Abstract

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Title of Doctoral Thesis: **Interactions of muscarinic receptors and choline esterases: Functional examinations of esterase inhibitors in the rat.**

The parasympathetic nervous system regulates a number of vital body functions. The neurotransmission within the parasympathetic nervous system is exerted by acetylcholine, a phylogenetic old neurotransmitter, which acts on nicotinic and muscarinic receptors. Whilst the nicotinic receptors are located in the ganglia, the muscarinic receptors are located on the glands and smooth muscles. The muscarinic receptors belong to the large group of G glycoprotein-coupled receptors. Five subtypes exist of the muscarinic receptor (M1-M5), which can be either excitatory or inhibitory depending on subtype. Within the synaptic cleft, acetylcholinesterase hydrolyses acetylcholine and the inhibition of acetylcholinesterase, by for instance pesticides, causes accumulation of acetylcholine at the synaptic cleft, which in turn causes overstimulation of cholinoceptors.

Muscarinic receptors and the acetylcholinesterase are important therapeutic targets in many disorders such as neurodegenerative disorders, disorders of the lower urinary tract, of the cardiovascular and of the respiratory tract. Muscarinic antagonists are sometimes used in drug therapies. However, because of the highly conserved orthosteric (primary binding) domain of muscarinic receptors they lack receptor subtype selectivity, which would be a beneficial feature in the perspective of adverse effects. Allosteric (secondary binding sites) modulators, which act on muscarinic receptor subtypes, have been developed and they may provide new possibilities

in future drug development. One drug of interest is obidoxime, which is suggested to be an allosteric modulator and which combines both esterase inhibition and antimuscarinic effects.

In the present thesis, three types of compounds have been used – a potent cholinesterase inhibitor (physostigmine), weak cholinesterase inhibitors which express muscarinic receptor antagonism (e.g. obidoxime) and a "selective" muscarinic M2 receptor antagonist (methoctramine). Obidoxime has a M2 "selective" antagonistic profile. The goal of the thesis was to determine the functional significance of muscarinic M2/M3 receptors in the state of acetylcholinesterase inhibition. The frequent occurrence of muscarinic M2 receptors on the smooth muscle within the respiratory and lower urinary tract has puzzled researchers for many years. Now, the pharmacological tools employed in the current thesis enabled us to study and discover new interaction mechanisms between muscarinic M2 and M3 receptors, which may be of utter significance for toxicity problems in the pharmacotherapy of esterase inhibition.

Methods: The functional studies were performed on isolated organs (rat atrium and urinary bladder strips) using *in vitro* organ bath experiments. In *in vivo* experiments, heart rate and urinary bladder pressure were studied in anaesthetized rats.

Key Findings: The results confirms that obidoxime exerts anti-muscarinic effects and that the muscarinic receptor inhibition profile shows M2 receptor selectivity. This antimuscarinic effect is much smaller than the effect of atropine. The results also indicate that the esterase inhibition and the muscarinic receptor antagonism occur at different concentrations and dose levels.

The current results also suggest a new role for the muscarinic M2 receptors, namely that they, at least at low intensity of cholinergic stimulation, also stabilize the bladder in the way of not being too sensitive towards acetylcholine. The findings should be considered when administering compounds that enhance cholinergic effects simultaneously with exerting muscarinic M2 receptor antagonism. Consequently, when using acetylcholinesterase reactivators, it seems reasonable to administer an oxime reactivator with less pronounced muscarinic M2 receptor affinity.

Conclusions: The present thesis shows that stimulation of muscarinic M2 receptors inhibits muscarinic M3 receptor-evoked contractile responses at low concentrations of acetylcholine in the synaptic cleft. The toxic effect of cholinesterase reactivators may be coupled to the previously described antimuscarinic M2 receptor profile. The muscarinic M2 and M3 receptor

crosstalk could thus be a counteracting mechanism in the treatment of acetylcholinesterase inhibition when using reactivators, such as obidoxime.

Key words: Atria, Urinary Bladder, Cholinoceptors, Acetylcholinesterase, Muscarinic Receptors, Acetylcholinesterase Inhibitors, Physostigmine, Obidoxime, Muscarinic Antagonists, Methoctramine, Atropine, Crosstalk, Muscarinic Agonists, Methacholine, Acetylcholine.