Mice with Blunted Hypoxic Ventilatory Response are Susceptible to Respiratory Disturbance during Hypoxia

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ADACHI, T., OGAWA, H., OKABE, S., KITAMURO, T., KIKUCHI, Y., SHIBAHARA, S., SHIRATO, K. and HIDA, W. *Mice with Blunted Hypoxic Ventilatory Response are Susceptible to Respiratory Disturbance during Hypoxia*. Tohoku J. Exp. Med., 2006, **209** (2), 125-134 —— Hypoxia causes a life-threatening situation, and the ventilatory response to hypoxia plays an important role in preventing death. We have hypothesized that persons with a blunted hypoxic ventilatory response may have a weak defense response to hypoxic episodes and be susceptible to fatal respiratory disturbances. However, precise correlations between the hypoxic ventilatory response and respiratory disturbances are not well understood. In the present study we examined the hypoxic and hypercapnic ventilatory responses in nine inbred mouse strains (A/J, AKR/N, BALB/c, C3H/He, C57BL/6, DBA/2, NZW, SWR/J, and 129Sv). Breathing frequency, tidal volume and minute ventilation of unanesthetized and unrestrained mice were assessed by whole body plethysmography. Age-matched mice were exposed for 3 min to 10% O_2 in N_2 gas or 10% CO_2 in hyperoxic gas to determine the acute ventilatory response to chemical stimuli. Basal respiratory variables and hypoxic ventilatory responses differed among the strains, but the hypercapnic ventilatory response did not differ. The hypoxic ventilatory response was the highest in AKR/N mice and the lowest in SWR/J mice. These findings suggest that genetic factors may have influenced the hypoxic ventilatory response but not the hypercapnic ventilatory response. To examine the effects of severe hypoxic stress on the respiratory cycle, we exposed the strain with the highest or lowest hypoxic ventilatory response to 6% O_2 in N_2 until the onset of apnea. The "appearance time of apnea", which is defined as the time from the hypoxic loading to the onset of apnea, was shorter in the SWR/J strain than in the AKR/N strain. We suggest that a lower hypoxic ventilatory response may be a risk factor for apnea under hypoxia. ——— blunted ventilatory reponse; hypoxic-induced apnea; genetics; respiration; animal

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The immediate hypoxic ventilatory response of vertebrates occurs in response to a lower fractional O_2 concentration, either in steps or progressively. Usually, a brief hypoxic exposure stimulates the peripheral chemoreceptors, resulting in a rapid increase in ventilation. This hypoxic ventilatory response may be important in some respiratory diseases that can trigger life-threatening episodes. Hunt et al. (1981) reported that infants with sudden infant death syndrome had lower hypoxic ventilatory responses than the control group. We found that persons with near fatal asthma showed lower hypoxic ventilatory responses than normal subjects and asthma patients without experience of near fatal asthmatic attack (Kikuchi et al. 1994). Furthermore, Hackett et al. (1988) reported that persons who experienced high altitude pulmonary edema showed lower hypoxic ventilatory responses than the control group. From these clinical studies, we hypothesized that persons with a blunted hypoxic ventilatory response may have a weak defense response to hypoxic episodes and be susceptible to fatal respiratory disturbances. However, to the best of our knowledge, there have been no reports showing that a blunted hypoxic ventilatory response is associated with respiratory disturbances, because it would be difficult to undertake this kind of investigation in a human study.

Tankersley et al. (1994) reported that there were differences in ventilatory behavior during exposure to hypoxia or hypercapnia among unanesthetized inbred mice, and suggested the presence of genetic influences on both responses. Therefore, it may be possible to identify mice strains with a blunted hypoxic ventilatory responses of inbred mice. Severe hypoxia could produce respiratory disturbance such as hypoxic-induced apnea (Guntheroth and Kawabori 1975). Thus it would be possible to examine the relationship between a blunted hypoxic ventilatory response and respiratory disturbance using mice.

In the present study we examined the ventilatory responses of 9 inbred male mouse strains that we could obtain, and identified two mouse strains with the highest and lowest ventilatory responses. Then, we studied the ventilatory responses in these two mouse strains during a prolonged exposure to 6% oxygen, showing that the mice with the lowest ventilatory response had shorter "appearance time of apnea" than the mice with the highest ventilatory response. We suggest that severe hypoxia might easily cause respiratory disturbances, such as apnea, in an animal with the blunted hypoxic ventilatory response.

