Red nucleus lesions abolish the biphasic respiratory response to isocapnic hypoxia in decerebrate young rabbits

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1. The respiratory response to isocapnic hypoxia (inspired O_2 fraction (F_{I,O_2}), 0·1–0·12) was measured in twelve vagotomized, paralysed, artificially ventilated young rabbits (aged 26·6 ± 0·4 days), following pre-collicular decerebration. Phrenic nerve efferent activity was used as an index of central respiratory output (RO). In hypoxia RO increased after 1–2 min (phase 1) but decreased over the subsequent 3–4 min to, or below, the pre-hypoxic control level (phase 2).

2. We used electrical stimulation to target areas in the mesencephalon which inhibit RO. Profiles of the response to stimulation were determined in a grid of electrode penetrations made mediolaterally and rostrocaudally at the level of the superior colliculi, in normoxia. Histology confirmed that stimulation in the red nucleus (RN) inhibited RO profoundly.

3. Electrolytic lesions were made bilaterally in RN inhibitory sites or in adjacent areas. The respiratory response to isocapnic hypoxia was measured again post-lesioning.

4. In six rabbits with bilateral lesions in the RN, phase 2 of the respiratory response was abolished and RO remained elevated throughout the hypoxic exposure. However, in six rabbits with *unilateral* lesions in the RN, or with bilateral lesions placed in areas *outside* the RN that did not inhibit RO on electrical stimulation, the respiratory response remained biphasic.

5. In both groups of animals, blood pressure increased during 1–3 min of hypoxia before decreasing to pre-hypoxic levels. This cardiovascular response remained biphasic irrespective of whether animals showed a biphasic respiratory response or a sustained increase in RO after lesioning.

6. We conclude that structures within the RN are crucial to the mechanism producing a fall in RO during isocapnic hypoxaemia in the neonate.

The mammalian neonatal respiratory response to hypoxia is biphasic. During the initial 1–2 min (phase 1) of the response, respiratory output (RO) increases but it falls to, or below, pre-hypoxic control levels over the next 5 min (phase 2). The biphasic respiratory response (BRR) has been widely reported and is shown by neonates of both altricial and precocial species (e.g. Woodrum, Standaert, Mayock & Guthrie, 1981; Blanco, Hanson, Johnson & Rigatto, 1984; Bureau, Lamarche, Foulon & Dalle, 1985; Eden & Hanson, 1987).

The mechanism of the increase in RO in phase 1 involves the peripheral chemoreceptors, as it is abolished by bilateral section of the carotid sinus nerves (Bureau *et al.* 1985). However, the processes underlying phase 2 of the response are not fully understood although various mechanisms have been proposed. Amongst these there is growing support from fetal, neonatal and adult studies for the hypothesis that a central nervous system (CNS) descending inhibitory mechanism, activated during hypoxia, underlies the fall in RO (see Moore & Hanson, 1994).

Fetal breathing movements (FBM) are abolished during isocapnic hypoxaemia (Boddy, Dawes, Fisher, Pinter & Robinson, 1974). The elimination of FBM appears to result from a CNS descending inhibitory mechanism, as in fetal sheep mid-collicular transection (Dawes, Gardner, Johnston & Walker, 1983) or bilateral electrolytic lesioning in the ventrolateral rostral pons (Gluckman & Johnston, 1987) abolishes the inhibition and allows peripheral chemoreceptor-

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mediated stimulation of FBM to occur during hypoxaemia (Johnston & Gluckman, 1992).

In the adult there is a fall in RO after prolonged hypoxic exposures (> 20 min) (Easton, Slykerman & Anthoninsen, 1986), although the magnitude of the fall is less than that in the neonate or fetus. However, following carotid sinus nerve section, RO falls rapidly during hypoxia in adult rats (Martin-Body, Robson & Sinclair, 1985). To investigate the mechanism of the fall in RO previous workers have administered hypoxia to the central nervous system in the absence of peripheral chemoreceptor stimulation. Ventilation with carbon monoxide (Lahiri, Mulligan, Nishino, Mokashi & Davies, 1981) or perfusion of the brain with hypoxaemic blood whilst the peripheral chemoreceptors are perfused with normoxaemic blood (van Beek, Berkenbosch, De Goede & Olievier, 1984) caused RO to fall in adult cats. Thus in adults RO is inhibited during hypoxia in the absence of peripheral chemoreceptor stimulation. In carotid sinus nerve denervated rats this inhibition is abolished by mid-collicular transection of the brainstem (Martin-Body, 1988), making it clear that the CNS rostral to this level is necessary for the decrease in RO during hypoxia in the adult.

