Contribution of Histamine Type-1 Receptor to Metabolic and Behavioral Control of Ventilation

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Abstract: Histaminergic neurons in the hypothalamus are well documented as being involved in the control of autonomic functions, such as the balance of energy metabolism and circadian rhythm. We tested the hypothesis that an activation of the histamine type-1 (H1) receptor is required for the control of ventilation during the course of a day in free-moving mice. Ventilation, aerobic metabolism, and electroencephalogram were measured by a whole-body-plethysmograph, a magnetic-type mass spectrometry system, and a telemetry system, respectively, in H1 receptor-knockout (H1RKO) and wild-type mice. Both genotypes showed daily oscillations in minute ventilation ($\dot{V}_E$) and oxygen consumption ($\dot{V}_{O_2}$), with greater values during the dark period compared to the light period. In the latter, H1RKO mice showed

increased $\dot{V}_E$ and CO_2 excretion ($\dot{V}_{\text{CO}_2}$) relative to wild-type mice, and $\dot{V}_E$ was comparable to the $\dot{V}_{\text{CO}_2}$ increase. However, there was no change in $\dot{V}_{O_2}$ in H1RKO mice, suggesting that differences in $\dot{V}_{\text{CO}_2}$ between genotypes are responsible for differences in $\dot{V}_E$ during the light period. During the dark period, $\dot{V}_{\text{CO}_2}$ was elevated in H1RKO mice compared with WT mice. Because there was no difference in $\dot{V}_E$, the ratio of $\dot{V}_E$ to $\dot{V}_{\text{CO}_2}$ was reduced in H1RKO mice. Electroencephalogram results suggested that this might be due to a depressed arousal state in H1RKO mice because the ratio of delta to theta band power spectrum densities was greater in H1RKO mice than in wild-type mice. We concluded that histamine modulates ventilation by affecting metabolism and arousal state via H1 receptors.

Key words: histamine type-1 receptor, metabolism, circadian pattern, arousal state, mice.

The respiratory pattern is generated by the intrinsic cellular mechanisms and the network system of the respiratory neuron in the lower brain stem, which is affected by metabolic and behavioral control systems. During both awake and sleep stages, the metabolic control of respiration is always activated. However, in the awake stage respiration is influenced by various intrinsic or extrinsic environmental factors, such as thermal stress, pain, and various emotional changes. These behavioral controls are believed to be generated in the higher center rather than in the brain stem [1]. It is especially important that the hypothalamus, which plays a role in the regulation of the autonomic nervous system, responds to the environmental factors.

Histaminergic neurons modulate many physiological functions, such as the arousal state [2, 3], locomotor activity [4], feeding and drinking [5, 6], lipid metabolism [7], and circadian rhythm [8, 9]. These functions are closely related to changes in ventilation and are predominantly mediated by histamine type-1 (H1) receptors rather than type-2 (H2) receptors. To date, postsynaptic H1 and H2

receptors and presynaptic type-3 autoreceptors, have been identified in the brain [10]. Histaminergic neuronal cell bodies are located in the tuberomammillary nucleus of the posterior hypothalamus and project ascending and descending axons to much of the brain, including the cerebral cortex, thalamus, hypothalamus, medulla oblongata, and other structures [11–13]. On the basis of these physiological and morphological characteristics, we hypothesized that histamine, acting via H1 receptors, might be involved in the regulation of ventilation as affected by metabolic rate and behavior.

We previously reported that histamine, acting via H1 receptors, has an important role in ventilatory responses to hyperthermia [14], hypercapnia [15], and hypoxia [16] in conscious mice. We suggested that histamine contributes via H1 receptors to change metabolic rate, thereby influencing ventilation during hypoxia [16]. Ventilation is surely also influenced by the effects of histamine on metabolism under normoxic conditions. Further, histamine, acting via H1 receptors, plays an important role in cortical activation and wakefulness [2]. These histaminergic ef-

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fects on ventilation could be elucidated by a long-term measurement of ventilation in free-moving H1 receptor knockout (H1RKO) and wild-type (WT) mice.

In the present study, we examined how histamine modulates ventilation via H1 receptors by 24 h measuring of ventilation, metabolic rate, and electroencephalogram (EEG) in H1RKO and WT mice.

