#532 Hyperpolarization-activated cyclic nucleotide-gated cation (HCN) Channels Constrain the Human Detrusor Contractility

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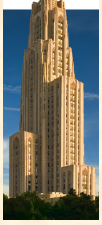


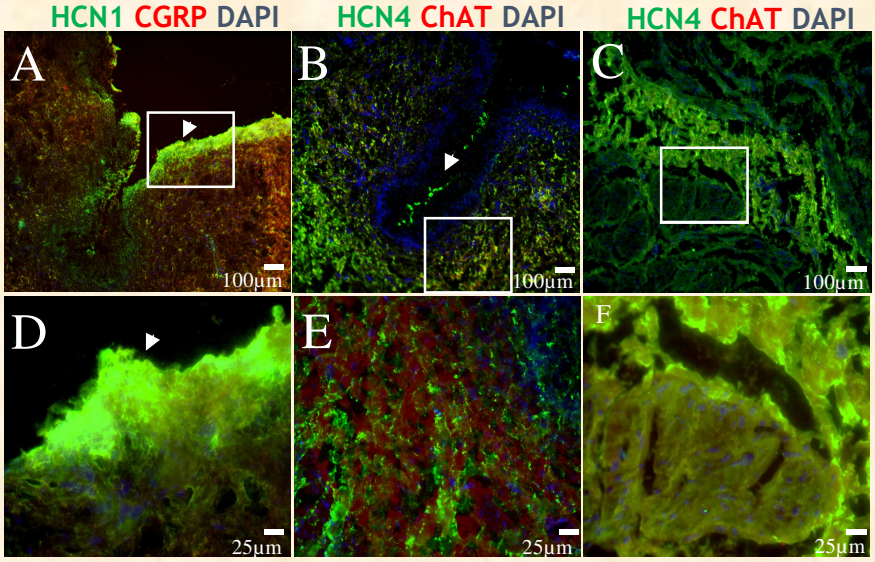
Introduction

HCN channels are activated by hyperpolarization instead of depolarization. HCN channels exist in 4 isoforms differing in activation kinetics and sensitivity to gating by cyclic adenosine monophosphate (cAMP), which is the intracellular second messenger for the two major classes of drugs approved for treating overactive bladder (OAB), namely beta 3 receptor agonists and muscarinic receptor antagonists. HCN channels typically open at potentials more negative to −50 mV[1] for regulating the intrinsic membrane excitability through modulation of low voltage gated Ca^{2+} channel activity. Although the expression of HCN channels in human bladder[2] is reported by several groups, their functional role is unclear. Here, we characterized the HCN1 and HCN4 immunoreactivity in urothelium, suburothelium and detrusor regions of human bladder and investigated the effect of HCN blocker, ZD7288 on the nerve evoked contractions of human bladder strips. ZD7288 is characterized by its high affinity for HCN channels with reported IC50 of 200nM and a IC50 of 50µM for directly blocking the T-type voltage gated calcium channels (VGCC)[2].

METHODS

Bladder from 3 deceased organ donors was obtained after ethical approval from the institutional committee. Urothelium intact and urothelium denuded detrusor strips were mounted in 37°C organ bath constantly gassed with 95% oxygen-5% carbon dioxide. Strips were stretched to 1g and equilibrated for 1h before isometric tension studies. Nerve-evoked contractions (tetrodotoxin-sensitive) were generated by electrical field stimulation (EFS: 5 ms pulses, 0.1-32Hz, 2s train at 20V) before and after addition of ZD7288 in nanomolar and micromolar range (10nM or 100 µM) or Neostigmine (1µM) separately and together. EFS frequency response curve were generated by stimulating at 0.1,0.5,1, 2, 4, 8, 16, and 32Hz (one stimulation at each frequency) at 15s intervals. Peak contractile responses after the addition of different drugs were normalized to the peak contractile response evoked at 32Hz stimulation in absence of any drug (control). A portion of bladder tissue was also preserved for double immunostaining of HCN channel isoforms, HCN1(1:200; Abcam) and HCN4 (1:300; Abcam) with neuronal markers, Calcitonin gene related peptide (CGRP) (1: 50; Santa Cruz) and choline acetyl transferase (ChAT) (1:100; Millipore).

RESULTS



Urothelium and sub urothelium **Detrusor**

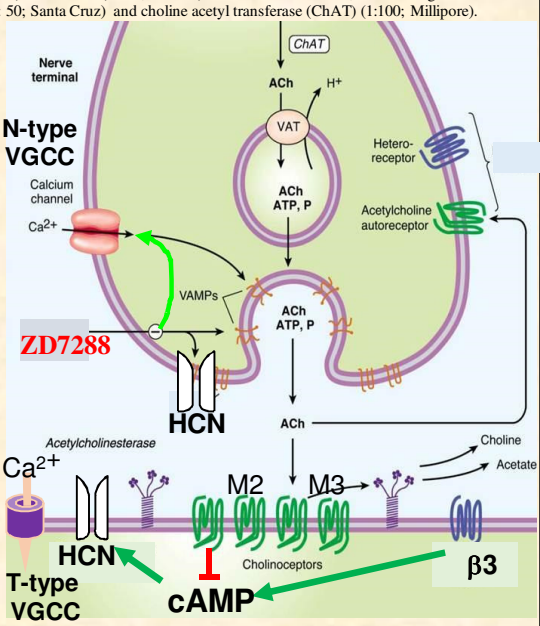


Figure 1: Double immunostaining of the separated mucosa (urothelium and sub-urothelium) and detrusor sections of human bladder revealed a co-localization of the HCN channel isoform HCN4 with ChAT in suburothelium and in detrusor. Co-localization of the HCN1 isoform with CGRP in urothelium, sub-urothelium and detrusor was also noted.

