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BRAIN TRANSECTIONS DEMONSTRATE THE CENTRAL ORIGIN OF HYPOXIC VENTILATORY DEPRESSION IN CAROTID BODY-DENERVATED RATS

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SUMMARY

1. The characteristics of hypoxic ventilation were studied in awake adult rats after brain transections about the intercollicular level. The results were compared with studies made before transection, 17–24 h after bilateral carotid body denervation effected by carotid sinus nerve section.

2. Transection at or below the intercollicular level converted the depressive hypoxic frequency response of control studies to a stimulatory response, increased the stimulation of tidal volume by hypoxia, and so converted the dominant pattern of ventilation in hypoxia from a depression to a progressive stimulation.

3. Transection above the intercollicular level failed to reverse the hypoxic frequency response but increased the stimulation of tidal volume by hypoxia. Consequently minute ventilation progressively increased as the severity of hypoxia increased.

4. The experiments demonstrate that in the adult rat depression of respiratory frequency by hypoxia after carotid body denervation requires the integrity of a region at or immediately above the intercollicular level. In contrast, the stimulation of tidal volume by hypoxia is markedly dependent upon precollicular structures.

5. The results are discussed in relation to the hypoxic depression of fetal breathing and the biphasic hypoxic ventilatory response of the newborn.

INTRODUCTION

Within the central nervous system hypoxia is considered to have a widespread depressive effect on neural activity. This is observed clinically as a loss of cognitive ability or as confusion (Gibson, Pulsinelli, Blass & Duffy, 1981) and is accompanied by changes in the EEG (Cooper, 1974).

Medullary neurones purportedly involved in the control of respiratory rhythm are not exempt from the depressive effects of hypoxia and often show reductions in firing rate as blood oxygen levels fall (St John & Wang, 1977). Some medullary respiratory neurones are excited by hypoxia but these are thought to be concerned with the reflexogenic pathway arising from hypoxic stimulation of the peripheral chemo-

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receptors (St John & Bianchi, 1985). A general depression of medullary neurones by hypoxia has been used to explain ventilatory depression in severe hypoxia in awake dogs (Morrill, Meyer & Weil, 1975) and cats (Ou, Miller & Tenney, 1976), the fall of ventilation after an initial excitation in prolonged hypoxia in man (Weil & Zwillich, 1976) and in newborn infants (Cross & Warner, 1951), the decrease in apneustic depth during hypoxia in cats with denervated peripheral chemoreceptors (St John, 1979) and hypoxic ventilatory depression after carotid endarterectomy in man (Wade, Larson, Hickey, Ehrenfeld & Severinghaus, 1970).

In the fetal lamb, where hypoxia most often causes apnoea, transection of the brain below the superior colliculus can prevent the apnoea (Dawes, Gardner, Johnston & Walker, 1983), and there is now evidence that this effect originates at a restricted site in the rostrolateral pons (Gluckman & Johnston, 1987). Further, in the newborn rabbit the decline in ventilation that occurs after 5 min of hypoxia can be prevented by brain transection at or below the intercollicular level (Martin-Body & Johnston, 1988). These studies suggest that hypoxic ventilatory depression involves a specific site in the central nervous system and does not simply reflect a widespread neural depression.

The present study explores the possibility that in adult animals hypoxic ventilatory depression is also mediated from a mid-brain or pontine site. The hypothesis has been investigated by studying the ventilatory response to hypoxia before and after brain transection between the levels of the rostral pons and the pre-colliculus in awake chemodenervated rats, in which ventilatory depression in hypoxia has already been characterized (Martin-Body, Robson & Sinclair, 1985, 1986).

METHODS

Experiments were performed on female Charles Wistar rats weighing between 252 and 340 g aged 113–136 days. Bilateral carotid sinus nerve section was performed under halothane anaesthesia as described previously (Martin-Body *et al.* 1985). In preparation for brain transection each rat was anaesthetized with halothane and orally intubated under direct vision; the tracheal tube was fixed to the lower incisors with thread and cyanoacrylate glue. This provided a route for easy administration of further halothane and permitted artificial ventilation at later stages. The head of the animal was then fixed in a stereotaxic frame. A parietal craniotomy was performed, the brain was transected with a blunt spatula at a level between the rostral pons and the pre-colliculus, and the cranial contents rostral to the transection removed by aspiration. Residual bleeding was well controlled by using the suction to close vessels. The halothane anaesthesia was immediately discontinued and the animal artificially ventilated for 30–60 min irrespective of the occurrence of spontaneous breathing. The cranium was loosely packed with cotton wool and the skin sutured. Body temperature was monitored throughout with a rectal thermistor and generally maintained during and after surgery by an infra-red heating lamp.

