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INVOLVEMENT OF PROTEASE-ACTIVATING RECEPTOR-2 IN FACILITATION OF NEUROMUSCULAR PURINERGIC TRANSMISSION IN THE GUINEA-PIG URINARY BLADDER

Hypothesis / aims of study

Protease-activated receptors (PARs) are a family of four G-protein coupled, seven-trans membrane receptors that are activated by proteolysis. PAR-2 is activated by trypsin and mast cell tryptase. It has been demonstrated that there is an increase in bladder mast cell number in the patients with interstitial cystitis. PAR-2 is expressed in the urothelium and detrusor muscle in the mouse urinary bladder [1]. Therefore PAR-2 may have physiological and pathophysiological role in the urinary bladder. However, the role of PAR-2 in the urinary bladder is not fully understood. Recent study showed that activation of PAR-2 stimulates release of prostaglandins from mucosal layer and thereby induces contraction in the rat urinary bladder [2]. The aim of this study was to investigate the role of PAR-2 on the electrical field stimulation-evoked neurogenic contraction in the guinea-pig urinary bladder.

Study design, materials and methods

Female guinea-pigs weighing 400-500 g were killed by cervical dislocation. Mucosa-free detrusor strips (10×2mm) were prepared from the dome of the urinary bladder. The strips were mounted between two parallel platinum electrodes in 10ml organ baths containing Krebs-Ringer solution, which was gassed with 95 % O_2 and 5% CO_2. Approximately 1g of resting tension was applied and was kept constant by re-adjustment during the equilibration period. Mechanical responses were recorded by means of an isometric transducer. After 1 h equilibration period, a calibration contraction was obtained to 40mM KCl in each strip. Following a 30 min washout period, strips were subjected to electrical field stimulation (EFS). Trains of electrical pulses (10 Hz for 5 sec, 0.5 msec pulse width, supramaximal voltage) were delivered at 1 min intervals. Following stabilization of the electrically evoked contractions (approximately 20 min), PAR-2 activating peptide, SLIGRL-NH_2 and Trypsin (bovine pancreas) were added cumulatively to the organ bath. The potentiations of the amplitude of EFS-evoked contraction by the drugs were expressed as the % increase to that in the absence of them. In preliminary experiments, atropine 10^{-6}M-sensitive (cholinergic) component of stimulated twitch contractions was 42%, while suramin 3×10^{-4}M-sensitive (purinergic) component was 40% of the total response. And these contractions were suppressed by 3×10^{-7}M TTX. To evaluate the effect of SLIGRL-NH_2 on cholinergic and purinergic component of EFS-evoked contraction, atropine 10^{-6}M or suramin 3×10^{-4} M was applied to the strips from 15 min before starting EFS.

Results

Cumulative addition of SLIGRL-NH_2 (10^{-6}M - 10^{-4}M) and also trypsin (10^{-8}M – 10^{-6}M) caused a concentration-dependent increase in the amplitude of twitch contractions induced by EFS (70.13±8.92% increase at 10^{-4}M ,n=6 and 293.75±37.2% increase at 10^{-6}M, n=4, respectively). SLIGRL-NH_2 had little effect on basal tonus. Whereas, trypsin (3×10^{-7}M – 10^{-6}M) induced tonic contraction (14.75±1.6% of 40 mM KCl at 10^{-6}M). In the case of atropine pretreatment, SLIGRL-NH_2 (10^{-6}M - 10^{-4}M) caused a concentration-dependent potentiation of twitch contractions induced by EFS with a similar potency of the control one (85.27±21.82% increase at 10^{-4}M,n=5). Whereas, in the suramin-pretreated strips, SLIGRL-NH_2 (10^{-5}M - 10^{-4}M) caused a concentration-dependent, but lesser potentiation than control (22.39±7.96% increase at 10^{-4}M,n=5).

Interpretation of results

PAR-2 activating peptide and trypsin potentiated the EFS-evoked twitch contraction in a dose dependent manner. These data indicate that activation of PAR-2 potentiates neurogenic contraction. Neurogenic contraction induced by EFS is mainly mediated by the release of acetylcholine and ATP in the guinea-pig urinary bladder. Pretreatment of atropine did not

affect the potency of the increased amplitude by PAR-2 activating peptide, however in the presence of P2X purinoceptor antagonist, suramin, the potentiation by PAR-2 activating peptide on EFS-evoked contraction was significantly inhibited. These data suggest that PAR-2 is involved in potentiation of purinergic component of EFS-evoked contraction.

Concluding message

PAR-2 is involved in potentiation of purinergic component of EFS-evoked contraction in the guinea-pig urinary bladder.

References

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