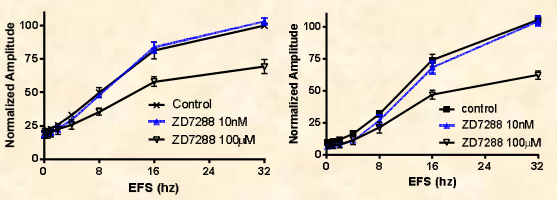
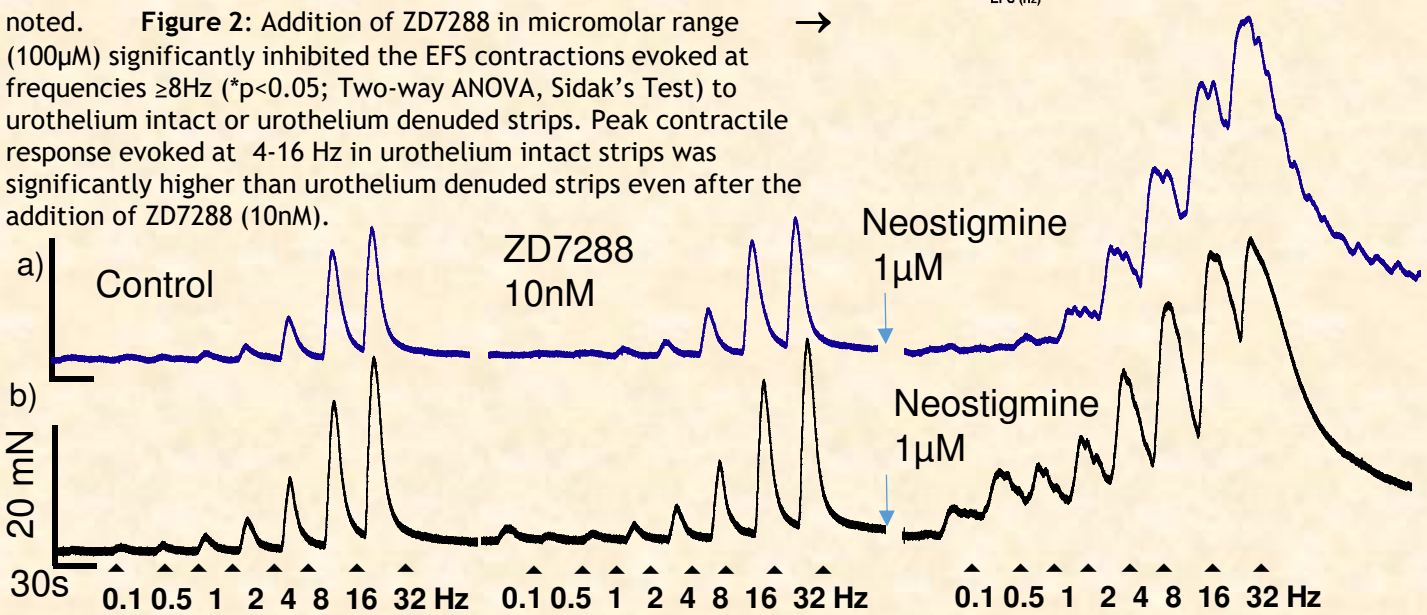


Figure 2: Addition of ZD7288 in micromolar range (100µM) significantly inhibited the EFS contractions evoked at frequencies ≥8Hz (*p<0.05; Two-way ANOVA, Sidak's Test) to urothelium intact or urothelium denuded strips. Peak contractile response evoked at 4-16 Hz in urothelium intact strips was significantly higher than urothelium denuded strips even after the addition of ZD7288 (10nM).



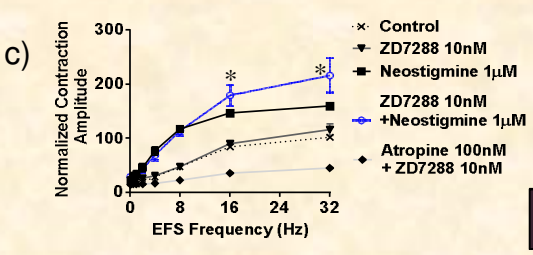


Figure 3: Panel a) and b) show traces of urothelium intact strips in presence and absence of ZD7288 (10nM) and Neostigmine 1µM. Panel C)- Force-frequency curve reveals significant enhancement of the Neostigmine 1µM response by ZD7288 (10nM) vs Neostigmine 1µM alone *p<0.05; Two-way ANOVA, Tukey's Test).

CONCLUSIONS

Collectively, this evidence suggests that HCN channels expressed in bladder serve a non-pacemaking role of constraining the human detrusor contractility through the modulation of spontaneous activity[2] and the evoked release of neurotransmitters. Findings suggest that agents capable of selectively opening and closing HCN channels in bladder can serve as therapeutic candidates for OAB and UAB, respectively.

REFERENCES

1. Xing Wu *et al.* Is ZD7288 a selective blocker of hyperpolarization-activated cyclic nucleotide-gated channel currents? Channels (Austin). 2012; 6(6): 438–442.2.
2. Kashyap *et al.* HCN channels modulate spontaneous and neurogenic contractions of human bladder. J. Urology. 2016; 195 (4): e797
3. Huang *et al.* HCN1 channels reduce the rate of exocytosis from a subset of cortical synaptic terminals. Sci Rep. 2017; 7:40257.