



Circadian expressions of cardiac ion channel genes in mouse might be associated with the central clock in the SCN but not the peripheral clock in the heart

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Significant circadian variations exist in the frequency of cardiac arrhythmia, but few studies have examined the relation between cardiac ion channels genes and biological clocks. We investigated this relation using suprachiasmatic nuclei lesion (SCNX) and pharmacological autonomic nervous system block (ANSB) mice. Significant 24-h variations were observed in the expression of clock genes *Per2*, *Bmal1*, and *Dbp* and ion channel genes *KCNA5*, *KCND2*, *KCHIP2*, and *KCNK3* in the control mice hearts. In the SCNX mice, all genes examined lost circadian rhythm. In the ANSB mice, the expressions of the three clock genes were dampened significantly but still had circadian rhythm, whereas the four ion channel gene expressions lost rhythm. Heart rate also lost circadian rhythm in both the SCNX and ANSB mice. These results suggest that some ion channel gene expressions might be regulated by the central clock in the SCN through the ANS but not the peripheral clock in the heart.

Keywords: arrhythmia; suprachiasmatic nuclei; autonomic nervous system; circadian rhythm

1. Introduction

In mammals, the master circadian pacemaker resides in the suprachiasmatic nuclei (SCN) of the hypothalamus, and slave oscillators exist in peripheral organs. Output signals from the master pacemaker drive and synchronize the circadian oscillation of various behavioural and physiological processes through autonomic and/or neurohumoural activation. When the SCN is destroyed, the temporal programs are disturbed (LeSauter and Silver 1998).

Apparent circadian variations exist in cardiac arrhythmia, and they have been posited to be associated with the circadian expression of ion channels or physiological responses to autonomic nervous function, evidenced by the multisynaptic autonomic

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connection from SCN neurons to the heart (Inoue and Zipes 1987; Scheer et al. 2001). Yamashita et al. (2003) demonstrated that two K⁺ channel genes (*KCNA5* and *KCND2*) in rat heart show significant circadian rhythm in their mRNA levels, and these circadian rhythms were modulated by a pharmacological block of the autonomic nervous system (ANS). However, there is no evidence of regulation of rat cardiac gene expression by the biological clock, and it is also unclear whether such a circadian expression of cardiac genes is a universal phenomenon. The goals of the present study were (1) to determine whether other cardiac ion channel genes, in addition to *KCNA5* and *KCND2*, also exhibit circadian oscillation in mouse heart, and (2) to evaluate the effect of an SCN lesion and ANS blockade on the expressions of the cardiac ion channels and clock genes, and electrocardiographic parameters.

2. Methods

Experimental animal care was conducted with permission from the Experimental Animal Welfare Committee of the Fujita Health University School of Medicine.

2.1. Animals

Ninety 4–6-week-old ddy mice (Chubu Kagaku Shizai Co., Ltd., Naogyo) were housed individually and maintained under a 12 h/12 h light/dark cycle (light on: 8 am, CT0; light off: 8 pm, CT12) for 2 weeks and allowed access to food and water ad libitum. The animals were sacrificed at 6-h intervals in a single day. The right and left atrium and apex of the left ventricle were quickly removed and frozen in the liquid nitrogen at -80°C . The samples for mRNA isolation were kept in RNA stabilization solution (RNAlater, Life Technologies, Carlsbad, CA, USA).

2.2. Telemetry transmitter implantation

Implantation of the telemetry transmitter in mice ($n = 18$) was performed with an intraperitoneal injection of ketamine/xylazine (125 mg/kg, 50 mg/kg). The transmitter was fixed on the back of the mouse with its two electrodes placed on the bilateral chest subcutaneously. The mouse was then put in an individual cage and placed on the transmitter's receiver board and allowed to recover. Each mouse's body temperature and electrocardiogram (ECG) were measured every 6 h. Heart rate was determined as the average of consecutive 20 beats of initiation of every 6 h. The QT interval was defined as the time between the QRS onset and the point at which the isoelectric line intersected a line drawn tangentially to the maximal downslope of the T wave. Data were collected by a DSI Dataquest A.R.T.2.3 system at the sampling rate of 1 kHz (Data Sciences International, New Brighton, MN, USA).

