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Enhancement of S1P-induced contractile response in detrusor smooth muscle of rats having cystitis

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Introduction: Sphingosine-1-phosphate (S1P) is a bioactive sphingolipid that causes smooth muscle contraction via $S1P_{1-3}$ receptors, both in physiological and pathological situations[1,2,3]. G-protein coupled receptors (GPCRs) have been known to activate smooth muscle contraction mainly via inositol 1,4,5-trisphosphate (IP_3). It has been later shown that cyclic adenosine diphosphate ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), two other major intracellular calcium mobilizing agents, may also participate in agonist-induced contraction[4,5]. The intracellular mechanism of S1P-induced contractile response and receptor subtypes were investigated in permeabilized detrusor smooth muscle of rats having cyclophosphamide induced cystitis.

Method: The study protocol was approved by the University Animal Ethics Committee (2014/34-6). Cyclophosphamide (150 mg/kg) was injected to rats (Sprague-Dawley,female,200-250g) intraperitoneally once a day on days 1, 4 and 7 to induce cystitis. Control group was injected with saline (NaCl 0.9 %) using the same protocol. Detrusor smooth muscle strips were mounted in 1 ml organ baths containing Hepes buffered modified Krebs' solution. Tissues were permeabilized with 40 µM β-escin for 30 min. Isometric contractions were expressed as % of 80 mM K^+. $S1P_2$ receptor protein levels were determined by Western blotting. Data were given as mean±S.E.M. $P<0.05$ was accepted as significant.

Results: S1P (50 µM)-induced contraction was significantly increased from 20±2% (control,n=11) to 43±6% in cystitis (n=8). This contraction was significantly inhibited by $S1P_2$ receptor specific antagonist JTE-013 (10µM) in control (13±1%,n=7) and cystitis (17±5%,n=6) groups. $S1P_2$ receptor protein expression was increased in cystitis group (59±16%,n=3). S1P-induced contraction was also significantly inhibited by $S1P_3$ receptor antagonist suramin (50µM) both in control (12±2%,n=6) and cystitis rats (14±4%,n=6). S1P-induced contraction was reduced by IP_3 receptor blocker, heparin (1mg/ml), in control (11±1%,n=6) and CYP-treated groups (12±3%,n=6). Moreover, bafilomycin (100nM), a vacuolar H^+-ATPase inhibitor, blocked S1P-induced contraction in both control (9±2%,n=6) and CYP-treated groups (12± 4%,n=6).

Conclusion: According to the present data, in interstitial cystitis, it seems that both $S1P_2$ and $S1P_3$ receptors are involved in S1P-induced augmented contractile response in permeabilized detrusor smooth muscle. Increased expression of $S1P_2$ receptor protein indicates the up regulation of S1P pathway in cystitis. Furthermore, our results suggest the involvement of both IP_3 sensitive sarcoplasmic reticulum stores and acidic lysosomal intracellular calcium stores in S1P-induced augmented contractile response in cystitis.

References: *[1]Watterson et al. (2007) **FASEB J.**2,2818-2828. [2]Aydin et al. (2010) **BJU Int.**106,562-572. [3]Q.Ballouhey et al. (2015) **IBJU.**41,1141-1147. [4]Boittin et al. (2003) **J. Biol. Chem.** 278,9602-9608. [5]Churchill et al. (2003) Cell. 111,703-708.*