

RESEARCH

Open Access



Histamine 1 receptor-G_{βγ}-cAMP/PKA-CFTR pathway mediates the histamine-induced resetting of the suprachiasmatic circadian clock

Yoon Sik Kim^{1,4}, Young-Beom Kim¹, Woong Bin Kim¹, Seung Won Lee¹, Seog Bae Oh², Hee-Chul Han¹, C. Justin Lee^{3*}, Christopher S. Colwell⁴ and Yang In Kim^{1*}

Abstract

Background: Recent evidence indicates that histamine, acting on histamine 1 receptor (H1R), resets the circadian clock in the mouse suprachiasmatic nucleus (SCN) by increasing intracellular Ca²⁺ concentration ([Ca²⁺]_i) through the activation of Ca_v1.3 L-type Ca²⁺ channels and Ca²⁺-induced Ca²⁺ release from ryanodine receptor-mediated internal stores.

Results: In the current study, we explored the underlying mechanisms with various techniques including Ca²⁺- and Cl⁻-imaging and extracellular single-unit recording. Our hypothesis was that histamine causes Cl⁻ efflux through cystic fibrosis transmembrane conductance regulator (CFTR) to elicit membrane depolarization needed for the activation of Ca_v1.3 Ca²⁺ channels in SCN neurons. We found that histamine elicited Cl⁻ efflux and increased [Ca²⁺]_i in dissociated mouse SCN cells. Both of these events were suppressed by bumetanide [Na⁺-K⁺-2Cl⁻ cotransporter isotype 1 (NKCC1) blocker], CFTR_{inh}-172 (CFTR inhibitor), gallein (G_{βγ} protein inhibitor) and H89 [protein kinase A (PKA) inhibitor]. By itself, H1R activation with 2-pyridylethylamine increased the level of cAMP in the SCN and this regulation was prevented by gallein. Finally, histamine-evoked phase shifts of the circadian neural activity rhythm in the mouse SCN slice were blocked by bumetanide, CFTR_{inh}-172, gallein or H89 and were not observed in NKCC1 or CFTR KO mice.

Conclusions: Taken together, these results indicate that histamine recruits the H1R-G_{βγ}-cAMP/PKA pathway in the SCN neurons to activate Ca_v1.3 channels through CFTR-mediated Cl⁻ efflux and ultimately to phase-shift the circadian clock. This pathway and NKCC1 may well be potential targets for agents designed to treat problems resulting from the disturbance of the circadian system.

Keywords: Calcium, CFTR, Chloride, Circadian rhythm, Histamine, NKCC1, Suprachiasmatic nucleus

Background

Histamine, a neurotransmitter/neuromodulator produced by tuberomammillary nucleus (TMN) neurons in the hypothalamus [1], has a crucial role in regulating brain arousal [2]. A large body of evidence indicates that histamine is also a potent regulator of the mammalian

circadian time-keeping system. Histaminergic fibers from the TMN densely innervate the master circadian clock suprachiasmatic nucleus (SCN) in the rat [3–5] and exogenously applied histamine exerts a phase-shifting effect on the circadian neural activity rhythms recorded in the hamster and mouse SCN slices maintained *in vitro* [6, 7] and on the circadian behavior rhythms manifested in rats [8]. Moreover, histamine synthesis inhibition disrupts circadian activity rhythms in the rat [8] and reduces the phase shifts of circadian activity rhythms induced by light in the hamster [9].

* Correspondence: cjl@kist.re.kr; yikim@korea.ac.kr

³Center for Neuroscience and Functional Connectomics, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea

¹Department of Physiology and Neuroscience Research Institute, Korea University College of Medicine, 126-1 Anam-dong 5-ga, Seoul 136-705, Republic of Korea

Full list of author information is available at the end of the article



It is well established that 4 different types of histamine receptors (i.e., H1R~H4R) mediate histaminergic actions and that the H1R, which is linked to the $G_{q/11}$ -phospholipase C (PLC) pathway, mediates a majority of excitatory actions of histamine in the central nervous system [2, 10]. The $G_{q/11}$ -PLC pathway leads to diacyl-glycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) production [2, 10]. While DAG can cause Ca^{2+} influx through the transient receptor potential cation channel (TRPC), IP_3 induces Ca^{2+} release from the internal stores via IP_3 receptor [10, 11].

Recently, we demonstrated that histamine, acting on H1R, increases intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in mouse SCN neurons by a novel mechanism driven by $Ca_v1.3$ L-type Ca^{2+} channels as well as Ca^{2+} -induced Ca^{2+} release from ryanodine receptor (RyR)-mediated internal stores and this is the molecular mechanism underlying the histamine-induced phase delay of circadian neural activity rhythm in the SCN [12]. In the present study, we sought to delineate the novel signaling mechanisms leading to L-type Ca^{2+} channel opening after H1R activation. Prior works have shown that histamine regulate Cl^- conductances in neurons [13, 14] and that, in some SCN neurons, the electrochemical gradient for Cl^- is set toward the extracellular side due to the Cl^- -importing activity of Na^+ - K^+ - $2Cl^-$ cotransporter isotype 1 (NKCC1) [15, 16]. Therefore, we hypothesized that histamine causes Cl^- efflux to elicit membrane depolarization needed for L-type Ca^{2+} channel activation. We considered the possibility that the cystic fibrosis transmembrane conductance regulator (CFTR) may mediate the histamine-induced Cl^- efflux because Allen Brain Atlas ([17], <http://www.brain-map.org>) indicates the presence of CFTR mRNA in the mouse SCN. Prior works also indicate that H1R is positively coupled through the $G_{\beta\gamma}$ protein to the cAMP signaling pathway [18, 19], which can lead to CFTR activation by stimulating protein kinase A (PKA) [20–24]. Here we present results indicating that the $G_{\beta\gamma}$ -cAMP/PKA-CFTR pathway links H1R to L-type Ca^{2+} channels and this pathway is essential for the phase delay of the circadian clock induced by histamine.

Methods

Study approval

The procedures of experiments employed in the current study were approved by the Animal Research Policies Committees of Korea University College of Medicine and Korea Institute of Science and Technology. Also, they conformed to the guidelines of National Institutes of Health of the United States of America.

Animals and housing

Male C57BL/6 mice (B6 mice, 3–6 week-old) bred in Korea Institute of Science and Technology were used for

Ca^{2+} imaging, Cl^- imaging, enzyme immunoassays (EIA) and extracellular single-unit recording experiments. In addition, male knock-out (KO) mice (3–6 week-old) for NKCC1 ($Slc12a2^{-/-}$) or CFTR (B6.129P2-Cftr^{tm1Unc/J}), obtained from Professor Min Goo Lee at Yonsei University (Seoul, Korea), were used in some of these experiments. Before being used, the mice were housed in group cages (4–6/cage) in a vivarium (22–24 °C) with a 12 h/12 h light/dark cycle for ≥ 1 week. The times of lights-on and -off in the vivarium were designated as zeitgeber time (ZT) 0:00 h and ZT 12:00 h, respectively.

Preparation of brain slices

Brain slices were prepared between ZT 10:00 h and ZT12:00 h. Under urethane anesthesia (1.25 g/kg, i.p.), the brain was quickly resected and put in aerated (95 % $O_2/5$ % CO_2) ice-cold artificial cerebrospinal fluid (ACSF), which was composed of (in mM) 124 NaCl, 1.3 $MgSO_4$, 3 KCl, 1.25 NaH_2PO_4 , 26 $NaHCO_3$, 2.4 $CaCl_2$ and 10 glucose. After being chilled in the ACSF for 1–2 min, the brain was cut into slices (300–400 μm thickness) in ice-cold ACSF using a vibroslicer (World Precision Instruments or Leica VT100S). The slices containing the SCN were selected and kept in aerated (95 % $O_2/5$ % CO_2) ACSF at room temperature (R/T, 30–60 min) before being used for SCN cell dissociation or being transferred to an electrophysiological recording chamber, which was continuously perfused with aerated (95 % $O_2/5$ % CO_2) ACSF (35 °C).

SCN cell dissociation

The hypothalamic slices incubated in aerated ACSF as above were used for SCN cell dissociation. The procedures for the cell dissociation were identical to those described previously [12].