2.3. SCN and ANS block

Bilateral thermal lesions of the SCN were performed stereotaxically (Narishige Co., Tokyo) under ketamine/hydrazine anaesthesia. A stainless steel electrode (0.35 mm diameter) was inserted in the SCN (0.2 mm posterior and 0.0 mm lateral to the bregma, 6.2 mm below the skull surface) using a lesion-making device (Ugo Basile Biological Research Apparatus; Ugo Basile, Comerio VA, Italy). A 2-mA current was passed for 4 s. The sham operation was performed by inserting the lesion needle into the brain

without passing a current. The body temperature, heart rate and body weight of the mice were monitored for 3 weeks after the lesion date. The mice that lost circadian rhythm in both heart rate and body temperature were used (Figure 1). A mouse was excluded from the study when its body weight increased more than 10 g or when it died less than 14 days before experiment. The autonomic nervous system block (ANSB) was performed by intraperitoneal injection of atropine (0.5 mg/kg) and propranolol (1 mg/kg) every 6 h for 2 weeks. Mice of this group did not receive an SCN lesion. On the 15th day, mice were sacrificed at 6-h intervals for sampling (24 mice in each group).

2.4. RNA preparation and real-time quantitative RT-PCR

The heart was removed from sham-operated, SCN-lesion and ANSB mice under ketamine/hydrazine anaesthesia. Total RNA was extracted from the samples by

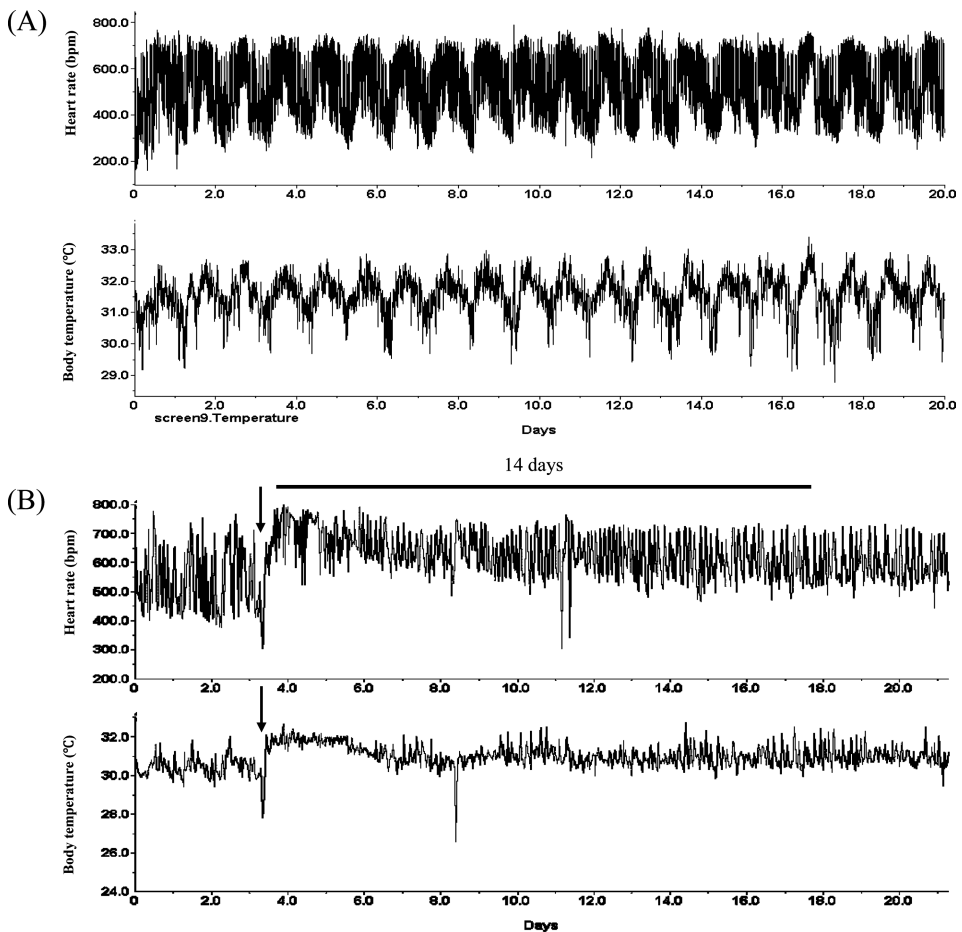


Figure 1. Confirmation of the successful suprachiasmatic nucleus (SCN) lesion. (A) Sham-operated mice: Substantial circadian rhythms in the heart rate and body temperature were observed. (B) SCN-lesioned mice: The arrow indicates the date that the SCN was lesioned. The SCN lesion was considered successful if the loss of circadian rhythms of heart rate and body temperature persisted for more than 14 days.

Table 1. Sequences of PCR primers and sequence-specific probes.

