310

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STRETCH, ACID, CAPSAICIN AND TACHYKININS AS STIMULI FOR ATP RELEASE FROM CULTURED PIG UROTHELIAL AND DETRUSOR MUSCLE CELLS.

Hypothesis / aims of study

ATP has been recognised as an important transmitter, which can act on afferent nerves or smooth muscle of bladder to modulate their responses to different stimuli. Previously, studies have demonstrated that ATP is released from cultured urothelial cells from cat [1], mouse [2] and human [3]. However further characterization of urothelial ATP release has been limited by the number of cells able to be cultured from these small bladder samples. The pig is considered to be an excellent model for the human bladder, but ATP release from cultured pig urothelial cells has never been investigated. The aim of the current study was to use primary cultures of pig bladder urothelium and smooth muscle, to address the hypothesis that ATP release is involved in response to stretch, acid, capsaicin and tachykinins.

Study design, materials and methods

Fresh pig bladders were obtained from the abattoir. The luminal surface of the bladder was exposed and digested with trypsin before the urothelium was scraped off and plated in a 48-well plate. Urothelial cells were cultured in RPMI supplemented with 10% FBS, antibiotics and epidermal growth factor (25 ng/ml) at 37ºC with 5% CO_2 until confluent (10-14 days). Detrusor muscle was macerated and digested with trypsin and collagenase, then pelleted and plated in a 48-well plate. Smooth muscle cells were cultured in RPMI (supplemented with 10% FBS and antibiotics) at 37ºC with 5% CO_2 until confluent (10-14 days).

Cultured cells were equilibrated with carbogenated Krebs solution. Basal ATP release was determined by exposure to fresh Krebs (pH 7.4). ATP release was stimulated by a 10 min exposure to Krebs solution containing acid (pH 6.4 and 5.5), capsaicin, neurokinin A or substance P (all at 1 μM), or to hypotonic media (50% Krebs). The supernatant was collected and released ATP measured using the Bio-luminescence ATP Assay kit (Sigma) and a luminometer (GloMax 20/20). Results are presented as the percentage of basal ATP release. Individual treatment groups were compared using a non-parametric Mann-Whitney test.

Results

The basal ATP released from cultured urothelium (19 ± 4.9 nM, n=22) was significantly higher than the basal ATP release from detrusor muscle cells (7.3 ± 2.4 nM, n=19, P = 0.028). Hypotonic media was an effective stimulus for ATP release in both urothelial (Fig 1A) and detrusor (Fig 1D) cultures.

Acidified Krebs (pH 6.4) stimulated ATP release from urothelial cell cultures (Fig 1B, P = 0.002) but not from detrusor cultures (Fig 1E, P > 0.05). Exposure to a lower pH (5.5) did not further enhance the amount of ATP released from urothelial cell cultures. ATP release from cultured urothelial cells in response to pH 6.4 was inhibited by the ASIC receptor antagonists amiloride (10 μM) and gadolinium (10 μM) (Table 1).

The TRPV1 agonist capsaicin stimulated ATP release in detrusor (Fig 1F, P = 0.004) but not urothelial (Fig 1C, P > 0.05) cell cultures. ATP release in response to capsaicin was inhibited by capsazepine (10 μM) in detrusor cells (Table 1).

Release of ATP by the tachykinins, neurokinin A and substance P, was no different from that seen to peptide vehicle alone (Table 1).

Table 1. Summary of ATP release from cultured pig urothelial and detrusor muscle cells

	Urothelium	**Detrusor**
pH 6.4	125 (114-144) % (n=12)	98 (70-118)% (n=13)
pH 6.4 + amiloride	89 (67-119) % (n=10)	ND
pH 6.4 + gadolinium	85 (64-126) % (n=10)	ND
DMSO (capsaicin vehicle)	119 (97-141) % (n=9)	99 (100-101) % (n=18)
Capsaicin	131 (116-149) % (n=9)	145 (110-147) % (n=14)
Capsaicin + capsazepine	ND	104 (77-130) % (n=10)
Peptide vehicle	118 (149-199)% (n=5)	139 (121-228) % (n=10)
Neurokinin A	127 (103-159) % (n=9)	117 (88-132) % (n=15)
Substance P	137 (110-164) % (n=8)	120 (96-165) % (n=14)