Ventilation was measured using a barometric plethysmograph in the manner we have described previously (Martin-Body *et al.* 1985). Tests of hypoxic ventilatory responses were made 17–24 h after bilateral carotid sinus nerve section, and again on the same day after transection. In studies undertaken before transection, the rat was lightly restrained in the plethysmograph breathing air for 30 min, after which duplicate records of ventilation were made, separated by 2 min of chamber reflushing. Then the chamber was flushed for 10 min with a mixture of 7 % O_2 in N_2 and ventilation was again recorded in duplicate. A similar protocol was followed to obtain measurements of the ventilatory response to less severe levels of hypoxia by administering successively, mixtures of 7·5, 10, 14 and 16 % O_2 in N_2. These concentrations were chosen because the ventilatory response at these levels permits a good approximation of the characteristics we have described using many more hypoxic levels (Martin-Body *et al.* 1985, 1986). The same protocol was followed for studies after transection except that the hypoxic gas mixtures were administered in the reverse order

because some animals fail to survive the most severe level of hypoxia. The first measurements of ventilation after transection were made between 1 and 1·5 h after discontinuation of the halothane anaesthesia.

The respiratory signal was recorded on FM tape (Martin-Body *et al.* 1985) and analysed by computer. Minute ventilation ($\dot{V}_E$), tidal volume (V_T), respiratory frequency (f), inspiratory time (T_I), expiratory time and mean inspiratory flow rate (V_T/T_I) were calculated on a breath-by-breath basis from twenty to thirty breaths after editing of sighs and movement artifacts. The mean, standard deviation and standard error of the mean (S.E.M.) were then computed. Differences in the ventilatory responses to hypoxia before and after transection were analysed using analysis of variance (ANOVA) procedures.

RESULTS

Control studies

The characteristics of the ventilatory response to hypoxia after bilateral carotid sinus nerve section are illustrated in Figs 2 and 3 by the data described with open circles (control data for rats which subsequently underwent brain transection). These results support and extend our previous descriptions of such responses (Martin-Body *et al.* 1985, 1986) by demonstrating the effect of hypoxia not only on V_T, f and $\dot{V}_E$ but also on T_I, T_E and V_T/T_I. Lowering of the inspiratory O_2 pressure (P_{I,O_2}) from 147 mmHg (normoxia) to 113 mmHg had no notable effects on any of these respiratory variables. However, a further lowering of the P_{I,O_2} to 96 mmHg resulted in a fall of f by about 15 breaths min^{-1}, a consistent depression of V_T by 0·1–0·2 ml, and a pronounced reduction in $\dot{V}_E$. In more severe hypoxia f remained depressed well below normoxic values whereas V_T and $\dot{V}_E$ increased to attain values above those in normoxia at P_{I,O_2} 52 mmHg. The sudden depression of f at P_{I,O_2} 96 mmHg was mainly attributable to a lengthening of T_E by 0·1–0·2 s; more severe hypoxia did not produce further significant changes. There was a slight lengthening of T_I at P_{I,O_2} 96 mmHg which was not sustained in severe hypoxia, and overall the changes in T_I were small. In consequence the drive to ventilation, indicated by mean inspiratory flow rate (V_T/T_I) closely mirrored the pattern of response of V_T to hypoxia.

Transection studies

Twelve animals underwent brain transection. The level of transection in each animal is shown on a sagittal outline of the brain of the adult rat (Paxinos & Watson, 1982) in Fig. 1. After transection there was a reversal of the frequency response to hypoxia in each of the cases where the transection level is indicated by a continuous line. The most rostral extent of these transections passed from the caudal regions of the superior colliculus dorsally, through the oculomotor nucleus, to the rostral edge of the interpeduncular nucleus ventrally. The most caudal transection passed immediately caudal to the inferior colliculus, through the pontine reticular formation to bisect the pontine nucleus ventrally.