MATERIALS AND METHODS

Animals

Nine inbred male mice (A/J, $n = 12$; AKR/N, $n = 13$; BALB/c, $n = 8$; C3H/He, $n = 11$; C57BL/6 $n = 10$; DBA/2, $n = 13$; NZW, $n = 8$; SWR/J, $n = 14$; and 129Sv, $n = 8$) matched for age (8 - 12 weeks) and weight (24 - 26 g), were supplied from the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine. Before the experiments, all animals were treated in a similar manner in terms of diet, environmental temperature, and light-dark exposure.

Measurement of ventilation

Ventilation was measured using the barometric method of plethysmography (Fig. 1) (Bartlett and Tenney 1970), which was basically the same method as described in previous papers (Mizusawa et al. 1994; Ogawa et al. 1995) except for the chamber volume used. An unanesthetized and unrestrained mouse was placed in a 700 ml Plexiglass animal chamber connected to a reference chamber of the same volume through a pressure transducer (model MP-45; Validyne, Northridge, CA, USA). To avoid CO_2 gas accumulation in the chamber, the animal chamber had inlet and outlet tubes, which were connected to a gas compressor and a vacuum pump, respectively, allowing constant background flow of humidified room air (500 ml/min). Calibration of the volume was repeated during each experiment using a gas-tight syringe (1 ml) to inject 0.1 ml into the chamber while the chamber was empty.

Breathing frequency (f) was calculated directly from the ventilation-induced pressure swings. Tidal volume (VT) was obtained as a function of the pressure difference between the two chambers. The equation to calculate VT is described elsewhere (Drorgaugh and Fenn 1955; Bartlett and Tenney 1970). The minute ventilation (V̇E) was calculated from f × VT. VT and V̇E were normalized with body weight (g).

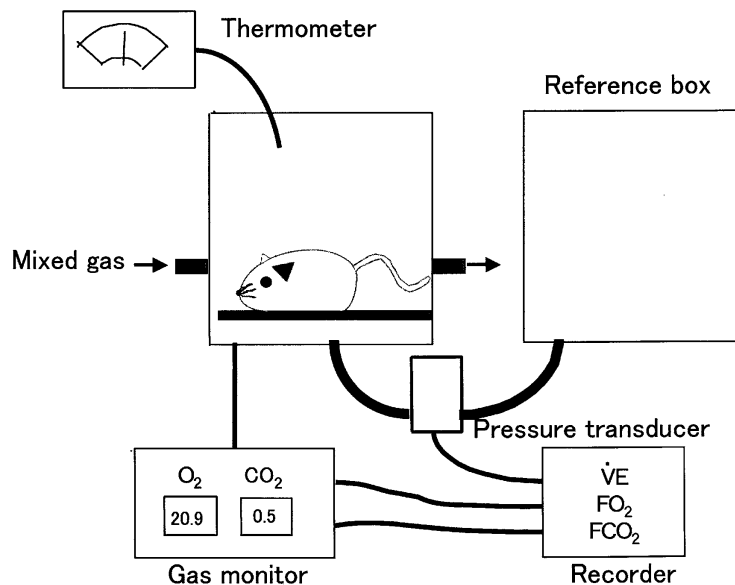The O_2 fraction concentration (FO_2) and the CO_2

Fig. 1. Schematic diagram of the experimental setup. Each unanesthetized mouse was allowed to acclimate in the chamber at least 30 min before the measurement. $\dot{V}E$, minute ventilation; FO_2, O_2 fraction concentration; FCO_2, CO_2 fraction concentration.

fraction concentration (FCO_2) in the chamber were continuously measured with the use of a gas analyzer (model no. 1H21A; NEC Sanei, Tokyo). FO_2, FCO_2 and pressure changes in the animal chamber were recorded on a strip chart recorder (Rectigraph-8S, NEC Sanei).