METHODS

Animals. H1RKO mice with a C57BL/6 mice background were provided by Dr. Takeshi Watanabe (Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan). H1RKO mice were backcrossed with C57BL/6 mice purchased from Japan SLC (Shizuoka, Japan), and these H1RKO and WT mice were maintained at Showa University under pathogen-free conditions. Genotyping was confirmed as described previously [17]. The mice were housed at a controlled temperature (24°C), exposed to a daily 12:12 h light-dark cycle, and provided with food and water *ad libitum*. The light period was defined as the 12 h from 08:00 to 20:00, and the dark period from 20:00 to 08:00; these periods were automatically controlled by switching the room light on and off.

Male mice of 9–10 weeks of age were used in all experiments, which were conducted at an environmentally controlled temperature (24–25°C). The study protocol was approved by the Showa University Animal Experiments Committee.

Fundamental procedures. Each mouse was placed in a whole-body single-chamber plethysmograph (WBP) (PLY3211; Buxco Electronics, Sharon, CT) to monitor ventilation and EEG or in a single-chamber box (120 × 70 × 60 mm) to monitor metabolism at 20:00 on the evening prior to experiments. The experiments were initiated at 08:00 and performed for 24 h. Because mice are nocturnal, they are active in the dark period of a day.

Measurement of visceral fat. The mice were measured for body weight (BW) and anesthetized with ketamine hydrochloride (3 mg/kg BW, i.p.). An incision was made in the abdominal wall, and visceral white adipose tissue, including mesenteric and retroperitoneal fat, was removed. The fat was then blotted with a piece of filter paper and weighed.

Measurement of ventilation. Ventilatory variables were measured by a WBP in unrestrained WT ($n = 9$) and H1RKO ($n = 8$) mice. The system is described in detail elsewhere [15]. In brief, the chamber received 1 l/min air-flow rate from a flow-pump reservoir system (PLY1020; Buxco Electronics). The pressure signal induced by ventilation was amplified and integrated by data analysis software (Buxco Electronics). A calibration volume of 1 ml of ambient air was introduced into the chamber with a syringe. Room temperature, humidity, and barometric pressure were routinely measured before experiments, and

these values were defined as the conditions in the chamber during experiments. To avoid additional stress, we did not monitor body temperature, which was estimated to be 37°C, on the basis of mean rectal temperature in a separate group of conscious mice. The chamber was masked from visual stimulation by a screen. Experiments were initiated at 08:00, and we continuously monitored respiratory rate (f_R , breaths/min), minute ventilation ($\dot{V}_E$, ml BTPS/min; BTPS, body temperature, and pressure saturated with water), tidal volume (V_T , ml BTPS), inspiratory time (T_I), expiratory time (T_E), and mean inspiratory flow (V_T/T_I). $\dot{V}_E$ and V_T were normalized per 10 g body weight (BW). These ventilatory variables were computed every 5 s and averaged for every 30 min.

Measurement of aerobic metabolism. The aerobic metabolism of WT mice ($n = 7$) and H1RKO mice ($n = 7$) was analyzed as follows: collected gases from the inflow and outflow of an animal chamber (120 × 70 × 60 mm) were analyzed by a magnetic-type mass spectrometry system (ARCO-1000; ARCO Systems, Chiba, Japan). O_2 consumption ($\dot{V}_{O_2}$, ml STPD/min; STPD, standard temperature and pressure, dry) and CO_2 excretion ($\dot{V}_{CO_2}$, ml STPD/min) were determined and normalized to kg BW. Data were computed every 15 s and averaged for every 30 min.

Electroencephalogram (EEG). WT mice ($n = 5$) and H1RKO mice ($n = 5$) were examined. EEGs were recorded with a telemetry system consisting of stainless steel electrodes (M1-2; Unique Medical Co., Tokyo, Japan), a telemetry unit (TA10ETA-F20; DSI, St. Paul, MN), and a receiver (Model RPC-1; DSI) along the lines of Weiergraber *et al.* [18]. The implantation of the EEG electrodes and the telemetry unit was performed under sodium pentobarbital (25 mg/kg, i.p.) anesthesia. To avoid infection, an antibiotic (cefazolin sodium hydrate, 400 mg/10 g BW i.p.) was administered before surgery. The anesthetized mice were placed in a stereotaxic apparatus, and a thermistor probe was inserted into the rectum. Body temperature was maintained at 37–37.5°C with an Animal Blanket Controller (AT1100; Nihon Kohden, Tokyo, Japan). A telemetry unit was implanted subcutaneously on the back via a 15–20 mm midline incision. EEG electrodes were then implanted onto the skull via a ~10 mm incision of the skin. After treatment with 10% H_2O_2 , the skull was drilled bilaterally at approximately 2 mm lateral and 2 mm caudal to the bregma. Two stainless steel electrodes were placed in the holes and were connected to lead wires tunneled subcutaneously from the telemetry unit. The electrodes were fixed to the skull by dental cement. The skin of the head and back was closed with 3-0 absorbable Vicryl. Each mouse was allowed to recover for at least 7 days before EEG measurement.

