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Short communication

Basolateral, but not apical, ATP inhibits vasopressin action in rat inner medullary collecting duct

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Abstract

Previous studies have shown that basolateral ATP inhibits vasopressin action in the renal collecting tubule. Although there is evidence for an apical $P2Y_2$ receptor in this tubule segment, it is not known whether apical ATP has similar effects. In the rat inner medullary collecting duct basolateral, but not apical, ATP (0.1–100 μM) reversibly inhibited vasopressin-induced increases in water permeability with an IC_{50} of 1.09 μM. Basolateral UTP, but not ADP, α,β-methylene-ATP or 2-methylthio-ATP also inhibited vasopressin action. It is concluded that basolateral but not apical $P2Y_2$ receptors inhibit vasopressin action in the collecting duct. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Purinoceptor; Vasopressin; Collecting tubule; Water permeability

1. Introduction

Extracellular nucleotides are now considered to be important autocrine and paracrine regulators of various physiological processes (Burnstock and Williams, 2000). The kidney is richly endowed with purinoceptors of both the metabotropic G protein-coupled P2Y class as well as the ionotropic P2X class (Schwiebert and Kishore, 2001). Functional and mRNA distribution studies have shown that purinoceptors are present in all the nephron segments (Bailey et al., 2000) suggesting an important role for extracellular nucleotides in the modulation of tubular transport. In the inner medullary collecting tubule (Kishore et al., 1995) and other collecting tubule segments (Rouse et al., 1994) basolateral ATP acting via the $P2Y_2$ receptor has been shown to inhibit the increase in water permeability caused by vasopressin. However, a recent study has shown the $P2Y_2$ receptors are located on both the basolateral and apical membrane of this tubule segment (Kishore et al., 2000). Since ATP and its metabolites are freely filtered by the glomerulus and are released across the apical membrane into the urine by tubule cells (Schwiebert, 2001), apical $P2Y_2$ receptors have the potential to play an important role in water transport in the collecting tubule. Accordingly, in the present study the effects of apical and basolateral ATP on vasopressin-induced osmotic water permeability in the rat inner medullary collecting duct were examined.

2. Materials and methods

Tubules were dissected from the lower one third of the inner medulla of kidneys removed from anesthetized male Sprague Dawley rats (250–300 g) and perfused at 37 °C as previously described in detail (Edwards and Brooks, 2001). All experiments were approved by our Institutional Animal Care and Use Committee. Tubules were bathed with a hypertonic solution consisting of (in mM) 210 NaCl, 25 $NaHCO_3$, 5 KCl, 1.5 $CaCl_2$, 1.2 $MgSO_4$, 2.3 Na_2HPO_4, 8 glucose, 5 alanine and 5 *N*-2-hydroxyethylpiperazine-*N*′-ethansulfonic acid and perfused with an isotonic solution that was identical to the bath except that it contained less NaCl (135 mM) and dialyzed [^{3}H]inulin (PerkinElmer Life Sciences, Boston, MA) which served as a volume marker. Solutions were gassed with 95% O_2/5% CO_2 to pH 7.4 and the bath was continuosly exchanged at 0.5 ml/min. Timed collections of perfusate were made using a constant-volume pipette, and osmotic water permeability (μm/s) was calculated according to Al-Zahid et al. (1977). At least three collections were made during each experimental period and were averaged to give a single value of water permeability. A similar protocol was followed for all experiments. Approximately 30 min after reaching 37°, collections for basal water permeability were made. The bath was then changed

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to one containing 10 pM vasopressin. About 30–40 min later, collections were made to determine vasopressin-dependent water permeability. The bath was then changed to one containing vasopressin and the purinergic compound to be tested. In some experiments, the purinergic compounds were added to the perfusate instead of the bath. Fifteen minutes later, collections were resumed.

All reagents were obtained from Sigma (St. Louis, MO). Statistical analysis was performed with Student's *t*-test for paired comparisons or by analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons.

3. Results

Addition of 10 pM vasopressin to the bath increased osmotic water permeability of inner medullary collecting ducts from 137 ± 10.9 to 978 ± 165 μm/s (Fig. 1). In the presence of vasopressin, addition of 10 μM ATP to the basolateral side of the tubule markedly and reversibly reduced osmotic water permeability to 210 ± 79 um/s ($P < 0.001$). In identical experiments, addition of 10 μM ATP to the apical side of the tubule had no effect on vasopressin-induced water permeability. Basolateral ATP (0.1–100 μM) produced a concentration-dependent inhibition of vasopressin-induced water permeability yielding an IC_{50} of 1.09 ± 0.36 μM (Fig. 1). Over this same concentration range, apically applied ATP failed to affect vasopressin-stimulated osmotic water permeability.

