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 H_2O) or hypotonic (140 mosmol/kg H_2O) 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.

Bailey MA (2004). Am J Physiol Renal Physiol 287 F789-796.

Shirley DG et al. (2003). J Am Soc Nephrol 14, SU-PO 072.

Wilson PD et al. (1999). J Am Soc Nephrol 10, 218-229.

Woost PG et al.(1996). Kidney Int 50, 125-134.

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

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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), $^{22}Na^+$ 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 Ap_4A) had no measurable effect on $^{22}Na^+$ 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 $P2Y_2$ and $P2Y_4$ receptors, homomeric $P2X_4$, $P2X_5$ and $P2X_6$ receptors, and the possibility of heteromeric $P2X_{4/5}$, $P2X_{4/6}$ and $P2X_{5/6}$ 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 $P2X_5$ and $P2X_{4/5}$. Ap_4A was a full agonist at $P2Y_2$ and $P2Y_4$ receptors and a partial agonist equipotent with ATP at $P2X_4$ and $P2X_5$. 2meSADP (1mM) was inactive at all the receptors tested.

In conclusion, given the agonist activity of BzATP and Ap_4A in vitro, and assuming a lack of function of homomeric $P2X_6$, it is unlikely that $P2Y_2$, $P2Y_4$, $P2X_4$, $P2X_5$, $P2X_6$ and $P2X_{4/5}$ receptors play a role in the inhibition of Na^+ reabsorption in sodium-restricted rats. In contrast, a role may exist for the heteromeric $P2X_{4/6}$ and/or $P2X_{5/6}$ receptors. Interestingly, Na^+ and Ca^{2+} influx through the $P2X_{4/6}$ (not $P2X_{5/6}$) 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 $P2X_{4/6}$ in Na^+-restricted rats, where increased expression of ENaC in the distal nephron would be expected.

Unwin RJ et.al. (2003) News Physiol. Sci. 18, 237-241

Shirley DG et.al. (2001) J. Am. Soc. Nephrol. 12, A3001

Turner CM et.al. (2003) Cells Tiss. Organs. 175, 105-117

Wildman SS et.al. (2003) J. Am. Soc. Nephrol. 14, SA-FC165

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