EEG data recorded for 24 h was transmitted to the receiver, picked up through an analog EEG output adapter (DSI), digitized with an analog-digital converter (Power-

Fig. 1. Ventilatory variables in histamine type-1 receptor-knockout (H1RKO) mice (filled circles) and wild-type (WT) mice (open circles). Values are means $\pm$ SE (WT, $n = 9$; H1RKO, $n = 8$). Clock time is plotted with open bars representing the 12-h light period and filled bars representing the 12-h

dark period. The significance between genotypes is shown for each period ($\#P < 0.05$, two-way ANOVA; n.s., not significant). A, respiratory frequency (f_R); B, tidal volume (V_T); C, minute ventilation ($\dot{V}_E$); D, inspiratory time (T_I); E, expiratory time (T_E); F, mean inspiratory flow ($\dot{V}_T/T_I$).

Lab; ADInstruments Pty Ltd. Charlotte, NC) at a sampling rate of 100 Hz, and then stored in a computer. Data analysis was performed offline with PowerLab software (ADInstruments). EEG data were filtered from 0.5 to 30 Hz, and motion artifacts were eliminated manually. EEG signals were then subjected to fast Fourier transformation analysis to yield power spectra with a 0.1-Hz resolution in a 0–49.6 Hz window. Data corresponding to the middle 8 h in each period were estimated for analysis and calculated for the percentage of total power. EEG estimation was performed according to standard criteria; the delta (δ) band was defined to be from 0.75 to 4.0 Hz, and the theta (θ) band from 6.25 to 9.0 Hz [19].

Statistical analysis. The data are expressed as mean $\pm$ SE. To compare ventilatory and metabolic variables between light and dark periods, we averaged values across

the middle 8 h of each period (i.e., from 10:00 to 18:00 in the light period and from 22:00 to 06:00 in the dark period). The differences in ventilatory and metabolic variables between light and dark periods were analyzed by a paired t -test. The genotype effect and time-course effect during each period were analyzed by two-way analysis of variance (ANOVA) on ventilatory variables ($\dot{V}_E$, f_R , V_T , T_I , T_E , and $\dot{V}_T/T_I$) and metabolic variables [$\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, respiratory exchange ratio (R)]. The averaged $\dot{V}_E$ in the mice, at each 30 min during the middle 8 h of each period, was plotted with corresponding $\dot{V}_{O_2}$ or $\dot{V}_{CO_2}$ at the same time. A discriminate analysis was performed to analyze differences among 4 groups of each genotype in each period. If differences were found, a Student's t -test was used to analyze differences between genotypes and between light and dark periods for the rate of $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$. Data cor-

Table 1. Ventilatory and metabolic variables.

	WT		H1RKO	
	Light	Dark	Light	Dark
f_R (breath/min)	170.5 ± 16.9	206.7 ± 22.3*	176.6 ± 13.1	196.3 ± 14.8*
V_T (ml/10 g BW)	0.053 ± 0.004	0.070 ± 0.005***	0.066 ± 0.004	0.08 ± 0.004***
V_E (ml/min/10 g BW)	8.99 ± 1.08	14.11 ± 1.94**	11.46 ± 1.39	15.34 ± 1.44***
T_I (s)	0.126 ± 0.008	0.113 ± 0.009***	0.126 ± 0.007	0.115 ± 0.007**
T_E (s)	0.295 ± 0.026	0.280 ± 0.028	0.279 ± 0.017	0.285 ± 0.017
V_T/T_I (ml/s/10 g BW)	0.439 ± 0.043	0.655 ± 0.075***	0.546 ± 0.060	0.734 ± 0.061***
R	0.800 ± 0.034	0.781 ± 0.039	0.935 ± 0.046	0.947 ± 0.041
$\dot{V}O_2$ (ml/min/kg BW)	41.5 ± 2.6	46.1 ± 3.8*	41.9 ± 2.4	45.6 ± 3.3*
$\dot{V}CO_2$ (ml/min/kg BW)	33.5 ± 2.8	35.8 ± 2.9*	39.2 ± 2.9	43.0 ± 2.9*