Acute hypoxic and hypercapnic ventilatory responses

Freely moving animals were placed in the plethysmograph (CHEST M.I., Inc., Tokyo) breathing room air and given sufficient time of at least 30 min to acclimate to the surroundings. After stabilization while breathing room air, control ventilation was measured for 15 min. For the ventilatory response to acute hypoxia, N_2 gas was mixed with background room air to maintain the chamber FO_2 at 10% and the $FCO_2 < 0.6\%$. The hypoxic ventilatory response was obtained under a poikilocapnic condition. For the ventilatory responses to hypercapnia, the FCO_2 in the chamber was adjusted to 10% CO_2 using a gas mixture of 40% $CO_2 \sim$ 60% O_2 in N_2 gas. The targeted fraction concentration of O_2 or CO_2 was obtained within 20 sec, and maintained for 3 min. The ventilatory responses to hypoxia were measured for 15 ~ 20 sec at the peak response during 3 min and those to hypercapnia were measured for the last 15 ~ 20 sec during 3 min (Mizusawa et al. 1994; Ogawa et al. 1995; Tabata et al. 2001; Adachi et al. 2004), with an interval of at least 30 min and in random order.

The mean values from the two trials were accepted as individual data. The response of each respiratory variable was assessed as the value during hypoxia or hypercapnia minus the corresponding basal value during room air breathing (Mizusawa et al. 1994; Adachi et al. 2004).

Sustained severe hypoxic ventilatory response

Mice of two strains (AKR/N; $n = 13$, and SWR/J; $n = 19$) were selected for the following reasons. Among the nine strains examined, AKR/N mice had the greatest hypoxic ventilatory response and SWR/J mice had the lowest hypoxic response. After control measurement of the ventilation, the animals were exposed to 6% O_2 in N_2 gas, which was adjusted by N_2 gas within 20 sec. The animals breathed the gas of this targeted fraction of O_2 until they exhibited respiratory disturbances such as apnea of 5 sec interval. After the loading of hypoxic gas, the animals breathed room air. We defined the "appearance time of apnea" as the time from the start point of breathing 6% O_2 to the point of occurrence of apnea lasting more than 5 sec. We compared the appearance time of apnea between the two strains.

These experiments were approved by the animal experiment review committee of Tohoku University School of Medicine.

All experimental procedures were carried out between a.m. 9:00 and p.m. 6:00 in a quiet and light

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room at an ambient temperature of 25°C.

Data were presented as means ± S.E. All data concerning the nine strains were analyzed by one-way analysis of variance (ANOVA), and the differences among strains were compared by post hoc Scheffe's test.

Comparison of the appearance time of apnea between the two strains was by unpaired Student's t-test. Differences between the mean values were considered significant when $p < 0.05$.

TABLE 1. Respiratory variables during room air breathing, and hypoxic and hypercapnic ventilatory responses of inbred murine strains.

Strain	n	Body weight, g	Breathing gas	$\dot{V}E$, ml/min/g	f, /min	VT, μl/g
AKR/N	13	24.8 ± 0.4	room air	2.75 ± 0.10	228.3 ± 7.5	12.1 ± 0.3
			10% O_2	5.35 ± 0.13*	312.1 ± 6.4*	17.2 ± 0.4*
			10% CO_2	8.40 ± 0.27*	342.4 ± 4.1*	24.6 ± 0.8
DBA/2	13	24.5 ± 0.3†	room air	2.59 ± 0.16†	221.1 ± 12.1	11.8 ± 0.4
			10% O_2	4.78 ± 0.21*	306.7 ± 6.7*	15.5 ± 0.5
			10% CO_2	7.46 ± 0.49	331.3 ± 10.0	22.2 ± 0.9†
BALB/c	8	24.4 ± 0.4†	room air	2.96 ± 0.11	233.3 ± 10.4	12.8 ± 0.6
			10% O_2	4.88 ± 0.25*	321.8 ± 5.6*	15.1 ± 0.6
			10% CO_2	8.50 ± 0.42	330.9 ± 12.3	25.7 ± 0.8
C3H/He	11	24.6 ± 0.4†	room air	2.55 ± 0.07†	217.0 ± 8.0†	11.9 ± 0.5
			10% O_2	4.33 ± 0.15	262.9 ± 6.3†	16.5 ± 0.4
			10% CO_2	6.83 ± 0.21	309.6 ± 4.4	22.0 ± 0.5†
A/J	12	24.3 ± 0.3†	room air	3.30 ± 0.16*	236.8 ± 10.0	14.0 ± 0.6*
			10% O_2	5.00 ± 0.26*	301.7 ± 7.6*	16.5 ± 0.7
			10% CO_2	8.29 ± 0.48	289.7 ± 6.1†	28.4 ± 1.3*
C57BL/6	10	23.1 ± 0.4†	room air	3.13 ± 0.20*	269.4 ± 10.3	11.7 ± 0.8
			10% O_2	4.72 ± 0.28	287.8 ± 13.6	16.4 ± 0.6
			10% CO_2	7.80 ± 0.43	342.1 ± 9.6*	22.7 ± 1.0†
129sv	8	27.3 ± 1.1*	room air	2.32 ± 0.13†	218.2 ± 9.0	10.6 ± 0.3†
			10% O_2	3.84 ± 0.14†	273.6 ± 6.3	14.0 ± 0.4†
			10% CO_2	7.27 ± 0.23	332.0 ± 14.9	22.2 ± 1.2†
NZW	8	25.2 ± 0.4	room air	2.15 ± 0.07†	215.7 ± 7.5	10.0 ± 0.3†
			10% O_2	3.54 ± 0.17†	259.7 ± 7.4†	13.6 ± 0.3†
			10% CO_2	6.10 ± 0.27†	291.1 ± 4.5†	21.0 ± 0.8†
SWR/J	14	23.1 ± 0.3†	room air	3.30 ± 0.09*	270.2 ± 9.8*	12.4 ± 0.6
			10% O_2	4.29 ± 0.09†	261.2 ± 6.2†	16.5 ± 0.4
			10% CO_2	8.72 ± 0.27*	344.4 ± 6.7*	25.3 ± 0.5

