



**PURINERGIC FACILITATION OF PARASYMPATHETIC ACTIVITY IN  
ISOLATED PIG DETRUSOR: INVOLVEMENT OF PREJUNCTIONAL P2X<sub>3</sub>  
RECEPTORS**

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Adenosin-triphosphate (ATP) is stored and co-released with acetylcholine in parasympathetic nerve terminals where it exerts the function of a neurotransmitter via activation of purinergic P2X<sub>1/7</sub> receptors. The purinergic system is being increasingly recognized to play an important role in sensory and motor functions of normal as well abnormal mammals urinary bladder. Immunohistochemical studies have determined that P2X<sub>1,2,4</sub> subunits are expressed on detrusor muscle-cells, P2X<sub>1-3,5</sub> on bladder nerves and P2X<sub>2,3,7</sub> subunits are located on bladder urothelial cells. The aim of this study was to investigate whether purinergic receptors are also involved in the control of the cholinergic neurotransmission in the urinary bladder. At this purpose, an isolated preparation of pig urinary bladder was used in electrically-evoked <sup>3</sup>H-acetylcholine (<sup>3</sup>H-ACh) release experiments to assess the presence and the function of P2X receptor subunits on parasympathetic nerve terminals.

Urothelium-deprived strips from pig detrusor were mounted isometrically in organ baths and superfused. After neural ACh stores were labelled with <sup>3</sup>H-choline, newly-synthesized <sup>3</sup>H-ACh release was electrically-evoked twice (S<sub>1</sub> and S<sub>2</sub>) and measured by liquid scintillation analyzer. Six trains of 9 pulses delivered at 20 Hz (0.1ms, 90V, 33s apart) produced smooth muscle contractions and a parallel overflow of radioactivity. Drugs effect was expressed as the S<sub>2</sub>/S<sub>1</sub> ratio in the presence of a drug in comparison with the equivalent ratio in control experiments. In the presence of TTX, both electrically-evoked <sup>3</sup>H-ACh release and contractile response was reduced by about 56%. The N-type Ca<sup>2+</sup>-channel blocker, ω-conotoxin GVIA significantly reduced the <sup>3</sup>H-ACh release (by about 71% inhibition at 1 μM). The N/P/Q-type Ca<sup>2+</sup>-channel blocker ω-conotoxin MVIIC did not produce any further inhibition at 100 nM. This suggests that N-type but not P/Q-type Ca<sup>2+</sup>-channels are likely involved in the release of ACh in isolated pig detrusor. ATP (100 μM) did not produce any significant facilitation (5-8%) of <sup>3</sup>H-ACh release. Conversely, in the presence of apyrase (a phosphatase III; 4U/ml) ATP (1-100 μM) facilitated the evoked <sup>3</sup>H-ACh release in a concentration-dependent manner (EC<sub>50</sub>=4.6) with a maximal effect by about 50%. Under such experimental conditions, the P2X<sub>1,7</sub> selective agonist Bz-ATP was not able to produce any significant facilitatory effect on <sup>3</sup>H-ACh release. At variance, α,β-meATP the putative P2X<sub>1,3,5</sub> agonist, relatively resistant to ecto-enzymes, induced on cholinergic neuro-transmission a marked concentration-dependent potentiation (by about 200%). This facilitatory effect was competitively antagonized by 1μM suramin, a putative P2X<sub>1,2,3,5</sub> antagonist. A pK<sub>B</sub> value of 6.4 was calculated. The concentration-response curve of α,β-meATP was not neither affected by 10μM NF023 (P2X<sub>1/3</sub> antagonist with a pK<sub>B</sub> affinity of 7.8 for P2X<sub>1</sub> subunit) nor shifted by 100 nM PPNDS, selective P2X<sub>1</sub> antagonist at nanomolar concentrations (pK<sub>B</sub>=7.8).

These preliminary findings are consistent with the notion that in pig detrusor ATP potentiates the cholinergic neurotransmission through the activation of P2X receptor and that the P2X<sub>3</sub> subunit is likely involved at prejunctional level in such a facilitation. Accordingly, the development of P2X<sub>3</sub> selective antagonists might contribute to the pharmacological armamentarium in the treatment of overactive bladder and urge incontinence.