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TWO LONG-LASTING CENTRAL RESPIRATORY RESPONSES FOLLOWING ACUTE HYPOXIA IN GLOMECTOMIZED CATS

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SUMMARY

1. Central respiratory response to acute (10 min) hypoxia, as measured by phrenic nerve activity, was determined in peripheral chemo-denervated cats.

2. Hypoxia was induced by ventilating cats for 10 min at reduced inspired oxygen levels (inspired O_2 fraction, $F_{I,O_2} = 0{\cdot}06$–$0{\cdot}15$). The degree of hypoxaemia was determined from an arterial blood sample and ranged from 'severe' (arterial O_2 pressure, $P_{a,O_2} < 26$ Torr) to 'mild' ($P_{a,O_2} > 35$ Torr). The respiratory response was monitored for 1 h following return to ventilation with 100% oxygen.

3. The results confirmed the finding of prolonged (> 60 min) inhibition of respiration upon return to hyperoxic conditions following severe hypoxia, as reported previously (Millhorn, Eldridge, Kiley & Waldrop, 1984). A new finding was a long-lasting (> 60 min) facilitation of respiration following exposure to less severe ($P_{a,O_2} > 35$ Torr) hypoxia.

4. Medullary extracellular fluid pH was measured in six cats. Changes in pH could not explain either the prolonged inhibition following severe hypoxia or the long-lasting facilitation observed following mild hypoxia.

5. Ablation studies were performed in order to determine the locations of the neuronal substrates for the inhibitory and facilitatory mechanisms. The results of this series of experiments indicate that the mesencephalon is necessary for activation of the inhibitory mechanism, while the facilitatory mechanism requires the presence of higher brain structures, notably the diencephalon.

6. Following removal of the diencephalon, the inhibitory response was seen following even mild hypoxic insults, i.e. those shown to produce facilitation in animals with intact brains. In the absence of the mesencephalon, neither prolonged inhibition nor prolonged facilitation could be produced following hypoxia.

7. It is proposed that there are two centrally mediated long-lasting responses to acute hypoxia. Facilitation is seen following mild hypoxia. Inhibition is more likely following severe hypoxia. However, both mechanisms appear to be triggered simultaneously and the output of the central respiratory controller reflects the influence of each.

INTRODUCTION

It is well known that hypoxia causes depression of respiration in glomectomized animals (Watt, Dumke & Comroe, 1942). The mechanism responsible for the depression is not well understood, but is believed to be due to a combination of

effects, including a direct depressive effect on central neurones by hypoxia (Cherniack, Edelman & Lahiri, 1970/71) and brain alkalosis resulting from an increased brain blood flow (Lee & Milhorn, 1975). Another possible mechanism involves the release of an inhibitory neurochemical. Support for this possibility comes from recent findings from our laboratory (Millhorn *et al.* 1984) which showed that brief exposure to severe hypoxia ($P_{O_2} < 30$ Torr), in addition to causing respiratory depression during the hypoxic episode, activated a central neural mechanism that caused sustained inhibition of phrenic nerve activity in glomectomized cats for more than an hour following cessation of hypoxia. The magnitude of hypoxic depression was reduced and the prolonged inhibitory effect was prevented in animals pretreated with theophylline, a specific antagonist of the purine nucleoside, adenosine (Daly, Burns & Snyder, 1981; Snyder, Katims, Annau, Burns & Daly, 1981). It was concluded that the long-lasting inhibition following hypoxia was mediated by adenosine and that adenosine was at least partially responsible for the acute depression of respiration during hypoxia.

The present work was undertaken to study further the mechanism that causes prolonged inhibition of respiration following exposure to hypoxia. We were especially interested in determining the degree of hypoxia required to evoke the long-lasting inhibitory response. In order to observe better the direct central effect of hypoxia upon respiration, the peripheral chemoreceptors were denervated by cutting the carotid sinus and vagus nerves. Our findings indicate that the long-lasting inhibitory response is more likely following hypoxia with P_{a,O_2} (arterial O_2 pressure) below 25 Torr, which we, for the present report, have termed 'severe'. A new finding was that following less severe hypoxia (P_{a,O_2} above 35 Torr), a persistent facilitation rather than inhibition of phrenic nerve activity was the predominant response.

A second series of experiments was performed, using gross ablation techniques, to determine the regions of the brain responsible for mediating these responses. The results from these experiments show that the mechanism responsible for the long-lasting inhibitory response is located in the mesencephalon, and that the diencephalon is the likely anatomical substrate for the facilitatory response.

METHODS

Animal preparations. Thirty-six adult cats weighing between 2·1 and 5·0 kg were studied. Twenty-four cats were studied with intact brains. In addition, twelve cats were studied following decerebration. In six cats, extracellular fluid (ECF) pH was measured on the ventral medullary surface.

All cats were anaesthetized with ether. Animals to be studied initially with intact brains were then given chloralose (40 mg kg^{-1}) and urethane (250 mg kg^{-1}) via a cannula introduced into the femoral vein. Cats to be studied initially decerebrate were in some cases given chloralose and urethane and were in other cases kept under deep surgical ether anaesthesia until the brain transection was complete. The combination of chloralose and urethane anaesthesia has been previously shown to be long-lasting (Eldridge, 1973) and therefore suitable for experiments such as these.

In each animal, the trachea was cannulated, airway P_{CO_2} continuously sampled, and CO_2 analysis performed by an infra-red analyser (Beckman LB-2). P_{CO_2} was controlled through the use of an electronic circuit which adjusted ventilator frequency as necessary to maintain P_{CO_2} within 0·5 Torr of the desired level (Smith, Mercer & Eldridge, 1978). A femoral artery was cannulated and arterial pressure monitored continuously via a strain gauge. Temperature was monitored by a rectal thermistor and controlled at 37·5 °C by an electronic circuit and a DC heating pad.

