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LOW AMPLITUDE RHYTHMIC CONTRACTIONS IN THE HUMAN DETRUSOR

Hypothesis / aims of study

Low amplitude rhythmic contractions (LARC) have been observed in detrusor smooth muscle (DSM) in mammals, including humans. Prior studies have quantified LARC amplitudes and frequencies in rabbit DSM and LARC response to tissue strain [1]. The urothelium and lamina propria (LP) are comprised of multiple cells types and prior research has identified multiple signalling pathways that exist in this intricate network [2]. Periodic calcium transients have also been identified emanating from urinary bladder interstitial cells in some mammals [3]. Our group has shown that LARC can contribute to detrusor preload and speculated that there may be a subset of patients with overactive bladder syndrome (OAB) mediated by derangements in LARC causing increased detrusor preload. This study aims to identify a possible cholinergic mechanism for LARC as well as the effects of urothelium & LP and tissue strain on LARC in human DSM (hDSM).

Study design, materials and methods

Part I After IRB approval, tissue strips of hDSM from patients undergoing cystectomy (n=4) were placed under low isometric tension (<2g) and equilibrated in Dulbecco's modified Eagle's medium (DMEM) to assess spontaneous LARC. Carbachol was then titrated at half log increments [0.01-10μM] to generate LARC if not seen spontaneously. **Part II** Tissue samples were obtained from additional patients (n=5) and dissected as follows to determine effects of urothelium and LP on LARC **(schematic, Figure 1)**: Group 1 - full thickness (urothelium, LP, and hDSM), Group 2 - hDSM with urothelium and LP almost completely dissected but still tethered at one point so that only hDSM was under tension, Group 3 - hDSM with dissected urothelium/LP within 1mm (both strips under tension), and Group 4 - hDSM only. Tissues were equilibrated under low (<2g) isometric tension in DMEM to assess for spontaneous LARC. Neostigmine was added at 3μM to amplify any endogenous cholinergic signalling and determine effects on LARC amplitude and frequency. All tissues were stretched at 0.5-1mm increments and allowed to re-equilibrate to determine effects of increasing tension on LARC amplitude and frequency (final strain = 300-350% of initial length). All tissues were then exposed to 1μM atropine to determine if LARC amplitude and frequency would be affected. Expected tension at 5 minutes post exposure to atropine was estimated by extrapolated log regression. Expected and actual tension measurements were compared for differences in each group.

Fast Fourier transform (FFT) analysis was performed on 200-400 second sections of tension data to identify LARC frequencies and amplitudes (**representative tracing, Figure 2A**). A predetermined frequency range of 0.026-0.300 Hz (1.6-18 cycles/minute) based on previous work was used to identify relevant frequencies. A relevant frequency was defined as having an associated amplitude which was both the maximum amplitude and greater than 2 standard deviations above the average amplitude in the above frequency range (**representative spectrum, Figure 2B**). A signal to noise (S/N) ratio was calculated for all FFT results as the maximum amplitude divided by the average amplitude over the aforementioned frequency range.

Results

Part I Spontaneous LARC was identified in 25% of hDSM strips. Titrated carbachol induced LARC in the remaining 75% of hDSM strips. Average LARC frequency was 0.048±0.010 Hz. A significant improvement in S/N ratio (0.044±0.005 increased to 0.13±0.009, p=0.001) was seen after addition of carbachol. There was no association between concentration of carbachol and LARC frequency. **Part II** Spontaneous LARC was identified in 40-60% of tissues (Cochran Q test between groups, p=NS) and increased to 80% in some groups after neostigmine exposure (Fisher's exact test for each group, p=NS). There was no difference in LARC frequency or amplitude before or after neostigmine, either between or within groups. LARC frequencies are shown in **Figure 3**. An increased S/N ratio was seen in Group 3 compared to the other groups after neostigmine exposure (ANOVA, p=0.03). An increase in frequency and amplitude of LARC was seen with increased strain in 60% of strips in Group 1 (R^2=0.68-0.97). Group 4 showed a parabolic correlation of LARC amplitude vs. strain in 60% of strips (R^2=0.51-0.99), similar to a smooth muscle active tension curve. At time of atropine exposure, 100% of tissue strips demonstrated LARC. After atropine exposure, 100% of Group 1, 60% of Group 2, 40% of Group 3 and 20% of Group 4 continued to demonstrate LARC (Fisher's exact test for Group 4 p<0.05, other Groups p=NS; Cochran Q test between groups approached significance, p=0.08). There was no change in LARC amplitude or frequency in the presence of atropine in Group 1. Group 4 demonstrated a significant decrease in actual preload to expected preload after atropine (paired t-test, p<0.05) but no other groups showed a significant change.

Interpretation of results

Cholinergic-mediated LARC was visualized in hDSM after exposure to carbachol, however there were no significant differences in spontaneous LARC and LARC in the presence of neostigmine suggesting that an endogenous cholinergic mechanism is not solely responsible. Increasing LARC amplitude and frequency seen with tissue strain in a subset of Group 1 suggests a tension-mediated LARC generator in the urothelium or LP. Based on published studies, it is tempting to speculate that interstitial cells could play this role [3]. Group 1 post-atropine exposure results demonstrate further that cell types in the urothelium or LP may be modulating LARC in addition to (or outside of) a cholinergic pathway. Furthermore, LARC appears to play a major role in maintenance of hDSM tension because it is decreased by atropine.

Concluding message

Elucidation of LARC generators from urothelium and LP may provide therapeutic targets in the future for modulation of LARC to decrease bladder wall tension in a subset of OAB patients

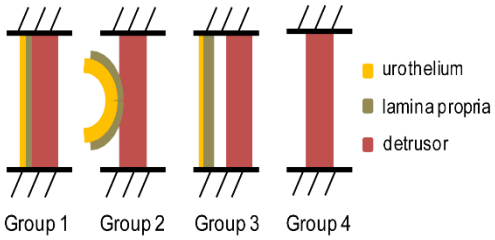

Figure 1

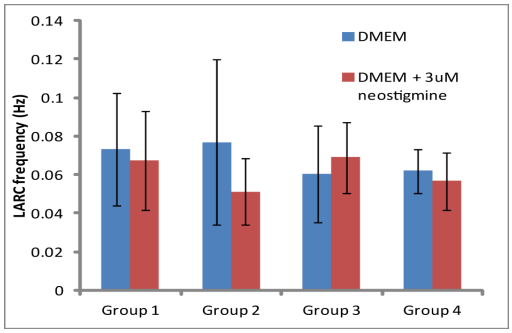

Figure 3

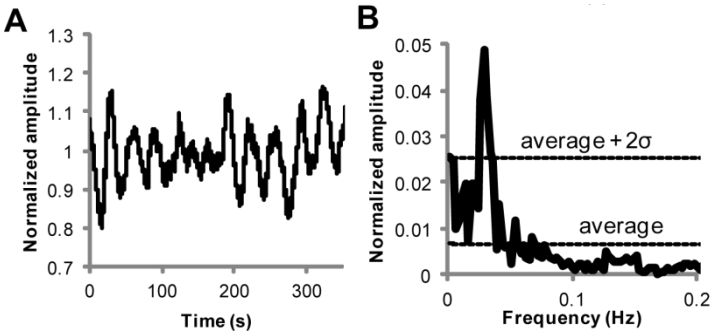

Figure 2

References

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