

## THE ROLE OF RHO-KINASE IN PHENYLEPHRINE AND 5-HT-INDUCED CONTRACTILE ACTIVITY IN THE PORCINE URETER

### Hypothesis / aims of study

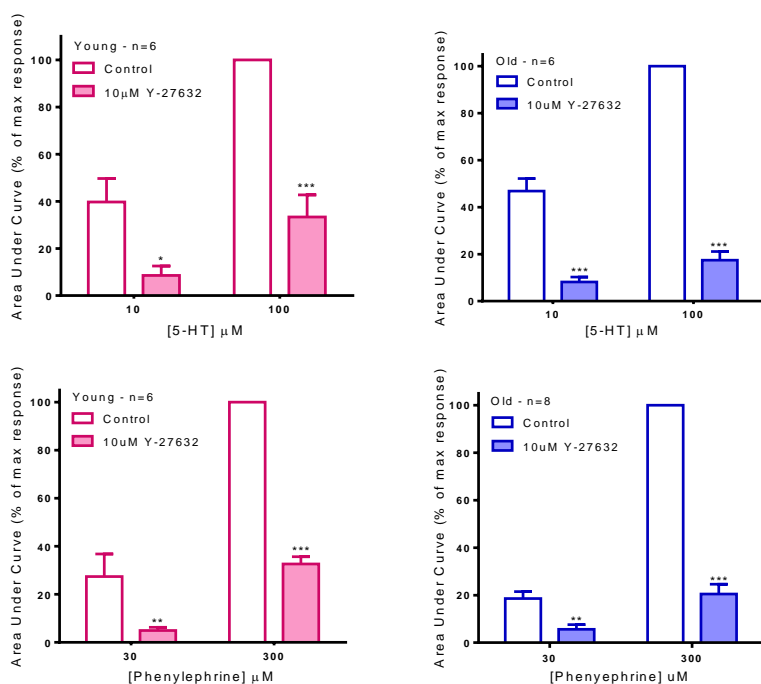
Ureteral calculus is frequently accompanied by ureteral colic, which is understood to be caused by constriction of the ureteral smooth muscle. Although the initiation of ureteral peristalsis is thought to be myogenic, the distal regions of the ureter are dependent on neurogenic mechanisms for modulation of contractility. Various modulators of the ureteral smooth muscle have been reported including  $\alpha$ -adrenergic, 5-HT and muscarinic receptor agonists [1]. Nonetheless, the precise intracellular mechanisms controlling ureteral contraction have not been clearly elucidated. In a previous study, we showed that porcine ureters from older animals generated greater contractions in response to  $\alpha$ -adrenergic receptor stimulation, and smaller contractions to 5-HT [2]. Since calcium sensitisation via the rho-kinase pathway has recently been proposed to play a significant role in smooth muscle contraction [3] our aim was to investigate the role of Rho-kinase in phenylephrine and 5-HT-induced contractile activity in isolated ureters from old and young pigs.

### Study design, materials and methods

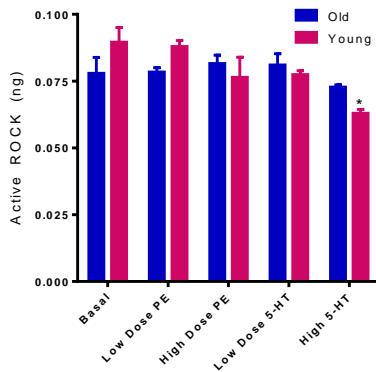
Contractile responses of isolated smooth muscle strips to EC50 (low) and maximal (high) doses of phenylephrine and 5-HT were examined in distal ureteral tissues from young (20 weeks) and old (56 weeks) pigs, in the absence and presence of Rho-kinase inhibitors, fasudil (30 $\mu$ M) or Y-27632 (10 $\mu$ M). Tissues developed spontaneous contractile activity and responses were expressed as area under the curve and normalized to tissue weight. Additionally, isolated ureteral smooth muscle strips from old and young pigs were homogenized and rho-kinase activity assay kit was utilised to measure rho-kinase activity at basal, phenylephrine- and 5-HT-induced (low and high doses) states.

### Results

The rho-kinase inhibitor fasudil (30 $\mu$ M) significantly attenuated ( $P < 0.0001$ ,  $n = 6$ ) ureteric smooth muscle contractions to low and high doses of phenylephrine by  $86.81 \pm 4.18\%$  and  $81.96 \pm 0.97\%$  vs control response in younger animals. In response to 5-HT, fasudil reduced contractions by  $83.69 \pm 2.97\%$  and  $77.67 \pm 3.16\%$  vs control response at low and high doses respectively ( $P < 0.0001$ ,  $n = 6$ ). In older animals, similar effects were observed, where contractions to low and high doses of phenylephrine were reduced by  $82.01 \pm 1.71\%$  and  $78.69 \pm 3.01\%$  vs control response ( $P < 0.0001$ ,  $n = 6$ ). Contractions to 5-HT were also reduced by  $80.16 \pm 3.43\%$  and  $79.31 \pm 3.24\%$  vs control response at low and high doses respectively ( $P < 0.0001$ ,  $n = 6$ ). The rho-kinase inhibitor Y-27632 (10 $\mu$ M) produced similar effects with both agonists in tissues from young and old animals. Active rho-kinase activity was similar in both age groups at basal and agonists-induced states.



**Figure 1** The effect of rho-kinase inhibitor Y-27632 on contractile responses to low and high doses of phenylephrine and 5-HT of porcine ureteral strips in old and young animals. Responses are represented as mean  $\pm$  SEM ( $*p < 0.05$ ,  $**p < 0.005$ ,  $***p < 0.001$  vs control) of 6 preparations for each group.



**Figure 2** Active rho-kinase levels at basal, low and high doses and phenylephrine-induced and 5HT-induced states in isolated porcine ureteral smooth muscle in old and young animals. Responses are represented as mean  $\pm$  SEM ( $*p < 0.05$  vs basal) of 3 preparation for each group.

#### Interpretation of results

This study is the first to show that rho-kinase mediates contractile responses to phenylephrine and 5-HT in isolated ureteral tissues of the pig. This effect of rho-kinase inhibitors was similar in tissues from both young and old animals despite different responses to various agonists with age. Basal levels of rho-kinase activity were similar agonist-induced levels, suggesting it is independent of  $\alpha$ -adrenoceptor or 5-HT receptor stimulation.

#### Concluding message

Rho-kinase plays an important role in ureteral contractility and may be a potential target for development of treatments aimed at relaxing ureteral smooth muscle.

#### References

1. Canda AE et al (2007) Urol Int 56:23-33
2. de Godoy MA and Rattan S (2007) Trends Pharmacol Sci 32:384-393
3. Lim et al (2013) Proceedings of ASCEPT ASM 2013 437:94

#### Disclosures

**Funding:** Bond University Pro-Vice Chancellor Scholarship **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Pig **Ethics Committee:** Bond University's Ethics Committee