Role of the parabrachial nucleus in ventilatory responses of awake rats

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1. The parabrachial nucleus (PBN) is thought to play an important role in cardiorespiratory control. However, the circumstances under which it affects ventilation are still not known. The purpose of the present study was to investigate how the PBN modulates the ventilatory responses to hypercapnia, hypoxia or a resistive load in awake rats with chemical lesions of the PBN.

2. In three groups of rats (with lateral PBN lesion, with Kölliker–Fuse nucleus lesion and control), ventilation was measured under various conditions.

3. There was no difference in the breathing of normal room air in any of the groups. However, the lesioned groups showed a reduced ventilatory response to hyperoxic hypercapnia (inspired CO_2 fractions (F_{I,CO_2}) of 3, 5, 8 and 10%) and to graded hypoxia (inspired O_2 fractions (F_{I,O_2}) of 16, 12, 10 and 8%) compared with the control group. The control group showed a biphasic response to sustained hypoxia (F_{I,O_2} at 10% for 30 min), known as 'hypoxic depression', while the lesioned groups showed moderate ventilatory exaggeration throughout hypoxia. In response to a resistive load, the lateral PBN lesion group showed no change in ventilatory compensation.

4. The PBN appeared to have a considerable influence on ventilation stimulated in various ways during wakefulness.

It has been suggested that the parabrachial nucleus (PBN) is one of the main relays for the transfer of a wide array of autonomic-related information from the more caudal levels of the neuraxis to supracollicular structures (Shannon & Lindsey, 1983; Milner, Joh, Miller & Pickel, 1984; Segers, Shannon & Lindsey, 1985; Berkley & Scofield, 1990). The PBN, in turn, is known to project to several autonomic nuclei interconnecting with the higher brain, and participates in cardiovascular and/or respiratory control (Fulwiler & Saper, 1984; Ward, 1988).

Compared with its neural connections, the functional roles of the nucleus are less clear. There are several interesting reports concerning the possible role of the PBN in respiration, but there is no consensus on its physiological role. Electrical stimulation or microinjection of glutamate into the ventrolateral regions of the lateral PBN caused tachypnoeic and hyperpnoeic responses (Takayama & Miura, 1993; Lara, Parkes, Silva-Carvhalo, Izzo, Dawid-Milner & Spyer, 1994), whilst some reports indicate that the PBN may participate in afferent-evoked inhibition of inspiration (Ling, Karius & Speck, 1993, 1994). These disparate findings may be partly due to the structural complexity of the PBN. In addition, considering its location and neural connections, the PBN may prove to be a respiratory modulator, particularly during wakefulness. For example, decerebration around the level of the PBN changes the hypoxic ventilatory response in rats (Martin-Body, 1988). Differences in anaesthetics and the depth of anaesthesia may greatly influence the PBN response to various stimulations (Caille, Vibert, Bertrand, Gromysz & Hugelin, 1979; Wagner, Eldridge & Dowell, 1991). It is therefore necessary to investigate the function of the PBN without anaesthesia. In the present study, two selected parts of the PBN (oriented to the lateral PBN and the Kölliker–Fuse (KF) nucleus) of rats were chemically lesioned, providing the first evidence that the PBN does contribute to the augmentation of ventilation not only during hypoxia but also in various other conditions in the awake state.

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METHODS

Animal preparations

All experiments were approved by the Animal Experiment Review Committee of the Tohoku University School of Medicine. Thirty-six male Sprague–Dawley rats weighing 200–250 g were used. For chemical lesioning of the PBN, rats were placed on a stereotaxic apparatus (Narishige, Tokyo, Japan) under Nembutal anaesthesia (38 mg kg^{-1}, I.P.). The initial dose was sufficient for the operation. The parts to be lesioned were chosen because the lateral PBN and the KF nucleus are supposed to have cardiorespiratory effects (Mraovitch, Kumada & Reis, 1982; Takayama & Miura, 1993). The skull was drilled, and a microinjection needle (150 μm, o.d.) was introduced stereotaxically 8·8 mm caudal to, 2 mm lateral to and 7 mm below bregma for the lateral PBN lesion, and 8·7 mm caudal to, 2·6 mm lateral to and 7·9 mm below bregma for the KF nucleus lesion (Paxinos & Watson, 1986). Chemical lesion was performed using kainate microinjection (36 ng dissolved in 100 nl saline over 2 min; Sigma Chemical Co.) (Housley & Sinclair, 1988). For the sham operation, the same volume of vehicle only was microinjected into the lateral PBN. In preliminary experiments, there was no significant difference in ventilatory response 1 week after the operation between vehicle microinjected into the lateral PBN or the KF nucleus area. Therefore, for the control group, vehicle was microinjected only into the lateral PBN in order to simplify the experiment. After microinjection, the needle was removed and the wound sutured. The animals were then kept warm and watched carefully until the anaesthesia had worn off completely. A prophylactic dose of penicillin (20 000 i.u., I.M.) was administered on the first three post-operative days. During the first 2–3 days the animals were fed with wet mash, but after that they resumed normal feeding and drinking. Subsequently they were kept on a 12 h light–12 h dark schedule with food and water available *ad libitum* for 1 week until the final experiments, by which time no unfavourable effects of surgery, such as hypophagia or loss of body weight, were present in any group of rats.

