S (Fig. 18), any positive reactions could not be obtained, except for weak positive reactions in the stratified squamous epithelium of the lingual mucous membrane (Fig. 17).

(B) Results obtained in the guinea pig tongue.

In the lingual mucous membrane of the male guinea pig stained with the RCA-I-G-S technique, the intercellular spaces of the basal and intermediate layers in the stratified squamous epithelium exhibited strong positive reactions, whereas such positive reactions were not varied in intensity with different loci as to the formation of filiform papillae (Fig. 19). In addition, the RCA-I-G-S reactions of the intercellular spaces of keratinized cells from the horny layer tended to be weaker in intensity than those in the rest of the epithelium (Fig. 19). The RCA-I-G-S technique resulted in relatively weak positive or negative reactions in the connective tissues of the lingual papillae and subpapillar lamina propria (Fig. 19).

In the root of the male guinea pig tongue, moderate positive RCA-I-G-S reactions were demonstrated in the acinar and ductal cells of the serous glands (Fig. 20). Further, acinar and ductal cells of the mucous glands together with accompanying crescent-shaped cells showed a variety of intensities of positive reactions, moderate, weak or doubtful, whereas some of these cells revealed negative reactions (Fig. 20). In the serous and mucous glands, in addition, the connective tissue elements such as the capsules, septa and septula exhibited weak positive reactions (Fig. 20).

In the male guinea pig tongue, the RCA-I-G-S technique gave rise to varying intensities of positive reactions (moderate, weak or doubtful ones) in the nerve fiber bundles, whereas the same technique resulted in moderate positive reactions of the interfibrillar spaces of the stratified muscle fibers and in doubtful or weak positive reactions of the muscle fibers (Fig. 21). In addition, the connective tissues surrounding the nerve and muscle fiber bundles showed weak RCA-I-G-S reactions (Fig. 21).

In the lingual mucous membrane of the male guinea pig, the Con A-G-S procedure resulted in staining patterns of all the histologic structures (the three layers of the epithelium and connective tissues), which were nearly comparable in intensity of positive reactions and negativity to those obtained in the lingual mucous membrane of the male rat (Fig. 22).

In the root of the male guinea pig tongue, the acinar and ductal cells of the serous glands together with striated muscle fibers surrounding the glands exhibited moderate positive reactions (Fig. 23). In contrast, the acinar and ductal cells of the mucous glands showed weak positive or negative and moderate or weak positive reactions respectively (Fig. 23).

In the male guinea pig tongue stained with the Con A-G-S procedure, strong or moderate positive reactions of the nerve fiber bundles and of the stratified muscle fibers were detected, whereas the connective tissue elements surrounding the nerve and muscle fiber bundles displayed weak positive, doubtful or negative reactions (Fig. 24).

In the tongue of female guinea pigs, nearly all the histologic structures such as mucous epithelium (Fig. 25), connective tissue elements (Figs. 25 & 26), serous and mucous gland cells, striated muscle (Fig. 26) and nerve fiber (Fig. 26) bundles were seen to show RCA-I-G-S reactions, which were more or less similar in intensity to those in the male guinea pig tongues.

In keeping with the RCA-I-G-S reactions mentioned, the Con A-G-S reactions of the epithelial (Figs. 27 & 28), connective (Fig. 27), muscular (Fig. 28) and nervous tissue elements in the female guinea pig tongues were comparable in intensity to those in the tongues of male animals.

In the lingual tissues of the guinea pig stained with the control procedures such as RCA-I-G β-D-gal-S (Fig. 29) and Con A-G α-MM-S (Fig. 30), positive reactions were not visualized, except for doubtful reactions in the stratified squamous epithelium of the lingual mucous membrane (Fig. 30).

Discussion

It has been well established that RCA-I and Con A bind specifically β-D-galactose (Yamada & Shimizu, 1977; Shimizu & Yamada, 1978; Pearse, 1985; Fujimori et al., 1988) and α-D-mannose or α-D-glucose (Yamada & Shimizu, 1976; Yamada, 1978; Shimizu & Yamada, 1978; Pearse, 1985; Fujimori et al., 1988) residues of carbohydrates
ND-DAB reactions in contrast with mucous acini, reported to exhibit unusually strong positive RCA-I-reactions (Fujimori et al., 1988). In the present study, the control procedures using a specific monosaccharide for each lectin such as RCA-I-G β-D-gal-S and Con A-G α-MM-S have been found to result in negative or doubtful reactions in nearly all the histologic structures examined. These results can be taken to substantiate that the specificities of the both lectin-G-S (LT-G-S) staining procedures are sufficient. In the lingual mucous epithelium of the rat and guinea pig, however, the control staining procedures for the both LT-G-S methods (RCA-I-G β-D-gal-S and Con A-G α-MM-S) gave occasionally rise to weak positive or doubtful reactions. The mechanism underlying such results of the control staining procedures can not be elucidated precisely, even though sulfur-containing proteins in the mucous epithelial cells could be concerned with sensitization during the course of physical development (Sasai, 1982; Fujimori et al., 1988).