Mean ventilatory responses for the seven animals concerned are detailed in Fig. 2*A*. Respiratory frequency increased progressively from 65·4 ± 4·0 min^{-1} in normoxia to 98·6 ± 8·6 min^{-1} at P_{I,O_2} 69 mmHg; there was a decline to 91·3 ± 8·8 min^{-1} at P_{I,O_2} 52 mmHg. Values in normoxia and mild hypoxia were 18–30 min^{-1} lower than control values but in moderate and severe hypoxia were 8–18 min^{-1} higher. V_T was higher than in control studies in normoxia and at all hypoxic levels. The increase in normoxic V_T reflected a heavy bias of the mean by results from studies involving the

three most caudal transections (indicated in Fig. 1 by continuous lines with †). In the absence of the data from these three animals an increased responsiveness of V_T to hypoxia was still observed despite normoxic V_T now being lower than in controls (Fig. 2*B*). The pattern of response of f was similar to that described for all seven animals. Transection also abolished the small but consistent decline in V_T at P_{I,O_2} 96 mmHg.

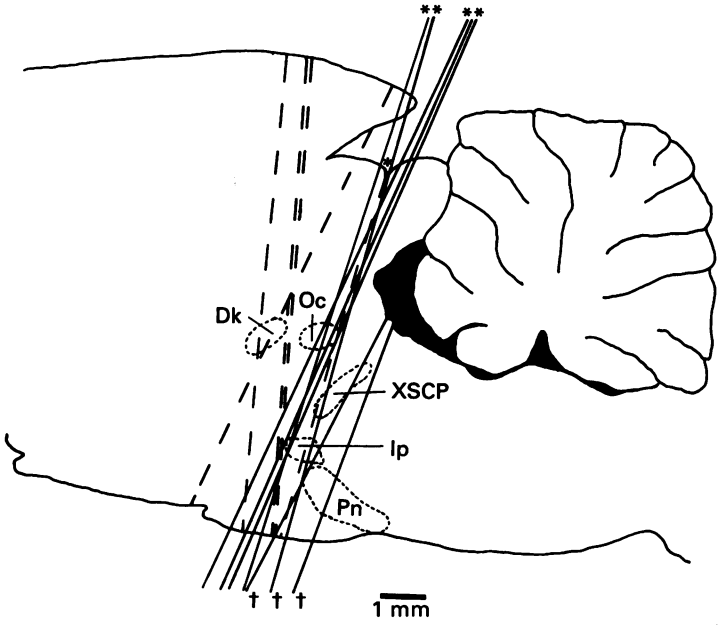

Fig. 1. Sagittal outline of the brain of the adult rat (Paxinos & Watson, 1982) indicating the level of transection in twelve animals. Continuous lines refer to animals whose ventilatory data is given as a group in Fig. 2*A* († the three most caudal transections); dashed lines refer to data given in Fig. 3. * Animals whose body temperatures increased during hypoxia after transection. Dk, nucleus Darkschewitsch; Ip, interpeduncular nucleus; Oc, oculomotor nucleus; Pn, pontine nucleus; XSCP, decussation superior cerebellar peduncle.

Consequent to the substantial stimulation of both f and V_T by hypoxia, $\dot{V}_E$ increased from $119{\cdot}4 \pm 11{\cdot}1$ ml min^{-1} in normoxia to $277{\cdot}3 \pm 30{\cdot}2$ ml min^{-1} at P_{I,O_2} 52 mmHg ($n = 7$). The increase in f during hypoxia was brought about by progressive reductions in T_E from values considerably longer than in controls during normoxia. T_I was shorter than in controls in normoxia and hypoxia but the variations in T_I were relatively minor. V_T/T_I was greater at all levels of hypoxia compared with the control studies, and showed no decline at P_{I,O_2} 96 mmHg (Fig. 2*A*).