Experimental preparations

Ventilation was measured using the barometric method of plethysmography as described elsewhere (Mizusawa *et al.* 1994). Briefly, rats were placed in a 7 l Plexiglass chamber which was connected to a reference chamber through a pressure transducer (model MP-45, ± 5 cmH_2O; Validyne, Northridge, CA, USA). Respiratory frequency (f) was calculated from the ventilation-induced pressure swings and tidal volume (V_T) was obtained as a function of the pressure difference between the two chambers. The animal chamber had inlet and outlet tubes for constant background flow of humidified room air (0·5–1·0 l min^{-1}) to keep the CO_2 fractional concentration (F_{I,CO_2}) in the chamber below 0·6%. The O_2 fractional concentration (F_{I,O_2}) and F_{I,CO_2} in the chamber were continuously measured by a gas analyser (model 1H21A; NEC San-ei, Tokyo, Japan).

Hypercapnic response. The F_{I,CO_2} was sequentially adjusted to 3, 5, 8 and 10% using a gas mixture of 10% CO_2–30% O_2 in N_2, each level being maintained for 2 min. Ventilation during the last 1 min of each period was used for the analysis.

Response to graded hypoxia. After ventilation was stabilized, N_2 gas was mixed with background room air through the inlet tube to change the chamber F_{I,O_2}, which was controlled at 16, 12, 10 and 8% sequentially. About 15 s was required for the stabilization of each F_{I,O_2} level. Each F_{I,O_2} level was given for 2 min, and the last 1 min of ventilation was sampled for the analysis.

Response to sustained hypoxia. After ventilation was stabilized, the chamber F_{I,O_2} was maintained at 10% by adding N_2 gas to the background room air. Thereafter, the chamber F_{I,O_2} was maintained at this level (10·0 $\pm$ 0·5%) for 30 min, while ventilation was continuously measured.

All the above experiments were performed in this order at intervals of more than 3 h. Resting ventilation and behaviour were used to assess the suitability of the rats to undergo the next experiment.

Response to resistive loading. This experiment was performed on the day after the hypoxic and hypercapnic tests. The rats were lightly anaesthetized with Brevital Sodium (an ultra-short-acting barbiturate, 45 mg kg^{-1}, I.P.) and tracheotomized. After intubation using a curved endotracheal catheter of polyethylene tubing (3 cm length, 2·5 mm o.d., 1·6 mm i.d.) rats were isolated in individual humidified cages for at least 5 h. The disappearance of the effects of the anaesthetic was assessed by the level of aversive responses shown by the rats to a noxious stimulus. After all anaesthetic effects had worn off, they were placed in the chamber to measure ventilation. After control sampling of ventilation, they were gently restrained and a narrow tube for resistive loading (26 mm length, 0·9 mm i.d.) was attached to the endotracheal tube. The length of the resistive tube was chosen to avoid changes in minute ventilation ($\dot{V}_E$) and arterial CO_2 tension during resting breathing. The rats were then placed in the chamber again for the measurement of ventilation. After 15 min, the resistive tube was extracted and ventilation was again recorded.

Behavioural observations were made throughout all ventilatory measurements.

Histological examination

At the end of the experiments, the brains were removed under ether anaesthesia and stored in 10% formalin and then in 30% sucrose. Serial frozen sections (50 μm) were cut and stained with Cresyl Violet for histological confirmation of the lesion sites.

Data analysis

For the hypoxic and hypercapnic responses, the differences between data obtained from control and PBN or KF nucleus lesioned rats were analysed by two-way analysis of variance (ANOVA) with repeated measurements. When significance was indicated, Student's *post hoc* unpaired t test was employed for point-by-point analysis. For the responses to sustained hypoxia and the resistive loading test, the change of ventilation in each group of rats was analysed by ANOVA with Student's *post hoc* paired t test. The point-by-point effects of PBN or KF nucleus lesioning were analysed by using Student's unpaired t test. Differences between mean values were considered significant when $P < 0.05$.