Target sequence	Accession no.	Assay ID of Primers and TaqMan [®] Probe	Amplicon length (bp)
<i>Per2</i>	NM_011066	Mm00478113_m1	73
<i>Bmall</i>	NM_007489	Mm00500226_m1	87
<i>Dbp</i>	NM_016974	Mm00497539_m1	57
<i>KCNA5</i>	NM_145983	Mm00524346_s1	61
<i>KCND2</i>	NM_019697	Mm00498065_m1	88
<i>KCHIP2</i>	NM_030716	Mm00518914_m1	74
<i>KCNK3</i>	NM_010608	Mm00807036_m1	128

using RNeasy[®] Fibrous Tissue Mini kit (Qiagen, Hilden, Germany) and then reverse-transcribed and amplified by using real-time TaqMan[®] technology and analysed on an ABI PRISM7900 (Applied Biosystems, Foster, CA, USA). The primer pairs were designed from the mouse sequences available in GenBank, including three clock genes (*Bmall*, *Per2*, and *DBP*) and four K⁺ channel genes: *KCNA5* (ultrarapid delayed rectifier K⁺ current), *KCND2* (α subunit of transient outward current), *KCHIP2* (β subunit of transient outward current), and *KCNK3* (cardiac two-pore background K⁺ channel). PCR was executed under the following conditions: cDNA synthesis at 25°C for 10 min and then 37°C for 120 min, PCR amplification for 40 cycles at in a two-step method with denaturation at 95°C for 15 s, and annealing and extension at 60°C for 1 min. The target clock gene cDNA was co-amplified with GAPDH cDNA in a single PCR tube. The PCR data are reported as the number of transcripts per number of GAPDH. The sequences of PCR primers and sequence-specific probes are presented in Table 1.

2.5. DNA microarray

Total RNA was isolated using an RNeasy kit (Qiagen, Valencia, CA, USA) and assessed for integrity using an RNA 6000 Nano Assay (Agilent Technologies, La Jolla, CA, USA). Sample amplification labelling and hybridization essentially followed the protocol recommended by the manufacturer. Briefly, high-quality RNA samples acquired from non-treated human umbilical vein endothelial cells (HUVEC) and Bmal/Clock-treated HUVEC were labelled with cyanine 3 (Cy3) and cyanine 5 (Cy5) using an Agilent Low-input RNA Fluorescent Linear Amplification kit. Hybridizations were performed on Agilent Human 1A (V2) Oligo Microarray slides, and a dye-swap experimental design was applied. Arrays were scanned on an Agilent Microarray Scanner (model GMS417 Array scanner, Affimetrix, Santa Clara, CA). The raw images were processed using Agilent's Feature Extraction software, and their analysis was conducted using Agilent Genespring software. The genes that were upregulated by fivefold or more in Bmal/Clock-treated HUVEC samples but not in nontreated HUVEC samples were selected.

2.6. Statistical analysis

The 24-h variations in the mean mRNA levels of the studied genes were double-plotted and analysed using an algorithm for least-squares cosine-curve fitting. The properties of the circadian rhythms were quantified by the following four parameters:

- (1) significance of rhythm (p -value for the hypothesis of zero-rhythm amplitude),
- (2) midline-estimating statistic of rhythm (MESOR, a rhythm-adjusted 24-h time-series mean),
- (3) amplitude (one-half of the peak-to-trough amplitude by fitting with a single cosine-curve approximation), and
- (4) acrophase (crest time of the peak fitted approximation to rhythm referenced to local midnight).

A circadian rhythm was considered to exist when the null hypothesis regarding the significance of rhythm was <0.05 . The gene expressions of 24-h components (MESOR, amplitude, and acrophase) among the three treatments (sham-operated, SCN_X, and ANSB) were analysed by one-way analysis of variance (ANOVA). After a demonstration of significant differences among treatments by ANOVA, post hoc comparisons between treatment pairs were made by the Bonferroni multiple comparison procedure. Quantitative data are expressed as means \pm standard deviation (SD) values. A two-tailed p -value of <0.05 was considered significant.

3. Results

3.1. Screening for candidate cardiac gene by DNA microarray

The DNA microarray showed that *KCNA5*, *KCND2*, *KCNK3*, *KCNJ12* and *ATP2A2*, *SLC9A3R2* were augmented by *Bmal1*/Clock induction (data not shown). Among those six genes, *KCNA5*, *KCND2*, *KCHIP2* and *KCNK3* exhibited significant circadian variation and were examined further.

3.2. Clock gene expression in the mouse heart

The sham-operated mice showed significant circadian rhythm in the three clock genes of the atrium (Figure 2A, Table 2). The SCN_X mice lost circadian variations of the expression in all clock genes. In the ANSB mice, the circadian rhythm of all clock gene expressions was preserved, and *Dbp* expression was significantly reduced. Also, the expression of the *Dbp* gene in both the SCN_X and ANSB mice was significantly lower than that in the sham-operated mice. Neither SCN_X nor ANSB influenced the expression level of *Per2*.