Each animal was placed supine on a table equipped with a rigid head mount. The carotid sinus nerves and vagi were identified visually and cut. Each animal was paralysed with gallamine triethiodide (3 mg kg^{-1} I.V.), followed by a continuous infusion at a rate of 3 mg kg^{-1} h^{-1}. A phrenic nerve root (C5) was exposed, desheathed, and placed on platinum recording electrodes. The electrodes were covered with a mixture of mineral oil and petroleum jelly. The electrodes used were built into a Teflon holder small enough to rest in a tissue well adjacent to the nerve. The only external attachments were flexible electrical wires. Consequently, it was possible to maintain a constant electrical coupling between the nerve and the electrodes for an extended period of time. With end-tidal P_{CO_2} and rectal temperature controlled as described, this preparation is sufficiently stable that there is no trend towards an increase or decrease in minute phrenic activity over an extended period of time (> 6 h, unpublished observations).

Measurement of ventral medullary extracellular fluid (ECF) pH. Six cats were prepared for pH measurement. With the animal in the supine position, the larynx and oesophagus were ligated, cut, and retracted rostrally to expose the muscle covering the occipital bone. This muscle was removed and the bone between the tympanic bullae was chipped away to reveal the ventral surface of the medulla oblongata at the level of and rostral to the rootlets of the twelfth cranial nerve. Bleeding was controlled with bone wax and Gelfoam. The dura was cut and retracted to allow access to the ventral surface of the medulla. pH measurement was made with a flat-surfaced electrode with a tip 2 mm across (Microelectrodes, Inc., Model MI-404, time constant = 1·5 s) and a microreference electrode (Model MI-401). Amplification was done with a custom-built isolation amplifier. The electrode was calibrated *in vitro* with standard phosphate buffers (pH 7·000 and 7·382). The pH and reference electrodes were positioned with a spring tension holder on the exposed ventral surface in an area devoid of major blood vessels. To test pH electrode placement and function, the ventilator was stopped briefly. In each animal, there was an appropriate acid shift in ECF pH following a delay of 4–6 s, representing the circulation time from lung to medulla. It has been demonstrated that respiratory responses are closely associated with medullary ECF pH rather than medullary cerebrospinal fluid pH (Kiley, Eldridge & Millhorn, 1985).

Decerebration. Twelve cats were decerebrated; three under ether anaesthesia and nine after chloralose and urethane anaesthesia. Decerebration was performed by suctioning away all tissue rostral to the superior colliculi or by transection of the neuraxis with a blunt spatula. The exact location of each transection was verified following the experiment. Decerebrations were described as high (rostral to the superior colliculi dorsally and in the vicinity of the third cranial roots ventrally), or low (mid-collicular rostrally and through the rostral aspect of the pons ventrally). Seven cats received high decerebrations, three received low decerebrations, and two cats were studied following high and, later, low decerebration.

Experimental protocols. Animals were ventilated with 100% oxygen. End-tidal P_{CO_2} was adjusted prior to each experimental period so that minute phrenic activity was between 30 and 40 normalized units. This normalization process, described more fully below, ensured that each experimental period of each cat studied began with equivalent central respiratory drive, as indicated by phrenic activity.

After all variables (phrenic activity, end-tidal P_{CO_2}, mean arterial pressure, rectal temperature, ECF pH) were stable, 3–5 min of control data were recorded. In some cases, an arterial blood sample was drawn at the end of the control period and P_{a,O_2} determined to ensure sufficient oxygenation of the blood. Each animal was then ventilated for 10 min with one of a number of hypoxic gas mixtures (inspired O_2 fraction, F_{I,O_2}, between 0·06 and 0·15, balance nitrogen). Data were recorded for the last 3 min of the hypoxic episode. At the end of 10 min of hypoxia, an arterial blood sample was taken and the extent of hypoxaemia determined. The animal was then returned to ventilation with 100% oxygen. Data were collected for 1 min, at 5 min intervals, for 1 h following the return to ventilation with 100% oxygen. In some cases, blood samples were drawn at 30 and at 60 min to ensure that blood oxygenation was similar to control levels. Blood samples were analysed on a Corning Model 165 or on a Radiometer (Copenhagen) blood gas analyser.

The complete protocol was performed one to four times in each animal. In some instances, the same F_{I,O_2} was used for each experimental run. In other cases, F_{I,O_2} was changed. In other animals, the F_{I,O_2} was not changed, but the animal underwent decerebration between runs.

Data analysis. Phrenic activity was amplified and integrated over 100 ms intervals by a sample-and-hold integrator (Gould). Integrated phrenic nerve activity, airway P_{CO_2}, arterial blood pressure, and the ECF pH were all recorded on a strip chart recorder (Gould). In addition, all variables were analysed on-line by a computer (DEC PDP-11/23) which produced immediate

reports including a breath-by-breath analysis of phrenic nerve activity with the corresponding end-tidal P_{CO_2}, mean arterial pressure, and pH for each breath. Averages for each variable were available for the period of data collection. It was thus possible to determine, quantitatively, the stability of the animal before proceeding with an experimental intervention. In order that comparison could be made between the phrenic responses of different animals, the integrated phrenic nerve activity was normalized. Briefly, end-tidal P_{CO_2} was lowered until phrenic activity was at the apnoic threshold. P_{CO_2} was then raised 20 Torr and the phrenic response measured. The peak integrated value of phrenic activity at this P_{CO_2} was assigned a value of 70 units on a scale with threshold activity equal to 0 units. The peak integrated phrenic activity has been shown to be the neural equivalent of tidal volume (Eldridge, 1975). The product of this normalized phrenic activity and the respiratory frequency, termed the normalized minute phrenic activity, was used as an indication of central respiratory drive which could be compared in different cats. P_{CO_2} was adjusted before each run so that the minute phrenic activity was between 30 and 40 normalized units. This normalization procedure is based upon the method of Eldridge, Gill-Kumar & Millhorn (1981).