RESULTS

Sites of lesion

Figure 1 shows the lesion sites of individual rats. Kainate was microinjected bilaterally and only rats whose rostral or medial lateral PBN or KF nucleus (at least unilateral) was histologically ascertained to be lesioned were used for the analysis. Thirty-six rats were microinjected in total, but only twenty-five (control, 8; lateral PBN lesion, 8; KF nucleus lesion, 9) were used for the data analysis. Data from the other eleven rats were excluded because neither the

bilateral PBN nor the KF nucleus region were lesioned. Kainate lesions produced near-complete loss of neuronal cell bodies which spread about 0·5 mm rostrocaudal. Lateral PBN lesions included most parts of the subnucleus lateralis, dorsomedialis and part of the subnucleus dorsolateralis of the PBN. KF nucleus lesions included most parts of the KF nucleus and part of the subnucleus lateralis or dorsomedialis of the PBN.

Effects on hypercapnic ventilatory response

Lesions in the lateral PBN and the KF nucleus did not affect ventilation during room air breathing, but produced consistent reductions in the ventilatory response to hypercapnia (Fig. 2). In control rats, ventilation increased in proportion to F_{I,CO_2}; $\dot{V}_E$ increased with increasing F_{I,CO_2} up to 441 ± 23 ml min^{-1} at 10% CO_2 exposure. On the other hand, $\dot{V}_E$ at 10% CO_2 was only 283 ± 12 ml min^{-1} in lateral PBN lesion and 268 ± 11 ml min^{-1} in KF nucleus lesion rats ($P < 0·01$ compared with controls for both groups; unpaired t test). The hypercapnic ventilatory response differed significantly between control and lateral PBN lesion rats, and between control and KF nucleus lesion rats ($P < 0·01$ in both cases; two-way ANOVA). In both groups of lesioned rats, reductions in both f and V_T seemed to contribute to the difference from controls. During the hypercapnic phase ($F_{I,CO_2} = 3$–10%), V_T of both groups and f of the KF nucleus lesion group were significantly lower than those of the control group (lateral PBN lesion

