359

Negoro H[1], Liou L[2], Spray D[3], Thi M[3], Ogawa O[4], Suadicani S[3]
1. *Kyoto University Graduate School of Medicine, and Albert Einstein College of Medicine,* ***2.*** *Cambridge Health Alliance,* ***3.*** *Albert Einstein College of Medicine,* ***4.*** *Kyoto University Graduate School of Medicine*

PANNEXIN 1 CHANNELS PARTICIPATE IN UROTHELIAL ATP RELEASE AND SIGNALING.

Hypothesis / aims of study

Urothelial cells respond to bladder distension with ATP release, and ATP signaling within the bladder and to the CNS has been proposed to communicate the degree of bladder fullness and modulate detrusor activity, which are essential for proper micturition. Urothelial ATP release occurs through both vesicular and non-vesicular mechanisms that involve activation of various channels and receptors, including P2 receptors (P2YR and P2XR).[1] Pannexin 1 (Panx1) channels are permeable to large molecules and have been shown to provide a conduit for mechanically-induced ATP efflux and to open upon $P2X_7R$ activation, thereby providing a mechanism for ATP-induced ATP release in various cell types.[2] In this study we investigated whether Panx1 channels participate in urothelial ATP release and signaling by providing a path for stretch- and P2R-induced ATP release.

Study design, materials and methods

1) Expression of Panx1 and $P2X_7R$ was determined in rat urothelium by immunostaining and in immortalized human urothelial cells (TRT-HU1) by PCR and immunoblotting.
2) Stretch-induced luminal ATP release was quantified from rat bladders instilled with saline with/without the Panx1 blocker mefloquine (MFQ 100nM) and bladders isolated from Panx1 and $P2X_7R$ deficient mice.
3) Panx1 functional expression and its interaction with $P2X_7R$ in TRT-HU1 were evaluated by assessing Panx1 opening via YoPro dye-uptake induced by hyposmotic shock, by quantifying mechanically-induced ATP release and by characterizing ATP signaling as transmission of intercellular Ca^{2+} waves (ICW).

Results

1) Panx1 and $P2X_7R$ are expressed in the bladder urothelium of rats and in TRT-HU1 cells.
2) Stretch-induced luminal ATP release was reduced by MFQ (7.7±0.2nM vs. 4.7±0.6nM; N=3 each; $P<0.01$) and was significantly lower in Panx1 and in $P2X_7R$ deficient mice compared to wild-type (WT) mice [WT 0.9±0.2nM vs. $Panx1^{-/-}$ 0.2±0.1nM, $P2X_7R^{-/-}$ 0.3 ±0.03nM; N=9, 4 and 7 respectively; $P<0.05$].
3) YoPro-uptake was inhibited by MFQ (100nM) and also by A438079 ($P2X_7R$ blocker; 10μM). Mechanically-induced ATP release from TRT-HU1 cells was reduced by Panx1 channel blockade, and was increased by exposure to low divalent cation solution (LDPBS), a condition that enhances $P2X_7R$ activation (7.0±2.0nM/μg protein vs. 26.1±5.4nM/μg protein, N=3 each; $P<0.05$). ICW spread was sensitive to exposure to LDPBS, to treatment with apyrase (ATP scavenger, 50U/mL) and MFQ. Radius of ICW in LDPBS was greater than in control conditions (246.2±3.7μm vs 145.8±7.5μm, N=6 fields; $P<0.01$) and such amplification of ATP signaling was prevented by MFQ (175.0±16.9μm, N=9 fields; $P<0.05$). In apyrase, ATP signaling was restricted to cells closer to the mechanically-stimulated cell in both control (24.5±4.2μm, N=6 fields) and LDPBS (49.1±9.5μm, N=4 fields) conditions.

Interpretation of results

Panx1 channels are involved in stretch-induced ATP release from isolated rat and mouse bladders. Panx1 channels are also functionally expressed in cultured human urothelial cells. They respond to mechanical stimulation, interact with $P2X_7R$ and contribute to intercellular Ca^{2+} signaling by releasing ATP. By providing a path for mechanically and $P2X_7R$-induced ATP release, Panx1 channels not only participate in urothelial mechanotransduction but also provide a mechanism of ATP-induced ATP release that amplifies urothelial signaling.

Concluding message

Panx1 channels participate in urothelial mechanotransduction and ATP signaling and are thus expected to play essential roles in regulation of bladder function.

References

1. Purinergic and muscarinic modulation of ATP release from the urothelium and its paracrine actions. Am J Physiol Renal Physiol 306:F286-F298, 2014
2. Connexins, pannexins, innexins: novel roles of "hemi-channels". Pflugers Arch 457(6):1207-1226, 2009

Disclosures

Funding: NIH DK081435 **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat, mouse and immortalized human cultured cells **Ethics Committee:** Albert Einstein College of Medicine Animal Experiment Committee