195

Zabbarova I[1], Salama G[1], Ikeda Y[1], Birder L[1], Kullmann F A[1], Kanai A[1]
1. *University of Pittsburgh School of Medicine*

RELAXIN TREATMENT REVERSES AGE RELATED BLADDER FIBROSIS

Hypothesis / aims of study

Age related impairment of bladder contractility has been associated with decreases in smooth muscle density and increases in collagen deposition, characteristics of fibrosis. Relaxin, a pleiotropic hormone, first identified for its role in reproduction and pregnancy, has been shown to be antifibrotic in a variety of tissues including heart and kidney [1,2]. We hypothesize that relaxin may be therapeutic in treating bladder underactivity in the elderly for which there is no effective treatment. Accordingly, the aims of this study were to test the effects of systemically administered relaxin on bladder smooth muscle function in aged and adult rats.

Study design, materials and methods

Adult (9 months old) and aged (24 months old) Fisher 344/Brown Norway F1 (F-344) male rats were used in this study. Six out of twelve rats in each age group were treated with relaxin (400 µg/kg/day) or vehicle which were infused by osmotic mini-pumps (ALZET) for 14 days, after which bladders were excised and cut from outlet to dome along the midline ventral and dorsal aspects to form two strips.

One strip was placed in a recording chamber with oxygenated Krebs solution. The base was pinned to a fixed platform and the dome connected to a tension transducer mounted on a programmable stepper motor. Bladder strips were stretched longitudinally in 500 µm increments and baseline tension allowed to stabilize (passive tension). Three field stimulation (20 Hz, 3 sec train, 0.5 ms pulse width, 15 V output) contractions were performed at each stretch to determine active force generation.

The other bladder strip was fixed in 10% PFA, embedded in paraffin, cut 5 µm thick, stained for collagen (Sigma HT251) and examined using bright field microscopy (total mag., 400X). The images were obtained at identical conditions and colour analysis was performed using the ImageJ software threshold function to discriminate collagen and smooth muscle content.

Differences between data sets were tested with Student's t-test, data represented as mean ± SD.

Results

In aged vehicle treated rats, passive tension began increasing at a smaller degree of stretch and active tension was less compared to relaxin treated aged animals (Figure 1). In contrast, there was no significant difference in passive tension while active tension increased in relaxin *versus* vehicle treated adult rats.

The collagen:tissue ratio was significantly greater in the bladders of aged vehicle *versus* relaxin treated rats (0.57 ± 0.09 *versus* 0.36 ± 0.08, respectively, $p < 0.05$), while there was no significant difference in vehicle and relaxin treated adult rat bladders (0.41 ± 0.09 *versus* 0.33 ± 0.09). Representative histological sections are shown in Figure 2.

Interpretation of results

The leftward shift in the passive length-tension curves in aged vehicle treated rats demonstrates a significant decrease in detrusor compliance due to increased collagen and decreased muscle content which was reversed by relaxin treatment. Relaxin also increased active tension in both adult and aged rats implying changes in intracellular Ca^{2+} dynamics and possible ion channel remodelling as reported in heart [3].

Concluding message

Relaxin may be therapeutically beneficial in treating age related bladder underactivity by decreasing collagen and increasing muscle content thereby improving bladder contractility.

Figure 1. Changes in bladder compliance demonstrated in length-tension curves.

