# On the Autonomic Control of Blood Flow and Secretion in Salivary Glands

Functional and morphological aspects on muscarinic receptor subtypes in different species

Akademisk avhandling
Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin vid Göteborgs universitet kommer att offentligen försvaras i hörsalen Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg,
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av

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Avhandlingen baseras på följande delarbeten:

**I** **Expression of muscarinic receptor subtypes in salivary glands of rats, sheep and man.**
Anders T Ryberg, Gunnar Warfvinge, Louise Axelsson, Ondrej Soukup, Bengt Götrick and Gunnar Tobin
*Published online in Archives of Oral Biology, 5 September 2007*

**II** **In vitro cholinergic effects and muscarinic receptor expression in blood vessels of the rat**
Anders T Ryberg, Hanna Selberg, Ondrej Soukup, Kathryn Gradin and Gunnar Tobin
*Submitted*

**III** **Distribution and function of muscarinic receptor subtypes in the ovine submandibular gland**
Gunnar Tobin, Anders T Ryberg, Scott Gentle and the late Anthony V Edwards
*Journal of Applied Physiology 2006 Apr;100 (4):1103-4.*

**IV** **In vivo effects of muscarinic receptor antagonists on the release of VIP in the ovine submandibular gland**
Anders T Ryberg, Ondrej Soukup, Gunnar Tobin
*Submitted*

# Abstract

Parasympathetic nervous activity is the principal stimulus for evoking fluid responses within salivary glands. Concomitantly to the onset of this response, the blood flow increases. The responses, in particular the vasodilatation, consist of an atropine-sensitive acetylcholine-mediated part and an atropine-resistant part mediated via non-adrenergic, non-cholinergic (NANC) transmitters. It has been generally agreed that the cholinergic effects are mediated by muscarinic M3 receptors. However, this view has been questioned, since most muscarinic receptors are expressed and muscarinic M1 receptors elicit functional effects in salivary glands. The distribution and function of the muscarinic receptors is not unravelled, neither according to secretion nor vasodilatation. The aim of this thesis has been to investigate the roles of different muscarinic subtypes in the control of blood flow and secretion in salivary glands.

In the thesis, the expression of muscarinic receptors in salivary glands and related blood vessels was investigated using immunoblotting and/or immunohistochemistry. Furthermore the effects of muscarinic stimulation and blockade were investigated on isolated vessels, on the secretion of saliva, on glandular blood flow and vessel permeability. The thesis includes observations on rats, sheep and man.

It is shown that M1 receptors contribute considerably, in addition to the functionally most significant M3 receptor, to the fluid secretory responses of rats and sheep. The M1 receptor is particularly apparent in seromucous and mucous glands, and of particular functional significance at low intense stimulation. Since the occurrence pattern was the same in human salivary glands, M1 receptors may be of significance in man also. Notably, in the human glands, inflammation increased the expression of muscarinic M5 receptors. In the arterial blood vessels muscarinic M1 receptors generally occurred in the endothelium, and muscarinic M5 receptors, and possibly M3 also, were detected in the smooth muscle. In venous endothelium muscarinic M1 and M4 receptors occurred, while M1 and/or M3 were expressed in the smooth muscle layer. Cholinergic stimulation generally caused arterial vasodilatation, which was mainly dependent on nitric oxide. The response was mediated by muscarinic M1 and possibly M5 receptors, in addition to the M3 receptor. The venous response included a contractile M1 mediated component that may preserve perfusion pressure during the secretory process. In tissue in close vicinity to the parenchymal tissues, M1 and in particular M4 receptors occurred. In the sheep, the increase of submandibular secretory and vasodilator responses to electrical stimulation of the parasympathetic nerve in the presence of muscarinic antagonists were explained by neuronal muscarinic M4 receptors. These receptors inhibited the release of transmitters as was shown for the NANC transmitter VIP. The role of muscarinic M5 receptors is unclear but may affect on the vascular response or more likely to be correlated to inflammation.

In general, the expression pattern and functions of the muscarinic receptors subtypes showed resemblance in the examined species. All muscarinic receptors occur in the salivary glands. In seromucous/mucous glands, muscarinic M1 receptors contribute substantially to the secretory response. In the vasculature, the muscarinic receptor subtypes interact, possibly via autocrine mechanisms, for preserving the hemodynamics in the glands.



Keywords: Muscarinic receptor subtype, neuronal, non-neuronal, expression, secretion, blood flow, salivary gland, human, rat, sheep