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Acid, but not capsaicin, is an effective stimulus for ATP release in the porcine bladder mucosa

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Abstract
Hypothesis / aims of study: Urothelial ATP release is thought to play an important role in bladder afferent signaling via activation of purinergic receptors on suburothelial afferent nerves. Stretch of the bladder mucosa is a well documented stimulus for ATP release in several species, including the pig [1]. In addition, in mouse bladder, capsaicin is also an effective stimulus for ATP release, acting via stimulation of vanilloid (TRPV1) receptors [2]. While acid is an agonist at the TRPV1 receptor, specialized acid sensing ion channels (ASICs) are also present in several organ systems [3]. Our aim was to characterize the ATP release from pig bladder mucosa in response to stretch, acid and capsaicin.

Keywords
acid, porcine, but, not, capsaicin, mucosa, effective, bladder, stimulus, atp, release

Disciplines
Medicine and Health Sciences

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231

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ACID, BUT NOT CAPSAICIN, IS AN EFFECTIVE STIMULUS FOR ATP RELEASE IN THE PORCINE BLADDER MUCOSA

Hypothesis / aims of study

Urothelial ATP release is thought to play an important role in bladder afferent signaling via activation of purinergic receptors on suburothelial afferent nerves. Stretch of the bladder mucosa is a well documented stimulus for ATP release in several species, including the pig [1]. In addition, in mouse bladder, capsaicin is also an effective stimulus for ATP release, acting via stimulation of vanilloid (TRPV1) receptors [2]. While acid is an agonist at the TRPV1 receptor, specialized acid sensing ion channels (ASICs) are also present in several organ systems [3]. Our aim was to characterize the ATP release from pig bladder mucosa in response to stretch, acid and capsaicin.

Study design, materials and methods

Pig bladders were obtained from an abattoir, transported on ice, and dissected within 3 h of removal from the animal. Mucosal strips were set up in 2 ml organ baths at 1g tension, in Krebs at 37ºC gassed with carbogen. They were left to equilibrate for 1 h, with washing every 15 min. After 1 h equilibration, bath fluid was changed and a sample collected for basal ATP release at 30 s. To measure stretch-evoked ATP release, strips were then washed and immediately stretched to 150% of their length. ATP was collected at 30 s after stretch. These strips were then returned to their original 1 g tension and left for a further 1 h before the procedure was repeated. In experiments where acid (pH range 6.6 to 5.0) or capsaicin (0.1 μM to 10 μM) was tested, strips were washed and then exposed to the agonist immediately after the wash. Fluid was again collected after 30 s. Further experiments investigated the receptors involved in the acid- and capsaicin-induced ATP release. Here, paired separate strips were used for the control response and antagonist incubated response. Antagonists used were the TRPV1 inhibitor, capsazepine (10 μM), ASIC receptor blockers, amiloride (0.3 μM) and gadolinium (100 μM), which were incubated with the strips for 30 min before agonist stimulation. ATP was measured using the Bioluminescence assay (Sigma), using a T20/20n luminometer. The final result as nmoles ATP / g strip weight was plotted and analysed using GraphPad Prism 3.0. Data are expressed as median (IQR). The Wilcoxon test was used for two paired groups. The Kruskal-Wallis test was used to compare three or more groups.

Results

Stretch caused an increase in median ATP release of 45.2 (18.0 – 304) nmoles/strip weight (n=18), compared with basal ATP release of 3.40 (1.43 - 9.70) nmoles/strip weight (Fig 1A). Stretch was a reproducible stimulus, such that three repeated stretch stimuli resulted in ATP release of similar magnitude each time. Acidified Krebs was an effective stimulus to release ATP (Fig 1B). At all three pH levels tested, the response was significantly ($P<0.0001$) higher than the corresponding basal release (1.03 nmoles ATP/strip weight, n=41), but there was no significant difference between the varying pHs. Release of ATP in response to acidified Krebs was inhibited by the TRPV1 antagonist capsazepine, and the ASIC antagonists amiloride and gadolinium (Table 1). Capsaicin (0.1 μM to 10 μM) was quite ineffective as a stimulus to release ATP (n=8-12) (Fig 1C). At 1 μM capsaicin, median ATP release was 0.0 (0.0 – 8.29) nmoles / g strip weight. Furthermore, capsaicin (1 μM) failed to contract pig detrusor muscle (n=4), although rat detrusor strips studied in parallel responded with marked contractions to the same solutions.

Interpretation of results

While stretch appeared to be the most effective stimulus for ATP release, our most striking and novel finding was that acidified Krebs was able to stimulate ATP release from the pig bladder mucosa (Table 1). The acid-evoked ATP release was partially mediated via the TRPV1 channel, as evidenced from inhibition by capsazepine. Acid-evoked ATP release was also mediated by the more specialised acid sensing channels, ASICs, as demonstrated by inhibition by low concentrations of the ASIC inhibitors, amiloride (0.3 μM) and gadolinium (100 μM). Our results provide evidence that both the ASIC and TRPV1 receptors are involved in mucosal ATP release in response to acid in the pig bladder mucosa. In pig bladder mucosa, ATP release in response to the specific TRPV1 agonist, capsaicin, was almost non-existent. The lack of effect of capsaicin, to either release ATP or to contract the porcine detrusor, is quite surprising, since it has opposite effects in other species.