RESULTS

Response of cats with intact brains to graded hypoxia

Figure 1 shows the effect on phrenic activity of severe hypoxia ($P_{a,O_2} = 25$ Torr) in one cat. End-tidal P_{CO_2} was kept constant at 26 Torr. During the control period the animal was ventilated with 100% O_2. Ventilation for 10 min with the hypoxic gas mixture led to apnoea. In this example, arterial pressure was unaffected by the hypoxia. The most important finding was that phrenic activity remained depressed for more than an hour after return to ventilation with 100% O_2. That the inhibition was not due to a general loss of responsiveness is evidenced by the brisk phrenic response to a small increase (2 Torr) in end-tidal P_{CO_2} accomplished immediately following the 60 min recovery period (panel at far right). The prolonged inhibitory response following hypoxia response is identical to that reported earlier by Millhorn *et al.* (1984).

The response of one cat to moderate hypoxia ($P_{a,O_2} = 38$ Torr) is illustrated in Figure 2. End-tidal P_{CO_2} was servo-controlled at 31 Torr. Again, phrenic nerve activity was depressed to the point of apnoea during exposure to hypoxia. However, within 15 min following the return to ventilation with 100% O_2, phrenic activity had increased above the control level. Moreover, it continued to increase for the remainder of the 60 min recovery period. The sustained facilitation of phrenic activity was a new and totally unexpected finding.

The averaged findings from glomectomized cats exposed either to severe ($P_{a,O_2} < 26$ Torr; $n = 4$), moderate ($P_{a,O_2} = 26$–35 Torr; $n = 15$) or mild ($P_{a,O_2} = 36$–65 Torr; $n = 13$) hypoxia are given in Fig. 3. The respiratory responses following return to ventilation with 100% O_2 were markedly different among the three groups. As expected, severe hypoxia caused long-lasting depression of phrenic minute activity. Phrenic activity showed a small degree of inhibition initially after moderate hypoxia, but had returned to the control level by 45 min. By far the most important finding was that after exposure to mild hypoxia, phrenic minute activity was enhanced above control level at 15 min and continued to increase throughout the remainder of the 60 min recovery period. In other words, exposure to mild hypoxia caused a long-lasting facilitation of respiration.

We were concerned that the persistent respiratory responses following hypoxia

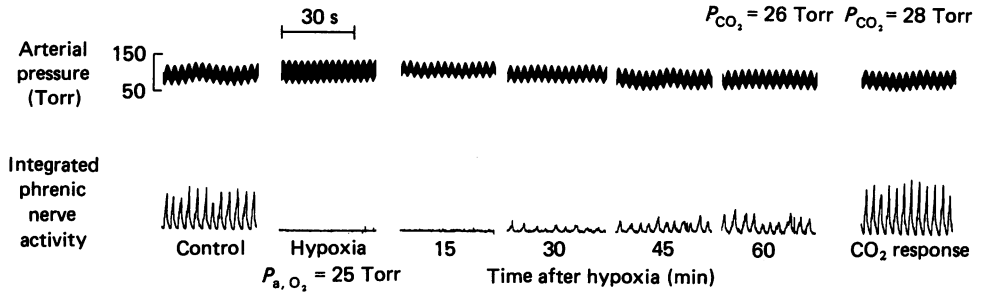

Fig. 1. Effect on phrenic activity of 10 min of severe hypoxia ($P_{a,O}$ = 25 Torr) in one peripheral chemo-denervated cat. Hypoxia caused depression of phrenic activity to the point of apnoea. Following return to hyperoxia, phrenic activity remained inhibited for 60 min. The brisk response to a 2 Torr increase in end-tidal P_{CO_2} immediately following 60 min of recovery indicates that respiratory-related networks are still functional and that the cat is still CO_2-responsive.

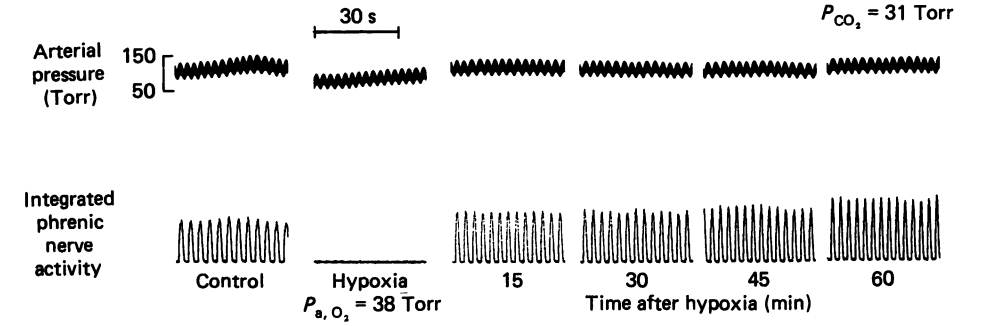

Fig. 2. Effect on phrenic activity of 10 min of mild hypoxia (P_{a,O_2} = 38 Torr) in one peripheral chemo-denervated cat. Hypoxia caused depression of phrenic activity to the point of apnoea. By 15 min following return to hyperoxia, phrenic activity was greater than during control and it continued to increase for the remainder of the 60 min recovery period.

