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CIRCADIAN REGULATIONS OF PIEZO1, TRPV4, CONNEXIN26 AND VNUT BY CLOCK GENES IN THE MOUSE BLADDER UROTHELIUM.

Hypothesis / aims of study

Clock genes exist in most cells and organs, and the products of *Per*, *Cry*, *Bmal* and *Clock* have the most important role to regulate circadian rhythm as representative clock genes (1). It has been known that lower urinary tract function is also regulated by clock genes (2) and we reported at ICS2015 that *Clock* mutant (*Clock*$^{\Delta 19/\Delta 19}$) mice showed phenotype of nocturia (NOC) and nocturnal polyuria (NP). Although the pathophysiologies of NOC are multifactorial and complex, we have reported at EAU16 and AUA16 that sensation of bladder fullness may change with circadian rhythm which are associated with expression of clock genes. These recognitions propose a new suggestion about the relationship between abnormalities in clock genes and NOC.

We hypothesis that clock genes regulate circadian rhythm of sensation of the bladder fullness through the circadian expression of transient receptor potential cation channel subfamily V member 4(*TRPV4*) and *Piezo1* as the mechano-sensor and vesicular nucleotide transporter(*VNUT)* and *Connexin26*(*Cx26*) as ATP release mediate molecules (ARMM) in the mouse bladder urothelium, which send signals of bladder sensation to the central nervous system via intracellular Ca^{2+} influx and ATP releasing. In the present study, we investigated expression rhythms in clock genes, mechano-sensors and ARMM in the mouse bladder urothelium, and gene transcription mechanisms in the mechano-sensors and ARMM by regulation of clock genes.

Study design, materials and methods

Male C57BL/6 mice aged 8-12 weeks (WT) and same aged male C57BL/6 *Clock*$^{\Delta 19/\Delta 19}$ were used. They were bred under 12-h dark/light (LD) conditions for 2 weeks. The light period started from 6 AM, which is zeitgeber time (ZT) 0. Mice were sacrificed every 4hr from ZT0. The mouse bladder urothelium were obtained to scrape the urothelium layer by knife.The gene expression rhythms of clock genes, mechano-sensors and ARMM were measured by quantitative RT-PCR (n= 4 for WT mice, n= 4 for *Clock*$^{\Delta 19/\Delta 19}$ mice at each time point) and Western Blot analysis in the mouse bladder urothelium. In order to investigate the functions of clock genes as a transcription factor in the *Piezo1, TRPV4, Cx26* and *VNUT* in the mouse urothelium, chromatin immunoprecipitation (ChIP) assays was performed at difference 2 time points (ZT4 and ZT12) using anti-Clock antibody (n = 9 for both Control group and anti-Clock Ab group at each time point). The experimental values were expressed as means ± SE. The statistical significances of differences between two groups were analysed using the Mann-Whitney's *u*-test. The One-way ANOVA and Two-way ANOVA with Bonferroni's test were used comparing differences among the time points in each group and at each time points between 2 groups, respectively.

Results

WT mice showed typical circadian mRNA-expressions of clock genes. Namely, a negative transcription factor such as *Per2* were low during active phase, and high during sleep phase, while a positive transcription factor such as *Bmal1* was high during active phase, and low during sleep phase. In contrast, *Clock*$^{\Delta 19/\Delta 19}$ mice showed loss of circadian mRNA-expressions of clock genes. In WT mice, mRNA-expressions in mechano-sensors and ARMM also showed circadian rhythms which were associated with circadian rhythms in clock genes. The peak times were observed at ZT12 and the nadir were at ZT4 (Fig1, Black). The protein expressions in mechano-sensors and ARMM were almost consistent with mRNA circadian expressions. In contrast, the all circadian rhythms in mechano-sensors and ARMM were disrupted in *Clock*$^{\Delta 19/\Delta 19}$ mice (Fig1, Red). The ChIP assays showed Clock bindings at promoter sequences of mechano-sensors and ARMM both ZT4 and ZT12 (Fig. 2).

As the results of deficiency of circadian regulation by clock genes, the absolute gene expression levels of mechano-sensors and ARMM were higher in *Clock*$^{\Delta 19/\Delta 19}$ mice than WT mice during sleep phase (from ZT4 to ZT8) (Fig 1).