Values are means ± SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ between light and dark periods for each genotype (Student's *t*-test). f_R , respiratory frequency; V_T , tidal volume; V_E , minute ventilation; T_I , inspiratory time; T_E , expiratory time; V_T/T_I , mean inspiratory flow; R, respiratory exchange ratio; $\dot{V}O_2$, O_2 consumption; $\dot{V}CO_2$, CO_2 excretion.

responding to the middle 8 h in each period were used to estimate EEG results. Comparisons of δ/θ ratios and percentages of total power spectrum density (PSD) in the δ and θ bands between genotypes and between light and dark periods were performed with Student's *t*-test. Significance was accepted at $P < 0.05$, and all analyses were performed with SPSS II software (SPSS Japan, Tokyo, Japan).

RESULTS

Ventilatory variables

Circadian pattern. Circadian profiles of ventilation measured for 24 h are shown in Fig. 1, and averaged values for the middle 8 h in the light and dark periods are shown in Table 1. The data clearly show oscillations. Respiratory frequencies V_T and V_E were significantly greater during the dark period than during the light period (WT, $P < 0.05$ for f_R , $P < 0.01$ for $\dot{V}_E$, $P < 0.001$ for V_T ; H1RKO mice, $P < 0.05$ for f_R , $P < 0.001$ for V_T and $\dot{V}_E$, paired *t*-test). T_I was shortened during the dark period compared to the light period. A significant difference between light and dark periods was found for T_I , but not for T_E , in both genotypes (WT, $P < 0.001$; H1RKO, $P < 0.01$, paired *t*-test). Therefore V_T/T_I was significantly greater in the dark period than in the light period (WT, $P < 0.001$; H1RKO, $P < 0.001$, paired *t*-test).

Genotype differences. Figure 1 also shows the genotype differences during each period. During the light period, genotype differences were clearly observed for V_T and $\dot{V}_E$, with significantly greater values in H1RKO mice than in WT mice ($P < 0.05$, two-way ANOVA). Respiratory frequency and its components T_E and T_I did not differ significantly between genotypes, and V_T/T_I was greater in H1RKO mice than in WT mice during the light period ($P < 0.05$, two-way ANOVA). During the dark period, respiratory variables did not differ between genotypes.

Metabolic variables

Circadian pattern. The circadian profiles of metabolic variables $\dot{V}O_2$, $\dot{V}CO_2$, and R are shown in Fig. 2. Averaged values for the middle 8 h in the light and dark periods are shown in Table 1. $\dot{V}O_2$ and $\dot{V}CO_2$ increased in both genotypes during the dark period ($P < 0.05$, paired *t*-test). R was constant in both genotypes throughout the measurement period.

Genotype differences. A genotype difference was found for $\dot{V}CO_2$ but not for $\dot{V}O_2$ during both the light and dark periods (Fig. 2). $\dot{V}CO_2$ was significantly greater in H1RKO mice than in WT mice ($P < 0.05$, two-way ANOVA) throughout the measurement period, which resulted in a significantly greater R in H1RKO mice than WT mice during both light and dark periods ($P < 0.05$, two-way ANOVA).