Ca^{2+} imaging

The Ca^{2+} imaging methods employed in this study were identical to those described previously [12]. $[Ca^{2+}]_i$ was expressed as the ratio of intensities of the fura-2 emission fluorescence of 510 nm, excited at 340 and 380 nm. All the Ca^{2+} -imaging experiments (as well as Cl^- -imaging study, below) were carried out between ZT 13:00 h and ZT 18:00 h.

Cl^- imaging

To measure changes of intracellular Cl^- concentrations ($[Cl^-]_i$), the Cl^- sensor N-(ethoxy carbonyl methyl)-6-methoxyquinolinium bromide (MQAE, Invitrogen, CA) was used. This sensor shows the highest fluorescence in the absence of Cl^- , and therefore, a decrease of the monitored fluorescence represents an increase in $[Cl^-]_i$, and vice versa. Dissociated SCN cells plated on poly-D-lysine-coated glass coverslips were incubated for 2 h in

HEPES-buffered saline that contained 2-mM MQAE and then rinsed with HEPES-buffered saline before being moved to an imaging chamber mounted on an inverted microscope (IX70, Olympus). MQAE was excited at 350 nm and emitted at 460 nm. Imaging data were acquired with the use of an intensified CCD camera (CasCade, Roper Scientific) and Metafluor software (Molecular Device). Changes in $[Cl^-]_i$ were calculated as ΔF with

$$\Delta F = \frac{(F-F_0)}{F_0}$$

where F is the fluorescence at each time point and F_0 is the average of the fluorescence measured at the first ten time points.

Microdissection of the SCN and cAMP measurement

Brain slices (350- μ m thickness) were prepared from B6 mice (4~5 week-old) as described above. After being treated with drugs (see Results) for 30 min starting at ZT 14:00 h, the slices were mounted on a silicon rubber stage chilled with dry-ice powder. The SCN on both the left and right sides were punched out from the frozen section with the use of a blunt needle (21 G) under a dissecting microscope. The SCN punches from each hypothalamic slice were pooled in 0.1-N HCl solution (300 μ l) and then sonicated with Bioruptor (COSMO BIO, Japan) at 4 °C. The concentration of cAMP was determined with the use of Cyclic AMP EIA kit (Cayman Chemical, MI) according to the manufacturer's protocol. The protein content was determined with Pierce™ BCA Protein Assay Kit (Thermo scientific, MA). Following centrifugation at 1,000 g for 10 min, the supernatant was diluted 1:2 with EIA buffer (Cayman Chemical). All SCN samples were acetylated prior to the determination of cAMP level by EIA. The assays were performed in duplicate, with measurement at $\lambda = 405$ nm on a micro plate reader (SPECTRA max plus 384, Molecular Devices, CA).

Experimental treatment and single-unit recording

On the first day, the slice was treated with histamine for 30 min from ZT 14:00 h. In certain experiments, histamine was applied to the slice along with bumetanide (NKCC blocker), gallein ($G_{\beta\gamma}$ blocker), CFTR_{inh}-172 (CFTR blocker), H89 (PKA blocker) or KT5720 (PKA blocker). The application of these drugs commenced at ZT 13:50 h and lasted for 50 min. Control slices were challenged neither with histamine nor with any of the blockers above.

On the second day, single-unit recordings were obtained extracellularly from the SCN at ZT 1:00–15:00 h. To detect the time at which the peak of circadian firing activity rhythm of SCN neurons occurs (i.e., a reliable

marker of the phase of circadian clock [25]), we plotted against ZT the average firing rates of randomly sampled single units for sequential 2-h periods with 1-h lags [25]. To quantify the phase shift resulting from a drug treatment, we measured the difference of the time-of-peak found in the experimental slice from the average time-of-peak of control slices [12, 25].

Drugs

Drugs and chemicals utilized in the present study were from Sigma-Aldrich (St. Louis, MO) or Tocris Bioscience (Bristol, UK). The solutions of histamine (100 μ M; [26–28]), 2-pyridylethylamine (100 μ M; [29–33]) and amthamine (20 μ M; [34–36]) were prepared in ACSF or HEPES-buffered saline. The solutions of bumetanide (10 μ M; [16, 37, 38]), CFTR_{inh}-172 (50 μ M; [39–41]), gallein (100 μ M; [42–44]), H89 (10 μ M; [45–47]), KT5720 (1 μ M; [48–50]), bicuculline (30 μ M; [51–53]) and strychnine (1 μ M; [54–56]) were prepared by diluting the stock solutions with ACSF or HEPES-buffered saline; the solvent of stock solutions was dimethyl sulphoxide and its final concentration was 0.01–0.003 %. The drug solutions were applied to SCN slices or dissociated SCN cells by peristaltic pump or gravity-fed bath-perfusion system [15].

Statistics

Numerical data are expressed as the mean \pm SEM. Student t -test and Mann–Whitney Rank Sum test were employed to compare two independent data sets with and without normal distribution, respectively. Paired samples having normal distribution were compared with paired t -test while paired samples without normal distribution with Wilcoxon Signed Rank test. One-way analysis of variance (ANOVA) and pairwise comparison with Student-Newman Keuls tests were performed to compare ≥ 3 independent data sets having normal distributions, while Kruskal-Wallis one-way ANOVA on ranks and pairwise comparison with Student-Newman Keuls tests were performed to compare ≥ 3 data sets lacking normal distributions. Chi-square test was used to determine whether there is a significant difference between the expected frequencies and the observed frequencies in one or more categories. $P < 0.05$ (two-sided) was considered significant.

Results

Histamine induces the efflux of NKCC1-accumulated Cl^- and, through this mechanism, activates L-type Ca^{2+} channels in SCN neurons

To test our hypothesis, we first examined how the NKCC inhibitor bumetanide affects the histamine (100 μ M, 30 s)-elicited rise in $[Ca^{2+}]_i$ in dissociated SCN neurons and whether NKCC1 KO mouse neurons would show an altered histamine response. Bumetanide (10 μ M) attenuated

the histamine-elicited Ca^{2+} rise in 41 of 105 cells (0.54 ± 0.06 to 0.20 ± 0.05) or converted the rise into fall in 40 of 105 cells (0.47 ± 0.04 to -0.31 ± 0.12) (Fig. 1a). In the remaining cells, it did not affect (7 of 105 cells; 0.77 ± 0.09 vs. 0.78 ± 0.09) or enhanced the histamine-elicited Ca^{2+} increase (17 of 105 cells; 0.32 ± 0.06 to 0.61 ± 0.08) (Fig. 1a). These effects of bumetanide were statistically significant [$t(104) = 8.302, p < 0.001$, paired t -test]. Likewise, histamine-evoked Ca^{2+} response was reduced in SCN neurons prepared from NKCC1 KO mice. In the KO mice, histamine application caused Ca^{2+} rise in 31 % of the neurons (28 of 90 cells from 2 mice) with an average increase of 0.30 ± 0.04 (Δ ratio 340/380). In wild-type mice, histamine application resulted in an increase in Ca^{2+} level in 60 % of the neurons (116 of 192 cells from 3 mice) with an average increase of 0.50 ± 0.03 (Δ ratio 340/380). Thus, in NKCC1 KO mice, the proportion of cells showing histamine-induced Ca^{2+} response was

significantly reduced [Chi-square(2) = 44.282, $p < 0.001$, Chi-square test] as well as the average magnitude of the response [$U = 2120, p < 0.001$, Mann-Whitney Rank Sum test]. Taken together, these results support the hypothesis that histamine induces the efflux of NKCC1-accumulated Cl^- in at least a subset of SCN neurons and through that mechanism increases $[Ca^{2+}]_i$.

