VESICULAR STORAGE AND RELEASE OF ATP IN A RAT PROXIMAL TUBULE CELL LINE

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Accumulating evidence indicates that extracellular nucleotides can influence solute and water transport in a variety of epithelia. Stimulation of apical P2 receptors in the renal proximal tubule inhibits bicarbonate reabsorption (Bailey, 2004), and nanomolar concentrations of ATP have been detected in proximal tubular fluid both in vitro (Wilson et al. 1999) and in vivo (Shirley et al. 2003). The source of ATP in tubular fluid remains speculative. The present study has explored the possibility that ATP is stored in proximal tubular cells as vesicles prior to its release. The antimalarial drug quinacrine has a high affinity for ATP, which, upon binding, fluoresces under exposure to UV light. These properties make it suitable for labelling intracellular ATP. In the present study, quinacrine was used to visualize potential intracellular stores of ATP in an immortalized cell line (WKPT-0293) derived from the S1 segment of the proximal tubule of Wistar-Kyoto rats (Woost et al. 1996).

Cells were grown to confluence (2-3 days) and were rinsed in Dulbecco-phosphate buffered saline (D-PBS). Cultures were then incubated with 1 ml of either isotonic (280 mosmol/kg H2O) or hypotonic (140 mosmol/kg H2O) D-PBS for 15 min at 37°C. During incubation, samples of the medium were taken at 1, 5 and 15 min, immediately centrifuged, and the supernatant snap frozen in liquid nitrogen. The luciferin-luciferase assay was used to determine ATP concentration. Immediately after the 15 min incubation period, the same cells were incubated with quinacrine (2mmol/l) in isotonic D-PBS, rinsed with isotonic D-PBS, then photographed.

Intense granular fluorescence was observed in cells maintained under isotonic conditions. In cells that had been subjected to hypotonic exposure, granular fluorescence was profoundly reduced. Extracellular ATP concentrations (mean ± SEM) under isotonic conditions were 4.0 ± 1.5, 4.6 ± 1.3 and 4.4 ± 1.5 nmol/l (n = 7) at 1, 5 and 15 min, respectively. Corresponding values during hypotonic exposure were 15.6 ± 6.0, 19.1 ± 5.8 and 25.4 ± 7.2 nmol/l (n = 7).

The rise in extracellular ATP and the reduction in granular fluorescence during hypotonic stimulation support the proposition that the fluorescence observed during incubation with quinacrine was attributable to ATP. The data suggest that proximal tubular epithelial cells may store ATP in the form of vesicles which, upon stimulation, can be released into the extracellular milieu.


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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PROGRAMMING OF NEPHRON NUMBER IN ADULT SHEEP BY MATERNAL NUTRIENT RESTRICTION IN EARLY GESTATION

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Maternal nutritional restriction during early gestation results in increased renal glucocorticoid sensitivity at term (Whorwood et al. 2001) and higher blood pressure in early adulthood (Gopalakrishnan et al. 2004). These adaptations may represent compensatory mechanisms as a consequence of decreased nephron number during nephrogenesis in utero (Wintour et al. 2003). In this study, it was hypothesised that maternal nutrient restriction, at the time of kidney development, would permanently reduce nephron number.

Ewes were randomly allocated to either control (C; 8.0 MJ/day) or nutrient restricted (NR; 4.0 MJ/day) diet from day 1 to 95 of gestation, with feed provision of 100% metabolisable energy requirements thereafter (Gopalakrishnan et al. 2004). Offspring delivered spontaneously at term, were ewe reared until weaning and, thereafter, fed at pasture until three years of age when they were humanely euthanased (intravenous sodium pentobarbitone, 170mg/kg) before kidney sampling. All animal procedures were performed under the UK Animals (Scientific Procedures) Act, 1986. Total renal nephron number was determined using an adaptation of an acid-hydrolysis method, renal glucocorticoid receptor abundance by RT-PCR and renal 11β-HSD2 enzyme activity by radiometric assay.