A two-way analysis of variance (ANOVA) revealed significant ($P < 0{\cdot}0001$) effects of treatment (transection) on all respiratory variables except f and V_T/T_I. The failure of f to show a significant treatment effect reflects the fact that the grand mean calculated across all P_{I,O_2} levels was unchanged by transection despite the reversal of the frequency characteristic. The treatment $*P_{I,O_2}$ interaction term, which provides an assessment of whether there are different response profiles before and after

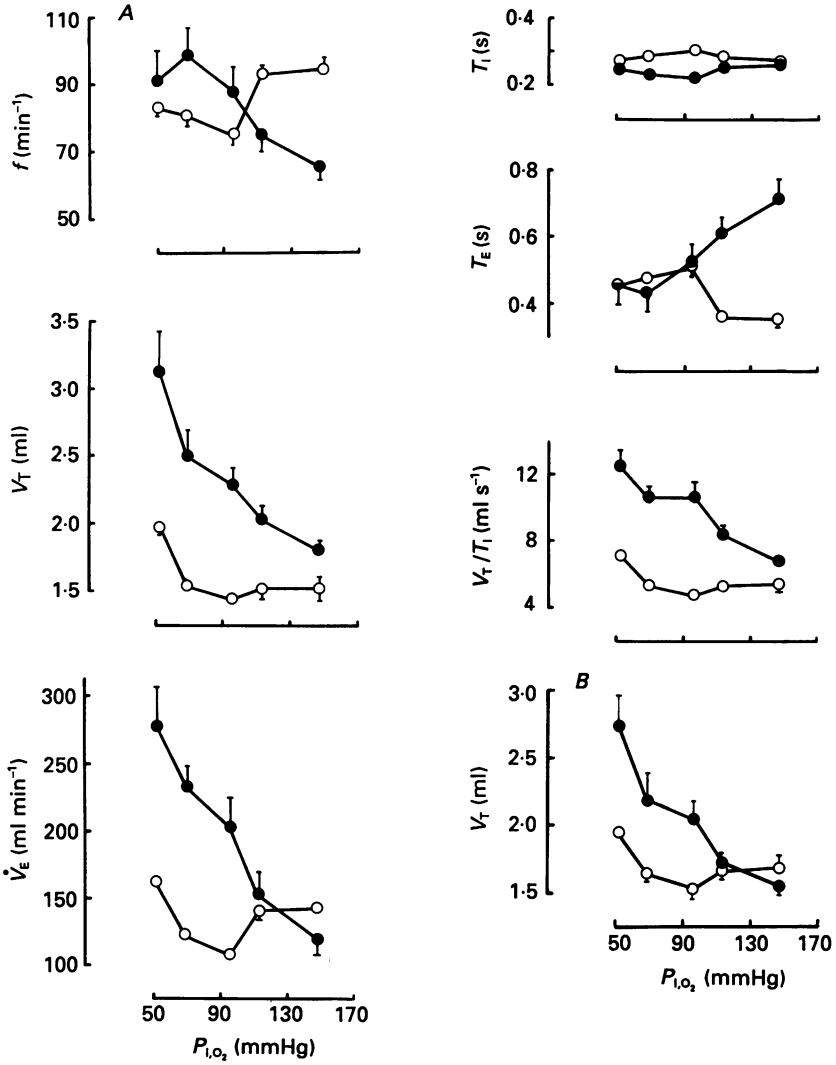

Fig. 2. Ventilatory responses at different levels of inspired oxygen (P_{I,O_2}) before (○) and after (●) transection of the brain, (*A*) at sites indicated by the continuous lines in Fig. 1 seven chemodenervated rats, and (*B*) at sites indicated by the continuous lines without the † in Fig. 1 four chemodenervated rats. Respiratory frequency (f), tidal volume (V_T), minute ventilation (V_E), inspiratory time (T_I), expiratory time (T_E) and mean inspiratory flow rate (V_T/T_I). Values are means ± S.E.M.; in some cases the S.E.M. falls within the point.

treatment, was significant for all variables. ($P < 0{\cdot}0001$ except for T_I where $P < 0{\cdot}001$).

The effects of transection on the ventilatory responses to hypoxia in the five animals whose transection levels are indicated by the dashed lines in Fig. 1 are detailed in Fig. 3. Transections were generally more rostral than those indicated by the continuous lines. Leaving aside the one exception, in the sagittal plane the most caudal extent of the transections bisected the superior colliculus, passed immediately

caudal to the nucleus of Darkschewitsch, and through the rostral portions of the interpeduncular nucleus ventrally. Such transections did not reverse the frequency response to hypoxia. However, the frequency characteristic was modified from that of controls by reductions of f at P_{I,O_2} 147 and 113 mmHg. There were also reductions in V_T, $\dot{V}_E$ and V_T/T_I in normoxia and mild hypoxia. The shape of the V_T response