In order to obtain more direct evidence that histamine causes Cl^- efflux in SCN neurons, we next performed Cl^- -imaging experiments using the fluorescent Cl^- sensor MQAE. Bath application of the H1R agonist 2-pyridylethylamine (100 μ M, 30 s) but not the H2R agonist amthamine (20 μ M, 30 s), resulted in a reliable decrease in $[Cl^-]_i$ in a subset of dissociated SCN cells (450 of 1121 cells from 26 mice) (Fig. 1b, left trace); the average peak amplitude of the H1R agonist-induced Cl^- response (ΔF) was 0.054 ± 0.003 ($n = 450$). In the remaining cells, H1R agonist produced either an increase

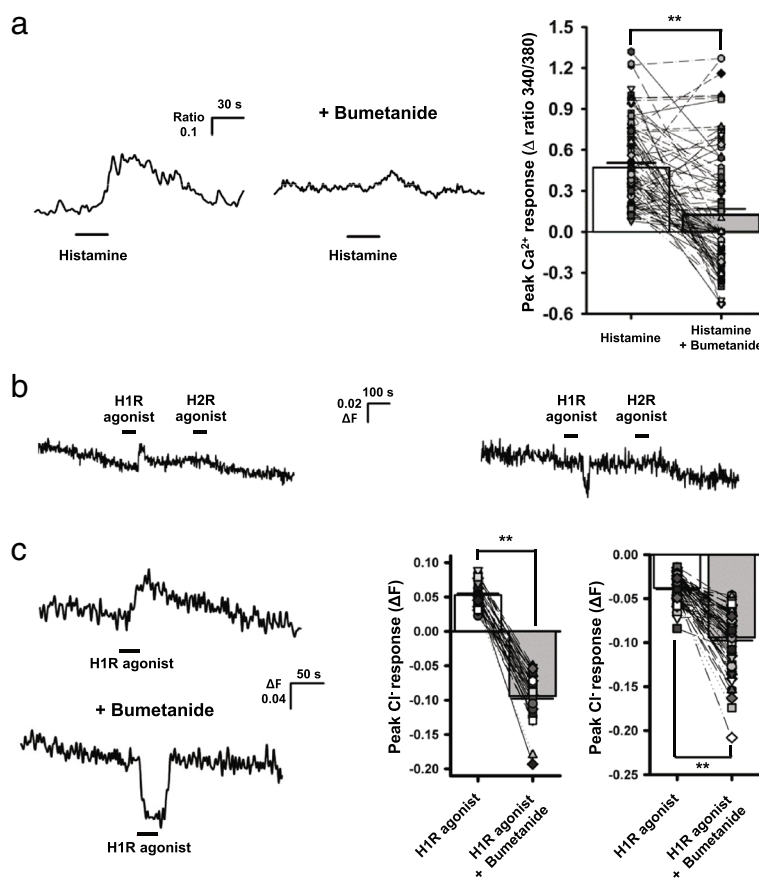


Fig. 1 Histamine induces the efflux of NKCC1-accumulated Cl^- , leading to intracellular Ca^{2+} rise in SCN neurons. **(a, left panel)** Traces from a Ca^{2+} -imaging experiment which show the effect of bumetanide on histamine-elicited increase in $[Ca^{2+}]_i$ in an SCN neuron. **(a, right panel)** Graphs summarizing the effects of bumetanide on the histamine-elicited Ca^{2+} responses in 105 SCN neurons from 3 mice. The bar charts indicate the mean (\pm SEM) peak Ca^{2+} responses. **b** Traces from Cl^- -imaging experiments show the effects of H1R agonist on $[Cl^-]_i$ in SCN cells. Up- and downward deflections of the trace denote decrease and increase in $[Cl^-]_i$, respectively. **c** Traces and summary graphs showing the effects of bumetanide on H1R agonist-induced decrease ($n = 81$) and increase ($n = 80$) in $[Cl^-]_i$ in SCN cells. The bar charts indicate the mean (\pm SEM) peak Cl^- responses. The symbols connected by lines in **(a)** and **(c)** denote data from the same cells. **: $p < 0.001$, paired t -test

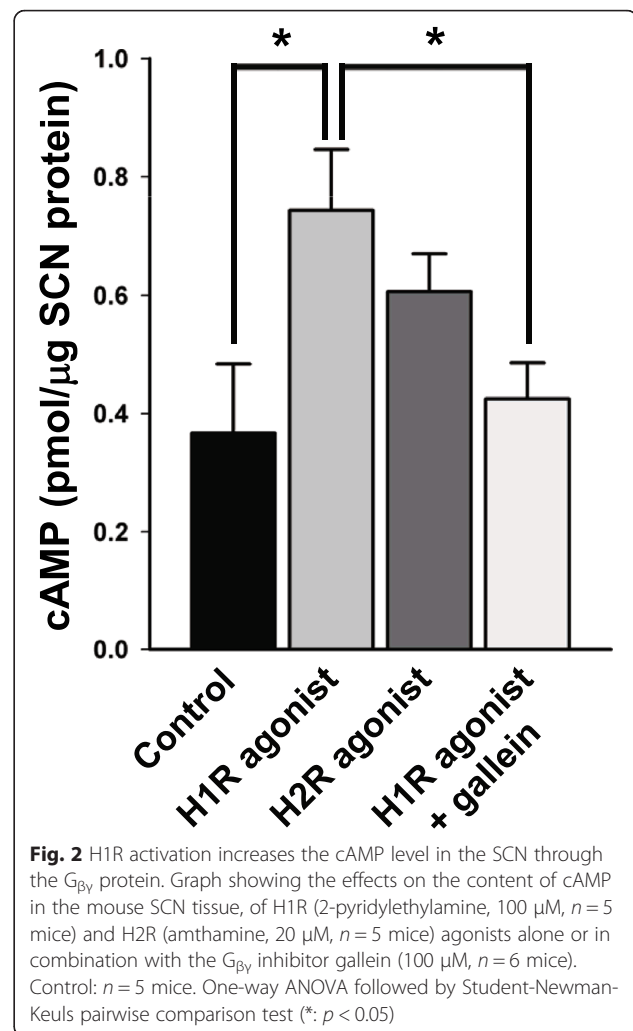
($\Delta F = -0.069 \pm 0.003$, $n = 375$) (Fig. 1b, right trace), or no change in $[Cl^-]_i$ ($n = 296$). After bumetanide (10 μM) treatment, the H1R agonist-induced decrease in $[Cl^-]_i$ was converted into an increase [$n = 81$ cells from 4 mice, $\Delta F = 0.053 \pm 0.003$ to -0.094 ± 0.003 ; $t(80) = 35.258$, $p < 0.001$, paired t -test] while the H1R agonist-induced increase in $[Cl^-]_i$ was enhanced [$n = 80$ cells from 4 mice, $\Delta F = -0.038 \pm 0.002$ to -0.094 ± 0.004 ; $t(79) = 15.929$, $p < 0.001$, paired t -test] (Fig. 1c). Collectively, these results indicate that histamine causes efflux of NKCC1-accumulated Cl^- in a subset of SCN neurons.

H1R- $G_{\beta\gamma}$ -cAMP/PKA pathway mediates histamine response

Previous work suggests that H1R is positively linked to cAMP signaling pathway through the $G_{\beta\gamma}$ protein [18, 19], and it is well known that cAMP is a strong PKA activator [57, 58]. Thus, we next explored the possibility that the $G_{\beta\gamma}$ protein arising from the activation of H1R promotes the cAMP production to activate PKA and ultimately to bring about histamine-induced responses in SCN neurons. To this end, we examined whether H1R activation leads to the increase in cAMP level in the SCN and, if so, the treatment of gallein ($G_{\beta\gamma}$ protein inhibitor) blocks this increase, and whether gallein and the PKA blocker H89 suppress the H1R agonist-elicited Ca^{2+} and Cl^- responses in dissociated SCN cells. We found that application of the H1R agonist 2-pyridylethylamine (100 μM , for 30 min starting at ZT 14:00 h), but not the H2R agonist amthamine (20 μM , for 30 min starting at ZT 14:00 h), resulted in a significant increase in cAMP content in the SCN and this effect was precluded by co-applied gallein (100 μM) [one-way ANOVA: $F(3,17) = 3.828$, $p = 0.029$, Student-Newman-Keuls pairwise comparison tests: Control vs. H1R agonist: $p = 0.037$, Control vs. H2R agonist: $p = 0.170$; Control vs. H1R agonist + gallein: $p = 0.635$; H1R agonist vs. H2R agonist: $p = 0.289$; H1R agonist vs. H1R agonist + gallein: $p = 0.043$; H2R agonist vs. H1R agonist + gallein: $p = 0.153$] (Fig. 2). Moreover, we discovered that both gallein (100 μM) and H89 (10 μM) generally suppressed the H1R agonist (100 μM , 30 s)-elicited Ca^{2+} and Cl^- responses (Fig. 3). Thus, taken together, these results suggested that the H1R- $G_{\beta\gamma}$ -cAMP/PKA pathway mediates histamine-elicited responses in SCN neurons.

