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 α-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 α-adrenergic receptor stimulation, and smaller contractions to 5-HT [2]. Since calcium sensitsation 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 (30µM) or Y-27632 (10µM). 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 (30µM) significantly attenuated (P<0.0001, n=6) ureteric smooth muscle contractions to low and high doses of phenylephrine by 86.81±4.18% and 81.96±0.97% vs control response in younger animals. In response to 5-HT, fasudil reduced contractions by 83.69±2.97% and 77.67±3.16% vs control response (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±1.71% and 78.69±3.01% vs control response (P<0.0001, n=6). Contractions to 5-HT were also reduced by 80.16±3.43% and 79.31±3.24% vs control response at low and high doses respectively (P<0.0001, n=6). The rho-kinase inhibitor Y-27632 (10µM) 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 ± 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 ± 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 α-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**

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