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ATP-ENGENDERED ATP RELEASE IN THE RAT URINARY BLADDER

Hypothesis / aims of study

It is well recognised that ATP plays a role as a neurotransmitter and as a neuromodulator in the nervous system (Burnstock, 1986). Recent findings have shown that stretch deformation of epithelial cells leads to release of ATP (Ferguson *et al.,* 1997). How the ATP is released remains to be determined. It appears likely that it involves a special ATP transport mechanism distinct from exocytic release from nerves. This system is thought to respond to physiological stimuli. However, ATP may also act as a nociceptive mediator. The aims of this study were 1) to study the effect of low and high dose ATP on release of ATP from the rat urothelium, and to determine whether age affected this release using different aged rats 2) to determine whether P2X receptor antagonists blocked the effect of ATP on the rat bladder and 3) to study the effect of selective P2X agonists on ATP release from the rat bladder.

Study design, materials and methods

Female Wistar rat bladders aged 16-18 days (55-100 g) (neonates), 16-18 week (300-800 g) (adult) and 18 month (1400-3000 g) (old) were removed and placed intact in 50 ml of oxygenated cold Krebs' solution and the ATP levels were allowed to equilibrate for a period of at 30 minutes before any drug addition. The pots were maintained at 37^{o}C in a water bath. Known concentrations of ATP and other drugs (Suramin, PPADS, α,β-Methylene ATP, ADP, 2-mSATP, and UTP) were added to the pots at appropriate concentrations, and the solution sampled at regular time intervals (90 minute s) for ATP. ATP was assayed using the luciferin-luciferase reaction, which utilises bioluminescence (Sigma, Poole, U.K.). The light produced is measured by a Turner TD-20e luminometer, and there is a linear relationship between the logarithm of generated light intensity and [ATP]. After all experiments were performed the bladders were gently blotted and weighed.

The initial rate of apparent ATP hydrolysis by the urothelium was calculated for 30 minutes immediately after ATP addition by linear regression of a line plotting [ATP] against time. This gave values with units of $\mu M min^{-1} g^{-1}$ (multiplying by pot volume and dividing by individual tissue sample masses). Results were expressed as means ± S.E.M. and the significance of differences in tissue response pre-drug addition and post-drug addition were compared.

Results

Addition of 3 μM ATP to the intact bladder resulted in an immediate rise in [ATP], with a peak at 15 minutes (Figure 1). The maximum [ATP] recorded represents a 2-fold increase from the basal level of [ATP] recorded before addition of ATP. Addition of 30 μM ATP also resulted in 2.5 fold increase in [ATP]. Most of this release is from the urothelium.

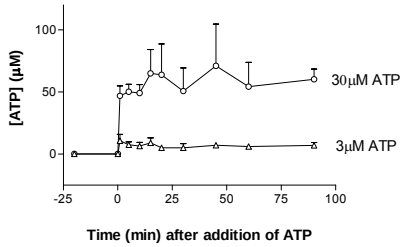

Figure 1 ATP induced ATP release in adult rat urinary bladders.

Several factors affected ATP release. In older rat bladders there was less ATP production and this was significant when compared to the adult bladders (P<0.0001). However there was no significant difference between adult and neonatal bladders. Time did not affect ATP release, whereas lack of glucose did in that less ATP was produced when there was no glucose in the solution. P2 antagonists, Suramin and 100 μM PPADS both reduced ATP release from the bladder, whereas P2 agonists showed an variable profile of activity:

ATP>>ADP>2methythio ATP (2-mSATP)>α,β-Methylene ATP (α,β-MeATP)>UTP

Interpretation of results

The large amounts of ATP released by the urinary bladders would appear to be non-physiological, though it was sustained over a prolonged period of time. ATP release is age-dependent, glucose-dependent, and ATP concentration-dependent. P2 receptor blockers and selective agonists were used to determine whether these effects were possibly mediated through P2X receptors, particularly $P2X_3$ receptors. The evidence would suggest that P2X receptors may be involved but not necessarily $P2X_3$ receptors.

Concluding message

P2 antagonists did block ATP release. However, the most potent agonist of ATP release, other than ATP, was ADP. Unexpectantly α,β-Methylene ATP (a significant agonist of $P2X_3$ receptors) resulted in a small release of ATP. One possible explanation is that there may be an ATP-ADP exchange system present, similar to that in mitochondria. At present, there is no direct evidence to link this ATP release with the polarised release of ATP, as shown by Ferguson *et al,* (1997). They showed that ATP was released in response to stretch on the basolateral side of the urothelium. ATP on the urinary side of the urothelium may have a separate role, which we do not yet understand, and maybe involved more specifically in nociceptive signalling. It is clear that this whole area needs extensive further investigation.

References

Burnstock, 1986 P2 purinoreceptors: Historical perspective and classification. In: *P2 purinoceptors: localisation, function and transduction mechanisms.* ed. Chadwick, D.J. and Goode, J.A. pp. 1-29. Chichester: Wiley.; Ferguson *et al.,* 1997 ATP is released from rabbit urinary bladder epithelial cells by hydrostatic pressure changes a possible sensory mechanism? *Journal of Physiology, London*, **505**, 503-511.