could have been the result of a change in the acid–base status of the brain. In order to test this possibility, we measured the pH of medullary ECF before, during, and after hypoxia in six glomectomized cats. The findings from one cat in which three different levels of hypoxia were tested are shown in Fig. 4. In *A*, mild hypoxia (P_{a,O_2} = 65 Torr) caused depression of phrenic activity to the point of apnoea and a slight alkaline shift in ECF pH that was most likely due to an increased blood flow as a result of vasodilatation of brain blood vessels (Kontos, 1981). Following hypoxia, phrenic activity showed a substantial facilitation that lasted for more than an hour. That the prolonged facilitation of respiration was not due to a persistent acidosis is evidenced by the finding that ECF pH had returned to the pre-hypoxia control level at 5 min after return to ventilation with 100% O_2 and remained at this level during the entire recovery period. In *B*, moderate hypoxia (P_{a,O_2} = 27 Torr) caused depression of phrenic activity and a small acid shift in ECF pH. After cessation of hypoxia, ECF pH increased to a new stable level that was alkaline relative to control pH. Phrenic nerve activity, on the other hand, returned to control level by 35 min

and remained at this level during the remainder of the experiment. Again, no cause-and-effect relationship between ECF pH and phrenic activity was found. Finally, the animal was exposed to a more severe hypoxia (P_{a,O_2} = 20 Torr) which caused an acid shift in ECF pH and a respiratory depression (*C*). Immediately upon return to ventilation with 100% O_2, phrenic activity increased due to the considerable

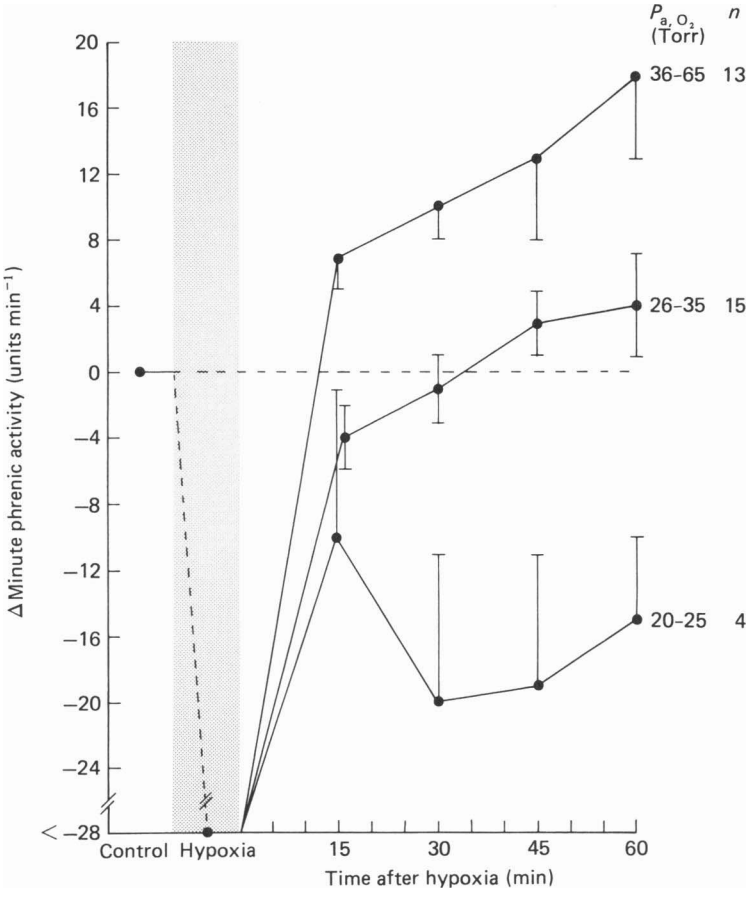

Fig. 3. Averaged results (mean ± S.E.M.) for effect on phrenic activity of 10 min of hypoxia in peripheral chemo-denervated cats. Severe hypoxia (P_{a,O_2} = 20–25 Torr, n = 4) resulted in respiratory inhibition lasting at least 60 min. Moderate hypoxia (P_{a,O_2} = 26–35 Torr, n = 15) was followed by a return to approximately the control level. Mild hypoxia (P_{a,O_2} = 36–65 Torr, n = 13) resulted in respiratory facilitation lasting at least 60 min.

transient acid shift. As ECF pH returned towards control level, the respiratory inhibition was revealed. By 25 min of recovery, the cat was apnoeic and remained thus for a further 25 min. Sixty minutes after the return to 100% O_2, phrenic activity was still considerably depressed despite the fact that ECF pH remained slightly more acid than the control level. These findings show that, although phrenic activity is influenced by ECF pH, neither the long-lasting facilitation nor prolonged inhibition of phrenic activity following hypoxia are due to a change in the acid–base status of the brain.