Relation between ventilation and metabolism. To clarify whether $\dot{V}_E$ was suitable for the metabolic demand or responsive to another requirement, the relationship between ventilation and metabolism was examined at each time point during a 24 h period. The averaged $\dot{V}_E$ value at each 30 min was plotted against $\dot{V}O_2$ or $\dot{V}CO_2$ at the same time, as shown in Fig. 3A and 3B, respectively. The averaged $\dot{V}_E$ value from each time point was divided by the corresponding averaged $\dot{V}O_2$ or $\dot{V}CO_2$ value; these are shown in Table 2. The plots of $\dot{V}_E$ against $\dot{V}O_2$ for all four groups were different ($P < 0.05$, discriminate analysis), with an upward shift in the curve during the dark period, compared to the light period, for both WT and H1RKO mice (Fig. 3A). These profiles of light and dark periods were supported by the results that $\dot{V}_E/\dot{V}O_2$ ratios during the dark period were greater than those during the light period in both genotypes (WT, $P < 0.001$; H1RKO, $P < 0.01$, paired *t*-test; Table 2). During both the light and dark periods, H1RKO mice showed greater $\dot{V}_E/\dot{V}O_2$ ratios than the WT mice ($P < 0.001$, light period; $P < 0.01$, dark period, Student's *t*-test).

Fig. 2. Metabolic variables in histamine type-1 receptor knockout (H1RKO) mice (filled circles) and wild-type (WT) mice (open circles). Values are means $\pm$ SE ($n = 7$). Clock time is plotted with open bars on the transverse axis representing the 12-h light period and filled bars representing the 12-h dark period. A significance between genotypes is indicated for each period ($\#P < 0.05$, two-way ANOVA; n.s., not significant). A, respiratory exchange ratio (R); B, O_2 consumption ($\dot{V}\text{O}_2$); C, CO_2 excretion ($\dot{V}\text{CO}_2$).

Fig. 3. Relation between minute ventilation ($\dot{V}_E$) and O_2 consumption ($\dot{V}\text{O}_2$) (A) and CO_2 excretion ($\dot{V}\text{CO}_2$) (B). Closed symbols, dark period; open symbols, light period. Triangles, wild-type (WT) mice; circles, H1 receptor-knockout (H1RKO) mice. Each symbol shows the average values in each 30 min during light and dark periods for both genotypes. Solid ellipses indicate groups of light and dark periods in WT mice, and dotted ellipses indicate groups of light and dark periods in H1RKO mice. Four groups were different in A and B ($P < 0.05$, discriminate analysis).

The plots of $\dot{V}_E$ against $\dot{V}\text{CO}_2$ were also different for all four groups ($P < 0.05$, discriminate analysis) with an upward shift in the curve during the dark period, compared to the light period, for both WT and H1RKO mice (Fig. 3B). As shown in Table 2, $\dot{V}_E/\dot{V}\text{CO}_2$ ratios were greater during the dark period in both H1RKO and WT mice than they were during the light period (WT, $P < 0.001$; H1RKO, $P < 0.01$, paired t -test). It is interesting that during the light period, the plots of $\dot{V}_E$ against $\dot{V}\text{CO}_2$ in H1RKO mice were shifted up and right compared to those in WT mice, with the same slope in both (Fig. 3B). In agreement with this, during the light period there was no difference in $\dot{V}_E/\dot{V}\text{CO}_2$ ratios between H1RKO and WT mice; during the dark period, however, H1RKO mice had lower $\dot{V}_E/\dot{V}\text{CO}_2$ ratios than WT mice ($P < 0.01$, Student's t -test; Table 2).

EEG

Power spectra for EEGs measured during the light and dark periods were analyzed separately. Representative EEG power spectra for each genotype are shown in Fig. 4. EEG PSDs for δ and θ bands are summarized in Table 3. During the light and dark periods, both genotypes showed two peaks in PSDs (δ band and θ band). Neither genotype showed a significant difference in PSD for δ band, θ band, or the δ/θ ratio between the light and dark periods. During the light period, no significant differences were found between genotypes in the percentage of PSD for the δ band, θ band, or δ/θ ratio. During the dark period, although the percentage of PSD in the θ band was not significantly different between H1RKO and WT mice, H1RKO mice showed a higher percentage of PSD in the δ band than WT mice did (Table 3), resulting in greater δ/θ ratios in H1RKO mice than in WT mice ($P < 0.05$, Student's t -test).

Body and visceral fat weights

Body and visceral fat weights at 9 weeks old are shown in Table 4. No difference was found between genotypes by BW and visceral fat (Student's t -test).

Table 2. Relation between ventilation and aerobic metabolism.