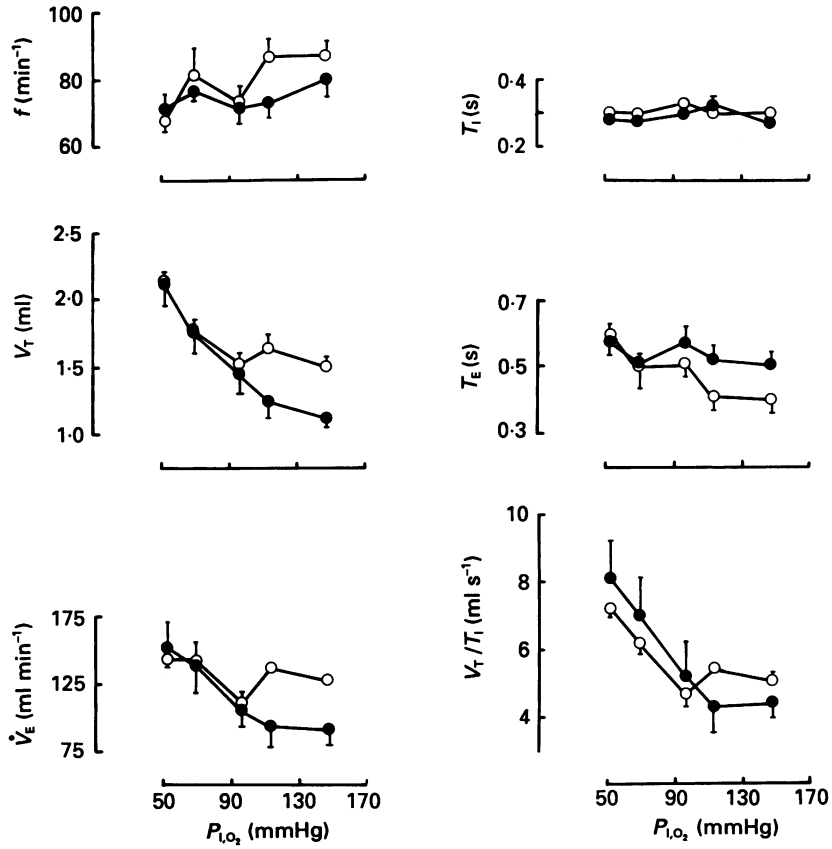

Fig. 3. Ventilatory responses to hypoxia before (○) and after (●) transection of the brain at sites indicated by dashed lines in Fig. 1, in five chemodenervated rats. Abbreviations as in Fig. 2. Values are means ± S.E.M.; in some cases the S.E.M. falls within the point.

curve was remarkably similar to that observed in animals which had a reversal of the hypoxic f response. In consequence, there was no depression of $\dot{V}_E$ in moderate hypoxia and decreases of P_{I,O_2} below 113 mmHg progressively increased $\dot{V}_E$. An ANOVA showed that the effects of transection were significant only with regard to V_T, $\dot{V}_E$ ($P < 0{\cdot}001$) and T_E ($P < 0{\cdot}02$). There were no significant treatment $*P_{I,O_2}$ interaction terms.

One animal within this group had a notably more caudal transection which might have been expected to result in a reversal of its frequency response to hypoxia. In this case the post-transection breathing frequencies on air were unusually high at 105 min^{-1}. Had f assumed more typical values of 80 min^{-1} then the expected reversal would have occurred. Indeed in hypoxia f varied from 85–95 min^{-1} which was similar

to the range of values observed in animals exhibiting a reversal of the frequency response to hypoxia. There was no obvious explanation for the anomalous air breathing values.

