Characterisation of intestinal muscarinic acetylcholine receptors using selective antibodies and muscarinic toxins

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Acetylcholine, acting at muscarinic receptors, is a major neurotransmitter in motility, secretomotor and vasomotor reflexes in the intestine. However, the roles of the different muscarinic receptor subtypes have not been adequately identified due to the poor selectivity of conventional agonists and antagonists[1]. The aims of the present study were to examine the distribution of M_1 and M_2 muscarinic receptors using immunohistochemical techniques, and to determine the functional significance of M_1 and M_4 receptors in the control of motility using highly selective muscarinic toxins from Green Mamba venom. Ileum segments were removed from guinea-pigs that had been stunned and killed by exanguination. M_1 receptor-immunoreactivity (M_1-IR) was restricted to myenteric and submucous neurons. In contrast, M_2-IR was present in myenteric and submucous neurons, throughout ganglia and fibre tracts, on circular and longitudinal muscle, and submucosal blood vessels. M_1-IR was present in 88% of myenteric and 91% of submucous neurons, while M_2-IR was found in 90% of myenteric and 97% of submucous neurons. The only myenteric neurons that were not M_1-IR comprised a subset (39%) of neurons that were also immunoreactive for calretinin, a marker of ascending interneurons and excitatory motor neurons. A proportion (23%) of calretinin-IR submucous vasodilator neurons were also not M_1-IR. Similarly, 47% of calretinin-IR myenteric neurons and 23% of calretinin-IR submucous neurons were not M_2-IR. Isolated organ bath experiments were used to determine the physiological role of muscarinic receptors in motility. The highly selective M_1 receptor antagonist, MT7 (1 nM), significantly inhibited ascending excitatory motility reflexes. Cholinergic contractions evoked by electrical field stimulation (EFS) were unaffected by MT7, however, MT3 (10 nM), a selective M_4 receptor antagonist, enhanced EFS contractions. These results demonstrate that M_1 receptors contribute to transmission in motility reflexes. They also indicate that presynaptic autoreceptors inhibiting the release of acetylcholine are of the M_4 rather than M_1 subtype, and suggest potential therapeutic application in the treatment of intestinal motility disorders.

(1) Caulfield, M.P. and Birdsall, N.J.M. (1998). International Union of Pharmacology. XVII. Classification of Muscarinic Acetylcholine Receptors. Pharmacological Reviews 50:279-290.