Values are means ± S.E. $\dot{V}E$, minute ventilation; f, breathing frequency; VT, tidal volume. Significant interstrain variability ($p < 0.05$) was determined for each dependent variable. Significant ($p < 0.05$) mean differences among strains are indicated by * or †, representing strains not different from one another but relatively high or low responses, respectively, within a strain distribution.

RESULTS

Room air breathing. The body weight and respiratory variables during air breathing are summarized in Table 1. There were significant differ-

ences in V̇E, VT or breathing frequency among the nine strains. However, there were no significant differences in these three parameters between AKR/N and SWR/J mice examined in the study of the severe hypoxic ventilatory response.

Acute hypoxic and hypercapnic ventilatory responses. Fig. 2 shows the hypoxic ventilatory
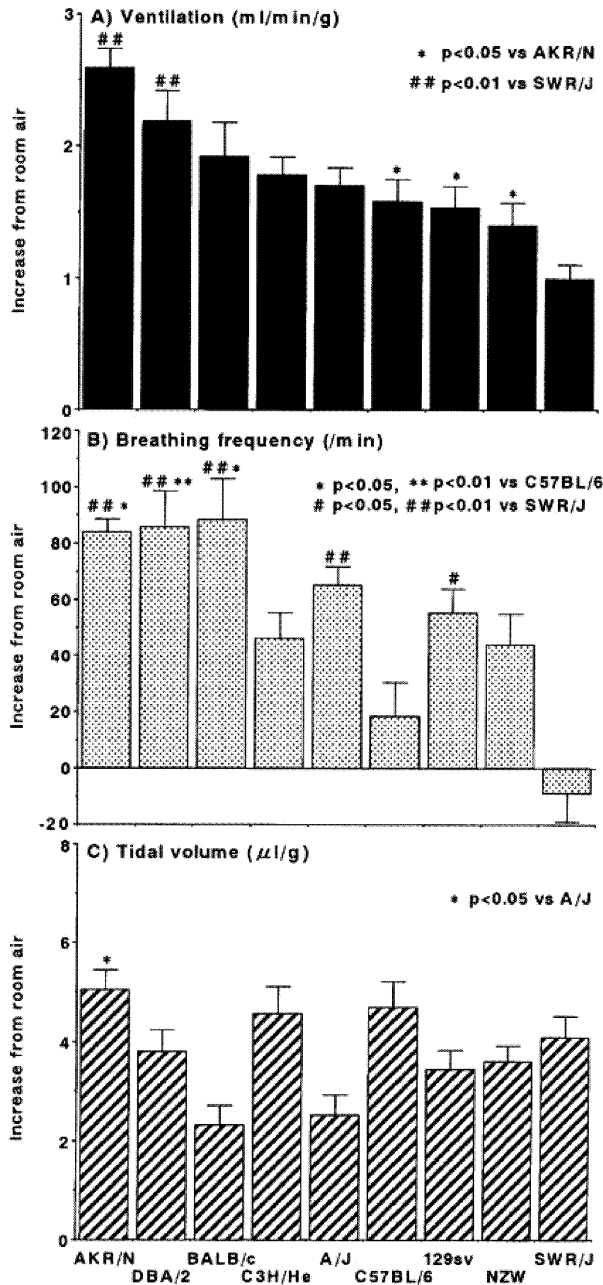