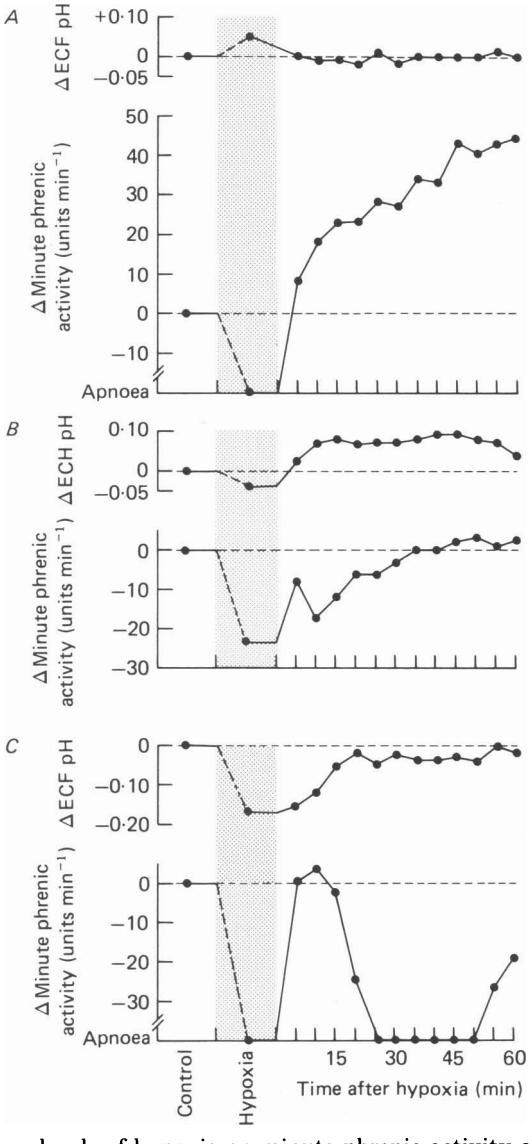

Fig. 4. Effect of three levels of hypoxia on minute phrenic activity and medullary ECF pH in one peripheral chemo-denervated cat. *A*, mild hypoxia (P_{a,O_2} = 65 Torr) resulted in respiratory facilitation while pH returned to the control level. *B*, moderate hypoxia (P_{a,O_2} = 27 Torr) was followed by a slight alkalosis while phrenic activity returned to the control level. *C*, severe hypoxia (P_{a,O_2} = 20 Torr) led to long-lasting respiratory inhibition to the point of apnoea despite a small acid shift in ECF pH.

As can be determined from Figure 3, there was a range of responses among cats rather than a common cut-off between inhibition and facilitation. Some animals showed inhibition with P_{a,O_2} values as high as 34 Torr, while others responded with facilitation to P_{a,O_2} values as low as 24 Torr. However, each of six animals which was tested using a range of P_{a,O_2} levels showed the same pattern of response: less severe

hypoxia led to facilitation while more severe hypoxia led to inhibition. Because we could not predict whether a given animal would respond with inhibition or with facilitation to a P_{a,O_2} near 30 Torr, it was necessary that each animal serve as its own control for the ablation experiments which constitute the second half of this study. It was therefore important to show that the responses in question were reproducible

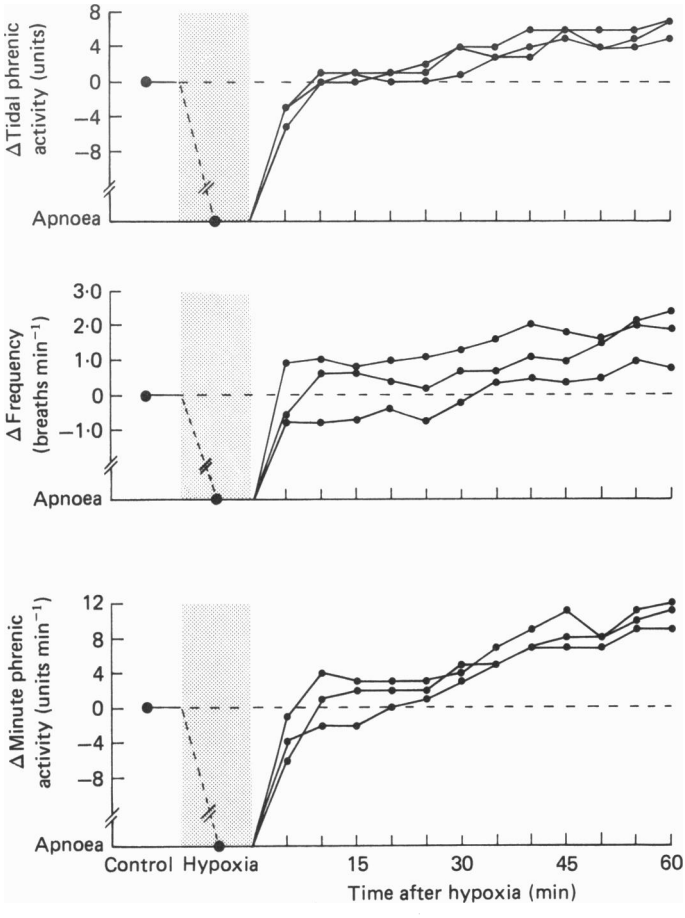

Fig. 5. Response of one peripheral chemo-denervated cat to three consecutive hypoxia experiments. In each, P_{a,O_2} was 40 Torr during the 10 min hypoxia. During each episode of hypoxia, phrenic activity was reduced to apnoea. Following each hypoxia, phrenic activity reached greater than control levels, with a similar degree of facilitation apparent during each 60 min recovery period.

and that the mechanisms involved did not saturate following a single exposure to hypoxia. Figure 5 shows the results from three experimental runs performed in one cat, all at the same P_{a,O_2}. Each exposure to hypoxia resulted in apnoea and subsequent sustained facilitation. Similar reproducibility was noted in all animals ($n = 10$) studied this way. This was an important finding because it provided the basis for experiments in which post-hypoxia respiratory responses were studied in individual cats before and after surgical transection of the neuraxis.