In the ventricle, the sham-operated mice showed significant circadian rhythm in the three clock genes (Figure 2B, Table 2). The SCN_X mice lost circadian variation of the expression of all three clock genes. In the ANSB mice, the circadian rhythm of the three clock gene expressions was preserved, and the *Dbp* expression was significantly phase-advanced. The MESOR values for *Bmal1* and *Dbp* were significantly different among the three treatment groups, whereas the expression of *Per2* was not influenced by the SCN_X or ANSB. The MESOR values for the three clock genes in the atria of the sham-operated mice were significantly larger than those in the ventricles (*Bmal1*, $p < 0.05$; *Per2*, $p < 0.05$; and *Dbp*, $p < 0.05$).

3.3. Effects of SCN_X and ANSB on ion channel gene expression

The sham-operated mice exhibited substantial circadian rhythms in all four cardiac ion channel genes (*KCNA5*, *KCND2*, *KCHIP2*, and *KCNK3*) in both the atrium and ventricle (Figure 3). The SCN_X eliminated the circadian rhythm of all of these

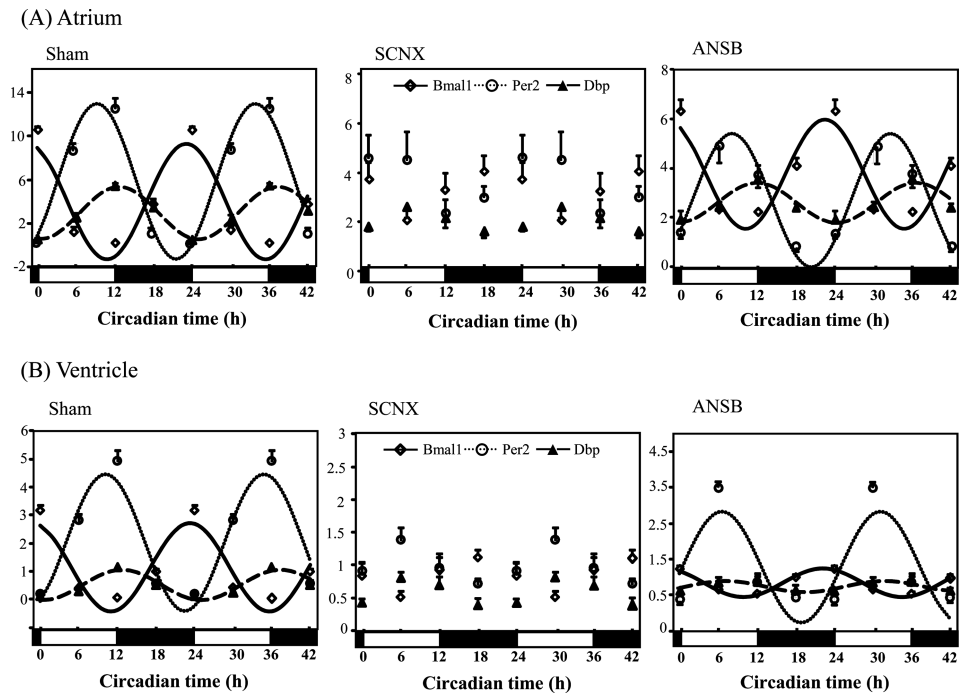


Figure 2. Influence of SCN and ANSB on the mRNA level of clock genes in the mouse heart (for each group, $n = 3-6$). Clock gene expressions in the atrium (A) and ventricle (B). All data were normalized against GAPDH and double-plotted. The solid line or dotted line indicates circadian rhythm fitted by least-squares cosine-curve fitting. Open diamonds, *Bmal1* expression; open circles and closed triangles, *Per2*; closed squares, *Dbp*. The open and closed bars under the x-axis represent the light and dark periods, respectively, in one day. The expression of all of the clock genes lost circadian rhythm in the SCN group and was dampened significantly in the ANSB group. ANSB, autonomic nervous system block; SCN, suprachiasmatic nucleus lesioned.

cardiac genes in the atrium (Figure 3A, Table 3). Of note, SCN increased the MESOR in *KCND2* and *KCNK3* expressions. ANSB, similarly, abolished circadian rhythms of all four ion channel genes, and the MESOR values of all cardiac gene expressions were increased significantly in the atrium.