Fig. 2. Hypoxic ventilatory responses of the nine murine strains. Significant differences were observed among the strains. Note that the hypoxic response of AKR/N is the highest and that of SWR/J is the lowest among the nine strains.
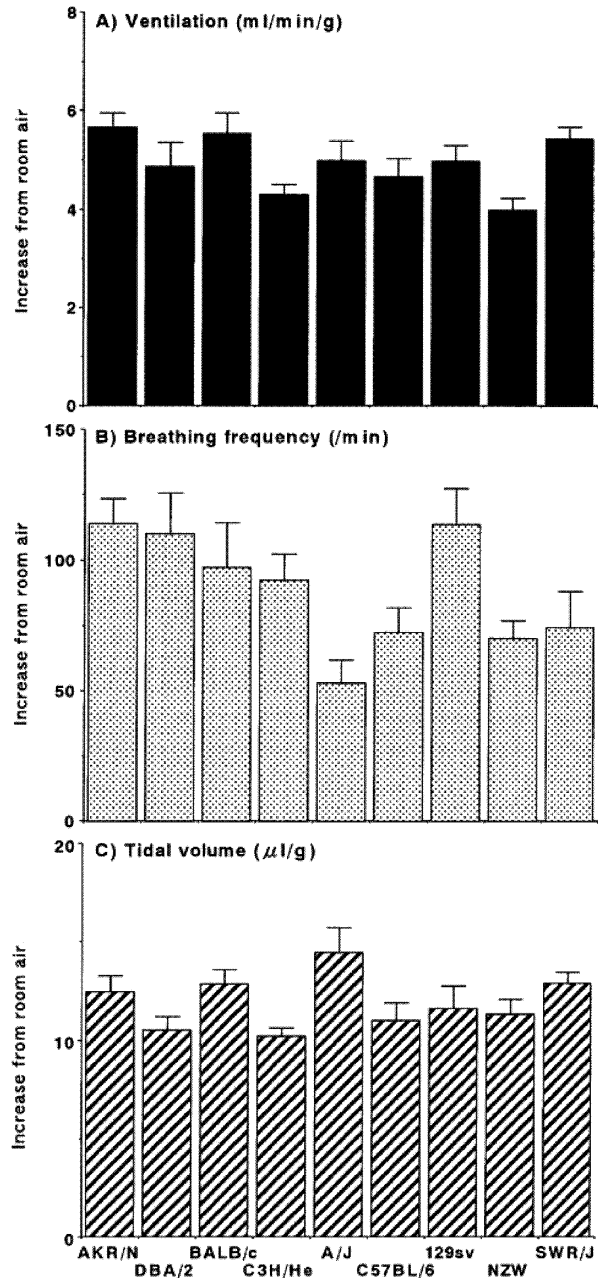

Fig. 3. Hypercanic ventilatory responses of the nine murine strains. No significant differences were observed among the strains.
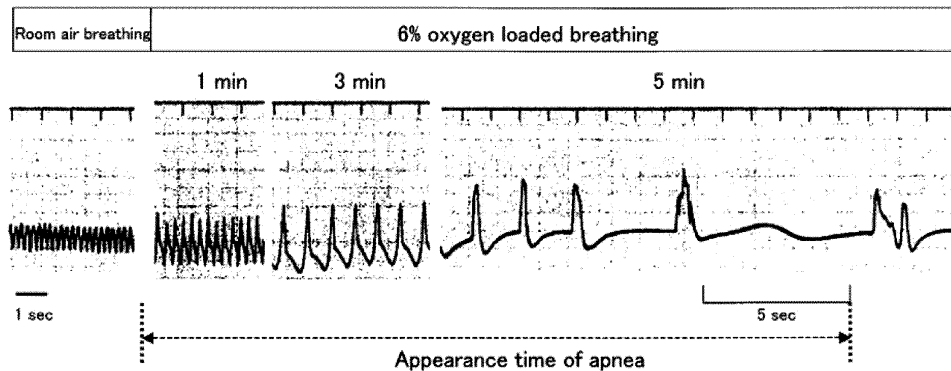
Fig. 4. Representative ventilatory response to severe hypoxic stress. Apnea over 5 sec was observed at 5 min after the onset of exposure of 6%O_2 in N_2 gas. This time was defined as "appearance time of apnea."