In the control studies hypoxia caused mean body temperature to decrease from $38{\cdot}19 \pm 0{\cdot}09$ to $35{\cdot}73 \pm 0{\cdot}17$ °C, the lowest temperatures consistently occurring at P_{I,O_2} 96 mmHg. Those at P_{I,O_2} 113 mmHg were only marginally higher at $36{\cdot}05 \pm 0{\cdot}19$ °C. For the seven animals whose frequency responses in hypoxia were reversed, body temperatures after transection ranged from $37{\cdot}62 \pm 0{\cdot}33$ °C in normoxia to $36{\cdot}79 \pm 0{\cdot}55$ °C at P_{I,O_2} 52 mmHg. These apparently higher temperatures were the result of increases in the body temperatures of four of the seven rats during hypoxia. Body temperatures in the animals whose hypoxic frequency responses were not reversed by transection were $35{\cdot}82 \pm 0{\cdot}43$ °C in normoxia and maximally decreased to $34{\cdot}47 \pm 0{\cdot}59$ °C at P_{I,O_2} 52 mmHg; one animal increased its body temperature during hypoxia. The large S.E.M.S after transection reflect the fact that two animals were deliberately not kept warm in the immediate post-operative period and consequently had body temperatures in the range 32–35 °C. Transection reversed the hypoxic f response in one of these animals but not in the other, suggesting that low body temperatures could not account for the ventilatory effects. The transection levels corresponding to the five animals with unusual variations in body temperature are those marked by asterisks in Fig. 1.

DISCUSSION

The most notable result of this study is that transection of the brain at or below the mid-collicular level in mature rats prevents the depression of respiratory frequency by hypoxia which occurs in the absence of excitatory input from the carotid bodies. The study also provides evidence that the responsiveness of tidal volume to hypoxia is determined, at least in part, by precollicular structures. These results have been demonstrated in awake animals, using an experimental design with each animal as its own control.

Interpretation of the results from transection studies can be difficult for several reasons: (1) it is difficult to reproduce precisely the level of transection, (2) as a consequence of local oedema, an indeterminable amount of tissue adjacent to the cut surface will be physiologically incompetent, (3) general neural trauma may be responsible for some of the effects, and (4) physiological systems other than the particular one under investigation will be affected, so that significant results may arise secondarily to non-respiratory disturbances.

That the animals were traumatized to some extent is clear from the fact that good survival rates were dependent on a period of artificial ventilation and oxygenation for about half an hour after the transection. This was the case regardless of the amount of blood loss; apparently poor post-operative condition was not necessarily attributable to cardiovascular decline. Nielsen, Bisgard & Mitchell (1986) reported that ventilatory support was necessary in some dogs after mesencephalic transections.

The possibility that the significant effects on the hypoxic response arose secondarily to disturbances to other systems cannot easily be discounted, although

the relatively systematic variation of the hypoxic response with the level of transection indicates otherwise. However, similarities in the anatomical organization of the CNS with respect to cardiovascular and respiratory reflexes has to be considered; blood pressure and heart rate were not measured but the fact that the animals were capable of surviving severe hypoxia suggests that the cardiovascular parameters were not extremely abnormal. Furthermore, studies on rabbits with rostral pontine or high mesencephalic transections show that during hypoxia blood pressure changes are similar to those seen in intact controls, although the patterns of response of heart rate, cardiac output and total peripheral resistance are different (Korner, Uther & White, 1969). The thermoregulatory system may also have important influences on respiratory output, and unusual variations in body temperature were noted in some animals after transections. However, there was no correlation between the reversal of the frequency response to hypoxia and actual body temperature or the changes of body temperature over the duration of the experiment.

The chronic nature of these experiments, the constraints laid by studies on awake animals, and the use of an animal with a relatively small blood volume imposed considerable problems with regard to making repeated blood gas measurements. Therefore, the ventilatory variables have each been presented in relation to P_{I,O_2} rather than arterial O_2 pressure (P_{a,O_2}). There will have been variations in P_{a,O_2} from animal to animal due to individual variations in the gas exchange characteristics of the lungs, but the consistency of the control responses in both groups of animals and the great similarities of these to previous data from this laboratory (Martin-Body *et al.* 1985, 1986) indicate that P_{I,O_2} is not an unreliable index of hypoxia. Further, in this study the statistical significance of the effects of transection have been established by consideration of the grand mean across all P_{I,O_2} levels for each ventilatory variable, and by consideration of the treatment $*P_{I,O_2}$ interaction terms, which measure the non-parallel nature of the ventilatory response characteristics. The former measure is not influenced by the use of P_{I,O_2} rather than P_{a,O_2} as an index of hypoxia providing the same ventilatory data is employed for the calculation. The interaction terms are certainly dependent upon the index chosen to measure the degree of hypoxia. However, given the argument that P_{I,O_2} is generally not an unreliable measure of hypoxia, given the magnitude of the changes from control in animals with transections at or below the intercollicular level, and given the great differences in respiratory responses depending on the level of transection, it is highly unlikely that use of P_{a,O_2} would change the conclusions of this study.