In the lingual mucous epithelium of the two rodent species studied, positive RCA-I-G-S reactions were detected primarily in the intercellular spaces, whereas positive Con A-G-S reactions were visualized in the cytoplasm of the epithelial cells (the cells of the intermediate layer exhibiting stronger positive Con A-G-S reactions than those of the cells of the basal layer). In view of the specificities of the both LT-G-S staining procedures, the intercellular substances and outer surface of the epithelial cell membranes are believed to contain an abundance of β-D-galactose residues of glycoproteins, whereas the intracellular cytoplasmic structures being thought to be rich in amounts of α-D-mannose or α-D-glucose residues of the glycoconjugates. In the epidermis of human (Uno, 1987; Takata et al., 1988) and mouse (Takata et al., 1988) skin, RCA-I reactive glycoproteins were reported to be localized in the epidermal cytomembranes, however, Con A-reactive glycoconjugates were found to be situated in the epidermal cell cytoplasm. Thus, it seems likely that the lingual mucous epithelium of mammals is comparable in functions of glycoproteins involved to the epidermis of their skin.

In the mucous membrane and serous and mucous lingual glands from the tongues examined in the present study, nearly all the connective tissue elements were shown to exhibit moderate or weak RCA-I-G-S and Con A-G-S reactions. In keeping with these results, likewise, the connective tissue elements of the rat skin and colon and porcine and equine submandibular glands were recorded to show moderate or weak RCA-I-G-S and Con A-G-S reactions (Fujimori et al., 1988).

In the lingual glands of the rat, serous acini were reported to exhibit unusually strong positive RCA-I-PO-DAB reactions in contrast with mucous acini, which showed relatively weak positive reactions (Schulte & Spicer, 1984). In line with these results, Fujimori et al. (1988) described that serous and mucous cells of the porcine and equine submandibular glands exhibited moderate positive and weak positive or negative RCA-I-G-S reactions respectively. In the present study, likewise, the lingual serous and mucous gland acini of the rat and guinea pig have been revealed to show strong or moderate and moderate to negative RCA-I-G-S reactions respectively. Thus, the lingual serous gland acini of the both rodents are believed to contain larger amounts of β-D-galactose-residues of glycoproteins, as compared with those in the lingual mucous gland acini. It remains, however, to be known how such variations in amounts of β-D-galactose residues of glycoproteins can be correlated with histophysiological functions of each type of the glandular acini.

In the equine submandibular glands, Fujimori et al. (1988) revealed that serous cells were reacted moderately with Con A-G-S, in contrast with mucous cells showing negative Con A-G-S reactions. In the lingual serous and mucous gland acini of the two rodent species examined here, likewise, moderate positive and weak positive to negative reactions were detected respectively. Thus, α-D-mannose or α-D-glucose residues of glycoproteins are larger in amount in the serous acini than those in the mucous acini. However, the histophysiological significances of such variations in amounts of the particular residues of glycoproteins can hardly be elucidated exactly.

In 1985, Streit et al. applied a RCA-I-PO-DAB staining procedure to rat peripheral nerves and disclosed that their nodes of Ranvier and outer myelin membrane exhibited strong or moderate positive and moderate or weak positive reactions respectively. In keeping with these results, the lingual nerve fiber bundles of the two rodent species examined were found to show strong, moderate or weak positive RCA-I-G-S reactions. In the sciatic nerves of the rat, Wood and McLaughlin (1975) demonstrated electron microscopically that interperiod lines of the myelin sheath were of positive Con A-PO-DAB reactions. In the rat and guinea pig tongues, likewise, the nerve fiber bundles were shown to react strongly or moderately with Con A-G-S. In view of the results of previous studies (Wood & McLaughlin, 1975; Streit et al., 1985), the positive LT-G-S reactions of the lingual nerve fiber bundles in the rat and guinea pig are thought to be due primarily to their myelin sheaths and their related structures. Thus, it seems possible that β-D-galactose and α-D-mannose or α-D-glucose-containing glycoproteins are concerned histophysiologically with insulation properties of myelin sheaths in mammalian peripheral nerve.
fibers. In 1981, Pena et al. performed lectin (RCA-I or Con A)-HRPO-DAB staining procedures in human quadriceps muscle fibers. According to their results, the sarcolemma and several annular profiles of the muscle fibers exhibited positive RCA-I-HRPO-DAB reactions, whereas the Con A-HRPO-DAB procedure resulted in positive reactions of the sarcolemma and fine punctate stainings of the muscle fibers. In agreement with such results, both the muscle fiber bundles and their interfibrillar spaces of the rat and guinea pig tongues were found to exhibit strong, moderate or weak positive LT (RCA-I or Con A)-G-S reactions. It can, however, not be determined precisely whether or not the LT-G-S reactive structures of the lingual muscle fibers are correlated substantially with the contractive activity of the fibers.