response of the 9 inbred mouse strains. The strains were placed in order of magnitude of the ventilatory response from highest to lowest. There was great diversity among the strains. AKR/N showed the highest hypoxic ventilatory response with increases in both f and VT. SWR/J showed the lowest hypoxic ventilatory response with an increase in VT. Interestingly, SWR/J did not show a significant change in f during acute hypoxic gas exposure. The difference of ventilation between these two strains was not due to the difference of tidal volume, but to the difference of respiratory frequency.

Fig. 3 shows the hypercapnic ventilatory response of the 9 inbred mouse strains. The displayed order is similar to the order in Fig. 2. There was no significant difference in the ventilatory response to hypercapnia among the strains.

Sustained severe hypoxic ventilatory response. Fig. 4 shows a representative trace of the breathing curve, which shows resting breathing and breathing at 1, 3 and 5 min after hypoxic loading. After 1 minute, the tidal volume increased. After 3 min, the respiratory frequency decreased and the tidal volume increased. After 5 min, the interval between breathing cycles was prolonged progressively, and apnea of more than 5 sec appeared. We obtained "appearance time of apnea" defined as the time from the hypoxic loading to the onset of apnea. In this case, appearance time of apnea was 5 min.
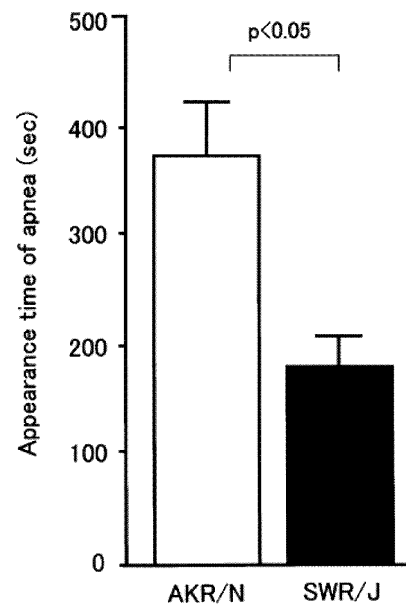
Fig. 5. Appearance time of apnea of AKR/N and SWR/J strains. Appearance time of SWR/J was significantly shorter than that of AKR/N.

Fig. 5 shows the appearance times of apnea of the AKR/N and SWR/J strains. The appearance time of SWR/J mice was shorter than that of AKR/N mice. This suggests that SWR/J mice with a lower hypoxic ventilatory response are prone to apnea within a shorter period under hypoxia. We could resuscitate the mice after the first apnea by exposing them to air after the hypoxic loading.

DISCUSSION

We studied the acute hypoxic and hypercapnic ventilatory responses of 9 inbred mouse strains and the effects of sustained severe hypoxic loading on the breathing pattern of two strains with the highest or lowest hypoxic ventilatory responses. We found great diversity among the strains in terms of the respiratory variables during air breathing and in the hypoxic ventilatory response. Such diversity among the strains corresponded with the results of previous papers, which showed variability in lung function (Reinhard et al. 2002) and airway responsiveness (De Sanctis and Drazen 1997) among inbred strains. The hypoxic ventilatory response in the AKR/N strain was the highest, and the hypoxic ventilatory response in the SWR/J strain was the lowest among the 9 strains examined in the present study. The hypercapnic ventilatory responses did not differ among the 9 strains. Furthermore, we found that hypoxic-induced apnea occurred after a shorter period in SWR/J mice than in AKR/N strain. These findings suggest that the hypoxic ventilatory response, but not the hypercapnic ventilatory response was determined by genetic factors, and that animals with a blunted hypoxic ventilatory response would have a weaker compensation in response to hypoxia.