Concluding message

Overall, these results indicate that while stretch and acid are effective stimuli for ATP release in the porcine bladder mucosa, capsaicin is ineffective. TRPV1 receptors in the porcine bladder mucosa appear responsive to acid but not to capsaicin.The pig has been a well known model for the human bladder, but our data now suggest that it should be used with caution, particularly for TRPV1-related studies.

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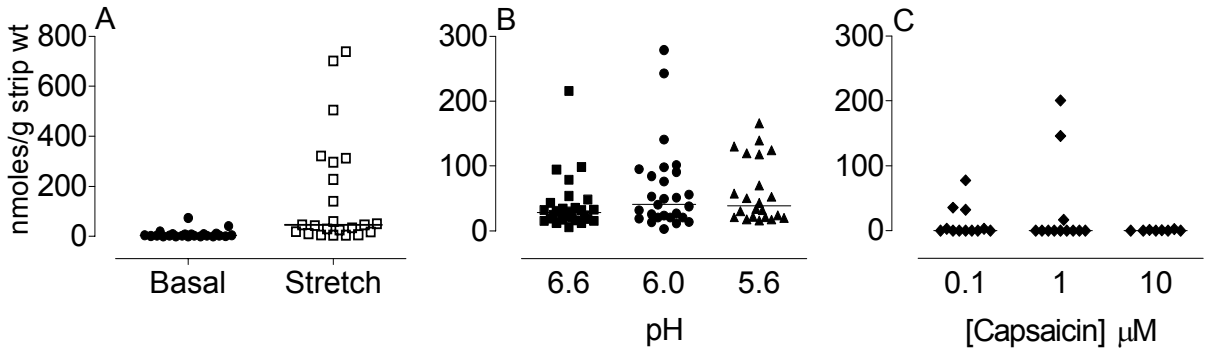

Figure 1. ATP release from pig bladder mucosal strips. A) Stretch to 150% of strip length elicited ATP release significantly higher than basal release (P<0.0001 Wilcoxon t-test; n=18 pigs). B) Acid-evoked ATP release. There was no significant difference in ATP release with varying pH (one-way ANOVA n=23-28). However at each point, the ATP release due to acid was significantly greater than the basal ATP release. C) Capsaicin failed to evoke ATP release at all three concentrations tested. Lines indicate medians.

Table 1. Acid-induced ATP release from pig bladder mucosa (nmoles/strip weight, n)

Antagonist	**pH 6.5**	**pH 6.0**	**pH 5.6**
None	28.0 (17.1 – 39.3) (28)	40.7 (20.9 – 93.2) (27)	38.6 (21.1 – 119) (22)
Amiloride	5.81 (2.44 – 13.3) * (12)	5.84 (1.77 – 25.5)* (10)	1.26 (0 – 1) (5)
Gadolinium	0.78 (0.23 - 1.51)* (8)	1.19 (0.63 - 4.97)* (10)	1.77 (0.68 - 4.46)* (10)
Capsazepine	2.22 (0.57 – 43.72) (6)	3.17 (2.10 – 13.28) * (7)	2.74 (1.51 – 20.6)* (7)

*, P<0.05 (Wilcoxon test)

Specify source of funding or grant	**NHMRC Australia**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**No**
Statement that no ethical approval was needed	**Pigs were killed at abattoir. Bladders were removed after death. Our UNSW Animal Ethics COmmittee has advised us that no ethical approval is required for such studies.**

232

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SPONTANEOUS CONTRACTILE ACTIVITY OF THE UROTHELIUM IS INCREASED BY MUSCARINIC AND PURINERGIC RECEPTOR STIMULATION

Hypothesis / aims of study

Detrusor overactivity is a condition resulting from spontaneous detrusor contractions during filling but the mechanisms involved are unclear. However, the urothelium/suburothelium (urothelium) influences the detrusor and this urothelial layer is capable of spontaneously generating its own phasic contractions. The aim of this study was to identify factors that may influence the frequency of these spontaneous urothelial contractions.

Study design, materials and methods

Isolated pig bladders obtained from the local abattoir were opened longitudinally and strips of urothelium 2cm long were taken from the anterior wall of the dome. The strips were mounted in Krebs-bicarbonate solution, maintained at 37°C and gassed with 5% CO_2 in oxygen. The frequencies of spontaneous contractions (cycles per minute), the amplitude of contraction (grams) and the resting basal tensions (grams) were recorded in the absence and presence of either carbachol (1μM), α,β-methylene ATP (10μM), phenylephrinine (1μM), clonidine (1μM), isoprenaline (1μM) or histamine (10μM).

Results

The urothelium exhibits spontaneous contractions within 10 minutes of being placed in the organ bath. This regular phasic activity was present throughout the course of the experiment (Figure 1).