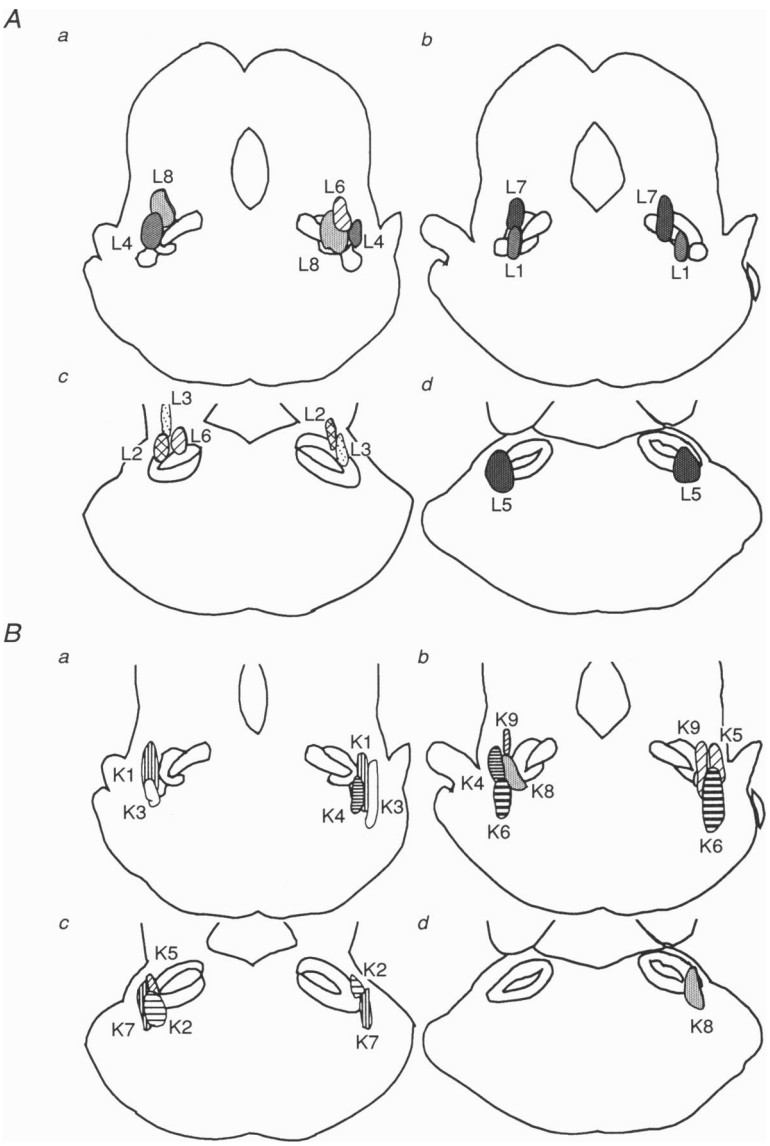

Figure 1. Lesion sites of rats

A, histological distribution of lateral PBN lesions in 8 rats (L1 to L8). *B*, sites of KF nucleus lesions in 9 rats (K1 to K9). Only rats used for the data analysis are shown. Line drawings in this figure are modified from Paxinos & Watson (1986). *a*, *b*, *c* and *d* represent −8·72, −8·8, −9·16 and −9·3 mm to bregma, respectively.

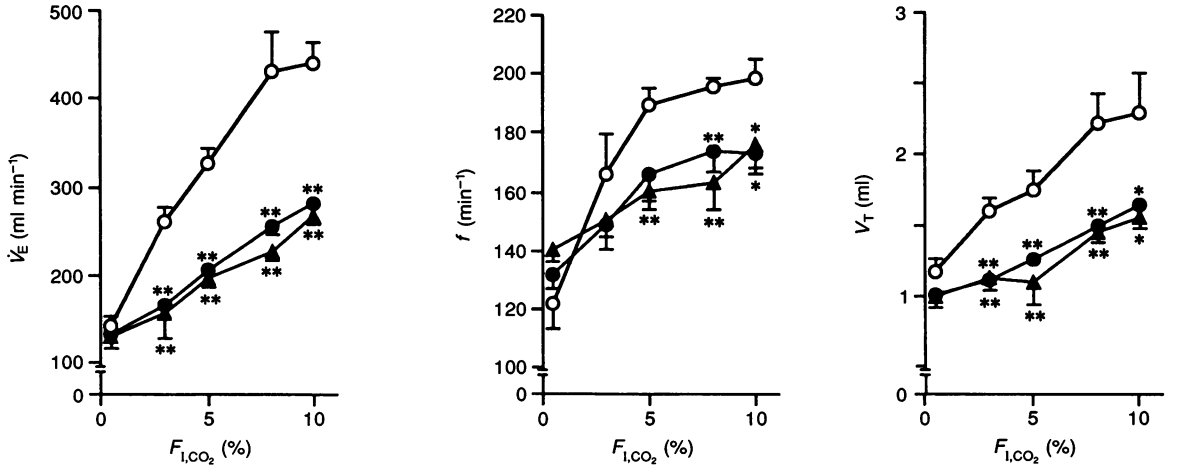

Figure 2. Ventilatory response to hyperoxic hypercapnia

Changes in $\dot{V}_E$, f and V_T are shown at an F_{I,O_2} of 30% and F_{I,CO_2} of 3, 5, 8 and 10%. ○, control; ●, lateral PBN lesion; ▲, KF nucleus lesion. Each point indicates the mean ± S.E.M. at each hypercapnic level. $\dot{V}_E$ and V_T differed significantly between control and lateral PBN lesion and between control and KF nucleus lesion rats ($P < 0{\cdot}01$ in all; two-way ANOVA). * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ compared with control values at the same point by unpaired t test.

group: V_T, $P < 0{\cdot}01$; KF nucleus lesion group: f, $P < 0{\cdot}05$ and V_T, $P < 0{\cdot}01$; two-way ANOVA). No significant difference was observed between lateral PBN lesion and KF nucleus lesion rats in all three parameters.

The behaviour of control rats during hypercapnic exposure included sniffing, rambling or gasping, although they remained still at the beginning of the experiments. These behaviours were observed especially during moderate or severe hypercapnia (F_{I,CO_2}, 8 and 10%). On the other hand, the two lesioned groups of rats were less active, even during severe hypercapnia. Some lesioned rats showed sniffing or rambling like the control rats, but most of the rats were still throughout the experiments.