In the ventricle, as in the atrium, both SCN and ANSB abolished the circadian rhythm of all four ion channel genes (Figure 3B, Table 3). The MESOR values of all gene expressions except *KCND2* were significantly increased in the ANSB mice, and the expression of *KCNA5* was significantly reduced in the SCN mice.

3.4. Effects of SCN and ANSB on cardiac electrophysiology

The sham-operated mice exhibited substantial circadian rhythms in the heart rate and QT interval, whereas in the SCN and ANSB mice, the circadian rhythms of the heart rate and QT interval were lost (Figure 4, Table 4). Interestingly, the MESOR values for heart rate in the SCN mice (669 ± 21 bpm) were significantly higher than those in the ANSB mice (466 ± 30 bpm, $p < 0.05$). The MESOR values for the QT interval in the ANSB mice were, therefore, longer than those in the SCN mice (51 ± 2 ms vs. 37 ± 1 ms, $p < 0.05$).

Table 2. Clock gene expressions in the mouse heart.

Gene	Treatment	Site	MESOR	Amplitude	Acrophase (h)
<i>Bmal1</i>	Sham	Atrium	4.0 ± 0.1 ^{§*}	5.4 ± 0.1 ^{§*#}	23.2 ± 0.3
		Ventricle	1.2 ± 0.1 ^{§*#}	1.7 ± 0.1 ^{§*#}	23.2 ± 0.2
	SCNX	Atrium	2.9 ± 0.3 [§]	1.5 ± 0.2 [§]	N/A
		Ventricle	0.7 ± 0.1 [§]	0.4 ± 0.1 [§]	N/A
	ANSB	Atrium	3.8 ± 0.2 [§]	2.2 ± 0.3 [§]	22.5 ± 0.1
		Ventricle	0.9 ± 0.1 [§]	0.4 ± 0.1 [§]	22.1 ± 0.4
<i>Per2</i>	Sham	Atrium	2.8 ± 0.3	2.5 ± 0.1 ^{§*#}	12.8 ± 0.4
		Ventricle	0.6 ± 0.1	0.5 ± 0.1 ^{§*#}	12.6 ± 0.3 [#]
	SCNX	Atrium	2.0 ± 0.2	1.3 ± 0.2 [§]	N/A
		Ventricle	0.6 ± 0.1	0.3 ± 0.1 [§]	N/A
	ANSB	Atrium	2.6 ± 0.4	0.9 ± 0.4 [§]	11.5 ± 1.2
		Ventricle	0.7 ± 0.1	0.04 ± 0.01 [§]	7.2 ± 2.3
<i>Dbp</i>	Sham	Atrium	5.8 ± 0.3 ^{§*#}	7.2 ± 0.6 ^{§*#}	9.9 ± 0.2
		Ventricle	1.9 ± 0.1 ^{§*#}	2.2 ± 0.2 ^{§*#}	9.9 ± 0.3 [#]
	SCNX	Atrium	3.6 ± 0.4 [§]	2.1 ± 0.4 [§]	N/A
		Ventricle	1.0 ± 0.1 [§]	0.5 ± 0.1 ^{§^γ}	N/A
	ANSB	Atrium	2.7 ± 0.2 [§]	2.4 ± 0.4 [§]	8.0 ± 0.4
		Ventricle	1.3 ± 0.1 [§]	1.5 ± 0.1 [§]	6.6 ± 0.1

Note: Data are mean ± SEM for 3–6 mice per group. MESOR, midline estimating statistic of rhythm; Sham, sham-operated; SCNX, suprachiasmatic nucleus lesioned; ANSB, autonomic nervous system block; N/A, not applicable. [§]One-way ANOVA $p < 0.05$, Bonferroni/Dunn, $p < 0.05$. *Sham vs. lesion; #Sham vs. ANSB; ^γLesion vs. ANSB.

4. Discussion

4.1. Major findings

We examined the clock gene and cardiac ion channel gene transcripts in the hearts of sham-operated, SCNX, and ANSB mice. Our major findings are as follows:

- (1) Besides *KCNA5* and *KCND2* (Yamashita et al. 2003), the other two cardiac genes, the expressions of *KCNK3* and *KCHIP2* also exhibited substantial circadian rhythms in the sham-operated mice.
- (2) In the SCNX mice, all three clock genes and all four ion channel genes lost circadian rhythm.
- (3) ANSB eliminated the circadian oscillation of all of the ion channel gene expressions but not the clock genes' expression.
- (4) The SCNX mice and the ANSB mice exhibited losses of circadian rhythm in the heart rate and QT interval.