	Light		Dark	
	WT	H1RKO	WT	H1RKO
V_E/V_{O_2}	0.22 ± 0.00	$0.29 \pm 0.00^{###}$	$0.31 \pm 0.00^{***}$	$0.35 \pm 0.00^{***\#}$
V_E/V_{CO_2}	0.27 ± 0.03	0.31 ± 0.02	$0.40 \pm 0.00^{***}$	$0.37 \pm 0.01^{***\#}$

Values are means $\pm$ SE. $^{**}P < 0.01$, $^{***}P < 0.001$: between light and dark periods in each genotype (paired *t*-test). $^{\#}P < 0.05$, $^{##}P < 0.01$, $^{###}P < 0.001$: between H1RKO mice and WT mice in each period (Student's *t*-test). V_E/V_{O_2} , a ratio of minute ventilation to O_2 consumption; V_E/V_{CO_2} , a ratio of minute ventilation to CO_2 excretion.

Fig. 4. Representative electroencephalogram (EEG) power spectra for light (A) and dark (B) periods. Solid lines, wild-type (WT) mice; dotted lines, histamine type-1 receptor-knockout (H1RKO) mice. Delta (0.75–4.0 Hz) and θ (6.25–9.0 Hz) powers are expressed as percent of total EEG power (0.5–30 Hz).**Table 3.** Power spectrum density.

	Light		Dark	
	WT	H1RKO	WT	H1RKO
δ power (%)	32.7 ± 6.2	36.0 ± 4.4	29.3 ± 2.7	$42.6 \pm 3.5^{\#}$
θ power (%)	32.0 ± 6.0	30.3 ± 2.3	33.8 ± 3.9	27.6 ± 1.4
δ/θ ratio	1.13 ± 0.39	1.23 ± 0.23	0.94 ± 0.17	$1.57 \pm 0.17^{\#}$

Data are expressed as a percentage of total EEG power (0.5–30 Hz). δ power is mean power density % in the δ band (0.75–4.0 Hz); θ power is that in the θ band (6.25–9.0 Hz). Values are means $\pm$ SE. $^{\#}P < 0.05$ between H1RKO mice and WT mice in the same period (Student's *t*-test).

DISCUSSION

In the present study, we monitored respiratory variables in WT and H1RKO mice over a period of 24 h. Both genotypes showed daily oscillations in ventilation with significantly higher values during the dark period than during the light period. During the light period, H1RKO mice showed greater $\dot{V}_E$ and $\dot{V}_{CO_2}$ compared to WT mice with an identical $\dot{V}_E/\dot{V}_{CO_2}$ ratio. This suggests that the greater $\dot{V}_E$ in H1RKO mice was responsible for greater $\dot{V}_{CO_2}$. During the dark period, however, H1RKO mice showed greater $\dot{V}_{CO_2}$ but unchanged $\dot{V}_E$ compared to WT mice, resulting in a decreased $\dot{V}_E/\dot{V}_{CO_2}$ ratio. EEG monitoring suggested that the decreased $\dot{V}_E/\dot{V}_{CO_2}$ ratio in H1RKO mice relative to WT mice could be responsible for the depressed arousal state during the dark period.

We identified daily oscillations in ventilation and metabolic rate depending on light and dark cycles. Respiratory frequency, V_T , $\dot{V}_E$, $\dot{V}_{O_2}$, and $\dot{V}_{CO_2}$ increased in both gen-

Table 4. Body and visceral fat weights.

	WT	H1RKO
Body weight (g)	26.51 ± 0.59	26.43 ± 0.44
Visceral fat weight (g)	0.90 ± 0.07	1.01 ± 0.07
<i>n</i>	9	11

Values are mean $\pm$ SE in 9-week-old mice. There is no statistical significance between genotypes at each weight (Student's *t*-test).

otypes during the dark period, which is consistent with the nocturnal behavior of mice (Figs. 1 and 2). There was no phase advance or delay in daily oscillations between genotypes. In experiments performed in free-running conditions in constant darkness, the animals followed their internal rhythm without entrainment by light or other external cues. In terms of this condition, it is reported that histamine is involved in phase shifts of circadian activity [8]. Present results, however, showed that the H1 receptor

does not contribute to the circadian rhythm entrained by light in ventilation and metabolism.