Effects on hypoxic ventilatory response

Response to graded hypoxia

Hypoxia caused ventilatory augmentation in control rats, as found in previous studies (Tenney & Ou, 1977; Mizusawa

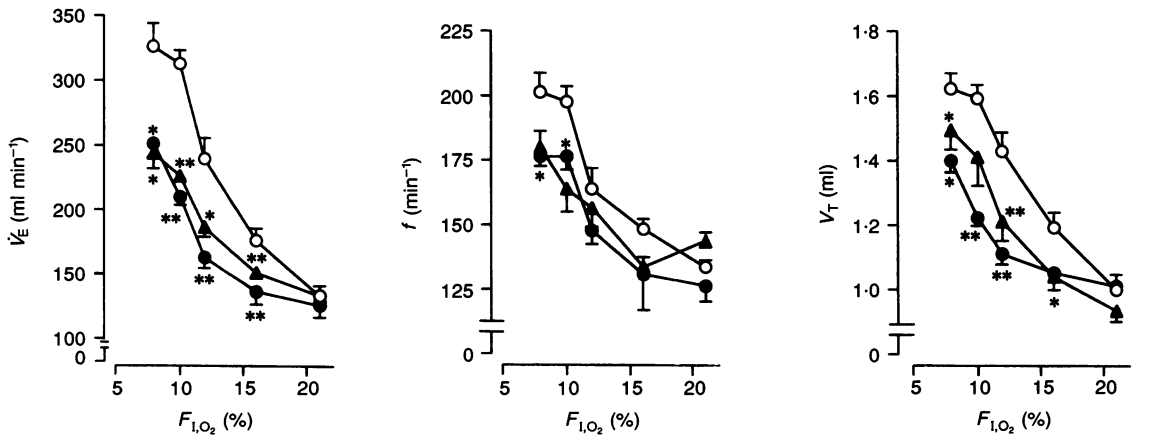

Figure 3. Ventilatory response to graded hypoxia

Rats were exposed to graded hypoxia, with F_{I,O_2} levels of 21, 16, 12, 10 and 8%. Symbols are the same as in Fig. 2; each point indicates the mean ± S.E.M. during each hypoxic level. $\dot{V}_E$ and V_T differ significantly between control and lateral PBN lesion rats ($P < 0{\cdot}01$ in both; two-way ANOVA) and between control and KF nucleus lesion rats ($P < 0{\cdot}01$ and $P < 0{\cdot}05$, respectively; two-way ANOVA). Between control and lateral PBN lesion rats, f also differed significantly ($P < 0{\cdot}01$; two-way ANOVA). * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ compared with control values at the same point by unpaired t test.

et al. 1994). As in the hypercapnic conditions described above, measurements established that the lateral PBN or KF nucleus lesions produced reductions of $\dot{V}_E$ at moderate hypoxia, but not in air (Fig. 3). As a whole, the hypoxic ventilatory response of the two lesioned groups differed significantly from that of sham-lesioned rats ($P < 0{\cdot}01$ in both cases; two-way ANOVA). For example, at 8% F_{I,O_2}, the $\dot{V}_E$ in control, lateral PBN and KF nucleus lesion groups was 325 ± 18, 251 ± 10 and 243 ± 12 ml min^{-1}, respectively. Both V_T and f had a tendency to decrease in lesioned groups, while the decrease of f in the KF nucleus lesion group was insufficient to reach statistical significance (lateral PBN lesion group: f, V_T, $P < 0{\cdot}01$; KF nucleus lesion group: V_T, $P < 0{\cdot}05$; two-way ANOVA). In the lesioned groups, the difference of ventilation was mainly due to a decrease in V_T. The behaviour of control rats during graded hypoxia was almost the same as during hypercapnia, i.e. sniffing, rambling and gasping, although these responses seemed to be weak compared with the hypercapnic test. In the lesioned groups of rats these responses were weaker still, and no obvious difference between the two lesioned groups was observed.

Response to sustained hypoxia

During 30 min exposure to hypoxic gas, ventilation was continuously recorded and the mean value of every 5 min period was used for the analysis (Fig. 4). Control rats showed a biphasic ventilatory response to sustained hypoxia, as noted before (Mizusawa *et al.* 1994). During the first 5 min, $\dot{V}_E$ increased sharply up to twice the basal level and subsequently declined. V_T, especially, declined to a level near that of the air-breathing controls after 15 min hypoxic exposure. This phenomenon is considered to be the so-called 'hypoxic depression' or 'hypoxic roll-off' which has been observed in awake humans and animals (Edelman & Neubauer, 1991; Eldridge & Chen, 1992). In the present study, this depression seemed to be due to a decrease in both V_T and f. As in the hypoxic response, sustained hypoxia caused different effects on lesioned rats compared with control rats. In the two groups of lesioned rats, hypoxia caused less $\dot{V}_E$ augmentation than in control rats in the first 10 min. However, in lesioned rats, this increase was maintained throughout the 30 min of hypoxic provocation and there was very little decline in ventilation. Because of the absence of ventilatory decline in lesioned rats, ventilation between control and lesioned rats did not differ 15 min after the initiation of hypoxia, although it did in the first 10 min.

