Abstract. Aim To analyze and compare the expression of MTNR1A receptor in normal and pathological major and minor salivary glands. Materials and Methods: Twenty samples of major and minor salivary glands and 10 with Warthin’s tumor were studied. Expression of the MTNR1A receptor (goat polyclonal antibody raised against a peptide mapping at the N-terminus of MEL-1A R of human origin) was analyzed. Results: The excretory ducts of major salivary glands demonstrated intense intracytoplasmic positivity but scant cytoplasmic membrane positivity for MTNR1A. The studied Warthin’s tumors showed intense cytoplasmic positivity for MT1 receptor in all cylindrical epithelial cells lining spaces and a less intense positivity in basal cells. The lymphoid component accompanying the tumor was negative for MT1 receptor. Conclusion: Intense intracytoplasmic positivity for the MTNR1A receptor in the excretory ducts of human major and minor salivary glands and Warthin’s tumor was found. The intense expression of MTNR1A receptors observed in this study in the excretory ducts of major and minor salivary glands may be related to salivary regulation.

Most saliva is produced by the major salivary glands, which vary between different species in number, size, and location. Some hormones can be found in saliva, with physiological consequences such as regulation of inflammatory processes, the promotion of cell proliferation, and contribute to a rapid wound healing in epithelia.

Melatonin (N-acetyl-5-methoxytryptamine) is a lipophilic hormone that is primarily synthesized and secreted by the pineal gland (1) and is widely distributed throughout the human body (2). It exerts chronobiotic, immunomodulatory, oncostatic and antioxidant actions, among others, via direct and indirect mechanisms (3). Melatonin is also synthesized in the digestive system by enterochromaffin cells of the intestinal mucosa in response to food intake (4), sometimes reaching concentrations up to 400-fold higher than those produced by the pineal gland (5).

After its release into the blood, melatonin enters the oral cavity by passive diffusion in the saliva. Melatonin concentrations in the saliva are 15-33% increased compared to those in plasma, since 70% of plasma melatonin is bound to albumin and does not enter the saliva to any appreciable extent. Hence, salivary melatonin represents the percentage of circulating melatonin that is not albumin-bound, i.e. free melatonin. Evaluating the levels of salivary melatonin is a reliable technique for monitoring circadian rhythms (2).

Two types of melatonin receptor, MTNR1A and MTNR2A have been reported in the cytoplasmic membrane of human cells (6). The MTNR1A gene is located at chromosome 4 and MTNR2A gene at chromosome 11 (7). These receptors, which are coupled to heterotrimeric guanine nucleotide binding proteins (G-proteins), are found in high concentrations in the pituitary, the suprachiasmatic nucleus of the hypothalamus, the retina, and ependymal cells of the choroid plexus (8, 9). MTNR1A has been implicated in the inhibition of melatonin in the suprachiasmatic nucleus of the hypothalamus and in the effect of melatonin on MCF-7 breast cancer cells (10). Experimental findings in rats suggest that the MTNR2A receptor mediates the action of melatonin in the retina and induces a phase shift of the circadian rhythm in the suprachiasmatic nucleus of the hypothalamus (11).

Results of an immunoblotting study of MTNR1A and MTNR2A receptors in the parotid gland of rat suggested that melatonin may be involved in salivary regulation via direct action on its receptors and via nitric oxide (12). However, no
Data have been published on the immunohistochemical expression of melatonin receptors in normal and pathological human salivary glands. The objective of the present study was to analyze the expression of MTNR1A receptors in normal major and minor salivary glands and in Warthin’s tumor from humans.

Materials and Methods

Patients’ samples. The study included samples of major salivary glands from 10 patients, minor salivary glands from 10 patients and 10 Warthin’s tumor. Structures corresponding to the major (parotid, submaxillary, sublingual) and minor (palatal and labial) salivary glands were preserved in all cases and utilized in the study. The median age of patients was 44 years (range, 33-66 years). All surgical procedures were carried out between 9 am and 1 pm. Written informed consent was obtained from all patients, and the study was approved by the Ethics Committee of our institution.