Response of cats decerebrated at different levels to hypoxia

These experiments were undertaken in an attempt to determine the brain regions responsible for mediating the long-lasting inhibitory and facilitatory responses following hypoxia. Figure 6 shows the results from a chloralose–urethane-anaesthetized cat exposed to the same degree of hypoxia (P_{a,O_2} = 40 Torr) when the

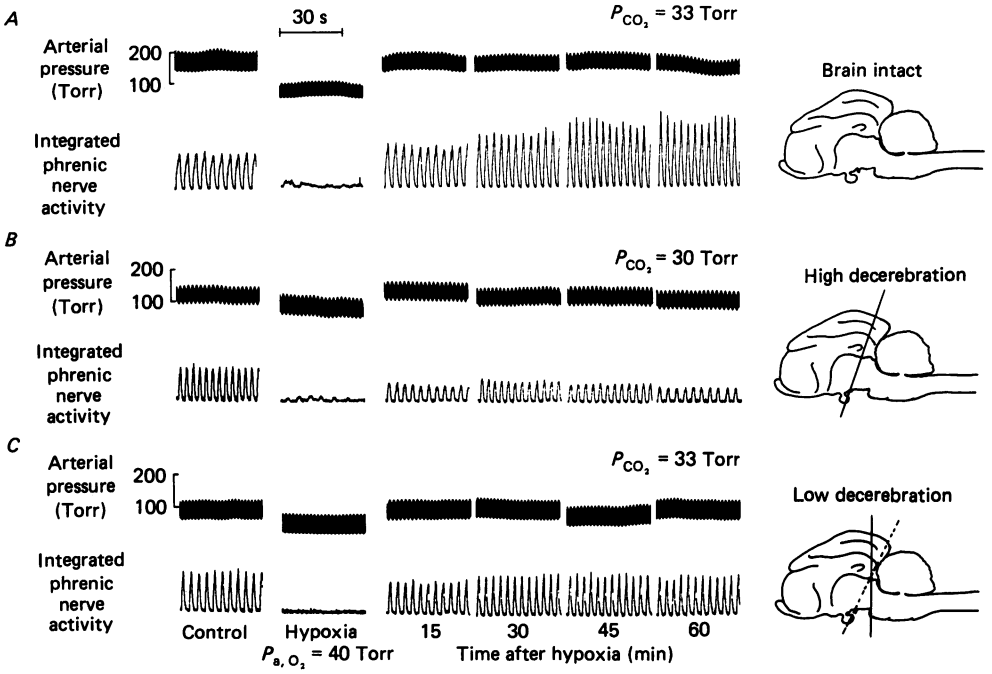

Fig. 6. Response of one peripheral chemo-denervated cat to mild hypoxia before and after ablations. *A*, with brain intact, a P_{a,O_2} of 40 Torr resulted in marked facilitation of phrenic activity. *B*, the response of the same cat to the same degree of hypoxia after a high decerebration. In this case, the response was long-lasting inhibition of phrenic activity. *C*, the response of the same cat to the same degree of hypoxia following a second transection, this time a low decerebration. The response during hypoxia was similar to that in *A* and *B*. Following hypoxia, however, neither facilitation nor inhibition was seen. Instead the phrenic activity returned to the control level.

brain was intact (*A*), following high decerebration (*B*) and after low decerebration (*C*). In all three cases the hypoxia caused hypotension and approximately the same magnitude of respiratory depression, i.e. apnoea or near apnoea, during the last 3 min of exposure. In contrast, the responses following return to ventilation with 100% O_2 were markedly different. When the animal was intact (*A*), the respiratory response following hypoxia was a persistent facilitation, so that at 60 min the phrenic activity was considerably elevated above the original control level. A high decerebration was then performed and after all variables had become stable, the experiment was repeated. The exact location of the transection of the neuraxis is shown by the line in the diagram in *B*. The response following hypoxia was a sustained inhibition rather than the facilitation measured when the brain was intact.

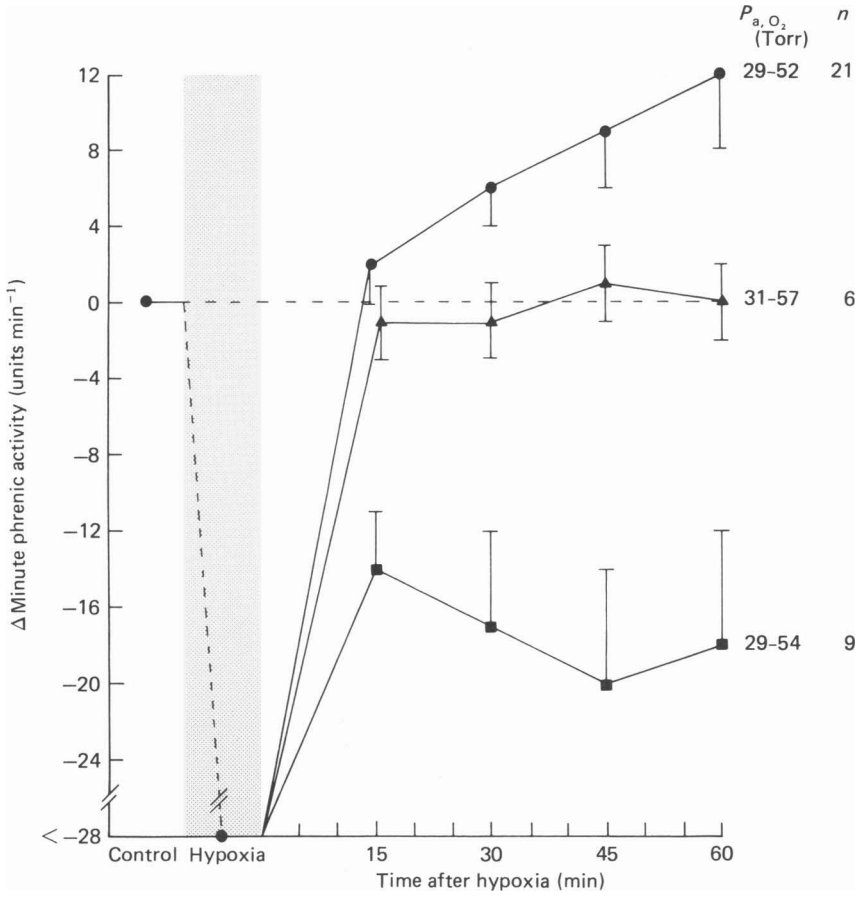

Fig. 7. Averaged results (mean ± S.E.M.) for effect on phrenic activity of 10 min of moderate to mild hypoxia in peripheral chemo-denervated cats before and after high and low decerebrations. In animals with intact brains (●), hypoxia was followed by facilitation of phrenic activity. Following high decerebration (■), moderate to mild hypoxia resulted in long-lasting inhibition of phrenic activity. Following low decerebration (▲), moderate to mild hypoxia did not result in any long-lasting change in phrenic activity. Rather, phrenic activity returned to control and remained there for the 60 min recovery period.