Ventilatory responses during hypoxia in decerebrate animals with peripheral chemoreceptor denervation have been reported previously in dogs (Bouckaert, Heymans & Samaan, 1938; Nielsen *et al.* 1986), rabbits (Wright, 1935; Korner *et al.* 1969) and cats (Selladurai & Wright, 1933). Except in the study by Korner and colleagues, the transections were apparently intercollicular although sufficient methodological details were not always given to be certain. The failure of these studies to report alterations in the response to hypoxia could therefore reflect either the transection level, or species differences. Korner *et al.* (1969) reported results in three rabbits with carotid and aortic nerve sections and pontine transections. A decrease of P_{a,O_2} from 52 to 41 mmHg led to an increase in respiratory frequency but

further lowering of P_{a,O_2} to 31 mmHg resulted in a fall; calculations from their data indicate a similar pattern of response in V_T. These responses partially resemble those we report here in that f increased progressively on lowering of the P_{I,O_2} from 96 to 69 mmHg (P_{a,O_2} about 59 and 48 mmHg respectively) but then declined at P_{I,O_2} 52 mmHg when the P_{a,O_2} would be about 33 mmHg (blood gas data, unpublished observations from this laboratory). We did not observe a decline in V_T at P_{I,O_2} 52 mmHg. Unfortunately Korner *et al.* did not deafferent rabbits with higher transections.

The experiments reported recently by Gallman & Millhorn (1988) provide supportive evidence for a role of supra-pontine structures in hypoxic ventilatory control, but these were concerned with post-hypoxic effects. The authors demonstrated that the long-lasting inhibition of phrenic nerve activity after a brief exposure to severe hypoxia in carotid body-denervated cats required the mesencephalon for its activation. Evidence that long-lasting inhibition is not a factor in the present study is obtained from the data on rats where the transection failed to reverse the hypoxic frequency response; in these cases the ventilatory characteristics were qualitatively similar before and after transection despite reversal of order of administration of the hypoxic gases. Also, in a previous study from this laboratory no effects of the sequence of administration of hypoxic gases on the ventilatory pattern of carotid body-denervated rats could be detected (Martin-Body *et al.* 1985).

Within the CNS hypoxia has long been thought to be a generalized depressant of neuronal activity (e.g. Gesell, 1939). In the medulla and pons there is a limited distribution of excitatory influences from the peripheral chemoreceptors (St John & Bianchi, 1985); not infrequently hypoxia depresses neuronal activities (St John & Wang, 1977). Most investigators have therefore attributed ventilatory depression in hypoxia to widespread decreases in neuronal activity. The results of the present study suggest that such an explanation is unlikely, firstly because the level of transection determined whether there was a reversal of hypoxic frequency depression and secondly, because after transection hypoxia resulted in marked increases in tidal volume and respiratory frequency. The theory that hypoxic ventilatory depression arises from a reduction in the stimulus to the central CO_2 chemoreceptor by hypoxic-induced overperfusion of the brain stem relative to metabolic rate (Neubauer, Santiago, Posner & Edelman, 1985) now also seems unlikely. But it is important to note that this study has not produced evidence that low oxygen *per se* is the active agent at the neuronal level, and neither has it shown that the receptive element is located near the intercollicular level. Rather, the study has demonstrated the integrity of this region is required for the expression of hypoxic respiratory depression in chemodenervated rats.

There is some evidence that hypoxic ventilatory depression results from alterations in neurotransmitter or neuromodulator levels within the central nervous system. For example, Millhorn, Eldridge, Kiley & Waldrop (1984) showed that post-hypoxic ventilatory inhibition was prevented in animals pre-treated with the adenosine antagonist, theophylline. Endogenous opioids may also mediate part of the ventilatory depression in acute hypoxia (Neubauer, Posner, Santiago & Edelman, 1987). Thus, the release of inhibitory neurotransmitters or neuromodulators onto

respiratory neurones through activation of suprapontine mechanisms is a likely manner by which hypoxia depresses ventilation.