On the other hand, ventilatory variables during the light period were different between genotypes. During the light period, $\dot{V}_T$ and $\dot{V}_E$ were significantly greater in H1RKO mice than in WT mice, resulting in greater $\dot{V}_T/T_I$ ratios in the former (Table 1). $\dot{V}_T/T_I$ is known to be an indicator of an inspiratory motor drive that is a neural output of the respiratory center [20]. Because the peripheral administration of histamine has little effect on lung mechanics in mice [21], a greater $\dot{V}_E$ is centrally generated by an increase in inspiratory motor drive. Thus a deletion of H1 receptors promotes inspiratory motor drive, increasing $\dot{V}_T$ and $\dot{V}_E$ during the light period. In our study, in which we used the ventilation data could be affected by the difference between the actual body temperature and our preset temperature. This limitation is worrisome if there is a genotype difference in resting body temperature. Resting body temperature varies with the level of metabolic rate, particularly in a small animal such as mice and newborns [22]. Although there was no genotype difference in $\dot{V}_{O_2}$ as noted in the next paragraph, consecutive measuring of body temperature using a telemetry system might be necessary for accurately measuring ventilation.

To estimate metabolic involvement in the increase of ventilation, we investigated $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ for 24 h. We showed that H1RKO mice had altered metabolisms compared with WT mice. Oxygen consumption did not show a genotype difference; however, $\dot{V}_{CO_2}$ was greater in H1RKO mice than in WT mice, resulting in a greater R (Fig. 2). Differing $\dot{V}_{CO_2}$ and R values between genotypes indicate that main energy substrates in metabolism may be changed. It has been reported that hypothalamic histamine accelerates peripheral lipid metabolism via sympathetic nerve pathways [7] and that central histamine infusion increases uncoupling protein (UCP)-1 expression in brown adipose tissue and UCP-3 expression in white adipose tissue [23]. Moreover, a depletion of H1 receptor caused mature-onset obesity, which was accompanied by hyperphagia and a decreased expression of UCP-1 mRNA [24]. These findings show that histamine, acting via H1 receptors, plays important roles in lipid metabolism and in the regulation of feeding. It is conceivable that H1RKO mice reduce their lipid metabolism; in the present study, however, BW and visceral white adipose tissue weight were not different between genotypes at 9 weeks old. That is, any reduced lipid metabolism was not reflected in BW at this age, but it could possibly cause obesity in aged H1RKO mice. These results are consistent with the growth curves of H1RKO and WT mice evaluated by Masaki *et al.* [23]. These traits in H1RKO mice might directly affect the aerobic metabolism to increase $\dot{V}_{CO_2}$ and R throughout a day and thus show a predominant glycolytic metabolism.

In contrast to the clear genotype difference in aerobic metabolism during both periods, the genotype difference in ventilation was limited to the light period. We therefore examined the relationships between $\dot{V}_E$ and $\dot{V}_{O_2}$ and between $\dot{V}_E$ and $\dot{V}_{CO_2}$. If ventilation is increased relative to the metabolic rate, hyperventilation is indicated. The condition may be induced by many factors, including the conscious state or emotional changes, other than metabolic factors. During the light period, H1RKO mice showed greater values for both $\dot{V}_{CO_2}$ and $\dot{V}_E$ than WT mice did, indicating that increased $\dot{V}_E$ might be followed by increased $\dot{V}_{CO_2}$ (Fig. 3B). In agreement with this, the $\dot{V}_E/\dot{V}_{CO_2}$ ratio in H1RKO mice was similar to that in WT mice (Table 2).

During the dark period, however, ventilatory variables were not different between genotypes (Fig. 1), whereas $\dot{V}_{CO_2}$ was greater in H1RKO mice than in WT mice, as was seen during the light period (Fig. 2). Consequently, the $\dot{V}_E/\dot{V}_{CO_2}$ ratio was lower in H1RKO mice than in WT mice, suggesting low ventilation in comparison with the metabolic demand during the dark period. In that period, the active phase of mouse behavior, $\dot{V}_E/\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{CO_2}$ ratios were increased in both genotypes compared with those recorded during the light period. It is commonly accepted that changes in metabolic rate following exercise or heat generation closely relate to alveolar ventilation, but the present results suggest that the matching of alveolar ventilation to metabolic rate is not complete. Greater $\dot{V}_E/\dot{V}_{O_2}$ during the dark period than during the light period is also observed in rats [25], and a small but significant decrease in end-tidal P_{CO_2} is observed in resting awake humans [26]. Thus hyperventilation during the dark period deserves attention in both genotypes, in which $\dot{V}_E$ may be stimulated by factors relating to arousal state. We can suppose that emotional and arousal factors might affect ventilation without metabolic requirements. During the dark period, the $\dot{V}_E/\dot{V}_{CO_2}$ ratio in H1RKO mice was attenuated compared to that in WT mice, whereas the relative increase of $\dot{V}_{CO_2}$ was similar in both the light and dark periods. That is, $\dot{V}_E$ was reduced with respect to the increased $\dot{V}_{CO_2}$ during the dark period in H1RKO mice on the basis of mechanisms other than metabolism. We focused the arousal state as a contributing factor to reduced ventilation during the dark period in H1RKO mice.

