Localization of different molecular forms of cholinesterases and their role in human tooth morphogenesis

A THESIS PRESENTED
BY

B. G. Tharanga Lakmali Nandasena

In partial fulfillment of the requirement for the degree of

MASTER OF PHILOSOPHY

of the

UNIVERSITY OF PERADENIYA
SRI LANKA

November, 2007

ABSTRACT

Introduction: Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are two types of cholinesterases (ChE) which are classically known for their hydrolytic action at cholinergic synapses of the brain and neuromuscular junction. However, expression of ChE in non-neuronal sites suggests the biological role of ChE is not limited to cholinergic neurotransmission. An association of this enzyme with proliferative and morpho-differentiating tissue has been reported in several species. A recent study demonstrating ChE in continuously erupting guinea pig teeth suggested an involvement ChE in tooth development. However, the precise action of this intriguing enzyme in tooth development is not entirely elucidated.

Aim: The present study is carried out with the aim of localizing ChE in tooth germs of limited erupting teeth (human), to determine the precise type of ChE (AChE or BuChE or both), and their precise distribution in tooth germs, and to find out the possible mechanisms of the action of this enzyme.

Material and Methods: Two fetuses, 4 stillbirths and 2 neonates free from any visible deformities and whose mothers' medical history revealed no illnesses were selected for the study. Dissected mandibles and maxillae were fixed by immersion in 4% paraformaldehyde in 0.1M phosphate buffer followed by decalcification in 4% EDTA. Decalcified jaws were sectioned and processed for preparation of paraffin and frozen sections to be used for routine histology, immunohistochemistry and enzyme histochemistry respectively.

Results: AChE activity was observed in the cells of the cervical loop, inner enamel epithelium (IEE), outer enamel epithelium (OEE) and stratum intermedium (SI) of deciduous tooth germ in the "late bell" stage and those in the "early bell" stage of permanent teeth. Distinct AChE activities were identified in preameloblasts and the papillary layer of deciduous tooth germs. However, secretory ameloblasts and odontoblasts were free of ChE activity. Epithelial cells in the lingual side of the permanent tooth germs in "bud" and "cap" stages also depicted strong activity for AChE while rest of the cells were devoid of staining. BuChE was localized in the degenerating dental lamina.

Immunoreactions for nicotinic receptor (nAChR) were localized in the core region of cervical loop, OEE, SI and stellate reticulum of deciduous tooth germs while IEE showed no reaction. Cells of the permanent tooth germs and degenerating dental lamina were devoid of immunoreaction for nAChR.

Discussion and conclusions: This study revealed action of AChE primarily on enamel organ of tooth germ and BuChE on degenerating dental lamina. Localization of AChE at different sites of enamel organ, which is devoid of nerve elements, implies non-neuronal functions of AChE in human tooth development. Action of ChE during tooth development may be mediated through its unique structural features or the receptor mediated catalytic action.