4.2. Regulation of the cardiac gene expression

It is well known that the master pacemaker controlling circadian rhythm is located in the SCN in mammals. In addition, accumulated evidence indicates that various peripheral tissues including cardiovascular cells (Ko and Takahashi 2006) also possess circadian oscillators, and that impairment of the function of such clock systems may be associated with some cardiovascular diseases (Maemura et al. 2000; McNamara et al. 2001; Nonaka et al. 2001; Young et al. 2001a; Durgan et al. 2005; Curtis and Fitzgerald 2006; Young 2006; Viswambharan et al. 2007).

The results of the present study suggest that circadian expressions of cardiac genes might be related to the biological clock in the SCN. In the SCNX mice, the

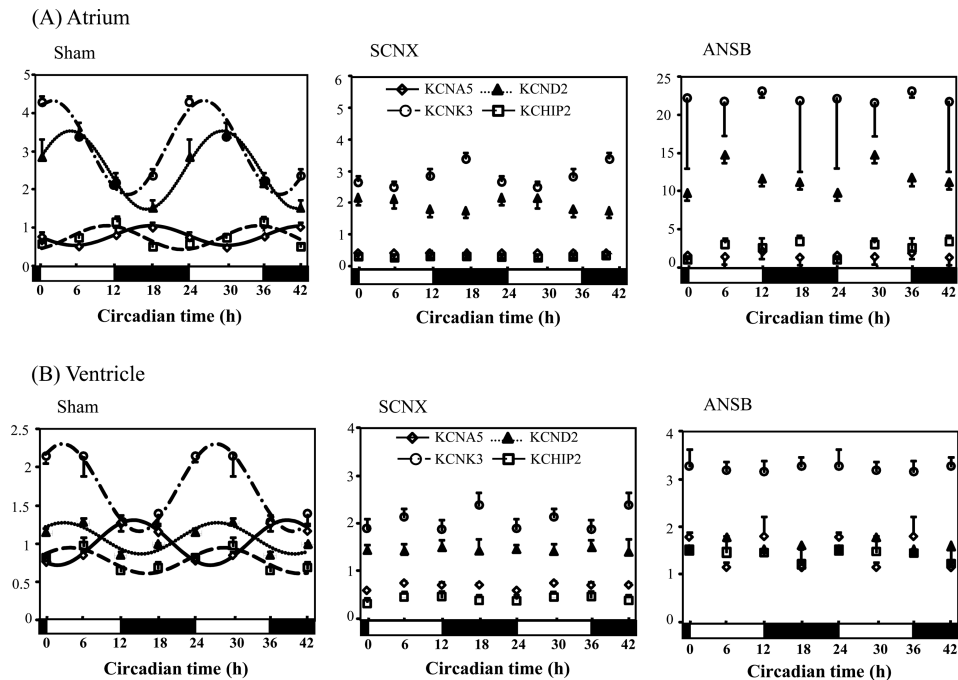


Figure 3. Cardiac ion channel gene expressions were similarly altered in the SCNX and ANSB mice (for each group, $n = 3-6$). Cardiac ion channel gene expressions in the atrium (A) and ventricle (B). All data were normalized against GAPDH and double-plotted. The solid line or dotted line indicates circadian rhythm fitted by least-squares cosine-curve fitting. Open diamonds, *KCNK3*; closed triangles, *KCND2*; open squares, *KCHIP2*; open circles, *KCNK3*. The open and closed bars under the x -axis represent the light and dark periods, respectively, in one day. All four ion channel gene expressions lost circadian rhythm in the SCNX and ANSB mice.

expressions of the clock genes and cardiac ion channel genes examined were arrhythmic. The MESOR and amplitude values in most of the cardiac genes were reduced significantly, which suggests that the ion channel gene expressions were “flattened” in the absence of a central clock. However, whether the central clock in the SCN directly influenced the cardiac ion channels or did so through peripheral clock genes in the heart remains unknown.

Evidence has accumulated supporting both neural and humoral control of peripheral rhythms, and this indicates that the SCN regulates the expression of circadian oscillations in various peripheral organs by diverse pathways. By using a model system, SCNX mice parabiotic-linked to intact partners, Guo et al. (2005) found that circadian rhythms of clock gene expressions were recovered in the liver and kidney of the SCNX mice, indicating that blood-borne cues from intact mice were enough to entrain the peripheral clock in these organs. On the other hand, clock gene expressions remained arrhythmic in the heart, muscle and spleen in the SCNX mice, and Guo et al. theorized that the heart might rely more heavily on neural signals.

This study suggested that the ANS is an important pathway for the SCN to transmit time cues to the heart. As expected in the present study, ANSB also resulted in arrhythmic ion channel gene expressions in the heart. On the other hand, unexpectedly, the three clock genes' expressions were still rhythmic in ANSB mouse

Table 3. Cardiac ion channel gene expressions in the mouse heart.

