Involvement of hydrogen sulfide in human urothelium

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The urothelium has been historically viewed as a barrier against the bladder urine content. However, to date a number of dynamic qualities of urothelium are well recognized (Ferguson DR et al., 1997; Lazzeri M. et al., 2006). Indeed, several data indicate that urothelial cells exhibit specialized sensory and signaling properties that could allow them to respond to urothelial signaling molecules that acting on different receptor/ion channels and secrete a numbers of transmitters or mediators. In particular, the expression of the protein receptors and the related mRNAs for all muscarinic (M) receptors subtypes have been found in rat and human urothelium (Mansfield KJ et al., 2007; Kim JC et al., 2008). Anyway, the physiological significance of what appears to be a cholinergic signal system in the urothelium is unclear. On the other hand, L-cysteine/hydrogen sulfide (H_2S) pathway was recently discovered in human bladder (Fusco F et al., 2012). Indeed, it has been reported that cysthationine-γ-lyase (CSE) and cysthationine-β-synthase (CBS), the main enzymes involved in H_2S biosynthesis are expressed in human bladder and that H_2S relaxes human bladder strips, contributing to the bladder homeostasis (Fusco F et al., 2012). Here, we have investigated if the M receptor activation causes H_2S production in human urothelium. In order to achieve our results we used human bladder biopsies obtained from patients affected with benign prostatic hyperplasia that underwent open prostatectomy as well as urothelial T24 cells. In this regard, we contracted with carbachol human bladder strips with and without urothelium. Carbachol-induced contraction was significantly higher in human bladder strips without urothelium compared with the effect in presence of urothelium. In addition, a concentration-response curve with carbachol (10 nM-30 μM) was performed in human bladder strips with urothelium, before and after the incubation with vehicle, DL-propargylglycine (PAG; 10 mM), or aminooxyacetic acid (AOAA; 1 Mm) CSE and CBS inhibitors, respectively. Only the CSE inhibition was able to increase the carbachol-induced contraction. Further experiments conducted in both human urothelium and T24 cells, revealed that carbachol (0.1,1,10 μM) increased H_2S production compared with vehicle and this effect was reversed by inhibition of CBS or CSE. Moreover on the basis of in vitro results on bladder strips, where CSE inhibition significantly increased the contraction induced by carbachol, we performed experiments by using T24 cells stable silenced for CSE. Interestingly, carbachol stimulation in these cells did not affected H_2S production. We have also investigated which M receptor was involved in carbachol increase in H_2S production by using a selective antagonists in both human urothelium and T24 cells such as telenzepine (M1 antagonist, 10 nM), AFDX (M2 antagonist, 100nM), 4-DAMP (M3 antagonist, 100nM) or PD102807 (M4 antagonist, 100nM). M1 and M3 antagonists significantly reduced H_2S production induced by carbachol (1μM) in human urothelium, while only telenzepine, M1 antagonist, significantly reduced carbachol-induced H_2S production in T24 cells. In conclusion we have shown that carbachol increases H_2S production through M1 and/or M3 receptors activation in human urothelium and T24 cells. This study may open new frontiers of knowledge of the physiology of human bladder that to date results still unclear and contextually may suggest novel pharmacological approaches in the management of overactive bladder.

References

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