Observed behaviour during hypoxic exposure was almost the same as during the hypercapnic response. Lesioned rats remained rather still throughout hypoxia but control rats showed instability and restlessness during moderate or severe hypoxia. Sustained hypoxic exposure for 30 min had different effects on the behaviour of the control rats. During the first 10 or 15 min of hypoxia they showed instability and restlessness, sniffing, rambling or gasping. However, in the latter half of hypoxia, most of them ceased rambling and crouched; this behavioural transition closely resembled the biphasic ventilatory response to sustained hypoxia. On the other hand, sustained hypoxia did not seem to affect the behaviour of lesioned rats. Some lesioned rats sniffed in the first 5 min of hypoxia, but most of them remained calm, as during room air breathing.

Effects on resistive loaded breathing

With an appropriate recovery period after the tracheotomy and intubation, all rats seemed to be in the same condition as before the operations with regard to ventilation and behaviour. Intubation itself did not affect the resting breathing in control or lesioned rats, at least in terms of f, V_T and $\dot{V}_E$. To attach the narrow tubes for resistive load into

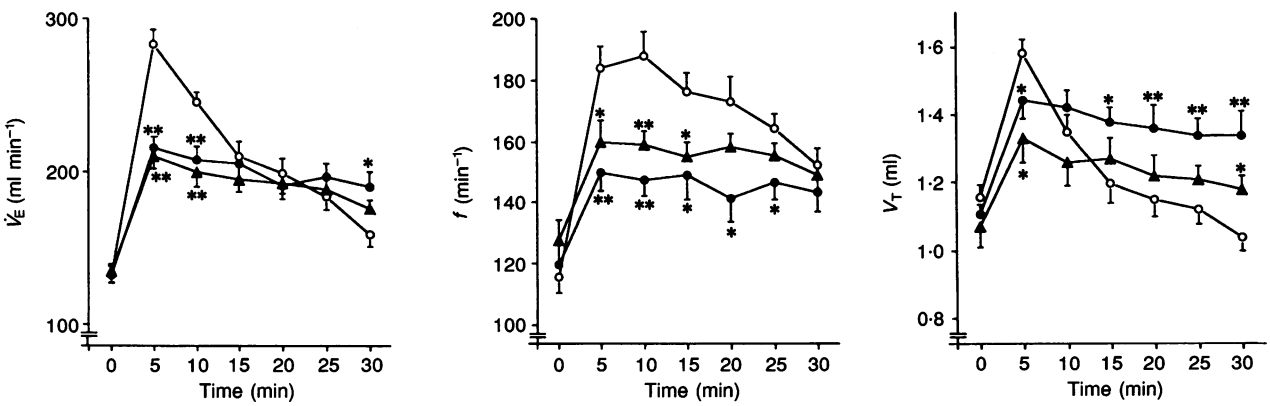

Figure 4. Ventilatory response to sustained hypoxia

Ventilatory response to sustained hypoxia (F_{I,O_2}, 10% for 30 min). Symbols are the same as in Fig. 2. Each point indicates the mean value during 5 min ± S.E.M. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, compared with control at the same point by unpaired t test.

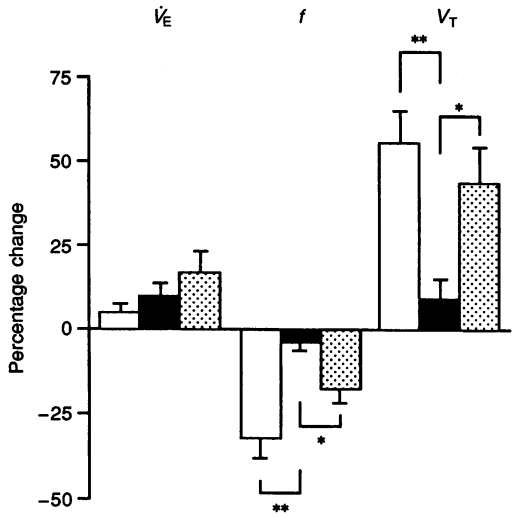

Figure 5. Ventilatory response to resistive loading
Immediately after resistive loads were applied, all rats showed an increase in ventilation. However, after 10 min all ventilation returned to the basal level. Hence ventilatory measurements were done at 10 min after resistive loading. Percentage changes in ventilatory parameters from preload values ($\dot{V}_E$, left; f, centre; V_T, right) are shown. □, control; ■, lateral PBN lesion; ▨, KF nucleus lesion. The responses of the PBN lesion group differed from the KF nucleus lesion and control groups, both of which showed ventilatory compensation to resistive loading, i.e. increase in V_T and decrease in f. * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