Gene	Treatment	Site	MESOR	Amplitude	Acrophase (h)
<i>KCNA5</i>	Sham	Atrium	0.8 ± 0.1 ^{§*#}	0.4 ± 0.1 ^{§*}	15.6 ± 0.6
		Ventricle	1.0 ± 0.1 ^{§*#}	0.3 ± 0.1	13.2 ± 0.2
	SCNX	Atrium	0.5 ± 0.1 ^{§?7}	0.1 ± 0.1 [§]	N/A
		Ventricle	0.7 ± 0.1 ^{§?7}	0.1 ± 0.1	N/A
ANSB	Atrium	1.5 ± 0.2 [§]	0.3 ± 0.3 [§]	N/A	
	Ventricle	1.5 ± 0.1 [§]	0.2 ± 0.1	N/A	
<i>KCND2</i>	Sham	Atrium	2.8 ± 0.1 ^{§*}	1.2 ± 0.2 ^{§*}	5.4 ± 0.4
		Ventricle	1.1 ± 0.1 [§]	0.2 ± 0.1	3.0 ± 0.3
	SCNX	Atrium	1.8 ± 0.1 ^{§?7}	0.6 ± 0.1 ^{§?7}	N/A
		Ventricle	1.5 ± 0.1 [§]	0.2 ± 0.1	N/A
ANSB	Atrium	11.7 ± 0.8 [§]	2.9 ± 0.4 [§]	N/A	
	Ventricle	1.6 ± 0.1 [§]	0.1 ± 0.1	N/A	
<i>KCHIP2</i>	Sham	Atrium	0.7 ± 0.1 ^{§#}	0.4 ± 0.1 [§]	11.1 ± 0.9
		Ventricle	0.8 ± 0.1 ^{§*#}	0.3 ± 0.2	12.2 ± 1.1
	SCNX	Atrium	0.4 ± 0.1 ^{§?7}	0.1 ± 0.1 ^{§?7}	N/A
		Ventricle	0.6 ± 0.1 ^{§?7}	0.2 ± 0.1	N/A
ANSB	Atrium	2.4 ± 0.5 [§]	0.9 ± 0.6 [§]	N/A	
	Ventricle	1.4 ± 0.1 [§]	0.1 ± 0.1	N/A	
<i>KCNK3</i>	Sham	Atrium	3.1 ± 0.1 ^{§#}	1.2 ± 0.1 ^{§#}	1.7 ± 0.8
		Ventricle	2.2 ± 0.1 ^{§#}	0.4 ± 0.1 [§]	16.8 ± 2.5
	SCNX	Atrium	3.0 ± 0.1 ^{§?7}	0.7 ± 0.1 ^{§?7}	N/A
		Ventricle	2.0 ± 0.1 ^{§?7}	0.5 ± 0.1 ^{§?7}	N/A
ANSB	Atrium	22.0 ± 3.2 [§]	10.5 ± 2.3 [§]	N/A	
	Ventricle	3.2 ± 0.1 [§]	0.1 ± 0.1 [§]	N/A	

Note: Data are mean ± SEM for 3–6 mice per group. Abbreviations are as in Table 1. [§]One-way ANOVA $p < 0.05$, Bonferroni/Dunn, $p < 0.05$. *Sham vs. lesion; #Sham vs. ANSB; ?Lesion vs. ANSB.

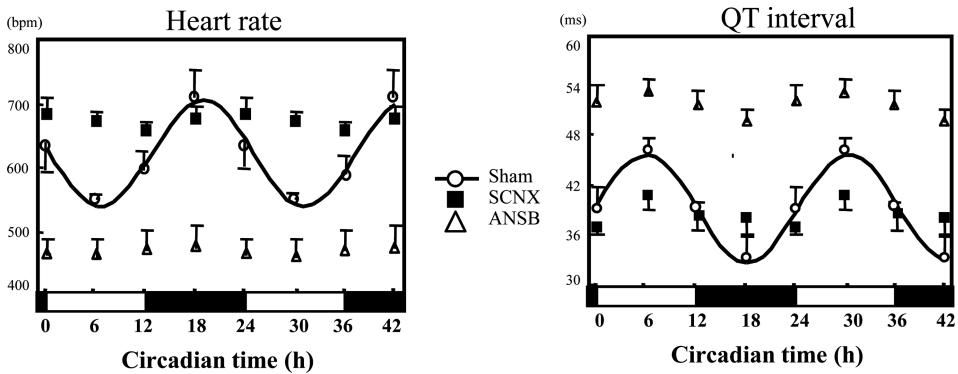


Figure 4. Heart rate and QT interval lost circadian rhythm in both SCNX and ANSB mice (for each group, $n = 3-6$). Heart rate (beats per min) and QT interval (ms) were recorded every 6 h and analysed by the least-squares cosine-curve fitting. The solid line indicates circadian rhythm fitted by least-squares cosine-curve fitting. Open circles, sham-operated mice; open triangles, ANSB mice; closed squares, SCNX mice. The open and closed bars under the x-axis represent the light and dark periods, respectively, in one day. The heart rate lost circadian rhythm in both SCNX and ANSB mice. The mean heart rate in the SCNX mice was significantly higher than that in the ANSB mice.