When the low decerebration was performed, mid-collicularly dorsally and extending ventrally through the rostral-most part of the pons (*C*), the post-hypoxia phrenic activity returned to the original control level, i.e. failed to show either long-lasting facilitation or inhibition. Evidence that the low decerebration did not affect the pontine respiratory group (i.e. nucleus parabrachialis medialis) is the normal respiratory rhythm observed during the control period in *C*.

Figure 6 also gives evidence for continued CO_2 responsiveness. Following the facilitation shown in *A*, P_{CO_2} was lowered from 33 to 30 Torr in order to lower minute phrenic activity. Conversely, following the inhibition shown in *B*, P_{CO_2} was increased in order to raise minute phrenic activity. Thus, similar levels of phrenic activity were established prior to each hypoxic episode.

The averaged responses to hypoxia measured in cats with intact brains and after decerebration are shown in Fig. 7. Included in the intact group are all runs from Fig. 3 with P_{a,O_2} values in the same range as the decerebrate runs. In animals with intact brains (●), phrenic activity had increased to a level well above the control level by 30 min after hypoxia, and continued to increase during the remainder of the 60 min recovery period. After high decerebration (■), the same range of hypoxia caused prolonged inhibition of phrenic activity. Following mid-collicular (low) decerebration (▲), phrenic nerve activity returned to the original control level by 15 min after hypoxia and remained at this level for the remainder of the 60 min recovery period. These findings suggest that the facilitatory response is mediated by regions rostral to the brain stem and that the inhibitory response emanates from a mechanism located within the mesencephalon.

DISCUSSION

This study was initially undertaken to characterize further the long-lasting inhibition of respiration following severe hypoxia in glomectomized animals described previously by Millhorn *et al.* (1984). In this regard, we found that animals with intact brains showed the long-lasting inhibition of respiration after exposure to severe hypoxia ($P_{a,O_2} < 30$ Torr). To our surprise, less severe hypoxia ($P_{aO_2} > 35$ Torr) often activated a central neural mechanism that caused facilitation of respiration that persisted for more than 1 h after return to hyperoxic conditions. To our knowledge, this is the first evidence for a central nervous system mechanism that is activated directly by hypoxia and causes a persistent facilitatory effect on respiration.

Ablation experiments were performed for the purpose of determining the regions of the brain responsible for mediating both the long-lasting inhibitory and facilitatory responses. Transection of the neuraxis just rostral to the mesencephalon (high decerebration) prevented the long-lasting facilitation of respiration following moderate to mild hypoxia. This finding provides evidence that the mechanism responsible for the long-lasting facilitation is located rostral to the mesencephalon. In addition, we discovered that following high decerebration, even mild levels of hypoxia ($P_{a,O_2} > 35$ Torr) caused long-lasting inhibition of respiration. The inhibitory response was prevented by mid-collicular transection of the neuraxis (low decerebration). We conclude therefore that the mechanism responsible for the prolonged inhibition of respiration following hypoxia is located in the mesencephalon.

We have considered various factors that might affect interpretation of our results. Since our animals were paralysed and ventilated artificially with a respirator servo-controlled by end-tidal P_{CO_2}, changes in P_{CO_2} can be ruled out as causal. We measured medullary ECF pH in six cats and found that ECF pH either returned to control or changed following hypoxia in a direction that could not possibly explain the respiratory response. That the long-lasting inhibition following severe hypoxia was not due simply to decreased responsiveness of the respiratory control system was evidenced by the brisk respiratory response to a small increase in end-tidal P_{CO_2}.

The most significant new finding of the present study was that hypoxia activated a higher brain mechanism which, in turn, caused long-lasting facilitation of

respiration. It has been generally accepted that hypoxia has a direct inhibitory effect on central respiratory control networks and can only stimulate respiration via the carotid and aortic body chemoreceptors, although this tenet has been challenged. Martin-Body, Robson & Sinclair (1986) reported some residual hypoxic response in awake rats with complete peripheral chemo-denervation. They suggested that this response was due to unknown peripheral chemoreceptors or to an as yet unidentified central mechanism, and further commented that a central response of this type would be masked by the depressive effect of anaesthesia upon respiration. Miller & Tenney (1975) found that in awake, peripheral chemo-denervated animals hypoxia causes rapid breathing (tachypnea) rather than depression of breathing. They hypothesized that the diencephalon was the site of the tachypneic response and attributed the depression of respiration most often seen during hypoxia to anaesthesia. In a later study, Tenney & Ou (1977) published findings which supported this hypothesis. They discovered that hypoxia causes tachypnea in unanaesthetized decorticate cats, but mid-collicular decerebration abolishes the tachypneic response. Their results are similar to our present finding that post-hypoxia facilitation is observed in intact, but not in decerebrate, cats. In addition, preliminary observations from this laboratory indicate that post-hypoxic facilitation can be produced in unanaesthetized decorticate cats. We therefore suggest that the diencephalon may be the forebrain region of greatest importance in the facilitatory mechanism.

