ONLINE SUPPLEMENT:

ADENOSINE ACTIVATES A2b RECEPTORS AND ENHANCES CHLORIDE SECRETION IN KIDNEY INNER MEDULLARY COLLECTING DUCT CELLS

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Short Title: Adenosine Induces Chloride Secretion in IMCD Cells

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EXPANDED METHODS

Ussing Chamber Measurements

Cell sheets were mounted between the Lucite half chambers of the Ussing chamber apparatus and bathed in Krebs-Henseleit solution (in mmol/L: 140 NaCl, 25 NaHCO₃, 5 KCl, 5 glucose, 2 CaCl₂, and 1 MgCl₂) and gassed with a mixture of 95% O₂ and 5% CO₂. Transepithelial voltage (Vₜₑ) across cell sheets was clamped to 0 mV, and a set voltage pulse of 1 mV was applied across cell sheets for 200 ms every 20 s. The short-circuit current (Iₛₑ) and transepithelial resistance (Rₜₑ) across cell sheets were continuously recorded using Acquire and Analyze Software (Physiological Instruments, San Diego, CA).

Western Blot Analysis

Seventy micrograms of protein from mIMCD-K2 lysates were resolved by 12% SDS-PAGE and transferred to PVDF membranes (BioRad, Hercules, CA). The membranes were immunostained with 1 µg/ml of anti-A2b receptor antibody (Alpha Diagnostics International, San Antonio, TX) for detection of A2b receptor immunoreactive protein. Membranes were incubated with horseradish peroxidase conjugated-secondary antibody (Amersham Biosciences, Piscataway, NH) and processed, as described previously.

cAMP Assay

Intracellular cAMP levels were measured using the Parameter cAMP ELISA assay kit (R & D Systems, Minneapolis, MN) after the apical side of cell sheets were treated with 10⁻⁵ mol/L adenosine or vehicle control. Cell sheets containing ~ 10⁶ cells were lysed, and cAMP concentrations were determined per manufacturer instructions.
REFERENCES


**Table S1.** Affinity of adenosine receptor agonists and antagonists for the four classes of adenosine receptors.

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Figure S1. Sidedness of adenosine-induced $I_{sc}$ across mIMCD-K2 cell sheets. Adenosine was added to either the apical or the basal side of paired cell sheets mounted in an Ussing chamber. $\Delta I_{sc}$, change in $I_{sc}$. Values are mean ± SE ($n = 13$ filters). *$P < 0.05$. 
Figure S2. Effect of apical adenosine treatment on intracellular cAMP levels in mIMCD-K2 cells. Cell sheets treated with apical adenosine $10^{-5}$ mol/L (Ado) had significantly higher intracellular cAMP levels than cells treated with vehicle control. Values are mean ± SE (n = 6 cell sheets). *$P < 0.05$. 

**Figure S2.**
Figure S3

Estimated surface area of mouse IMCD

Single collecting duct:
Internal diameter = 20 µm [2]
Length = 5.3 mm = 5300 µm [3]

\[2\pi rh = 2 \times \pi \times 10 \text{ } \mu\text{m} \times 5300 \text{ } \mu\text{m} = 333,142.8 \text{ } \mu\text{m}^2 = 0.3331428 \text{ } \text{mm}^2\]

The number of collecting ducts per kidney in a rodent model = 10,000 [4]

Surface area of mouse IMCD:

\[0.3331428 \text{ } \text{mm}^2 \times 10,000 \text{ } \text{collecting ducts} \times 2 \text{ } \text{kidneys} = 6662.856 \text{ } \text{mm}^2\]

= 66.63 cm²

Estimated Cl⁻ flux across mouse IMCD epithelium per day

Typical \(I_{sc}\) increase with \(10^{-5}\) mol/L adenosine = 1.2 µA/cm²
\(I_{sc}\) increase across mouse IMCD epithelium = 1.2 µA/cm² x 66.63 cm² = 80 µA
Using Faraday's constant, 26 µA = 1 µEq/hr, 80 µA = 3.07 µEq/hr

3.07 µEq/hr x 24 hrs = 74 µEq of Cl⁻ secreted across mouse IMCD epithelium

Estimated Effect on Daily Fractional Excretion of Chloride (FE_{Cl⁻})

Filtered Cl⁻ load in one day:
Serum [Cl⁻] = 110 mEq/L = 110 µEq/mL
Creatinine clearance in a C57BL/6 mouse under high salt diet = 179 µl/min [5]
110 µEq/mL x 1 mL/1000 µL x 179 µL/min x 1440 min = 28,354 µEq Cl⁻ filtered

\[\text{FE}_{\text{Cl}^-} = \frac{74 \text{ } \mu\text{Eq of Cl}^- \text{ secreted}}{28,354 \text{ } \mu\text{Eq Cl}^- \text{ filtered}} = 0.261\%\]

Figure S3. Mathematical modeling of the magnitude of adenosine-stimulated chloride flux extrapolated to the in vivo setting. Calculations listed are for the estimated adenosine-stimulated chloride flux across mouse IMCD epithelium and the estimated effect of adenosine-stimulated chloride secretion in the IMCD on daily fractional excretion of chloride (FE_{Cl⁻}).