MUSCARINIC CONTROL OF PARASYMPATHETIC ACTIVITY IN ISOLATED PORCINE AIRWAYS: INVOLVEMENT OF M_1/M_2 MUSCARINIC AUTORECEPTORS

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The effects of muscarinic receptor (MR) ligands on electrically-evoked ^{3}H-acetylcholine (ACh) release and smooth muscle contraction were evaluated in an isolated porcine airways preparation. Lungs of mature white pigs (>5 mo, carcass wt 180-200 kg) were obtained from local abattoir and branches of 5-6 mm I.D. were dissected from the bronchial tree. The mucosa was removed from the tissue by rubbing the luminal surface with a moisted stem-pipe cleaner, a procedure not affecting the neuromuscular integrity of the preparation as evidentiated by microscope analysis. Muscular strips (4 mm width, 15-18 mm lenght, 40-60 mg wet weight) were isolated and set up in organ baths under isometric tension. Neural stores were labelled with ^{3}H-choline and newly-synthesized ^{3}H-ACh release was evoked twice (S_1 and S_2) by trains of 1800 pulses of electrical field stimulation (EFS) delivered at 10 Hz (0.5 ms, 9V). EFS produced smooth muscle contraction and a parallel overflow of tritiated compounds. Both ^{3}H-overflow (EEO: 5,586 ± 860 Bq) and contraction (EEC: 1,360 ± 130 mN· g^{-1} of tissue, n=26) induced by S_1 were abolished by 300 nM tetrodotoxin and ω-conotoxin GVIA (5 μM), suggesting their neural origin. Drug effects were evaluated as % variation of S_2/S_1 ratio in comparison to control experiments (S_2/S_1 = 0.71± 0.03).

Hexamethonium (ganglionic blocker; 10 μM) and eserine (AChesterase inhibitor; 10-100 nM) did not induce any significant variation of EEO. Conversely, 3μM eserine enhanced both EEO and EEC (37% and 68% maximal increase vs. control experiments, respectively; $p<0.01$). The MR agonist bethanechol produced a dual effect on ^{3}H-ACh release: facilitation (1 nM-1 μM) and inhibition (1-100 μM; pEC_{50} 5.21). At variance, muscarone and oxotremorine, two M_2/M_4 subtype-preferring agonists, concentration-dependently (1 nM-10 μM) inhibited the evoked ^{3}H-ACh release (pEC_{50} 7.70, 7.50, respectively; maximal inhibition 80%). Atropine (1-1000 nM), non-selective muscarinic antagonist, produced a non-concentration-related decrease of EEO by about 15%. Conversely, it was markedly reduced in a concentration-dependent manner (0.1-100 nM range) by the M_1 antagonist pirenzepine as well as by atropine in the presence of 100 nM eserine (maximal inhibitory effect by about 50%; pIC_{50} 8.20 and 7.9, respectively). Under these experimental conditions, tripitramine and AFDX 116, M_2/M_4 subtype-preferring antagonists, but not MT-3 (M_4 selective), produced at nanomolar concentrations a facilitatory effect (pEC_{50} 8.30 and 5.93, respectively). Based on the comparison of our estimates with affinities to MR subtypes, the muscarinic feedback mechanisms controlling ACh release in porcine airways have been cha-racterized conclusively. Facilitatory M_1 and inhibitory M_2 autoreceptors localized at cholinergic nerve terminals exert a complex regulation of parasympathetic activity, depending on the level of ACh in the neural cleft. A similar neural muscarinic-mediated control was observed in human bronchi at prejunctional level (1). Since MRs expressed in humans and pig are highly similar (2), porcine bronchi can represent a reliable in vitro model for the development of new MR blockers more effective in the treatment of peripheral airways diseases.

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2- Eglen R., Hedge S. and Watson N. (1996) Pharmacol. Rev. 48: 531-565.