Although the reports discussed above appear to compare favourably with the present study, extreme care must be taken in drawing conclusions based on these comparisons since the experimental designs of the studies were different. In particular, Tenney and co-workers (Miller & Tenney, 1975; Tenney & Ou, 1977) and Martin-Body *et al.* (1986) measured the respiratory response during hypoxia, whereas we were concerned primarily with post-hypoxia effects. It should also be mentioned that in the present study we observed substantial depression of phrenic activity during hypoxia in unanaesthetized decerebrate cats. This finding does not support the conclusion of Miller & Tenney (1975) that anaesthesia is the cause of the depression of breathing during hypoxia.

There have been other studies not related to respiration that indicate a direct excitatory effect of hypoxia on central nervous structures. For example, Schiff & Somjen (1985) found that hippocampal cells were hyperexcitable for up to 45 min after a brief exposure to hypoxia. There have also been reports of neuronal hyperexcitability following post-hypoxic epilepsy (Madison & Niedermeyer, 1970) and monoclonus (Lance & Adams, 1963). An important aspect of these studies was that the hyperexcitability persisted for a relatively long time following the hypoxic insult. Although these studies suggest the possibility of hypoxic excitation as the mechanism for the respiratory facilitation we report, it is also possible that the mechanism involves disinhibition, i.e. inhibition of a tonic inhibitory input to the central respiratory network.

The new finding concerning the long-lasting inhibition following severe hypoxia is that the mechanism responsible for mediating the effect appears to be located in the rostral part of the mesencephalon. This is based on our finding that the prolonged inhibitory effect is present following transection of the neuraxis just rostral to the

superior colliculi, but not after mid-collicular decerebration. In a previous report (Millhorn *et al.* 1984), we presented findings which show that the inhibitory effect was prevented in animals pre-treated with theophylline, an antagonist of adenosine (Daly *et al.* 1981; Snyder *et al.* 1981). There is evidence that adenosine concentration in the brain increases during hypoxia (Rubio, Berne, Bockman & Curnish, 1975; Winn, Rubio & Berne, 1981; Zetterstrom, Vernet, Ungerstedt, Tossman, Jonzon & Fredholm, 1982) and that adenosine causes inhibition of neuronal activity (Kostopoulos, Limacher & Phillis, 1975; Kostopoulos & Phillis, 1977; Phillis, Edstrom, Kostopoulos & Kirkpatrick, 1979). Thus, there is reason to believe that the long-lasting inhibition is mediated by a mesencephalic mechanism via the release of adenosine onto respiratory neurones. It is therefore interesting that the mesencephalon has recently been shown immunohistochemically to contain neurones with a high concentration of adenosine (Brass, Newby, Wilson & Snyder, 1986). We have no information concerning neurotransmitters or modulators that might be involved in mediating the long-lasting facilitatory response.

Two pieces of evidence suggest that in the intact animal the long-lasting facilitatory and inhibitory mechanisms are activated simultaneously by hypoxia. First, levels of hypoxia that caused long-lasting facilitation of respiration when the brain was intact caused a persistent inhibition following transection of the neuraxis just above the superior colliculi. This finding suggests that both mechanisms are activated by moderate hypoxia, but that the facilitatory mechanism is predominant. Second, when animals are pre-treated with theophylline prior to exposure to severe hypoxia, long-lasting facilitation rather than inhibition is measured (Millhorn *et al.* 1984). We can now interpret this to mean that theophylline prevents manifestation of the inhibitory effect, and by doing so, unmasks the facilitatory effect which is present even at severe levels of hypoxia.

No attempt was made to determine exactly how long either the inhibitory or facilitatory effects last following cessation of hypoxia. Suffice it to say that once activated, the response (inhibitory or facilitatory) was still very much evident at the end of the 60 min post-hypoxia measurement period. It is entirely possible therefore that the time course for both mechanisms is several hours following activation by brief (10 min) exposure to hypoxia. We also wish to emphasize that in the intact animal the exact P_{a,O_2} at which either the long-lasting inhibitory or facilitatory response becomes manifest varies from animal to animal.

The physiological significance of the facilitatory and inhibitory long-lasting mechanisms was not elucidated by the present study. However, in animals with intact peripheral chemoreceptors, the mechanisms described in this study might play an important role in the overall response to hypoxia and offer explanations to several unexplained phenomena. For example, the long-lasting facilitatory mechanism adds a secondary stimulus to that elicited by input from the peripheral chemoreceptors during moderate hypoxia. Such a mechanism might explain the secondary respiratory stimulus associated with acclimatization to hypoxia (Dempsey, Forster & DoPico, 1974). Furthermore, the long-lasting nature of the facilitatory mechanism offers a viable explanation for the continued hyperventilation after return to normoxic conditions (Dempsey *et al.* 1974). Millhorn, Eldridge & Waldrop (1980*a*, *b*) reported recently the existence of a brain-stem serotonergic mechanism that is

activated by afferent input from the carotid bodies and not by hypoxia in glomectomized animals. Once activated this mechanism also stimulates respiration for a long time (hours). It is therefore possible that these two long-lasting facilitatory mechanisms interact to provide the additional respiratory stimulus required for acclimatization to hypoxia.

The long-lasting inhibitory mechanism may explain other phenomena associated with the respiratory response to hypoxia. If predominant, this mechanism would subtract from the respiratory response elicited by carotid body stimulation. Therefore, it might explain the decrease in ventilation that sometimes occurs after the initial increase evoked by carotid body stimulation (Watt *et al.* 1942; Weil & Zwillich, 1976; Kagawa, Stafford, Waggener & Severinghaus, 1982). The inhibitory mechanism might also provide an explanation for the so-called biphasic respiratory response in neonates (Cross & Oppe, 1952), i.e. an initial carotid-body-mediated stimulation followed by a profound inhibition of respiration.

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