Immunohistochemical studies. Samples were fixed in 10% buffered formalin for 24 h and embedded in paraffin. Paraffin-embedded 4-μm sections were then de-waxed, hydrated, and heat-treated at 95°C for 20 min in 1 mM EDTA buffer, pH 8, for antigenic unmasking. Sections were incubated for 30 min at room temperature with goat polyclonal antibody raised against a peptide mapping at the N-terminus of MTNR1A of human origin; it was applied at a dilution of 1:500 (Santa Cruz Biothecnology, Santa Cruz, California, USA). The immunohistochemical study was carried out on an automatic immunostainer (Autostainer 480; LabVision Fremont, CA, USA), by an indirect polymer-peroxidase-based method followed by development with diaminobenzidine (Masvision; Master Diagnóstica, Granada, Spain).

The MTNR1A cytoplasmic staining pattern was graded as weakly positive (+), moderately-positive (++) or strongly-positive (+++); samples of brain and retina tissue were used as positive controls.

Results

Normal parotid gland. The serous cells that make up the acini exhibited focal intracytoplasmic positivity (+) for MTNR1A in thick clumps, with no evidence of positivity in the cytoplasmic membrane. The myoepithelial cells surrounding the serous cells were not MTNR1A-positive. The excretory ducts (lobar and lobular) demonstrated intense intracytoplasmic positivity (++++) but scant cytoplasmic membrane positivity for MTNR1A (Figure 1).

Pathological parotid glands (Warthin’s tumor). The epithelial component. This presented intense (+++) positivity for MTNR1A receptor in the cytoplasm and a lesser positivity (+) in the cell membrane; positivity (+) was also observed in the cytoplasm of basal cells (Figures 2 and 3).

Lymphoid component. This did not stain for MTNR1A although a slight positivity (+) was detected in some histiocyte cells in lymphoid follicles; lymphocytes located among epithelial cells were negative for MTNR1A.

Submaxillary gland. This gland comprises of mixed acini with a predominance of serous cells. MTNR1A positivity in the serous component was similar to that in the parotid gland (+). However, the mucous cells, which were less abundant, exhibited scant positivity in the cytoplasmic membrane and reticular positivity in the cytoplasm. The myoepithelial cells around the serous cells were not MTNR1A-positive. The excretory ducts (lobar and lobular) showed intense cytoplasmic positivity for MTNR1A (+++), with weaker positivity in the cytoplasmic membrane.

Sublingual gland. Mucous cells were more abundant and exhibited MTNR1A positivity (+++) in the cytoplasmic membrane and the peripheral portion of the cytoplasm, and reticular positivity in the cytoplasm. The myoepithelial cells around the serous cells were not MTNR1A-positive. The excretory ducts (lobar and lobular) exhibited intense cytoplasmic positivity (+++ for MTNR1A and weaker positivity for MTNR1A in the cytoplasmic membrane (Figure 4).
Figure 1. Normal parotid gland, immunohistochemical study of MTNR1A expression in the excretory ducts (diffuse ++++) and acini (granular +) (original magnification ×200).

Figure 2. Pathological parotid gland (Warthin’s tumor), immunohistochemical study of MTNR1A expression in the epithelial component (diffuse +++), (original magnification ×40).
Figure 3. Pathological parotid gland (Warthin’s tumor), immunohistochemical study of MTNR1A expression in the epithelial component (diffuse +++), (original magnification ×400).