In terms of its respiratory responses to hypoxia, the neonate lies between the fetus and the adult. Mid-collicular transection of the brainstem abolishes the fall in RO during hypoxia in newborn rabbits (Martin-Body & Johnston, 1988) and rats (Hanson & Williams, 1989). In contrast, following pre-collicular transection of the brainstem the response remains biphasic, indicating that sites in the rostral pons or mesencephalon (caudal to the hypothalamus) mediate the fall in RO during hypoxia (Hanson & Williams, 1989). Furthermore, focal cooling of a dorsal pontine region, including the locus coeruleus and adjacent reticular formation at the level of the middle cerebellar peduncle, abolishes the fall in RO reversibly in newborn sheep (Moore, Parkes, Noble & Hanson, 1991).

Putting the foregoing studies together, we felt that the role of mesencephalic structures in mediating the fall in RO in hypoxia merited further investigation. In adult cats and rabbits electrical stimulation in, or in the region of, the red nucleus (RN; Bassal & Bianchi, 1982; Gallman, Lawing & Millhorn, 1991) or in the rubrospinal tract (Schmid, Böhmer & Fallert, 1988) inhibits RO during normoxia. Our initial studies (Ackland, Waites, Noble & Hanson, 1995) showed clearly that electrical stimulation in the RN also inhibits RO in young rabbits. Preliminary results of the effects of electrolytic lesions in the RN also supported its role in mediating inhibition of RO during isocapnic hypoxia (Ackland, 1995; Ackland *et al.* 1995). In the present study, we tested the hypothesis that the RN plays a key role in the respiratory response of young rabbits to hypoxia by comparing their responses before and after placement of electrolytic lesions in the mesencephalon. We predicted that, if the RN was important in mediating the fall in RO in hypoxia, then this fall would be abolished by lesions placed in the RN bilaterally, but not by lesions placed in adjacent areas of the mesencephalon. We believe that this is the first definitive study indicating the role of the RN in the neonatal respiratory response to hypoxia.

METHODS

Twelve young (pre-weaning) New Zealand White rabbits of either sex, weighing 0·56 ± 0·02 kg (mean ± S.E.M.) were studied at a mean postnatal age of 26·6 ± 0·4 days (range 28–35 days).

Preparation of animals

Rabbits were sedated (ketamine hydrochloride; 25 mg kg^{-1} I.M.), anaesthetized (halothane; 2–2·5% in air), tracheotomized and artificially ventilated. Catheters were placed in the right femoral artery and vein. Femoral arterial blood pressure was recorded and instantaneous heart rate was derived from this signal. The vagi were sectioned bilaterally at a mid-cervical level. Deep surgical anaesthesia was maintained continuously, as determined by lack of the hindlimb withdrawal, palpebral and cardiovascular reflexes. End-tidal oxygen, carbon dioxide and halothane were analysed (Ohmeda RG5250) continuously.

Animals were placed in a stereotaxic frame so that the zygomatic toothbar line was inclined 51 deg from the base of the instrument. Parietal craniotomy was followed by pre-collicular decerebration. Bleeding was controlled using Oxycel (Becton Dickinson, Oxford, UK) and anaesthesia was discontinued after decerebration. The animals were paralysed with gallamine triethiodide (15 mg kg^{-1} initially and then as required). Arterial blood gas/acid–base status were measured (Instrumentation Laboratory, Warrington, UK; pH/blood gas analyser 1302 series) and controlled within the normal range. Any negative base excess was corrected by intravenous infusion of sodium bicarbonate. Rectal temperature was measured and maintained at 38·5–39·0 °C with a thermostatically controlled heat blanket (CPF 8185 homeothermic blanket control, Harvard Apparatus, UK).

The right phrenic nerve was identified by a dorsal approach, cut and placed upon bipolar electrodes under paraffin oil to prevent desiccation. Phrenic nerve activity, used as an index of central RO, was amplified, filtered (bandwidth, 5 kHz), integrated (EMG integrator; NL 703; time constant, 100 ms) and recorded on a chart recorder (Gould ES 1000).

Experimental protocol

Experiments started 1½–2 h after discontinuation of anaesthesia. Then the respiratory response to 8 min of isocapnic hypoxia (fraction of inspired O_2 (F_{I,O_2}), 0·10–0·12) was recorded. Arterial blood samples (0·1 ml) were taken during the air breathing control period after 4 min of hypoxia for analysis of blood gases/pH. After 1 h recovery a concentric bipolar electrode (RNE-100, Rhodes Metal) was used to stimulate (150–300 μA, 0·1 ms pulses, 100 Hz for 5–10 s; A320R-C, World Precision Instruments) at various depths, in 0·5 or 1·00 mm steps, in a series of electrode penetrations made in rows across the superior colliculus to locate sites in the region of the RN eliciting maximal inhibition of RO. Once these sites had been identified, lesions were made at five to ten points within a 1 mm^3 area circumscribing them by passing 0·5–1·0 mA currents for 20–30 s. Electrical stimulation and lesioning were carried out bilaterally at inhibitory sites. If no clear inhibitory site could be found on one or both sides, lesions were placed at the appropriate depth so that these animals could serve as controls.