hearts. The present results clearly demonstrated that removal of the sympathetic input led to a disrupted circadian expression in ion channels but was not enough to eliminate the daily rhythmicity of clock genes within a tissue. In light of the combined data from

Table 4. Heart rate and QT interval.

Parameter	Treatment	MESOR	Amplitude	Acrophase (h)
Heart rate (bpm)	Sham	622 ± 9 ^{§#}	109 ± 21 ^{§*#}	19 ± 1.4
	SCNX	669 ± 21 [§]	20 ± 4 [§]	N/A
	ANSB	466 ± 30 ^{§7}	9 ± 6 [§]	N/A
QT interval (ms)	Sham	40 ± 1 ^{§*#}	8 ± 2 ^{§*#}	5 ± 1.1
	SCNX	37 ± 1 [§]	3 ± 1 [§]	N/A
	ANSB	51 ± 2 ^{§7}	3 ± 1 [§]	N/A

Note: Data are mean ± SEM for 3–6 mice per group. Abbreviations are as in Table 1. [§]One-way ANOVA $p < 0.05$, Bonferroni/Dunn, $p < 0.05$. ^{*}Sham vs. lesion; [#]Sham vs. ANSB; ⁷Lesion vs. ANSB; N/A, not applicable (aperiodic).

the SCNX and ANSB mice, it is reasonable to speculate that a clock system in the SCN but not in the heart might be associated with the circadian cardiac ion channel gene expression. More evidence must be gathered to test this hypothesis.

4.3. Changes in cardiac electrophysiology in accord with those of cardiac gene expression

We examined ECG parameters in the SCNX and ANSB mice. Our results demonstrated that the heart rate and QT interval had substantial circadian rhythms in the sham-operated mice, whereas the SCNX and ANSB abolished such rhythms, similar to the changes in the ion channel gene expressions.

Diurnal variations in several cardiovascular parameters (e.g., blood pressure, heart rate) have been attributed primarily to circadian variations in environmental stimuli (Turton and Deegan 1974; Muller et al. 1989). The biological clock system provides a means by which the heart can predict diurnal variations in the environment to produce optimized responses, enabling the cardiovascular system to prepare for a given stimulus, thereby optimizing the appropriate response. Attenuation of this molecular mechanism would therefore impair such an ability of the heart.

Young et al. (2001b) reported that pressure overload-induced hypertrophy altered the circadian clock in the rat heart, resulting in attenuation in the clock output genes. They also demonstrated that the phases of the clock genes isolated from streptozotocin-induced diabetic rats heart were altered compared to those observed for control hearts (Young et al. 2002). These two studies indicate that the biological clock system might be damaged under pathological conditions. Knutsson et al. (1986) showed that nightshift workers had an increased incidence of heart disease when compared with dayshift co-workers. Although there is no confirmed casual relation, the impairment in the circadian clock may cause the heart to fail to anticipate the changes during a day. Our study using an SCNX and ANSB animal model provides some indirect evidence supporting this hypothesis.

4.4. Study limitations

There are some limitations in the present study. The corresponding proteins to clock and cardiac genes and current density of cardiac ion channels were not measured. We cannot determine whether the protein and electrophysiological properties are

also influenced by other aspects of the study treatments such as feed restriction or light control. Light stimulation was shown to partly modulate the expression of the circadian variation in *KCNA5* in the rat heart [4]. The mouse is a nocturnal animal, and its circadian rhythm may be completely different from that of human beings.

5. Conclusions

In the present study, we observed that the cardiac ion channel genes *KCNA5*, *KCND2*, *KCHIP2*, and *KCNK3* showed circadian expressions, suggesting that the central clock in the SCN but not a peripheral clock in the heart might be involved in the regulation of these cardiac ion channels, and the ANS might mediate such regulation. Further studies on the pathways by which the biological clock transmits its circadian signals to the heart might open a new avenue to our understanding of cardiac arrhythmia and give birth to some new therapy methods.

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