the intubation tube the rats had to be restrained for a moment, causing them to struggle slightly. Therefore, soon after the attachment, ventilation of all rats increased by almost the same degree (approximately 50% increase in $\dot{V}_E$). Both f and V_T of all rats increased during the first 5 min. Ten minutes after the attachment, they appeared calm and $\dot{V}_E$ returned to preload values. However, the breathing pattern was entirely changed compared with preload in control rats. Slow and deep breathing was observed: V_T increased up to $55 \pm 9\%$ and f decreased $32 \pm 6\%$ compared with preload values. On the other hand, in the lateral PBN lesion group the breathing pattern had fully returned to preload values within 10 min. There were significant differences in both f and V_T between control and lateral PBN lesion groups ($P < 0{\cdot}01$ in both) (Fig. 5). The KF nucleus lesion group showed almost the same ventilatory pattern as the control rats during resistive loading. V_T increased ($44 \pm 11\%$) and f decreased ($-18 \pm 5\%$), as in control rats, resulting in no difference between KF nucleus lesion rats and controls in all three parameters. In all rats, locomotion and body movement increased immediately after attachment of the resistive loads. After a few minutes, control and KF nucleus lesion groups crouched and gasped for breath; the lateral PBN lesion group behaved almost the same way except that they showed ordinary locomotion during the latter half of the resistive loading test. In three rats of each group twofold resistive loads (using a double length of the narrow tube) were given. The result was almost the same as above: ventilation increased soon after attachment and slow and deep breathing was observed only in control and KF nucleus lesion rats 10 min after the resistive loads, but not in the lateral PBN lesion group. In all rats that had resistive loads, the narrow tubes were extracted after 15 min. Ventilatory patterns returned to preload values after their removal.

DISCUSSION

The main findings of the present study are as follows: (1) lateral PBN or KF nucleus lesions had no effects on room air breathing; (2) the lesioned groups, however, showed attenuated ventilatory responses to hypoxia and hypercapnia; (3) a biphasic respiratory response to sustained hypoxia was observed in control rats while neither lesioned group showed a ventilatory decline throughout sustained hypoxia; (4) in response to resistive loads, the control and KF nucleus lesion groups showed an increase in $\dot{V}_E$ and a decrease in f, while the lateral PBN lesion group showed less compensation; and (5) throughout the experiments, behavioural responses such as sniffing or gasping seemed to be reduced in the lesioned groups.

The supracollicular structure has numerous effects on ventilation in conscious animals (Caille *et al.* 1979). Several studies have provided evidence that cerebral involvement in the response to hypercapnia is of importance (Murphy, Mier, Adams & Guz, 1990). The finding that the hypercapnic ventilatory response is reduced during sleep leads us to the idea that the absence of cerebral participation may contribute to this reduction. The results of the present study are compatible with this study. A lesion in the PBN seemed to eliminate higher brain participation in the ventilatory response to chemical stimuli.

Whether the supracollicular structure has an inhibitory or excitatory effect on ventilation during hypoxia cannot yet be definitively answered. Decortication caused a hyperexcitable ventilatory response to acute hypoxia in cats, which returned to pre-lesion levels after decerebration (Tenney & St John, 1980), while in adult rats the integrity of a region at or immediately above the intercollicular level seemed to be essential to the hypoxic ventilatory response (Martin-Body, 1988). All the differences may have resulted from variations in the lesion site and side-effects of acute brain lesions, which could be excluded in the present study

by using a very restricted lesion and by allowing an appropriate recovery period. That the lesion attenuated the hypoxic ventilatory response might indicate that unanaesthetized higher brain centres may have excitatory effects on ventilation during hypoxia.

With regard to sustained hypoxia, two noteworthy studies using fetal lambs (Gluckman & Johnston, 1987; Johnston & Gluckman, 1993) reported that PBN lesioning eliminated hypoxic ventilatory depression, indicating that the PBN (especially the KF nucleus region) might be essential to the fetal hypoxic ventilatory response. Methodological differences make it difficult to compare this study with ours, but the essence seems to be the same: lambs whose KF nucleus had been lesioned did not show the depression phase during sustained hypoxia; neither did PBN lesioned rats.