In the tongues of the rat and guinea pig, the LT (RCA-I or Con A)-G-S reactions of various histologic structures involved in the mucous membrane, lingual glands, nerve fibers and muscle fibers were not significantly varied in intensity with the sex of the animals. Such results can be regarded as indicating that male and female sexual hormones do not exert any substantial effects upon the histophysiological functions of the LT-G-S reactive glycoproteins in the histologic structures mentioned.

In the rat tongue, the LT (RCA-I or Con A)-G-S reactions of the lingual serous gland acini were relatively stronger in intensity, as compared with those in the guinea pig tongue. Such a difference between the two rodent species may be taken to reflect qualitatively stronger in intensity, as compared with those reactions of the lingual serous gland acini were relatively stronger in intensity, as compared with those in the guinea pig tongue. Such a difference between the two rodent species may be taken to reflect qualitatively stronger in intensity, as compared with those in the guinea pig tongue. Such a difference between the two rodent species may be taken to reflect qualitatively stronger in intensity, as compared with those

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Explanation of Figures

Plate I

Fig. 1. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a rat. Stained with hematoxylin-eosin. ×200

Fig. 2. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a rat. Stained with hematoxylin-eosin. ×200

Fig. 3. Parts of the nerve and muscle fiber bundles in the tongue of a rat. Stained with hematoxylin-eosin. ×200

Fig. 4. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a guinea pig. Stained with hematoxylin-eosin. ×200

Fig. 5. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a guinea pig. Stained with hematoxylin-eosin. ×200

Fig. 6. Parts of the nerve and muscle fiber bundles in the tongue of a guinea pig. Stained with hematoxylin-eosin. ×200
Plate II

Fig. 7. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a male rat. Stained with RCA-I-G-S. ×200

Fig. 8. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a male rat. Stained with RCA-I-G-S. ×200

Fig. 9. Parts of the nerve and muscle fiber bundles in the tongue of a male rat. Stained with RCA-I-G-S. ×200

Fig. 10. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a male rat. Stained with Con A-G-S. ×200

Fig. 11. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a male rat. Stained with Con A-G-S. ×200

Fig. 12. Parts of the nerve and muscle fiber bundles in the tongue of a male rat. Stained with Con A-G-S. ×200
Plate III

Fig. 13. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a female rat. Stained with RCA-I-G-S. ×200

Fig. 14. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a female rat. Stained with RCA-I-G-S. ×200

Fig. 15. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a female rat. Stained with Con A-G-S. ×200

Fig. 16. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a female rat. Stained with Con A-G-S. ×200

Fig. 17. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a male rat. Stained with RCA-I-G β-D-gal-S. ×200

Fig. 18. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a male rat. Stained with Con A-G α-MM-S. ×200
Plate IV

Fig. 19. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a male guinea pig. Stained with RCA-I-G-S. ×200

Fig. 20. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a male guinea pig. Stained with RCA-I-G-S. ×200

Fig. 21. Parts of the nerve and muscle fiber bundles in the tongue of a male guinea pig. Stained with RCA-I-G-S. ×200

Fig. 22. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a male guinea pig. Stained with Con A-G-S. ×200

Fig. 23. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a male guinea pig. Stained with Con A-G-S. ×200

Fig. 24. Parts of the nerve and muscle fiber bundles in the tongue of a male guinea pig. Stained with Con A-G-S. ×200
Plate V

Fig. 25. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a female guinea pig. Stained with RCA-I-G-S. ×200

Fig. 26. Parts of the nerve and muscle fiber bundles in the tongue of a female guinea pig. Stained with RCA-I-G-S. ×200

Fig. 27. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a female guinea pig. Stained with Con A-G-S. ×200

Fig. 28. Parts of the serous and mucous glands with interglandular muscle fibers in the root of the tongue of a female guinea pig. Stained with Con A-G-S. ×200

Fig. 29. Parts of the nerve and muscle fiber bundles in the tongue of a male guinea pig. Stained with RCA-I-G β-D-gal-S. ×200

Fig. 30. Parts of the mucous epithelium forming filiform papillae and subjacent connective tissues in the tongue of a male guinea pig. Stained with Con A-G α-MM-S. ×200