Investigation of the basal spontaneous activity, the effect of cholinergic receptor modulators and obstruction on the pig upper urinary tract.

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Introduction

The initiation and propagation of the peristaltic wave along the different ureteric segments have not been wholly and sequentially described. Is there a specific role for each of the ureteric segments during peristalsis and whether this behavioural difference can be affected by cholinergic modulation and ureteric obstruction by stones ?

Hypothesis and aims of the study

We hypothesise that there are physiological differences in the contractile properties of the pelvicalyceal system (PCS), renal pelvis (RP), upper ureter (UU), middle ureter (MU) and lower ureteric (LU) segments and cholinergic modulation has a different effect on the various segments of the ureter during basal contraction and ureteric obstruction due to stones.

Aims and objectives

1- Investigate the basal spontaneous activity of the different segments of the upper urinary tract.

2- Describe contractile behaviour between the upper, middle and lower ureteric segments in response to cholinergic modulatory agents.

3- Develop an in vitro model of ureteric obstruction.

4- Compare the upper and lower ureteric segments basal contraction with and without intraluminal stones.

Investigate the effect of cholinergic agonist - carbachol on stone passage in the upper and lower ureteric segments.

Materials and Methods

There are three experimental settings which test each element of the hypothesis and will be referred to as EXP 1, EXP 2 and EXP 3.

- EXP 1 - investigation of the basal contractile activity of the pelvicalyceal system, renal pelvis, upper, middle and lower ureteric segments.
- EXP 2 - Investigation of the effect of cholinergic receptor modulators on the upper, middle and lower ureteric segments.
- EXP 3- The effect of obstruction on ureteric contractility; comparison between the upper and lower ureteric segments.

The ureteric segments were retrieved from freshly slaughtered pigs aged 6 to 8 months. All the ureteric segments were mounted in glass organ baths and attached to a force transducer and were perfused with carb-oxygenated Krebs solution at 36±1°C and equilibrated under a resting tension of 1.5– 2g for 60 min. The tension was monitored via isometric force transducers (Pioden Controls Ltd, UK) and a PowerLab data acquisition system using LabChart software (ADInstruments, UK).

The amplitude of contraction was measured as the force generated by the tissue for a 5-minute interval, and the frequency of contraction was measured as the number of contractile events per 5 minutes. Statistical analysis was performed using Students T-test, one-way ANOVA and followed by a post hoc Tukey test. All data are expressed as the mean ± SEM.

For EXP 1- The tissue segments were observed for basal spontaneous activity without drug stimulation.

EXP 2- Cholinergic receptor modulators, Carbachol (CCh, 10 µM) and atropine (AT) (1µM) were added to the tissue segments in the organ bath with 10-15 minutes' exposure to each drug. The effect on the basal spontaneous activity of the upper, middle and lower ureteric segments was investigated.

EXP 3- The upper and lower ureteric segments were tested with a stone made of compressed aluminium foil and 10µmol Carbachol and the other two segments were tested as control with the observation of the basal spontaneous activity and 10µmol Carbachol. The stone dimensions were uniformly round and 6mm in size.