Maternal nutrient restriction in early gestation, followed by restoration of nutrient intake in mid-late gestation, resulted in a lower total nephron number in offspring at 3 years of age (C (n=7): 998 [807-1088]; NR (n=6): 350 [271-372] x 103 nephrons/kidney; P<0.05, median [interquartile ranges], Mann Whitney U-test) Although glucocorticoid receptor abundance was unaffected (C: 33.9 [27.3-54.8]; NR: 37.3 [29.7-46.4] mRNA:18S rRNA; NS), the enzyme activity of 11β-HSD2 was reduced (C: 0.54 [0.48-0.57]; NR: 0.44 [0.39-0.53] pmol/min/mg protein; P<0.05).

In conclusion, persistent renal effects, with a decrease in nephron number and increase in the potential sensitivity to glucocorticoids, are programmed in sheep by maternal nutrition in early fetal development. This may contribute, in part, to raised blood pressure in later life.


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INDIRECT EVIDENCE FOR REGULATION OF SODIUM REABSORPTION IN SODIUM-RESTRICTED RATS BY AN ATP-GATED HETEROMERIC P2X RECEPTOR

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Epithelial Na⁺ channels (ENaC) play a crucial role in Na⁺ transport in the distal nephron, where they co-localize with ATP-gated P2 receptors. Evidence suggests that ATP may indirectly regulate ENaC by first activating luminal P2 receptors (Unwin et al. 2003). Previously, using in vivo microperfusion of the rat collecting duct (CD), 22Na⁺ urinary recovery was measured to assess Na⁺ reabsorption following P2 receptor activation. Although the non-hydrolysable ATP analogue ATPγS had no effect in control animals, ATPγS inhibited Na⁺ reabsorption in Na⁺-restricted rats (Shirley et al. 2001). Other non-hydrolysable analogues (2meSADP, BzATP and Ap₄A) had no measurable effect on 22Na⁺ uptake (unpublished observation).

In the present study, we have investigated the selectivity and potency of this series of ATP analogues on recombinant ionotropic P2X and metabotropic P2Y receptors found in rat CD, expressed in Xenopus oocytes and studied under voltage-clamp conditions, in order to compare in vitro agonist profiles with our in vivo findings in Na⁺-restricted rats. Immunohistochemical and RT-PCR studies have identified P2Y₂ and P2Y₄ receptors, homeric P2X₄, P2X₅ and P2X₆ receptors, and the possibility of heteromeric P2X₄/5, P2X₄/6 and P2X₅/6 receptors in rat CD (Turner et al. 2003). For all the above recombinant P2 receptors, agonist concentration-response curves (100nM-1mM) were constructed. ATPγS was found to be a full agonist equipotent with ATP at all receptors tested. BzATP was found to be a partial agonist at P2X₃ and P2X₄/5. Ap₄A was a full agonist at P2Y₂ and P2Y₄ receptors and a partial agonist equipotent with ATP at P2X₄ and P2X₅. 2meSADP (1mM) was inactive at all the receptors tested.

In conclusion, given the agonist activity of BzATP and Ap₄A in vitro, and assuming a lack of function of homomeric P2X₆, it is unlikely that P2Y₂, P2Y₄, P2X₄, P2X₅, P2X₆ and P2X₄/5 receptors play a role in the inhibition of Na⁺ reabsorption in sodium-restricted rats. In contrast, a role may exist for the heteromeric P2X₄/6 and/or P2X₅/6 receptors. Interestingly, Na⁺ and Ca²⁺ influx through the P2X₄/6 (not P2X₅/6) ion channel, following its activation by extracellular ATP, results in the down-regulation of ENaC (Wildman et al. 2003). Our findings suggest a potential regulatory role for P2X₄/6 in Na⁺-restricted rats, where increased expression of ENaC in the distal nephron would be expected.

Shirley DG et.al. (2001) J. Am. Soc. Nephrol. 12, A3001
Turner CM et.al. (2003) Cells Tiss. Organs. 175, 105-117

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