The role of the higher brain centre in the response to resistive loading remains obscure. In awake humans, a resistive load leads to an increase in tidal volume and a decrease in respiratory frequency (Kikuchi, Hida, Chonan, Shindoh, Sasaki & Takishima, 1991). Because these changes did not occur under anaesthesia (Whitelaw, Derenne, Couture & Milic-Emili, 1976), higher brain centres may be involved in changes in the breathing pattern during resistive loading (Cherniack & Altose, 1981). In awake men, the ventilatory response to resistive loading may be due to behavioural control of breathing to reduce the sensation of dyspnoea (Kikuchi *et al.* 1991). The results of the present study seem to be compatible with these findings: higher brain centres are essential to the usual ventilatory response to resistive loads.

What is the mechanism of attenuation of ventilatory response to chemical and mechanical stimulation in the lesioned rats? The fact that the lesions did not affect the resting ventilation and the lesioned rats showed less restlessness and nervousness leads us to propose that the lesion disrupted the route conveying the peripheral information to higher brain regions such as the limbic system or cortex and, possibly, prevented the appearance of 'respiratory sensation' (Eldridge & Chen, 1992). The sensation of respiration is not perceived until unpleasant respiratory stimulation occurs. Then higher brain regions that sense such breathing discomfort may in turn strengthen ventilation during respiratory stimulation (Murphy *et al.* 1990). This hypothesis seems to coincide with the evidence that sustained hypoxia has a biphasic effect on ventilation and that the sensation of dyspnoea accompanies this ventilatory output, i.e. initially augmented and subsequently dulled (Chonan, Hida, Okabe, Chung, Kikuchi & Takishima, 1992). Receiving information from the periphery and processing it as a 'sensation' in brain centres seems to be indispensable to a normal ventilatory response. PBN lesioning may interrupt this normal processing to attenuate the response. There is also the possibility that the lesion may have disrupted not the afferent, but the efferent, pathway from higher brain regions to the medullary respiration centre. However, considering that behaviour during stimulation was also affected by the PBN lesioning, it is more probable that it was the afferent projection from the periphery that was disrupted.

The possibility that the behavioural differences themselves caused the ventilatory differences cannot be ignored. However, there are several reasons why this is unlikely to have been the case in the present study. First, the behavioural responses observed in control rats were not vigorous and they did not keep moving around. Second, with regard to the hypercapnic ventilatory response, exercise itself does not change the steepness of the rise in the ventilatory increment in proportion to the arterial CO_2 increment (Martin, Weil, Sparks, McCullough & Grover, 1978), while in the present study the lesioning reduced the steepness of the rise in the ventilatory response.

We intended to clarify the regional role of the PBN by lesioning two representative parts, but failed to demonstrate obvious differences between the two lesions. There may be several reasons for this. The PBN is so compact and complicated that it may be impossible to completely differentiate between the lateral PBN and KF nucleus lesions. Moreover, given the incompleteness of the micro-injection technique, some injection sites overlapped (Fig. 1) and this may be one of the reasons for the apparent homogenous function of these two parts. Further studies are needed to clarify these points.

The rostral dorsolateral pons has been suggested as being a pneumotaxic centre (Ling *et al.* 1993, 1994). Lesioned rats may simply lose the ability to breathe deeply. However, in most of their studies Ling and co-workers investigated regions caudal to the colliculus, and they used cats. In rats, the rostral dorsolateral pons is also thought to work as a pneumotaxic centre (Wang, Fung & St John, 1993), although the region investigated here was more rostral than ours. Moreover, as shown in their hypercapnia study, even lesioned rats could increase ventilation by up to 300 ml min^{-1}. It appears that in our study, the lesioned rats did not increase ventilation in response to stimulation, not because they could not but because they were unaware of any respiratory stimulation through hypercapnia, hypoxia and resistive loading.

If the PBN plays an important role in the ventilatory response to various ventilatory stimulations, we will be able to apply this evidence to many unresolved problems. Why a biphasic ventilatory response occurs during sustained hypoxia is unknown. We consider that the immediate exaggerated response through higher brain centres is essential to the biphasic ventilatory response. In addition, there is the problem of patients with asthma who have dulled sensitivity to air-hunger or dyspnoea and who

have a reduced hypoxic or hypercapnic ventilatory response (Kikuchi *et al.* 1994). A disturbance in the PBN may account for this phenomenon. The results of the present study may be useful for the elucidation of these problems.

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