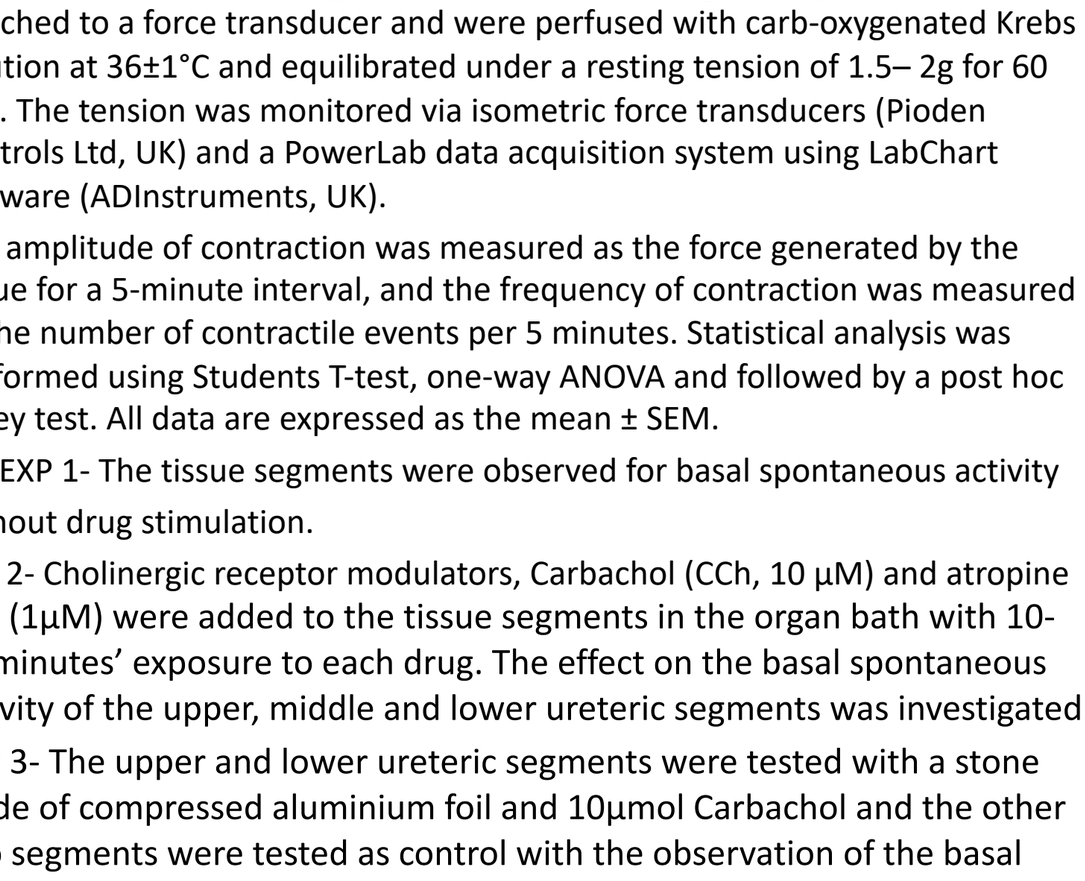

Fig.1 (a) - Bar chart demonstrating the amplitude of contraction of all the ureteric segments. The amplitude of contraction is represented in grams per milligrams of tissue. The stars sign (***) above the chart indicates a statistically significant result (*P<0.001*). The renal pelvis and the lower ureter both show a significantly higher amplitude of contraction.

Fig.1 (b) - Bar chart demonstrating the frequency of contraction of all the ureteric segments. The frequency of contraction is represented as the number of events in 5 minutes. The stars sign (***) above the chart indicates a statistically significant result (*P<0.001*). The pelvicalyceal system and the renal pelvis show a significantly higher frequency of contraction than the upper, middle and lower ureteric segments. The upper ureter demonstrates higher frequency than the middle and lower ureteric segments.

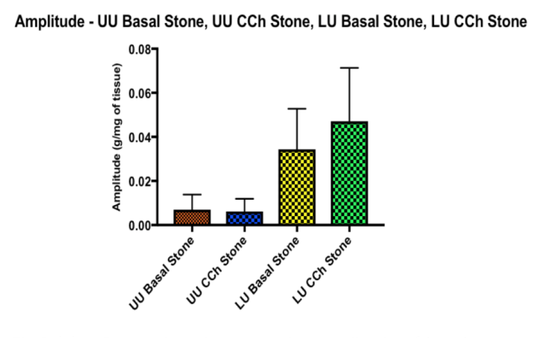

Fig.2 (a) – Comparison between the amplitude of basal contraction of the UU segments (+) stone versus the UU(+)stone and stimulated with CCh 10µmol, compared with the basal contractions of LU segments (+) stone and the amplitude of the LU(+)stone that has been stimulated with CCh 10µmol.

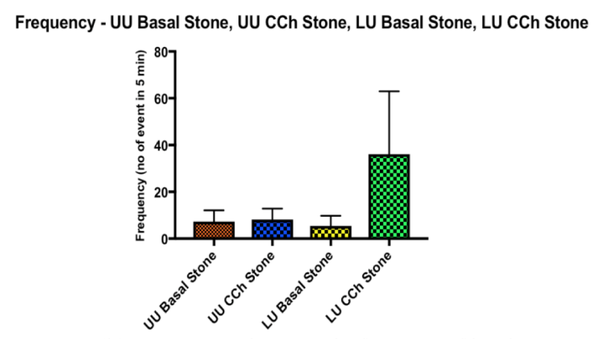

Fig. 2 (b) – Comparison between the frequency of basal contraction of the UU segments (+) stone versus UU segments with (+) stone and stimulated with CCh 10µmol, compared with basal contractions of LU segments (+) stone and the frequency of the LU segments (+) stone that has been stimulated with CCh 10µmol.