After a period of *ca* 20 min the respiratory response to 8 min of isocapnic hypoxia (F_{I,O_2}, 0·10–0·12) was measured again.

At the end of each experiment the animal was killed by an overdose of sodium pentobarbitone I.V. and the brainstem was removed and fixed in 10% formalin. The brains remained in formalin for at least 48 h before being sectioned on a freezing microtome into 150 μm thick transverse sections, mounted onto microscope slides and stained with Cresyl Violet. The locations of electrical stimulation sites were then identified by histological reconstruction of the electrode tracks and the sites of lesions were determined.

Analysis of the respiratory response to hypoxia

Integrated phrenic nerve activity was analysed to determine inspiratory time (T_I), expiratory time (T_E) and peak phrenic activity (PPA). These values were used to calculate breath frequency ($1/(T_I + T_E)$(60 breaths min^{-1})) and PPA × breath frequency. Mean arterial blood pressure (MAP) was calculated as diastolic pressure + 1/3(systolic pressure − diastolic pressure). For the determination of all cardiovascular and respiratory variables, data collected during ten respiratory cycles were averaged after 2 min of control (pre-hypoxia) and after each minute of the hypoxic exposure. As we could not demonstrate that the data were normally distributed, they were expressed as medians and interquartile ranges (IQR, 25th–75th centiles). Statistical comparisons of control values (1 min before hypoxia) were made with pre-control values (2 min before hypoxia) and values after 2 min hypoxia and 8 min hypoxia. Both non-parametric (Wilcoxon's ranked sign test) and parametric (Student's paired t test) statistical comparisons were used, the latter being more stringent. Bonferroni corrections were applied when multiple comparisons were made. $P < 0·05$ was considered significant. A respiratory response was considered biphasic when RO increased significantly from control values during minutes 1–2 of the hypoxic exposure and then decreased to be not significantly different from control values by minute 8 of the hypoxic exposure. Arterial blood gases and pH (pH_a) are presented as mean values ± S.E.M.

RESULTS

The RO response to hypoxia in decerebrate neonatal rabbits

The respiratory response to 8 min isocapnic hypoxaemia was found to be biphasic in all twelve decerebrate rabbits (see Fig. 4*A* and *C*). There were no consistent differences in the response with age in the group. RO (PPA × frequency (f)) increased to a peak after 2 min of hypoxia ($P < 0·03$)