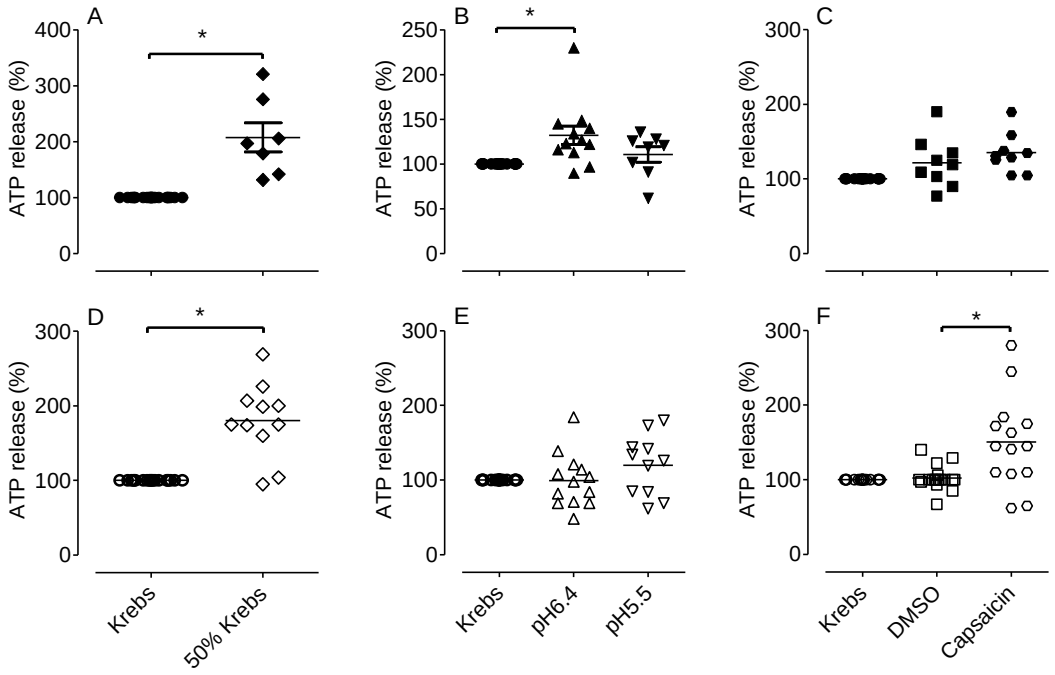

Figure 1. ATP release from cultured pig urothelial (closed symbols, A, B and C) and detrusor muscle (open symbols, D, E and F) cells. Cells were incubated with hypotonic media (A, D), acidified Krebs (B, E) and capsaicin (C, F). ATP release is presented as a percentage of basal release. *, $P < 0.05$ (Mann-Whitney test)

Interpretation of results

ATP release was increased by hypotonic media (stretch stimulus) in both urothelial and detrusor muscle cell cultures. ATP release was stimulated by exposure to acidified Krebs in urothelial cells (but not muscle cells), and was inhibited by the ASIC receptor antagonists, amiloride and gadolinium. Acid-induced ATP release in the bladder mucosa may play an important role in signaling of bladder afferent sensations through ASIC receptors in the urothelium.

It was interesting that in the pig bladder the detrusor muscle cells but not the urothelial cells responded to capsaicin. This is unexpected, as murine urothelial cells have previously been shown to release ATP in response to capsaicin [2].

ATP release was not induced by the tachykinins, neurokinin A and substance P, in either urothelial or detrusor muscle cultures; this indicates that the tachykinins are not strong stimuli for ATP release in these cells.

Concluding message

This study has for the first time characterised ATP release from primary cultures of pig urothelium and detrusor muscle. The advantage of the pig as an animal model is that large numbers of cultured cells can easily be generated, which make it ideal for investigating the stimuli for release of ATP and other mediators from the bladder urothelium. This is particularly important given the increasing evidence that ATP released from the bladder urothelium is important for the modulation of bladder functions.

References

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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 local Animal Ethics Commitee advised that ethical approval was not required for these studies.**