



**MUSCARINIC CONTROL OF PARASYMPATHETIC ACTIVITY IN ISOLATED  
PORCINE AIRWAYS: INVOLVEMENT OF M<sub>1</sub>/M<sub>2</sub> MUSCARINIC  
AUTORECEPTORS**

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The effects of muscarinic receptor (MR) ligands on electrically-evoked  $^3\text{H}$ -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 moistened stem-pipe cleaner, a procedure not affecting the neuromuscular integrity of the preparation as evidenced by microscope analysis. Muscular strips (4 mm width, 15-18 mm length, 40-60 mg wet weight) were isolated and set up in organ baths under isometric tension. Neural stores were labelled with  $^3\text{H}$ -choline and newly-synthesized  $^3\text{H}$ -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  $^3\text{H}$ -overflow (EEO:  $5,586 \pm 860$  Bq) and contraction (EEC:  $1,360 \pm 130$   $\text{mN} \cdot \text{g}^{-1}$  of tissue,  $n=26$ ) induced by  $S_1$  were abolished by 300 nM tetrodotoxin and  $\omega$ -conotoxin GVIA (5  $\mu\text{M}$ ), suggesting their neural origin. Drug effects were evaluated as % variation of  $S_2/S_1$  ratio in comparison to control experiments ( $S_2/S_1 = 0.71 \pm 0.03$ ).

Hexamethonium (ganglionic blocker; 10  $\mu\text{M}$ ) and eserine (AChE inhibitor; 10-100 nM) did not induce any significant variation of EEO. Conversely, 3  $\mu\text{M}$  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  $^3\text{H}$ -ACh release: facilitation (1 nM-1  $\mu\text{M}$ ) and inhibition (1-100  $\mu\text{M}$ ;  $\text{pEC}_{50}$  5.21). At variance, muscarone and oxotremorine, two  $M_2/M_4$  subtype-preferring agonists, concentration-dependently (1 nM-10  $\mu\text{M}$ ) inhibited the evoked  $^3\text{H}$ -ACh release ( $\text{pEC}_{50}$  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%;  $\text{pIC}_{50}$  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 ( $\text{pEC}_{50}$  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 characterized 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.

1- Rackè K. and Matthiesen S. (2004) Pulmon. Pharmacol. Ther. 17: 181-198.

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