The targeted fractional concentration of the hypoxic response was 10% O_2 in N_2. This hypoxic level is generally accepted in studies of small animals. On the other hand, the target CO_2 concentration, 10% CO_2 in over 40% O_2, might be higher. In additional experiments, we studied the graded hypercapnic ventilatory response using 5% CO_2, 10% CO_2 and 12% CO_2 in over 40% O_2 in an additional 6 AKR/N mice and 6 SWR/J mice. We found a linear response up to 12% CO_2 (data not shown). Therefore, the 10% CO_2 applied in this study seemed to be acceptable.

In the present study, we could not measure the arterial oxygen tension (PaO_2) or arterial CO_2 tension from the arterial blood. The reason for this is that this series of experiments was performed under a freely moving and unanaesthetized condition and blood sampling would have been technically difficult. Instead, we assessed the chamber FO_2 or FCO_2 instead of PaO_2 or $PaCO_2$.

Furthermore, as we did not monitor the cardiovascular parameters, it was difficult to determine whether cardiac arrest occurred before the occurrence of apnea in the study of the sustained hypoxic ventilatory response. However, as the mice recovered from apnea after air breathing or the heart beat was recognized by touching, the apneic episodes during severe hypoxic loading could be expected to have occurred independently of near arrest.

Tankersley et al. (1994) studied the inter-strain variance of the ventilatory response to normal air, hypoxia and hypercapnia among eight inbred strains of mice. They found significant inter-strain differences in ventilation during air breathing and in the ventilatory response to two chemical loadings, and these inter-strain differences might have been influenced by genetic determinants. The present study seemed to support partly the study by Tankersley et al. (1994). Several mouse strains of the present study were the same as those used in Tankersley's study, such as DBA/2, BALB/c, C3H/He, C57BL/6 and A/J. The order of the increase in the hypoxic ventilatory response in these strains was compatible with their study, except for the case of C57BL/6. The reasons for some of the differences between the two reports may be as follows. One possibility may be the different time domains in the hypoxic response, which are thought to reflect different physiological systems (Huey and Powell 2000). Another possibility may be differences in the phenotyping strategy in terms of the length of exposure or degree of loaded gas. Furthermore, there were differences in the time of day that the studies were performed, the degree to which the mechanical aspects of control were measured and the mouse strains that were used.

Han et al. (2001) reported the ventilatory behavior after 8% oxygen in C57BL/6 and A/J mice. They did not find a difference in the V̇E response between the two strains. However, they found greater increase in f and less increase in VT in the A/J strain compared to the C57BL/6 strain.

The ventilatory behavior after hypoxic exposure in the C57BL/6 and A/J mice used in the present study was consistent with Han et al. (2001). Furthermore, our study may support clinical studies by Kawakami et al. (1982, 1984), who reported that genetic factors might influence the ventilatory response to hypoxia but not that to hypercapnia, as judged from studies concerning hypoxic and hypercapnic ventilatory responses in twins.

The underlying mechanisms of the hypoxic ventilatory response have been elucidated in terms of the neurotransmitters or neuromodulators in the central nervous system. The nitric oxide (NO) in the nucleus tractus solitarii plays an important role on the acute phase during hypoxic stress, and this works as a retrograde messenger in an excitatory transmitter, L-glutamate, releasing a positive feedback system that contributes to the augmentation of ventilation during hypoxia (Mizusawa et al. 1994; Ogawa et al. 1995). The facilitation of the L-glutamate release system by NO might contribute to the prevention of abrupt changes in ventilation.

Recently, we have reported that mice lacking heme oxygenase-2 (HO-2) show attenuated ventilatory responses to hypoxia with normal responses to hypercapnia (Adachi et al. 2004), suggesting that HO-2 might play a role as an oxygen sensor or a heme-containing regulator (Shibahara 2003). Incidentally, the degree of the blunted hypoxic response seen in SWR/J mice is similar to that seen in HO-2 (-/-) mice. Thus, SWR/J mice may provide a suitable alternative to HO-2 (-/-) mice. Furthermore, the SWR/J mouse strain characteristically carries the Cys variation in the juxtamembrane domain of the c-Met receptor tyrosine kinase, proven to functionally modulate tumorigenesis (Zaffaroni et al. 2005). In this context, the SWR/J mouse has been known as a strain highly susceptible to lung tumorigenesis (Rabstein et al. 1973). It is therefore tempting to speculate that c-Met and its ligand, hepatocyte growth factor, might be involved in oxygen sensing.