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Figure.1 mRNA-expression rhythms of mechano-sensors and ARMM

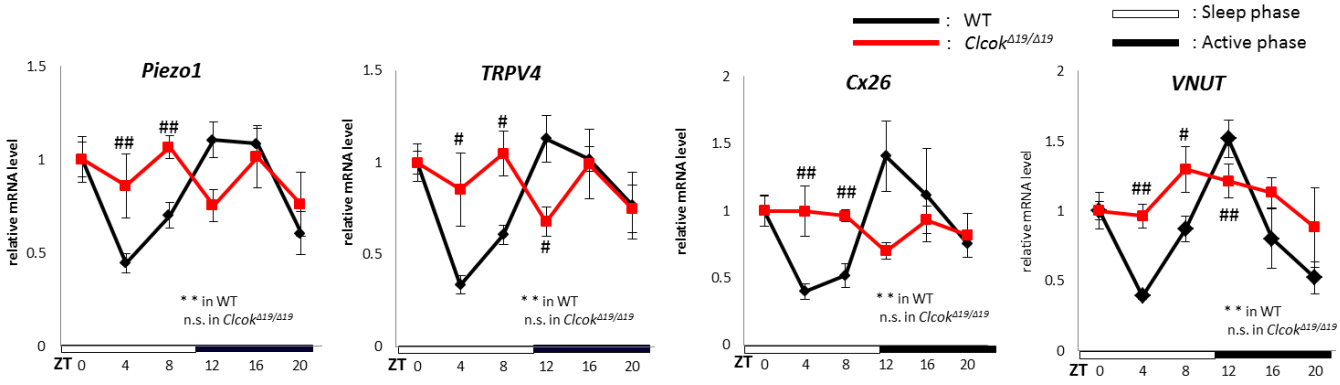
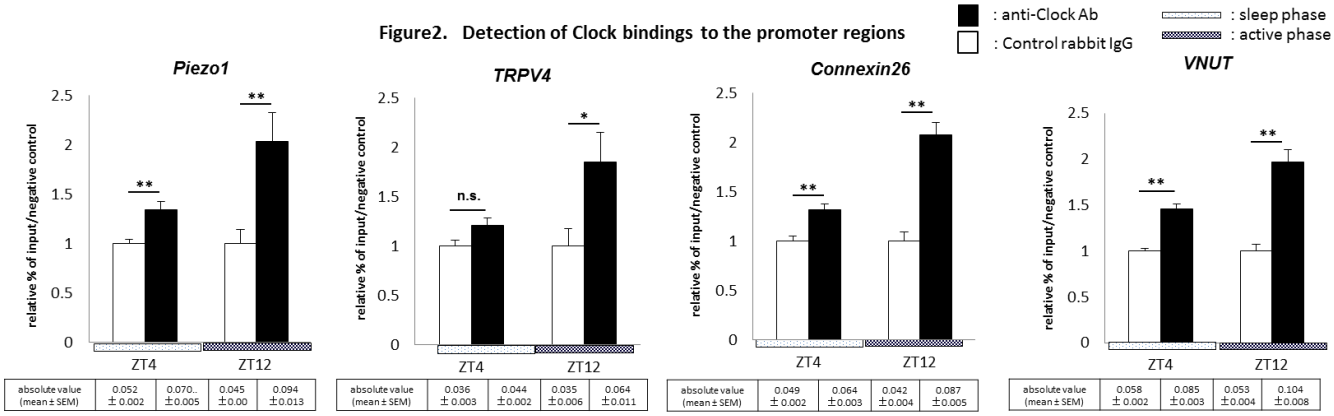


Figure2. Detection of Clock bindings to the promoter regions



Interpretation of results

The expressions of mechano-sensors and ARMM are regulated by clock genes in the mouse bladder urothelium. These are high during active phase and low during sleep phase in WT mice. In contrast, *Clock*$^{\Delta 19/\Delta 19}$ mice lost these circadian expressions. Moreover, the gene expression levels of mechano-sensors and ARMM were higher in *Clock*$^{\Delta 19/\Delta 19}$ mice than WT mice during sleep phase because of the deficiency of circadian gene regulations.

Concluding message

This present study suggests that the bladder sensation may have the circadian rhythm due to the circadian expressions of mechano-sensors and ARMM in the bladder urothelium, which were sensitive during active phase and insensitive during sleep phase. The disruption of circadian rhythms of these channels could lead to the hyper sensitivity of bladder fullness during sleep and be one of the causes of NOC, which observed in the voiding behavior of *Clock*$^{\Delta 19/\Delta 19}$ mice.

References

1. Okamura, H.,et al. Adv Drug Deliv Rev, 62: 876, 2010
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Disclosures

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