EEG monitoring was carried out for 24 h, and its power density for the δ and the θ bands was calculated. During the dark period, the mean power density (%) for the δ band was higher in H1RKO mice than in WT mice, showing an increased δ/θ ratio. It is reported that the wake stage is increased during the dark period in both genotypes and that the amounts and circadian profiles of sleep and wakefulness were unchanged even in H1RKO mice [27]. An increased power spectrum density of δ band is the most important sign of cortical inactivation, occurring predominantly during natural slow-wave sleep. Furthermore, there is an agreement that θ rhythms recorded from the ro-

dent frontoparietal neocortex are originally propagated from the hippocampus [28] and that hippocampal θ rhythms during waking constitute the most important EEG sign of an attentive state [29]. Therefore our result of an increased δ/θ ratio suggests that the arousal state is depressed during the dark period in H1RKO mice. In fact, it is reported that knockout mice for the histamine synthesizing enzyme histidine decarboxylase (HDC) showed a decrease in cortical EEG power in θ -rhythm and an increase in δ -rhythm during waking [30]. It is generally understood that H1 receptor antagonists have little effect on wakefulness during nocturnal sleep in humans, although antihistamines lead to marked drowsiness, an increased tendency to sleep, and impaired performance during the day [31]. We believe that the depressed arousal state is involved in the reduced $\dot{V}_E/\dot{V}_{CO_2}$ ratio during the dark period.

It is known that PSDs in δ and θ bands are varied for the sleep-awake stage, such as wakefulness, paradoxical sleep, and slow-wave sleep. The total length of each sleep-awake stage is not changed in H1RKO mice [27] or HDC mutant mice [30]; however, the sleep-awake cycle is changed in duration and in number of episodes [30]. For example, HDC mutant mice show reduced durations but increased episode numbers of each wakefulness and slow-wave sleep stage. Cortical EEG power density in HDC mutant mice shows increased δ band and decreased θ band, which are demonstrated especially in wakefulness. Short-term wakefulness during the dark period may be accompanied by drowsiness and affect the power density of the δ and θ bands and their ratio. Although the present study lacked in the discrimination of sleep-awake stage, δ/θ ratio increased in total stages during the dark period. It is apparent that H1RKO mice have lower vigilance during their active phase.

We should consider that circadian patterns of respiration may be secondarily caused by circadian changes in behavior, in which histamine H1 receptor may be involved. Previous studies indicated that H1RKO mice show a significant decrease in locomotor activity during the dark period [17, 32]. However, because $\dot{V}_{O_2}$ did not differ between WT and H1RKO mice, the decrease in locomotion did not account for the metabolic rate in the present study. In agreement with this, circadian changes in ventilation, which do not depend on the animal's activity, are also reported in rats [25]. Thus a depressed arousal state reduces ventilation independently from the metabolic rate.

Blood pressure, which is 10 mm Hg higher in rats during the dark period than during the light period, is another important factor effective in circadian changes of respiration [33]. Since an increase in blood pressure has an inhibitory effect on $\dot{V}_E$ [34], the $\dot{V}_E$ during the dark period might be influenced by blood pressure. A recent study showed that H1RKO mice do not differ from WT mice in

systolic blood pressure and diastolic blood pressure [35]. However, histamine plays a role in cardiovascular control via H1 receptors [16, 36]; therefore H1 receptors are attributable to the changes in blood pressure during the dark period. Further studies are needed to examine a role for histamine in the relationship between respiration and blood pressure during a dark period.

In conclusion, histamine plays an important role for ventilatory control by affecting metabolism and arousal state via H1 receptors.

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