Figure 4. Normal sublingual gland, immunohistochemical study of MTNR1A expression in the acini (granular ++) (original magnification ×200).
Minor salivary glands. These glands exhibit cell variability as a function of their localization: in the palate they are mucous glands, whereas in the tongue, lip and oral mucosa, they are mixed salivary glands, with a variable proportion of serous-mucous components (predominantly serous in the lip). Regardless of the site of these glands, the mucous and serous components exhibited the same pattern of positivity for MTNR1A. Mucous cells revealed variable positivity, ranging from light (+) to moderate intensity (++) in the cytoplasmic membrane, with focal intracytoplasmic positivity with a reticular pattern. In serous cells, positivity was granular and was found in thick intracytoplasmic clumps. The myoepithelial cells around the serous cells were not MTNR1A-positive. The system of ducts (lobar and lobular) exhibited variable positivity for MT1 (++), with less intense expression in intercalated ducts (+) (Figure 5).

Discussion

The melatonin receptor, MTNR1A has been found in multiple sites in the human body, and also in cancer of the prostate, breast, bone and gallbladder, and melanoma. To the best of our knowledge, our study is the first report, which compares MTNR1A expression in Warthin’s tumor and in normal major and minor salivary glands.

The biological role and clinical relevance of the MTNR1A in normal and tumor tissues are poorly understood. MTNR1A was found to modulate the proliferation of malignant cells and was reported to mediate the effects of melatonin on growth suppression and gene modulation in breast cancer cells (13). Warthin’s tumor is a multicystic lesion containing liquid and granular materials that are secreted by the tumor cells. The tumor cells exhibit intense expression of the cytokeratins.
associated with columnar differentiation and it has been reported that activation of MTNR1A increases phosphorylation of mitogen-activated protein kinase and MEK1-2 and ERK 1/2, probably leading to induction of synthesis of filamentous structures of non-neuronal tissues (14). We suggest that the MTNR1A may actively participate in synthesizing cytokeratin in Warthin’s tumor cells.

Melatonin is not only produced by the pineal gland, and its synthesis at other sites, such as the gastrointestinal apparatus, can be up to 400-fold higher (15). Melatonin levels increase considerably with food intake (16), which has been related to the stimulation of duodenal production of bicarbonate as a defence mechanism against gastric chlorhydric acid, increased pancreatic amylase (17, 18) and cholecystokinin (19, 20). The action of melatonin in the oral cavity would appear to be related to the increase in melatonin produced by enterochromaffin cells during food intake rather than to the physiological circadian cycle of melatonin production (21-22).

The presence of melatonin increases the secretion of salivary amylase by direct action, with no mediation of the sympathetic or parasympathetic nervous systems nor of intestinal peptides (12). However, melatonin has not been associated with an increase in salivary volume. The intense expression of MTNR1A observed in this study in the excretory ducts of major and minor salivary glands may be related to salivary regulation. Melatonin may be involved in the secretion of sodium, potassium, chloride and bicarbonate ions in salivary ducts, similar to its regulatory role in the production of duodenal bicarbonate. The finding of a more intense expression of MTNR1A in the ducts than in the acini suggests that the antioxidant effect of melatonin is of little importance in the ducts as it is independent of the presence of receptors. However, MTNR1A expression is weaker in the acini, where the antioxidant effect may therefore be more intense, especially in the mucous component. It has been reported that MTNR1A expression is inversely-related to the concentration of melatonin and is in fact higher in its absence (23, 24). Accordingly, the present finding that MTNR1A positivity is high in excretory ducts and low in acini would suggest a lower concentration of melatonin in ducts than in acini.

In conclusion, although further immunohistochemical studies are warranted to elucidate the role of melatonin and its receptors in salivary secretion and their influence as inflammatory or tumorous diseases of the oral cavity (25-27), melatonin may participate in the modulation of absorptive and/or secretory processes of salivary secretion and in the pathophysiology of Warthin’s tumor.

Conflicts of Interest

None.

References

7. Slaugenhaupt SA, Roca AL, Liebert CB, Gusella JF and Repert SM: Mapping of the gene for the Mel1a-melatonin receptor to human chromosome 4 (MTNR1A) and mouse chromosome 8 (MTNR1A). Genomics 27: 355-357, 1995.


Received July 23, 2012
Revised October 4, 2012
Accepted October 5, 2012