CFTR mediates histamine-induced Cl^- efflux

In order to better understand the histaminergic regulation of Cl^- in SCN neurons, we then explored the possible role of CFTR, the PKA-activated Cl^- channel. Specifically, we examined the effects of the CFTR inhibitor CFTR_{inh}-172 on histamine (or H1R agonist)-induced responses in SCN cells. We found that the application of CFTR_{inh}-172 (50 μM) blocked both Cl^- efflux [ΔF :



0.111 ± 0.035 to 0.003 ± 0.003 , $n = 41$ cells from 4 mice; $W = -861$, $p < 0.001$, Wilcoxon Signed Rank test; Fig. 4a and b, left panel] and influx [ΔF : -0.108 ± 0.011 to 0.002 ± 0.002 , $n = 38$ cells from 4 mice; $W = 741$, $p < 0.001$, Wilcoxon Signed Rank test; Fig. 4b, right panel] induced by 2-pyridylethylamine (H1R agonist; 100 μM , 30 s) in SCN cells. Furthermore, we discovered that CFTR_{inh}-172 attenuated significantly the histamine (100 μM , 30 s)-elicited Ca^{2+} rise in SCN neurons [0.53 ± 0.05 to 0.15 ± 0.03 , $n = 31$ cells from 3 mice; $t(30) = 6.849$, $p < 0.001$, paired t -test] (Fig. 4c). Collectively, these results indicated that CFTR mediates histamine-induced Cl^- fluxes in SCN neurons.

Histamine resets the circadian clock through the $G_{\beta\gamma}$ -cAMP/PKA-CFTR pathway

Our previous work has indicated that, in order to reset the circadian clock, histamine raises $[Ca^{2+}]_i$ in SCN neurons by activating the $Ca_v1.3$ L-type Ca^{2+} channels through H1R, and secondarily by causing Ca^{2+} -induced Ca^{2+} release from RyR-mediated internal stores [12].

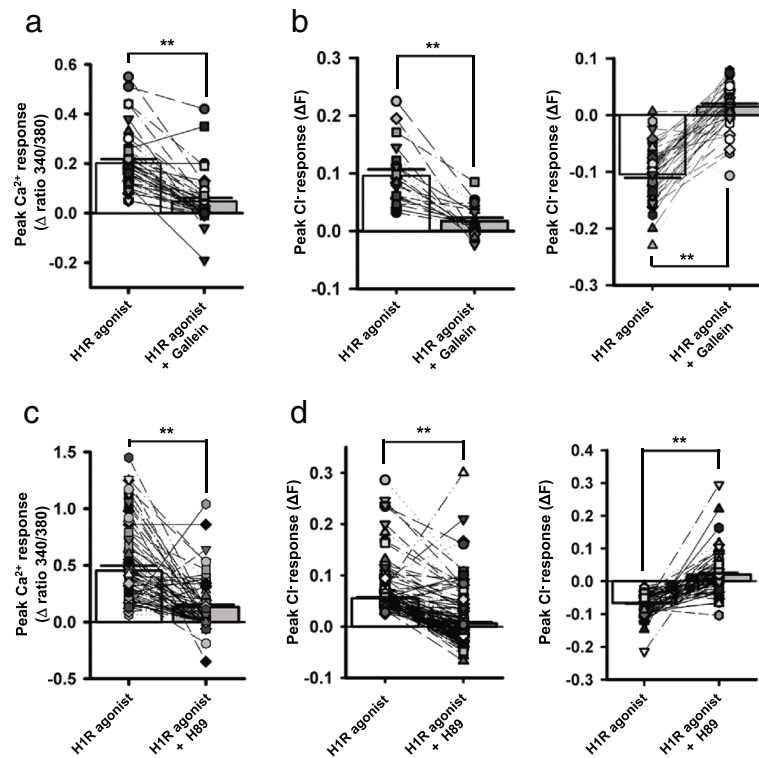


Fig. 3 $G_{\beta\gamma}$ -cAMP/PKA signaling pathway plays a crucial role in H1R agonist-elicited Ca^{2+} rise and Cl^{-} fluxes. **a** Summary graphs showing the effects of the $G_{\beta\gamma}$ blocker gallein on H1R agonist-elicited increase in $[Ca^{2+}]_i$. The bar charts indicate the mean (\pm SEM) peak Ca^{2+} responses. **: $p < 0.001$ [$n = 47$ neurons from 3 mice, $t(46) = 10.409$, paired t -test]. **b** Summary graphs showing the effects of gallein on H1R agonist-induced Cl^{-} efflux [left panel, $n = 21$ cells from 4 mice, $t(20) = 6.783$, $p < 0.001$, paired t -test] and influx [right panel, $n = 50$ cells from 4 mice, $t(49) = -14.644$, $p < 0.001$, paired t -test]. The bar charts indicate the mean (\pm SEM) peak Cl^{-} responses. **: $p < 0.001$. **c** Summary graphs showing the effects of the PKA inhibitor H89 on H1R agonist-elicited increase in $[Ca^{2+}]_i$. The bar charts indicate the mean (\pm SEM) peak Ca^{2+} responses. **: $p < 0.001$ [$n = 90$ neurons from 3 mice, $t(89) = 7.272$, paired t -test]. **d** Summary graphs showing the effects of H89 on H1R agonist-induced Cl^{-} efflux [left panel, $n = 277$ cells from 8 mice, $t(276) = 19.895$, paired t -test] and influx [right panel, $n = 90$ cells from 8 mice, $t(89) = -11.458$, paired t -test]. The bar charts indicate the mean (\pm SEM) peak Cl^{-} responses. **: $p < 0.001$. The symbols connected by lines in (a-d) denote data from the same cells

Meanwhile, the results described above suggest that the $G_{\beta\gamma}$ -cAMP/PKA-CFTR pathway is responsible for linking H1R to L-type Ca^{2+} channel. To further test this hypothesis, we investigated how the pharmacological blockade or genetic removal of $G_{\beta\gamma}$, PKA, CFTR or NKCC1 altered the histamine-elicited phase delay of circadian neural activity rhythm in the mouse SCN slice. In the control slices, the peak of the circadian neural activity rhythm occurred at ZT 6.00 ± 0.33 h ($n = 6$; Fig. 5a and k). In the histamine (100 μ M)-treated slices, the peak was phase-delayed about 6 h to ZT 11.67 ± 0.21 h ($n = 6$; Fig. 5b and k) by the next cycle [6, 12]. This histamine effect on the circadian phase was almost completely blocked by the co-applied inhibitors of $G_{\beta\gamma}$ (gallein, 100 μ M), PKA (H89, 10 μ M; KT5720, 1 μ M), CFTR (CFTR_{inh}-172, 50 μ M) and NKCC (bumetanide, 10 μ M) (Fig. 5c-f and k), but not by the GABA_A receptor antagonist bicuculline (30 μ M) or the glycine receptor antagonist strychnine (1 μ M) (Fig. 5g, h and k). In addition, the histamine-elicited phase delays were absent in KO

mice lacking CFTR and NKCC1 (Fig. 5i-k). In control experiments, we found that application of the blockers alone or the genetic loss of CFTR and NKCC1 did not alter the phase of the circadian rhythm. The time of peak electrical activity of SCN neurons recorded in the slices treated with either gallein [ZT 6.5 ± 0.3 h, $n = 4$; $p = 0.242$, $t(8) = -1.265$, t -test], H89 [ZT 5.8 ± 0.3 h, $n = 4$; $p = 0.610$, $U = 14.5$, Mann-Whitney Rank Sum Test], CFTR_{inh}-172 [ZT 5.5 ± 0.3 h, $n = 4$; $p = 0.242$, $t(8) = 1.265$, t -test] or bumetanide alone [ZT 6.5 ± 0.3 h, $n = 4$; $p = 0.242$, $t(8) = -1.265$, t -test] was not significantly different from that of neurons recorded in drug-untreated, control slices (ZT 6.00 ± 0.33 h, $n = 6$). Likewise, the time of peak electrical activity of SCN neurons recorded in the slices from CFTR KO [ZT 6.3 ± 0.5 h, $n = 4$; $p > 0.999$, $U = 12$, Mann-Whitney Rank Sum test] or NKCC1 KO mice [ZT 6.0 ± 0.4 h, $n = 4$; $p > 0.999$, $U = 12$, Mann-Whitney Rank Sum Test] was not significantly different from that of neurons recorded in the slices from wild-type animals (ZT 6.00 ± 0.33 h, $n = 6$).

