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ß3-adrenoceptor inhibition of cholinergic bladder nerves requires retrograde adenosine release triggered by EPAC/PKC/ENT1 pathway in the human and rat detrusor

Eur Urol Suppl 2019;18(8):e3083

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Introduction & Objectives: The molecular mechanism underlying efficacy of β_3-adrenoceptor (β_3-ADR) agonists (e.g. mirabegron) to control overactive bladder is unknown. Confocal microscopy data demonstrated that β_3-ADR are mainly located on the plasma membrane of human smooth fibers. Yet, this fails to explain the inhibitory effect of β_3-ADR agonists on cholinergic bladder neurotransmission unless a retrograde signaling mediator is involved. Our group explained this paradox by showing that β_3-ADR activation triggers adenosine outflow through type I equilibrative nucleoside transporters (ENT1) and the nucleoside inhibits nerve-evoked acetylcholine release via A_1 receptors activation (Silva et al, 2017, Am J Physiol Renal Physiol. 313:F388-F403). β_3-ADR positively couple to adenylate cyclase yielding cyclic AMP (cAMP) accumulation.

Materials & Methods: Here, we investigated the most relevant cAMP responsive element, either protein kinase A (PKA) or the exchange protein directly activated by cAMP (EPAC), participating in β_3-ADR-induced inhibition of [^{3}H]Acetylcholine ([^{3}H]ACh) release and adenosine outflow from urothelium-denuded detrusor samples from cadaveric human organ donors and Wistar rats. The presence of β_3-ADR, EPAC and ENT-1 in smooth muscle cells of the human and rat urinary bladders was confirmed by immunofluorescence confocal microscopy.

Results: The EPAC inhibitor, ESI-09 (10 μM), prevented β_3-ADR-induced ENT-1-mediated adenosine release from human and rat detrusor strips caused by mirabegron (0.1 μM) and isoprenaline (1 μM), respectively. ESI-09 (10 μM), but not the PKA inhibitor, H-89 (10 μM), attenuated inhibition of [^{3}H]ACh release from stimulated (10 Hz, 200 pulses) detrusor strips produced by β_3-ADR agonists, the adenylyl cyclase activator (forskolin, 3 μM), and the EPAC stimulator (8-CTP-2Me cAMP, 20 μM). Isoprenaline-induced inhibition of [^{3}H]ACh release was also prevented by blocking protein kinase C (PKC, with 5 μM chelerythrine and 30 nM Go6976) or by inhibiting ENT1 (with dipyridamole, 0.5 μM). PKC activation with PMA (10 μM) mimicked the inhibitory effect of isoprenaline and mirabegron in isolated rat and human detrusors, respectively. Pretreatment with ESI-09 (10 μM) and chelerythrine (5 μM), but not with H-89 (10 μM), prevented the reduction of the voiding frequency caused by isoprenaline (0.1-1000 nM) and forskolin (0.03-10 μM) in urethane-anaesthetized rats.

Conclusions: Data suggest that β_3-ADR-induced inhibition of cholinergic neurotransmission in human and rat urinary bladders involves activation of an EPAC/PKC pathway downstream cyclic AMP production resulting in adenosine outflow via ENT-1 and retrograde activation of A_1 receptors.

Work supported by FCT (UID/BIM/4308/2019). IS was in receipt of a PhD fellowship by FCT (SFRH/BD/88855/2012).