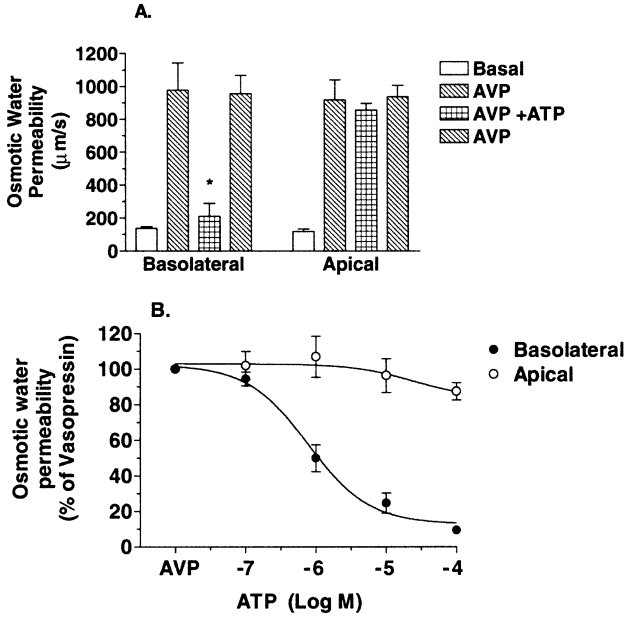

Fig. 1. Effects of basolateral and apical ATP on vasopressin-stimulated osmotic water permeability. (A) Tubules were sequentially exposed to no vasopressin (basal), 10 pM vasopressin (VP), vasopressin and basolateral or apical ATP 10 μM (VP + ATP) and again to vasopressin alone. Each bar is the mean ± SEM of five tubules. * Significantly different ($P < 0.001$) from both vasopressin periods. (B) Concentration–response to basolateral ($n = 4$) or apical ($n = 4$) ATP. Results are expressed as a percentage of water permeability in the presence of 10 pM vasopressin which was 1068 ± 100.5 μm/s.

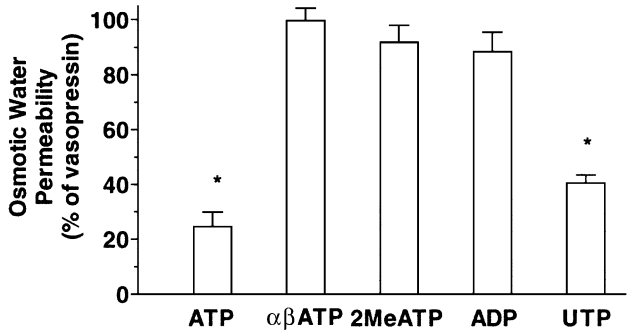

Fig. 2. Effect of basolateral purinergic and pyrimidinergic agonists on vasopressin-stimulated osmotic water permeability. Tubules were exposed to vasopressin (10 pM) follwed by vasopressin and 10 μM of ATP, α,β-methylene-ATP (α,β-ATP), 2-methylthio-ATP (2MeATP), ADP or UTP. Each bar is the mean ± SEM of four to seven tubules. Results are expressed as a percentage of vasopressin-stimulated water permeability which was 930 ± 66 μm/s. * Significantly different ($P < 0.003$) compared to vasopressin alone.

In additon to ATP, a number of other nucleotides were tested for their ability to inhibit vasopressin-stimulated osmotic water permeability when applied to the basolateral surface of the tubule. Of the nucleotides tested at 10 μM, only UTP caused a significant inhibition of vasopressin action (Fig. 2). ADP, α,β-methylene ATP and 2-methylthio-ATP were without effect.

4. Discussion

Functional studies have demonstrated that basolateral ATP inhibits the hydrosmotic response to vasopressin in the collecting tubule via $P2Y_2$ receptors (Rouse et al., 1994; Kishore et al., 1995). A recent immunohistochemical study has shown that $P2Y_2$ receptors are also present on the apical membrane of rat inner medullary collecting duct cells at a higher density than on the basolateral membrane (Kishore et al., 2000). In renal tubule cells, ATP and its metabolites appear to be preferentially released into the tubule lumen and also appear in the urine (Schwiebert, 2001). Therefore, apical purinoceptors may play an important role in the regulation of salt and water transport in the collecting tubule. Apical effects of ATP on vasopressin action in the collecting tubule have not been reported previously. Therefore, the purpose of this study was to compare the effects of apical and basolateral ATP on the response to vasopressin in the rat inner medullary collecting tubule.

In confirmation of previous reports (Rouse et al., 1994; Kishore et al., 1995), basolateral ATP reversibly inhibited vasopressin-induced increases in osmotic water permeability. The similar effect of UTP and the lack of effect of ADP, α,β-methylene ATP and 2-methylthio-ATP are consistent with activation of $P2Y_2$ receptors (Burnstock and Williams, 2000). In contrast to the effects of basolateral ATP, apical

administration of ATP under the identical conditions had no effect on vasopressin-stimulated water transport. These results were surprising in view of the histochemical demonstration of apical $P2Y_2$ receptors in the rat inner medullary collecting duct (Kishore et al., 2000). The reasons for these differing results are not clear but may relate to the heterogeneity of the collecting duct. For example, there are at least three morphological and functional distinct segments of the inner medullary collecting duct, $IMCD_{1-3}$ (Sands and Knepper, 1987). The present study utilized the terminal portion of the inner medullary collecting duct or $IMCD_3$. It is not clear which segment was used to perform the immunohistochemical studies (Kishore et al., 2000).

While apical $P2Y_2$ receptors may not be involved in the regulation of vasopressin action in the terminal inner medullary collecting duct, there is ample evidence that apical and basolateral purinoceptors do regulate salt transport in the collecting tubule. However, there are both species differences as well as differences within the various segments of the collecting tubule system to $P2Y_2$ receptor stimulation. For example, apical, and to a lesser extent, basolateral $P2Y_2$ agonists, increase intracellular calcium in the perfused mouse cortical collecting tubule (Deetjen et al., 2000). However, in the same tubule segment from the rabbit, basolateral but not apical $P2Y_2$ agonists increase intracellular calcium (Deetjen et al., 2000). In a mouse cortical collecting tubule cell line, apical and basolateral $P2Y_2$ receptors inhibit sodium absorption and stimulate chloride secretion (Cuffe et al., 2000) while in a mouse cell line originating from the inner medullary collecting duct, apical but not basolateral $P2Y_2$ receptors inhibit sodium absorption and stimulate chloride secretion (McCoy et al., 1999). These studies point to the complexity of purinergic receptor distribution and biology in the kidney, the importance of which is only beginning to be understood (Schwiebert, 2001; Schwiebert and Kishore, 2001).

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