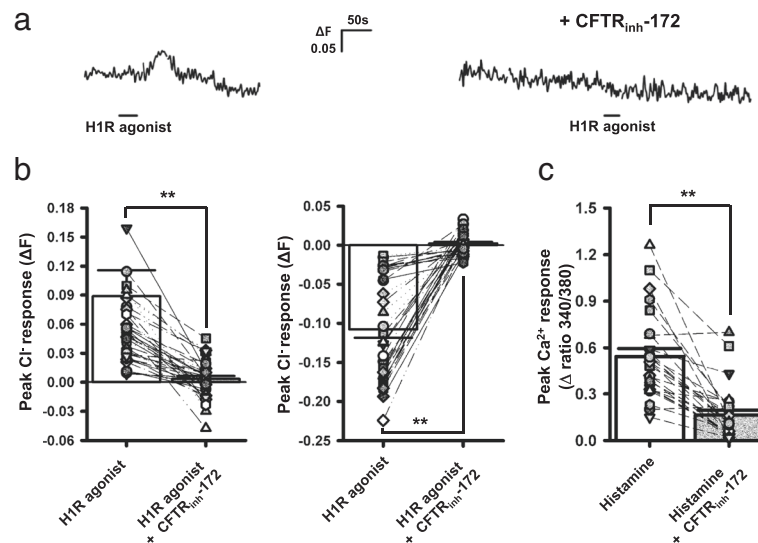


Fig. 4 The CFTR inhibitor CFTR_{inh}-172 suppresses histamine-elicited Cl⁻ efflux and Ca²⁺ rise in SCN cells. **a** Traces from Cl⁻-imaging experiments that show the inhibitory effects of CFTR_{inh}-172 on 2-pyridylethylamine (H1R agonist)-induced Cl⁻ efflux in an SCN cell. **b** Summary graph showing the effect of CFTR_{inh}-172 on H1R agonist-induced Cl⁻ efflux ($n = 41$ cells from 4 mice; *left panel*) and influx ($n = 38$ cells from 4 mice; *right panel*) in SCN cells. **c** Summary graph showing the effect of CFTR_{inh}-172 on histamine-elicited increase in [Ca²⁺]_i in SCN neurons ($n = 31$ neurons from 3 mice). The bar charts in **(b)** and **(c)**, respectively, indicate the mean (\pm SEM) Cl⁻ and Ca²⁺ responses elicited by H1R agonist or histamine in the absence or presence of CFTR_{inh}-172. The symbols connected by lines in **(b)** and **(c)** denote data from the same cells. **, $p < 0.001$

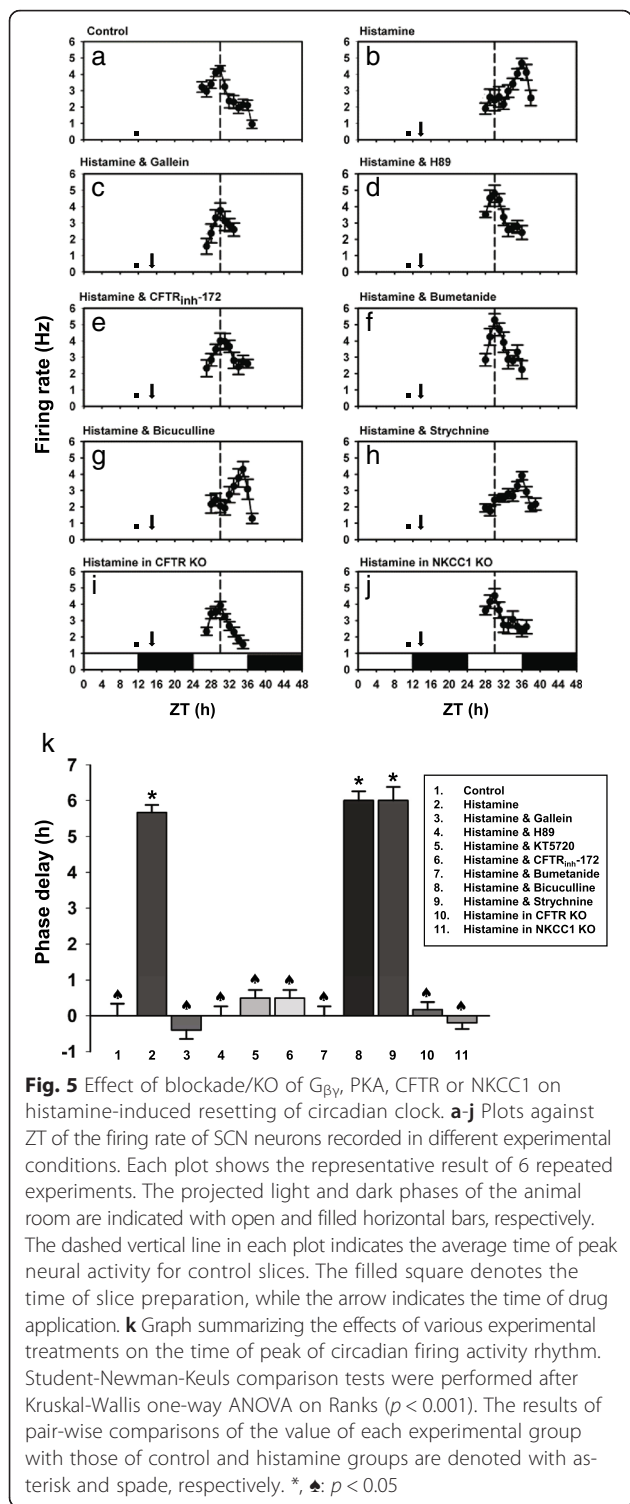
Thus, taken together, the results from the functional assay further supported our hypothesis that the G_{βγ}-cAMP/PKA-CFTR pathway links H1R to Ca_v1.3 L-type Ca²⁺ channels in SCN neurons, leading to the resetting of the circadian clock (Fig. 6).

Discussion

In a recent communication [12] we reported that, in order to reset the circadian clock in the mouse SCN, histamine increases [Ca²⁺]_i in SCN neurons by activating the Ca_v1.3 L-type Ca²⁺ channels through H1R, and secondarily by causing Ca²⁺-induced Ca²⁺ release from RyR-mediated internal stores. Moreover, we provided evidence that IP₃ and DAG, the downstream of PLC, are not involved in the H1R-mediated rise in [Ca²⁺]_i. In the current study, we explored the signaling mechanism that links the H1R to the Ca_v1.3 channels and obtained the results indicating that, in a subset of SCN neurons loaded with Cl⁻ by NKCC1, histamine causes Cl⁻ efflux through H1R to produce membrane depolarization needed for the activation of Ca_v1.3 channels. CFTR is well known as anion channel whose malfunction leads to dysregulation of epithelial fluid transport in the lung, pancreas and other organs, resulting in cystic fibrosis [22, 24, 59]. In this study, we present evidence that CFTR mediates the histamine-elicited Cl⁻ efflux in SCN cells while the G_{βγ}-cAMP/PKA pathway downstream of H1R activates the CFTR. Studies have provided evidence that histamine-activated Cl⁻ channels are expressed in

mammalian brain [13, 14] and that CFTR mRNA exists in the mouse SCN ([17], <http://www.brain-map.org>). Also, it has been reported that H1R is positively coupled through the G_{βγ} protein to the cAMP signaling pathway [18, 19], which can activate CFTR by up-regulating PKA [20–24]. Nevertheless, the H1R-mediated signaling pathway that involves CFTR has never been reported. To the best of our knowledge, the current study is the first to provide the evidence for the existence of such a signaling pathway. Also, this study is the first to present evidence indicating that CFTR has a functional role in the circadian time-keeping system.

In the current study we noticed that, even after the blockade of CFTR, a significant proportion of SCN neurons still responded to histamine with an increase in [Ca²⁺]_i although the magnitudes of the responses were significantly smaller. This finding suggests that mechanism(s) other than the membrane depolarization arising from CFTR-mediated Cl⁻ efflux also contributes to the L-type Ca²⁺ channel activation by histamine. The possible mechanisms include the direct activation of the Ca²⁺ channel by PKA [60–63] and the blockade of leak K⁺ channels through the H1R-coupled G_{q/11} protein [2], which can produce the membrane depolarization needed for the activation of the Ca²⁺ channels. Whether these mechanisms operate in SCN neurons for the histaminergic activation of L-type Ca²⁺ channels and, if so, whether they work along with the “CFTR” mechanism in the same cells await further investigation.