After the appropriate transections a marked stimulation of respiratory frequency occurred which was related to the severity of the hypoxia. This tachypnoea superficially resembles that of the unanaesthetized chemodenervated cat although in this animal it is accompanied by low tidal volumes (Miller & Tenney, 1975; Gautier & Bonora, 1980). These authors have concluded that the tachypnoea is diencephalic in origin, perhaps indicating it is not of the same origin as that described here. In the rat the role of subsidiary glomus tissue in the neck and abdomen (Martin-Body *et al.* 1985, 1986) in mediating the post-transection tachypnoea should be considered, although the contribution of this secondary glomus tissue to the excitatory hypoxic response appears to be substantially less than that required to account for the increase in respiratory frequency after transection. However, in the absence of hypoxic respiratory depression the total contribution of the subsidiary peripheral chemoreceptors to ventilatory output in hypoxia may be greater than previously measured. Another mechanism which might account for post-transection tachypnoea is lactic acidosis, but the fact that f was increased even in mild hypoxia (P_{I,O_2} 113 mmHg) makes it an unlikely explanation.

The spontaneous rise in body temperature noted in some rats is apparently a consequence of the release, from inhibition, of sympathetic drive to brown fat (Rothwell, Stock & Thexton, 1983). The precise site which mediates the inhibition is not known but Rothwell *et al.* (1983) reported that pre-pontine transection led to a spontaneous rise in rectal temperature whereas transections 1–4 mm anterior to the pons did not. Results of the present study are in general agreement with these findings, although rats with the most caudal transections did not show unusual variations in body temperature during hypoxia or high basal rectal temperatures. Discrepancies between the studies most likely arise from variations in damage to remaining brain tissue.

The results from the present study can explain the apparent paradox that hypoxia depresses breathing in the fetus and yet stimulates breathing in the adult. In both cases it is now clear that a site in the rostral pons or caudal mesencephalon is involved in the depression of respiratory motor output during hypoxia. In the adult, this inhibition is overridden by a powerful excitation arising from the peripheral chemoreceptors, particularly the carotid bodies. Since the carotid bodies of the fetal lamb actively respond to hypoxaemia (Blanco, Dawes, Hanson & McCooke, 1984) but hypoxia depresses ventilatory output, then it must be concluded that at this stage of development the neuronal substrate of the medulla is unable to transmit the incoming afferent activity to the motoneurones of respiration. Thus, differences between the hypoxic ventilatory responses of the fetus and the adult probably reflect differences in the maturity of the brain stem with respect to the processing of afferent input from the peripheral chemoreceptors rather than differences in the underlying physiology. The response of the newborn to hypoxia falls somewhere between that of the fetus and the adult: at a given hypoxic stimulus, ventilation first rises as the carotid body excitation drives the respiratory motoneurones but then ventilation falls to or below air breathing levels. The fall has recently been shown to be prevented by transection of the brain at the level of the rostral pons (Martin-Body & Johnston, 1988).

Finally, some consideration should be given to specific anatomical structures which may mediate the hypoxic ventilatory depression of chemodenervated rats. The proximity of the transections to the nucleus parabrachialis medialis and the Kolliker–Fuse nucleus both of which contain a high density of respiratory-related neurones, may suggest a role for these nuclei. Indeed, it is presumed that the elevations of normoxic V_T in the three rats with very caudal transections resulted from damage to these structures, because previous studies have shown that there is a significant elevation of V_T and fall in f after 'pneumotaxic centre' ablation (St John, 1972). This region is also known to be involved in the integration of peripheral and central chemoreceptor stimuli. After bilateral punctate lesions of the pneumotaxic centre $\dot{V}_E$ responses to hypoxia were dependent upon the prevailing P_{A,CO_2} (St John, 1977). In particular, at P_{A,CO_2} 40 mmHg V_T in hypoxia approximated values found under the same conditions in decerebrate controls, whereas at P_{A,CO_2} 20–30 mmHg hypoxic V_T was always lower than in controls. Respiratory frequency in hypoxia was not sensitive to P_{A,CO_2}. Such responses do not parallel those reported here but the comparison may not be legitimate because in the animals of this study P_{A,CO_2} was determined by the level of spontaneous ventilation. The importance of the adjacent reticular formation in the mediation of hypoxic ventilatory depression should also be considered since electrical stimulation in the mesencephalon and pons but outside the classical respiratory centres can produce a variety of effects on ventilation (Kabat, 1936; Evans & Pepler, 1974; Coles, 1987). Studies are required to localize more precisely the region mediating hypoxic ventilatory depression.

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