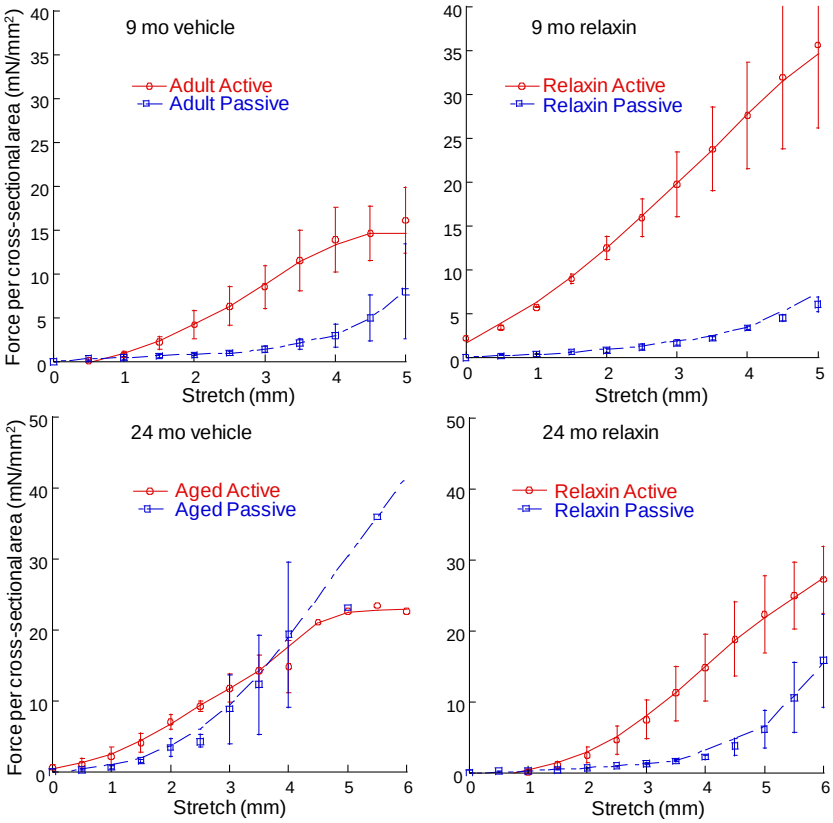
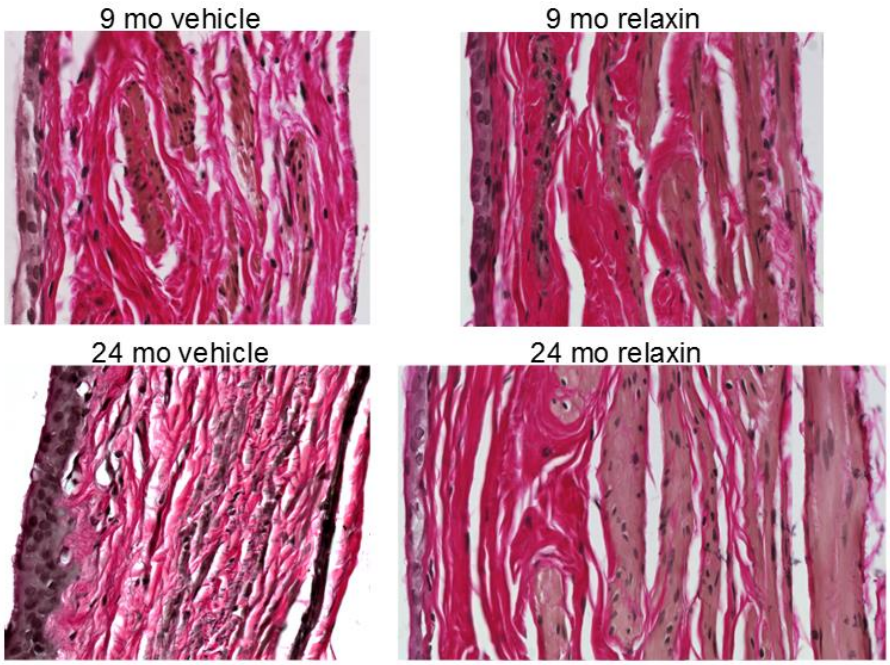
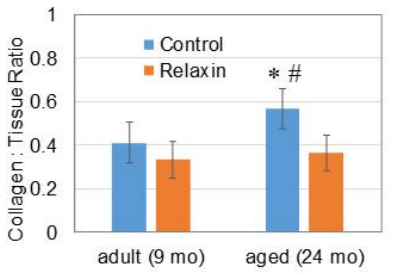


Figure 2. Collagen deposition in aged bladders and its reversal with relaxin.





* $p < 0.05$ *vs* aged relaxin treated bladders
\# $p < 0.05$ *vs* adult bladders

References

1. RG Bennett. Relaxin and its role in the development and treatment of fibrosis. Transl Res, 2009 Jul;154(1):1-6
2. A Parikh, D Patel, CF McTiernan et al. Relaxin suppresses atrial fibrillation by reversing fibrosis and myocyte hypertrophy and increasing conduction velocity and sodium current in spontaneously hypertensive rat hearts. Circ Res, 2013 Jul 19;113(3):313-21
3. BL Henry, B Gabris, Q Li et al. Relaxin suppresses atrial fibrillation in aged rats by reversing fibrosis and upregulating Na(+) channels. Heart Rhythm, 2016, Apr;13(4):983-91

Disclosures

Funding: NIH NIDDK DK093424, DK098361, DK071085 grants to A. Kanai **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** University of Pittsburgh Institutional Animal Care and Use Committee