The results of the current study indicate that NKCC1 is crucial for the histamine-elicited increase in $[Ca^{2+}]_i$ in SCN neurons and the subsequent resetting of the circadian clock in the SCN. By importing Cl^- , NKCC1 provides the driving force for the histamine-elicited Cl^- efflux through CFTR which produces membrane

depolarization needed for the activation of L-type Ca^{2+} channels. Our data do not suggest that histamine effects depend on GABAergic and/or glycinergic transmission, the polarity and strength of the postsynaptic responses of which can be regulated by NKCC1. This view is consistent with the finding that histamine caused $[Ca^{2+}]_i$ to rise in dissociated SCN neurons which were no longer under synaptic control. Moreover, it is consistent with the observation that both bicuculline and strychnine had no significant effects on the histamine-elicited phase delay of the circadian neural activity rhythm in the SCN slice.

The present study demonstrates that, in certain SCN cells (~33 % cells examined), H1R activation led to Cl^- influx, instead of efflux. This is presumably because of that, in these cells, the NKCC1 activity was low or absent such that the electrochemical gradient for Cl^- was set toward the intracellular side. At present, we do not know the functional significance of the histamine-induced Cl^- influx. However, it is unlikely that this Cl^- flux promoted histamine to phase-delay the circadian pacemaker in the SCN, since the blockade of NKCC1 by bumetanide, which converted the H1R agonist-induced Cl^- efflux into influx in some cells and enhanced the H1R agonist-induced Cl^- influx in other cells, resulted in a complete blockade of the histamine-elicited phase shift.

Significance

A variety of evidence indicates that histamine is a potent regulator of the phase of the circadian system. Prior work has shown that histamine application onto SCN neurons in the rat and hamster hypothalamic slices increases or decreases the electrical activities of these cells [64–66] while bath-applied histamine phase-shifts the circadian neural activity rhythms recorded in hamster and mouse SCN slices [6, 7, 27]. The inhibition of histamine synthesis by α -fluoromethylhistidine decreases the phase shifts of circadian activity rhythms induced by light in the hamster [9]. Injection of histamine into the cerebral ventricle in the rat leads to the phase shifts of circadian activity rhythms [8] and decreases the time needed to entrain activity rhythms to an abruptly advanced light–dark cycle [67]. In our own work, we demonstrated that histamine acts on H1R to alter the phase of the circadian clock in the mouse SCN and this effect depends on the increase of $[Ca^{2+}]_i$ which results from the activation of $Ca_v1.3$ L-type Ca^{2+} channels and Ca^{2+} -induced Ca^{2+} release from RyR-mediated internal stores [12]. In the present study, we further explored the signal transduction mechanisms and present evidence that the $G_{\beta\gamma}$ -cAMP/PKA-CFTR pathway is intercalated between H1R and the L-type Ca^{2+} channels. These results clearly delineate a novel signaling mechanism that underlies the histamine-induced resetting of the circadian clock.

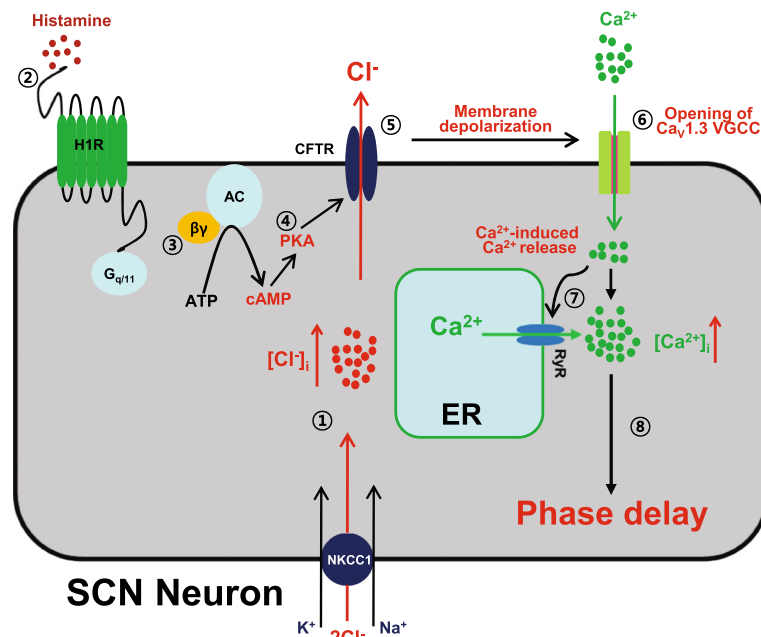


Fig. 6 Proposed signaling pathway for histamine-induced resetting of the circadian pacemaker in the SCN. ① A subset of SCN neurons are loaded with Cl⁻ by the action of the Cl⁻ importer NKCC1. ② H1R stimulation by histamine at early night results in the production of activated G_{βγ} protein in these cells. ③ The activated G_{βγ} protein stimulates adenylyl cyclase (AC) to produce cAMP from ATP. ④ PKA activated by cAMP opens the Cl⁻ channel CFTR. ⑤ The efflux of Cl⁻ through CFTR down the electrochemical gradient results in membrane depolarization. ⑥ In response to this membrane depolarization, the Ca_v1.3 L-type voltage-gated Ca²⁺ channel (VGCC) is activated. ⑦ The resulting Ca²⁺ influx through Ca_v1.3 VGCC induces Ca²⁺ release from the endoplasmic reticulum (ER) through RyR. ⑧ The consequent increase in [Ca²⁺]_i leads to phase delay of the circadian clock

More broadly, there are many remaining questions about the role of histamine in the generation of circadian rhythms *in vivo* and the functional significance of the phase control remains unclear. Among the complications is evidence for species-specific differences as Scott et al. [66] showed that the hamster SCN is almost devoid of histaminergic fibers and that histamine has only a weak phase-shifting effect on the free-running locomotor activity rhythm. In addition, the inhibition of histamine synthesis by α -fluoromethylhistidine or the KO of histidine decarboxylase gene disrupted the circadian activity rhythm in the rat [8] and lowered the levels of wheel-running and spontaneous locomotor activities in mice in both light–dark and dark–dark conditions [68]. These findings raise the possibility that histamine may be involved in the generation of the rhythms as well as phase control. The receptor mediating these effects is less clear. Studies on H1R KO mice found disrupted diurnal feeding rhythm [69] but essentially normal locomotor activity rhythms [70]. In contrast, the H3R KO mice exhibited a greatly reduced locomotor activity rhythm but normal rhythms in clock gene expression [70]. Unfortunately, the studies with the KO mice did not examine whether these genetic manipulations altered the photic regulation of the circadian cycle. Taken together, these data indicate that histamine is a potent regulator of the

circadian time-keeping system in some mammals and suggest that the H1R signaling is important for phase control while the H3R signaling may be more important for regulation of the amplitude of circadian rhythms in activity.

Conclusions

In summary, we present experimental results indicating that, in order to activate Ca_v1.3 L-type Ca channels in SCN neurons and ultimately to phaselay the circadian clock in the SCN, histamine recruits the H1R-G-cAMP/PKACFTR pathway in the SCN neurons loaded with Cl⁻ by NKCC1. This pathway and NKCC1 may well be potential targets for drugs designed to treat clinical problems resulting from the disturbance of the circadian system.

Ethics approval

The experimental procedures employed in the present study were approved by the Animal Research Policies Committees of Korea University College of Medicine and Korea Institute of Science and Technology.

Consent for publication

Not applicable.

Availability of data and materials

We do not have any special data or materials to share with readers.

Abbreviations

[Ca²⁺]_i: intracellular calcium concentration; [Cl⁻]_i: intracellular chloride concentration; ACSF: artificial cerebrospinal fluid; ANOVA: one-way analysis of variance; CFTR: cystic fibrosis transmembrane conductance regulator; DAG: diacylglycerol; H1R: histamine 1 receptor; IP₃: Inositol 1,4,5-trisphosphate; KO: knock-out; MQAE: N-(ethoxy carbonyl methyl)-6-methoxyquinolinium bromide; NKCC1: Na⁺-K⁺-2Cl⁻ cotransporter isoform 1; PKA: protein kinase A; PLC: phospholipase C; R/T: room temperature; SCN: suprachiasmatic nucleus; RyR: ryanodine receptor; TMN: tuberomammillary nucleus; TRPC: transient receptor potential cation channel; VGCC: voltage-gated calcium channel; ZT: zeitgeber time.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Y.S.K., S.B.O., H.C.H., C.S.C., C.J.L. and Y.I.K. conceived this project. Y.S.K., Y.-B.K., W.B.K. and S.W.L. performed the experiments and analyzed the results. Y.S.K., C.S.C. and Y.I.K. wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grants funded by the Korea government (MSIP) to Y. I Kim (NRF-2011-0022529 and No. 2014R1A2A1A11049900) and Korea University Grant (K1031781; Y.I.K), and by KIST institutional program, Project No. 2E25210 (C.J.L.), and Brain Research Program through the National Research Foundation of Korea funded by the Ministry of Science, ICT & Future Planning, NRF-2012M3C7A1055412 (C.J.L.), and WCI Program of National Research Foundation (C.J.L.). Y.S.K., Y.-B.K., W.B.K., S.W.L. and Y.I.K. were supported by the Brain Korea 21 Project from 2009 to 2014. Y.S.K. and C.S.C. received support from the O'Keefe Foundation.