On the other hand, in the decline phase of ventilation during sustained hypoxic loading, γ-aminobutyric acid (GABA), which is an inhibitory transmitter, in the NTS had an important role in the hypoxic ventilatory decline after sustained hypoxic loading (Tabata et al. 2001). Whether a GABAergic mechanism affects the onset of apnea under severe hypoxic stress is still unknown.

It was reported that the patients who had a history of near-miss death from sudden infant death syndrome (Hunt et al. 1981) and asthma attacks (Kikuchi et al. 1994), or who experienced high altitude pulmonary edema had blunted hypoxic chemosensitivity. From these clinical studies, we speculated that persons with a blunted hypoxic ventilatory response may have a weak defense response to hypoxic episodes and be susceptible to fatal respiratory disturbances. The present study in which animals with blunted hypoxic chemosensitivity had respiratory depression as indicated by hypoxic induced apnea, may suggest that the level of chemosensitivity plays a role in occurrence of apnea in sleep apnea syndrome.

Obstructive sleep apnea (OSA), which causes hypoxemia by repetitive apneas during sleep mainly due to upper airway obstruction (Miki et al. 1988; Hida et al. 1994; Taguchi et al. 1997; Hida 1998), results in rapidly progressive hypercapnia and hypoxia, which could initiate reopening of the upper airway and determine the postapneic ventilation together with nonchemical factors (Satoh et al. 1991; Kimoff et al. 1994). Satoh et al. (1991) reported that the slopes of the regression line obtained from the relationships between arterial oxygen saturation (SaO$_2$, %) induced by apnea and the postapneic ventilation during both rapid eye movement (REM) and non-REM sleep correlated well with the hypoxic ventilatory response (HVR) during wakefulness in OSA patients. These results suggest that the slopes might partly reflect hypoxic responsiveness during sleep in OSA patients. If so, we could compare HVR during sleep with that while awake in OSA. We found that the awake HVR was higher than that during both sleep stages, and that the HVR during REM sleep is less than that of non-REM sleep. Thus, the ventilatory response to hypoxic stress is lowest during REM sleep among the three states. Generally, respiratory distur-

bances are prone to occur during REM sleep more than during non-REM sleep. A lower hypoxic ventilatory response during REM sleep may have a relationship with the easier occurrence of apnea (Hida 1998).

During sustained hypoxia, ventilation is known to show a biphasic response, which is comprised of an initial stimulatory phase mediated by peripheral chemoreceptors and then a depressive phase caused by central and/or peripheral mechanisms in animals and in humans (Easton et al. 1986; Neubauer et al. 1987). The response to sustained hypoxia has been examined under moderate hypoxic conditions such as FiO_2 10%. In the present study we examined the time until the onset of apnea under severe hypoxic loading of 6% O_2 in N_2 gas. This method was basically similar to that used in a previous study by Scremin et al. (1980), except that they obtained the time from the start of exposure of the same level of hypoxic gas until the last gasp as the survival time. Severe hypoxia could cause apnea, that is, hypoxic-induced apnea (Guntheroth and Kawabori 1975; Fewell et al. 2005). Although the mechanism of hypoxic-induced apnea is not known, we speculate that central hypoxic depression may result from a positive feedback mechanism (Tabata et al. 2001) inducing apnea, or that hyperventilation during hypoxic loading may induce apnea in the same manner as post-hyperventilation apnea induced by hypocapnia below the apneic threshold (Meah and Gardner 1994).

The main findings of the present study were that genetic factors influenced the ventilatory responses to both the brief and sustained hypoxic loading but not the hypercapnic ventilatory response, and that a lower hypoxic ventilatory response could lead to hypoxic-induced apnea. As the present study was performed under prolonged hypoxic exposure in awake mice, our results may have limited value in explaining the mechanisms of intermittent apnea during sleep in sleep apnea syndrome. However, these findings may be helpful to explain fatal respiratory disturbances observed clinically under hypoxic conditions of various diseases such as chronic obstructive pulmonary disease (Ichinose 2003; Takemura

et al. 2005) and sleep apnea syndrome (Miki et al. 1988; Hida et al. 1994; Taguchi et al. 1997; Hida 1998).

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