Results

EXP 1- The pelvicalyceal system (n=32), renal pelvis (n=26) and the upper ureter (n=38) have demonstrated basal spontaneous activity in 100% of the specimens.

The middle (n=18) and lower ureteric (n=26) segments demonstrated spontaneous activity in 67% and 65% respectively.

The PCS fig.1 (a) demonstrated the lowest amplitude (0.0046±0.0009) of contraction but significantly ($p<0.001$) higher frequency (38.9±2.71) than the UU, MU & LU. The Renal Pelvis demonstrated significantly ($p<0.001$) higher amplitude (0.0182±0.0046) of contraction than the PCS (0.0046±0.0009), UU (0.0065±0.0012) and MU (0.0147±0.0015).

The frequency (21.26±1.56) of contraction in the UU, fig.1 (b) was lower than the PCS and the RP but significantly higher ($p<0.001$) than the MU (5.28±0.71) and LU (5.5±0.63).

The LU had significantly ($p<0.001$) higher amplitude (0.0262±0.0021) than the PCS, RP, UU and MU but lower frequency (5.5±0.63) than the PCS (38.9±2.71 ($p<0.001$), the RP and the UU segments.

EXP 2- All the upper ureteric segments (n=23), demonstrated basal spontaneous activity (BSA) (100%). The middle (n=24) and lower ureteric segments (n=24) demonstrated a lower percentage of spontaneous activity (65%) in both. Carbachol 10µM significantly enhanced the amplitude and the frequency of the spontaneous activity in the MU ($P<0.01$) (n=13) and LU ($P<0.001$) (n=17) segments. However, it did not affect the BSA of the UU segments (n=16). Atropine 1µM did not have an effect on the UU, MU and LU segments.

EXP 3- There was an increase in amplitude of contraction, fig.2 (a) in the upper ureteric segment plus stone (0.00695 ± 0.0026 g/mgT) in comparison with the UU basal contraction minus stone (0.00536±0.0027g/mgT). The UU CCh stimulated segments minus stone (0.004963654±0.0022) and the ureteric segment plus stone and Carbachol did also have an increase in amplitude (0.00614±0.0022 g/mgT) similar to the UU segment plus stone. The ureteric stone and Carbachol each had an impact on increasing the contractile frequency of the ureteric segments fig.2 (b). The contractile frequency of the UU segment minus stone (4.29 ±2.21E/5min) has shown the lowest frequency of contraction in comparison with the basal contractility of the UU segment plus stone (7.286±1.80 E/5min). The amplitude (0.02033±0.0086 g/mgT) of the non-stimulated LU segment minus stone was the lowest among all segments. The LU segment plus stone and stimulated with Carbachol demonstrated the highest amplitude (0.04708±0.0081 g/mgT) of contraction.

Discussion

This study has shown that the pelvicalyceal system had a significantly higher frequency of contraction in comparison with the other segments. The frequency of contraction gradually decreases from the pelvicalyceal system onwards. The renal pelvis and the upper ureter demonstrated a significantly higher frequency of contraction in comparison with the middle and lower ureteric segments. There was an inverse relationship between both the frequency and amplitude of contraction, as the frequency increases in a segment, the amplitude decreases. This was observed in all segments except the renal pelvis which is the only segment that demonstrated high frequency and amplitude of contraction to propel urine through the pelviureteric junction.

This study demonstrated that there is a difference between the basal and cholinergic modulated contractile behaviour of the pig ureter in organ bath experiments. The upper, middle and lower ureteric segments have demonstrated a difference in their response to both carbachol and atropine. Carbachol had a significant effect on the amplitude and frequency of contractility of the middle and lower ureteric segments. Obstruction affected the ureteric contractility which was different between the upper and lower ureteric segments. The effect of cholinergic modulation on obstruction especially in the lower ureter may have an effect on stone passage.

Conclusions

The difference in the contractile behaviour of the pelvicalyceal system, renal pelvis, upper, middle and lower ureter may indicate that each segment plays a different role in mediating the peristaltic wave and the effect of cholinergic modulation alters the frequency and amplitude of contractility which is similar to ureteric obstruction. Understanding the difference in the basal contractile behaviour of the upper urinary tract may provide insight into the development of a targeted approach in the management of ureteric conditions. Further research is required to investigate the effect of cholinergic modulation as a means for medical expulsive therapy.

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