Author details

¹Department of Physiology and Neuroscience Research Institute, Korea University College of Medicine, 126-1 Anam-dong 5-ga, Seoul 136-705, Republic of Korea. ²Pain Cognitive Function Research Center, Dental Research Institute and Department of Neurobiology and Physiology, Seoul National University, Seoul 110-749, Republic of Korea. ³Center for Neuroscience and Functional Connectomics, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea. ⁴Department of Psychiatry & Biobehavioral Sciences, University of California-Los Angeles, 760 Westwood Plaza, Los Angeles, CA 90024, USA.

Received: 22 March 2016 Accepted: 15 April 2016

Published online: 06 May 2016

References

- Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y, et al. Distribution of the histaminergic neuron system in the central nervous system of rats - A fluorescent immunohistochemical analysis with histidine-decarboxylase as a marker. *Brain Res.* 1984;295(1):13–25.
- Haas H, Panula P. The role of histamine and the tuberomammillary nucleus in the nervous system. *Nat Rev Neurosci.* 2003;4(2):121–30.
- Antle MC, Silver R. Orchestrating time: arrangements of the brain circadian clock. *Trends Neurosci.* 2005;28(3):145–51.
- Golombek DA, Rosenstein RE. Physiology of circadian entrainment. *Physiol Rev.* 2010;90(3):1063–102.
- Welsh DK, Takahashi JS, Kay SA. Suprachiasmatic nucleus: cell autonomy and network properties. *Annu Rev Physiol.* 2010;72:551–77.
- Cote NK, Harrington ME. Histamine phase shifts the circadian clock in a manner similar to light. *Brain Res.* 1993;613(1):149–51.
- Biello SM. Circadian clock resetting in the mouse changes with age. *Age.* 2009;31(4):293–303.
- Itowi N, Yamatodani A, Nagai K, Nakagawa H, Wada H. Effects of histamine and alpha-fluoromethylhistidine injections on circadian phase of free-running rhythms. *Physiol Behav.* 1990;47(3):549–54.
- Eaton SJ, Cote NK, Harrington ME. Histamine synthesis inhibition reduces light-induced phase shifts of circadian rhythms. *Brain Res.* 1995;695(2):227–30.
- Haas HL, Sergeeva OA, Selbach O. Histamine in the nervous system. *Physiol Rev.* 2008;88(3):1183–241.
- Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. *Nature.* 1999;397(6716):259–63.
- Kim YS, Kim YB, Kim WB, Yoon BE, Shen FY, Lee SW, et al. Histamine resets the circadian clock in the suprachiasmatic nucleus through the H1R-Ca(v)1.3-RyR pathway in the mouse. *Eur J Neurosci.* 2015;42(7):2467–77.
- Yang QZ, Hatton GL. Histamine mediates fast synaptic inhibition of rat supraoptic oxytocin neurons via chloride conductance activation. *Neuroscience.* 1994;61(4):955–64.
- Lee KH, Broberger C, Kim U, McCormick DA. Histamine modulates thalamocortical activity by activating a chloride conductance in ferret perigeniculate neurons. *Proc Natl Acad Sci USA.* 2004;101(17):6716–21.
- Choi HJ, Lee CJ, Schroeder A, Kim YS, Jung SH, Kim JS, et al. Excitatory actions of GABA in the suprachiasmatic nucleus. *J Neurosci.* 2008;28(21):5450–9.
- Farajnia S, van Westering TLE, Meijer JH, Michel S. Seasonal induction of GABAergic excitation in the central mammalian clock. *Proc Natl Acad Sci USA.* 2014;111(26):9627–32.
- Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, et al. Genome-wide atlas of gene expression in the adult mouse brain. *Nature.* 2007;445(7124):168–76.
- Marley PD, Thomson KA, Jachno K, Johnston MJ. Histamine-induced increases in cyclic-AMP levels in bovine adrenal medullary cells. *Brit J Pharmacol.* 1991;104(4):839–46.
- Maruko T, Nakahara T, Sakamoto K, Saito M, Sugimoto N, Takuwa Y, et al. Involvement of the betagamma subunits of G proteins in the cAMP response induced by stimulation of the histamine H1 receptor. *Naunyn Schmiedeberg Arch Pharmacol.* 2005;372(2):153–9.
- Tabcharani JA, Chang XB, Riordan JR, Hanrahan JW. Phosphorylation-regulated Cl⁻ channel in CHO cells stably expressing the cystic fibrosis gene. *Nature.* 1991;352(6336):628–31.
- Quinton PM. Physiological basis of cystic fibrosis. A historical perspective. *Physiol Rev.* 1999;79(1 Suppl):S3–S22.
- Gadsby DC, Vergani P, Csanady L. The ABC protein turned chloride channel whose failure causes cystic fibrosis. *Nature.* 2006;440(7083):477–83.
- Lee JH, Richter W, Namkung W, Kim KH, Kim E, Conti M, et al. Dynamic regulation of cystic fibrosis transmembrane conductance regulator by competitive interactions of molecular adaptors. *J Biol Chem.* 2007;282(14):10414–22.
- Cant N, Pollock N, Ford RC. CFTR structure and cystic fibrosis. *Int J Biochem Cell Biol.* 2014;52:15–25.
- Chen D, Buchanan GF, Ding JM, Hannibal J, Gillette MU. Pituitary adenylyl cyclase-activating peptide: A pivotal modulator of glutamatergic regulation of the suprachiasmatic circadian clock. *Proc Natl Acad Sci USA.* 1999;96(23):13468–73.
- Rotrosen D, Gallin JI. Histamine type I receptor occupancy increases endothelial cytosolic calcium, reduces F-actin, and promotes albumin diffusion across cultured endothelial monolayers. *J Cell Biol.* 1986;103(6):2379–87.
- Meyer JL, Hall AC, Harrington ME. Histamine phase shifts the hamster circadian pacemaker via an NMDA dependent mechanism. *J Biol Rhythms.* 1998;13(4):288–95.
- Shim WS, Tak MH, Lee MH, Kim M, Kim M, Koo JY, et al. TRPV1 mediates histamine-induced itching via the activation of phospholipase A(2) and 12-lipoxygenase. *J Neurosci.* 2007;27(9):2331–7.
- Aguilar MJ, Moralesolivas FJ, Rubio E. Pharmacological investigation into the effects of histamine and histamine analogs on guinea-pig and rat colon in vitro. *Brit J Pharmacol.* 1986;88(3):501–6.
- Dickenson JM, Hill SJ. Selective potentiation of histamine H1-receptor stimulated calcium responses by 1,4-dithiothreitol in DDT1MF-2 cells. *Biochem Pharmacol.* 1994;48(9):1721–8.
- Lundius EG, Sanchez-Alavez M, Ghochani Y, Klaus J, Tabarean IV. Histamine influences body temperature by acting at H1 and H3 receptors on distinct populations of preoptic neurons. *J Neurosci.* 2010;30(12):4369–81.
- Tabarean IV. Functional pharmacology of H1 histamine receptors expressed in mouse preoptic/anterior hypothalamic neurons. *Br J Pharmacol.* 2013;170(2):415–25.
- Gemba C, Nakayama K, Nakamura S, Mochizuki A, Inoue M, Inoue T. Involvement of histaminergic inputs in the jaw-closing reflex arc. *J Neurophysiol.* 2015;113(10):3720–35.

