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Oral presentation

Effects of stimulating the cGMP pathway on spontaneous activity in pacemaker cells isolated from the rabbit urethra

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Nitric oxide (NO) is the main inhibitory transmitter in the urethra [1] but little is known about how it mediates relaxation in this tissue. One possibility is that NO inhibits the Ca^{2+} oscillations underlying spontaneous electrical activity in the urethra and consequently reduces tone. We have recently demonstrated the presence of specialized, spontaneously active cells in the rabbit urethra that resemble interstitial cells of Cajal (ICC) in the gut. We hypothesized that urethral interstitial cells (IC) serve to drive the surrounding bulk smooth muscle and thus may contribute significantly to the generation of urethral tone and its modulation by neurotransmitters [2-4]. The purpose of the present study was to examine if spontaneous Ca^{2+} oscillations in isolated urethral IC were altered by NO donors and other activators of the cGMP pathway. Rabbits were humanely killed with pentobarbitone (I.V.) and their urethras removed. Cells were isolated as previously described [2] and incubated for 15 minutes at room temperature with the fluorescent Ca^{2+}-sensitive indicator Fluo-4AM (2 μM). Cells were then plated onto glass-bottomed petri dishes for 30 minutes before being perfused with Hanks solution. Single cell imaging was performed at 5–15 frames sec^{-1} using a Nipkow spinning disk laser confocal microscope. When cells were maintained at 37°C calcium waves usually initiated at one or more discrete sites and then propagated throughout the cell as recently reported [5]. Wave amplitudes were calculated as a ratio ($\Delta F/F_0$) by dividing the mean intensity of the ROI during the peak of the wave by the mean intensity during quiescent periods. The waves occurred at a mean frequency of 5.5 ± 0.4 min^{-1} and had peak amplitudes of 1.6 ± 0.2 ($\Delta F/F_0$, n = 42). These were abolished in the presence of ryanodine (30 μM, n = 12) or tetracaine (100 μM n = 12), suggesting that calcium waves were dependant on functional RyR. In contrast, application of the IP_3R blocker 2APB (100 μM) failed to reduce wave frequency (4.8 ± 0.5 compared to 4.1 ± 0.4 min^{-1}) but reduced the spatial spread of the Ca^{2+} wave suggesting that IP_3R were necessary for propagation of the waves. Application of the NO donor DEA-NO (30 μM), also failed to reduce the frequency of the Ca^{2+} waves but reduced their spatial spread from 83.25 ± 2.5 to 34.9 ± 5.6 μm (n = 5, $p < 0.05$), as did 8Br cGMP (1 mM; from 83.54 ± 17.23 to 28.26 ± 9.1 μm, n = 4, $p < 0.05$). A reduction in spatial spread also occurred on application of the protein kinase GI activator SP-8-BrcGMPs (25 μM). In 4 cells, Ca^{2+} waves propagated 103 ± 19.25 μm under control conditions compared to 39.2 ± 9.2 μm in the presence of SP-8-BrcGMPs (n = 4, $p < 0.05$).

Given the similarity between the effects of the IP_3R blocker 2-APB and the activators of cGMP/GK pathway, these data suggest that NO may mediate its effects by interfering with IP_3R and thus prevent propagation of the Ca^{2+} signal in rabbit IC.

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