34. Shayo C, Fernandez N, Legnazzi BL, Monczor F, Mladovan A, Baldi A, et al. Histamine H2 receptor desensitization: involvement of a select array of G protein-coupled receptor kinases. *Mol Pharmacol*. 2001;60(5):1049–56.
35. Liu YW, Li J, Ye JH. Histamine regulates activities of neurons in the ventrolateral preoptic nucleus. *J Physiol*. 2010;588(21):4103–16.
36. Copsel S, Garcia C, Diez F, Vermeulem M, Baldi A, Bianciotti LG, et al. Multidrug resistance protein 4 (MRP4/ABCC4) regulates cAMP cellular levels and controls human leukemia cell proliferation and differentiation. *J Biol Chem*. 2011;286(9):6979–88.
37. Roopun AK, Simonotto JD, Pierce ML, Jenkins A, Nicholson C, Schofield IS, et al. A nonsynaptic mechanism underlying interictal discharges in human epileptic neocortex. *Proc Natl Acad Sci USA*. 2010;107(1):338–43.
38. Cleary RT, Sun H, Huynh T, Manning SM, Li Y, Rotenberg A, et al. Bumetanide enhances phenobarbital efficacy in a rat model of hypoxic neonatal seizures. *PLoS One*. 2013;8(3):e57148.
39. Penmatsa H, Zhang WQ, Yarlagadda S, Li CY, Conoley VG, Yue JM, et al. Compartmentalized cyclic adenosine 3',5'-monophosphate at the plasma membrane clusters PDE3A and cystic fibrosis transmembrane conductance regulator into microdomains. *Mol Biol Cell*. 2010;21(6):1097–110.
40. Dekkers JF, Wiegierinck CL, de Jonge HR, Bronsveld I, Janssens HM, de Winter-de Groot KM, et al. A functional CFTR assay using primary cystic fibrosis intestinal organoids. *Nat Med*. 2013;19(7):939–45.
41. Arora K, Moon C, Zhang WG, Yarlagadda S, Penmatsa H, Ren AX, et al. Stabilizing rescued surface-localized delta f508 CFTR by potentiation of its interaction with Na⁺/H⁺ exchanger regulatory factor 1. *Biochemistry*. 2014;53(25):4169–79.
42. Wilson SR, Gerhold KA, Bifolck-Fisher A, Liu Q, Patel KN, Dong XZ, et al. TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nat Neurosci*. 2011;14(5):595–602.
43. Ferreira T, Wilson SR, Choi YG, Rizzo D, Dudoit S, Speed TP, et al. Silencing of odorant receptor genes by G protein $\beta\gamma$ signaling ensures the expression of one odorant receptor per olfactory sensory neuron. *Neuron*. 2014;81(4):847–59.
44. Stott JB, Povstyan OV, Carr G, Barrese V, Greenwood IA. G-protein $\beta\gamma$ subunits are positive regulators of Kv7.4 and native vascular Kv7 channel activity. *Proc Natl Acad Sci USA*. 2015;112(20):6497–502.
45. Alle H, Jonas P, Geiger JRP. PTP and LTP at a hippocampal mossy fiber-interneuron synapse. *Proc Natl Acad Sci USA*. 2001;98(25):14708–13.
46. Skeberdis VA, Chevaleyre V, Lau CG, Goldberg JH, Pettit DL, Suadicanio SO, et al. Protein kinase A regulates calcium permeability of NMDA receptors. *Nat Neurosci*. 2006;9(4):501–10.
47. Tang F, Lane S, Korsak A, Paton JFR, Gourine AV, Kasparov S, et al. Lactate-mediated glia-neuronal signalling in the mammalian brain. *Nat Commun*. 2014;5:3284.
48. Leonard AS, Yermolaieva O, Hruska-Hageman A, Askwith CC, Price MP, Wemmie JA, et al. cAMP-dependent protein kinase phosphorylation of the acid-sensing ion channel-1 regulates its binding to the protein interacting with C-kinase-1. *Proc Natl Acad Sci USA*. 2003;100(4):2029–34.
49. Lin SL, Johns-Farley NN, Lubinsky DR, Cowen DS. Coupling of neuronal 5-HT7 receptors to activation of extracellular-regulated kinase through a protein kinase A-independent pathway that can utilize Epac. *J Neurochem*. 2003;87(5):1076–85.
50. Dodge-Kafka KL, Soughayer J, Pare GC, Michel JJC, Langeberg LK, Kafiloff MS, et al. The protein kinase A anchoring protein mAKAP coordinates two integrated cAMP effector pathways. *Nature*. 2005;437(7058):574–8.
51. Williams SR, Buhl EH, Mody I. The dynamics of synchronized neurotransmitter release determined from compound spontaneous IPSCs in rat dentate granule neurones in vitro. *J Physiol*. 1998;510(2):477–97.
52. Roberto M, Madamba SG, Moore SD, Tallent MK, Siggins GR. Ethanol increases GABAergic transmission at both pre- and postsynaptic sites in rat central amygdala neurons. *Proc Natl Acad Sci USA*. 2003;100(4):2053–8.
53. Anstee QM, Knapp S, Maguire EP, Hosie AM, Thomas P, Mortensen M, et al. Mutations in the Gabrb1 gene promote alcohol consumption through increased tonic inhibition. *Nat Commun*. 2013;4:2816.
54. Lim R, Alvarez FJ, Walmsley B. GABA mediates presynaptic inhibition at glycinergic synapses in a rat auditory brainstem nucleus. *J Physiol*. 2000;525(2):447–59.
55. Sadlaoud K, Tazerart S, Brocard C, Jean-Xavier C, Portalier P, Brocard F, et al. Differential plasticity of the GABAergic and glycinergic synaptic transmission to rat lumbar motoneurons after spinal cord injury. *J Neurosci*. 2010;30(9):3358–69.
56. Choe KY, Olson JE, Bourque CW. Taurine release by astrocytes modulates osmosensitive glycine receptor tone and excitability in the adult supraoptic nucleus. *J Neurosci*. 2012;32(36):12518–27.
57. Gomperts BD, Kramer IM, Tatham PER. *Signal transduction*. 2nd ed. San Diego: Academic; 2009.
58. Alberts B, Johnson A, Lewis J, Morgan D, Raff M, Roberts K, et al. *Molecular biology of the cell*. 6th ed. New York: Garland Science; 2015.
59. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell*. 1993;73(7):1251–4.
60. Catterall WA. Structure and regulation of voltage-gated Ca²⁺ channels. *Annu Rev Cell Dev Biol*. 2000;16:521–55.
61. Hille B. *Ion channels of excitable membranes*. 3rd ed. Sunderland: Sinauer Associates; 2001.
62. Meuth S, Pape HC, Budde T. Modulation of Ca²⁺ currents in rat thalamocortical relay neurons by activity and phosphorylation. *Eur J Neurosci*. 2002;15(10):1603–14.
63. Mahapatra S, Marcantoni A, Zuccotti A, Carabelli V, Carbone E. Equal sensitivity of Cav1.2 and Cav1.3 channels to the opposing modulations of PKA and PKG in mouse chromaffin cells. *J Physiol*. 2012;590(20):5053–73.
64. Liou SY, Shibata S, Yamakawa K, Ueki S. Inhibitory and excitatory effects of histamine on suprachiasmatic neurons in rat hypothalamic slice preparation. *Neurosci Lett*. 1983;41(1-2):109–13.
65. Stehle J. Effects of histamine on spontaneous electrical activity of neurons in rat suprachiasmatic nucleus. *Neurosci Lett*. 1991;130(2):217–20.
66. Scott G, Piggins HD, Semba K, Rusak B. Actions of histamine in the suprachiasmatic nucleus of the Syrian hamster. *Brain Res*. 1998;783(1):1–9.
67. Itowi N, Yamatodani A, Mochizuki T, Wada H. Effects of intracerebroventricular histamine injection on circadian activity phase entrainment during rapid illumination changes. *Neurosci Lett*. 1991;123(1):53–6.
68. Abe H, Honma S, Ohtsu H, Honma K. Circadian rhythms in behavior and clock gene expressions in the brain of mice lacking histidine decarboxylase. *Brain Res Mol Brain Res*. 2004;124(2):178–87.
69. Yoshimatsu H. Hypothalamic neuronal histamine regulates body weight through the modulation of diurnal feeding rhythm. *Nutrition*. 2008;24(9):827–31.
70. Rozov SV, Porkka-Heiskanen T, Panula P. On the role of histamine receptors in the regulation of circadian rhythms. *PLoS One*. 2015;10(12):e0144694.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