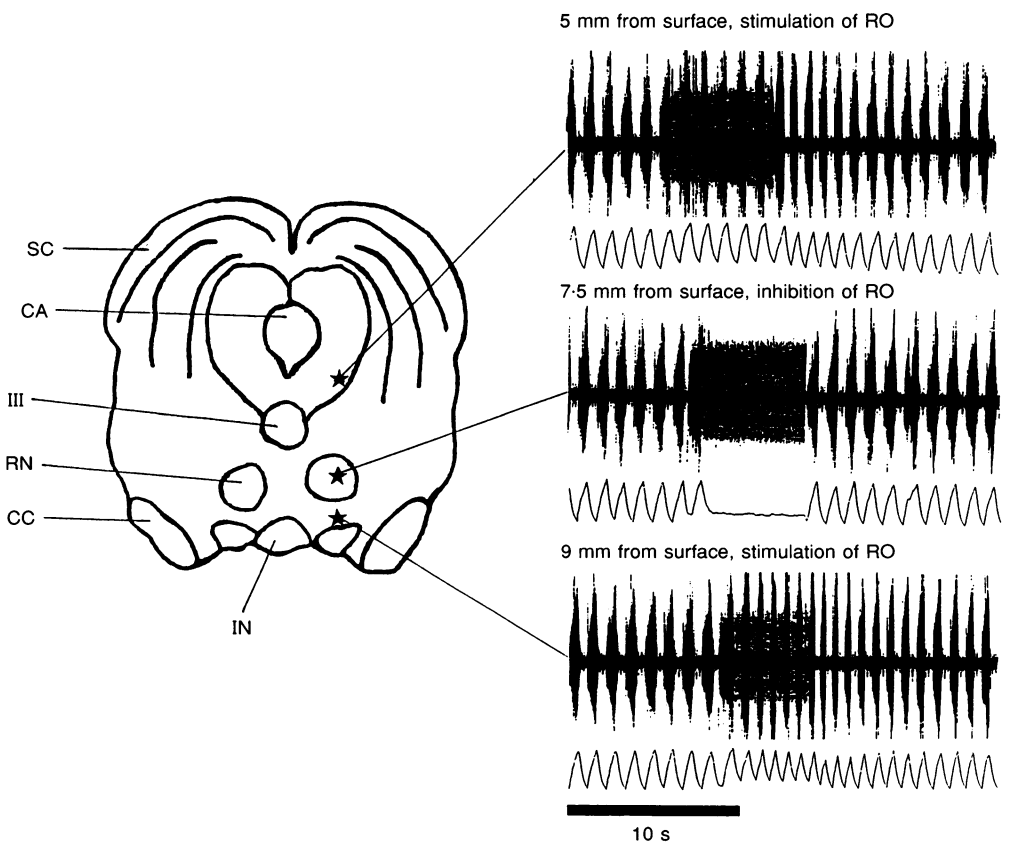

Figure 1. Electrical stimulation in the red nucleus inhibits neonatal respiratory output

Diagram (left) of cross-section of the mesencephalon, showing the sites of electrical stimulation at three depths. Traces (right) show in each case raw (upper) and integrated (lower) phrenic activity before, during and after electrical stimulation (seen from stimulus artifact) at the sites indicated. Note that stimulation in red nucleus inhibited respiratory output (RO), whilst stimulation above or below it produced a stimulation of RO. SC, superior colliculus; CA, cerebral aqueduct; III, oculomotor nucleus; RN, red nucleus; CC, crus cerebri; IN, interpeduncular nucleus.

Table 1. Arterial blood gases and pH_a during normoxia and after 4 min of hypoxia, before and after lesioning

Group		Before lesioning			After lesioning		
		pH_a	P_{a,CO_2}	P_{a,O_2}	pH_a	P_{a,CO_2}	P_{a,O_2}
RN	Normoxia	7·35 ± 0·01	41·7 ± 1·8	122·0 ± 11·7	7·3 ± 0·01	41·8 ± 2·5	117·2 ± 11·2
RN	Hypoxia	7·33 ± 0·02	41·3 ± 1·7	28·2 ± 1·2*	7·3 ± 0·02	43·5 ± 1·3	28·7 ± 1·1*
Non-RN	Normoxia	7·33 ± 0·02	41·0 ± 2·5	110·5 ± 11·9	7·32 ± 0·03	46·3 ± 2·0	108·8 ± 7·8
Non-RN	Hypoxia	7·32 ± 0·02	42·1 ± 2·9	27·0 ± 1·3*	7·3 ± 0·03	44·9 ± 3·2	29·8 ± 2·2*

RN, RN lesioned animals ($n = 6$); non-RN, lesions not in RN bilaterally ($n = 6$). * Different from value during normoxia by paired t test ($P < 0·05$). All values are means ± S.E.M.

before decreasing to, or below, pre-hypoxaemic control levels (nadir at 7–8 min, n.s. *vs.* control). The increase in RO (2 min) was a result of increased PPA ($P < 0·05$) with no change in breath frequency, whilst the fall in RO (minute 8) was a consequence of reductions in PPA and breath frequency ($P < 0·005$).

Mean arterial blood gas values (± S.E.M.) measured during control and hypoxic periods are given in Table 1. Values of arterial O_2 pressure (P_{a,O_2}) were less during hypoxia than normoxia in both groups of animals, both before and after lesioning. The presence or absence of a biphasic respiratory response was not related to the level to which P_{a,O_2} was reduced in hypoxia, either before or after lesioning. There were no differences between the values of pH_a and arterial CO_2 pressure (P_{a,CO_2}) throughout the experiment.

The respiratory response to electrical stimulation in the red nucleus during normoxia

Figure 1 shows examples of the effects on RO of electrical stimulation at varying depths within one electrode track. By moving the electrode tip as little as 1 mm above or below the inhibitory site in the RN, stimulatory responses could be evoked. As inhibitory effects could also be elicited from the RN when the electrode was withdrawn to it after having passed through it, it was clear that the penetration itself had not destroyed tissue to an important extent. Figure 2 shows the relationship between the experimentally determined co-ordinates for sites at which electrical stimulation evoked complete inhibition of RO (depth from the surface and distance from the mid-line of the superior colliculus) and the location of the RN as measured histologically in ten individual animals. These results show that the site at which inhibition of RO was produced coincides closely (within 1 mm) with the RN.

The effect of electrolytic lesioning

Although we aimed to lesion the RN bilaterally in all animals studied, this was not always possible. Data from animals with lesions outside the RN serve for comparison with data from animals with bilateral lesions in the RN. We have divided the animals into 'RN lesioned' and 'adjacent lesioned' groups using the following criteria. In 'RN

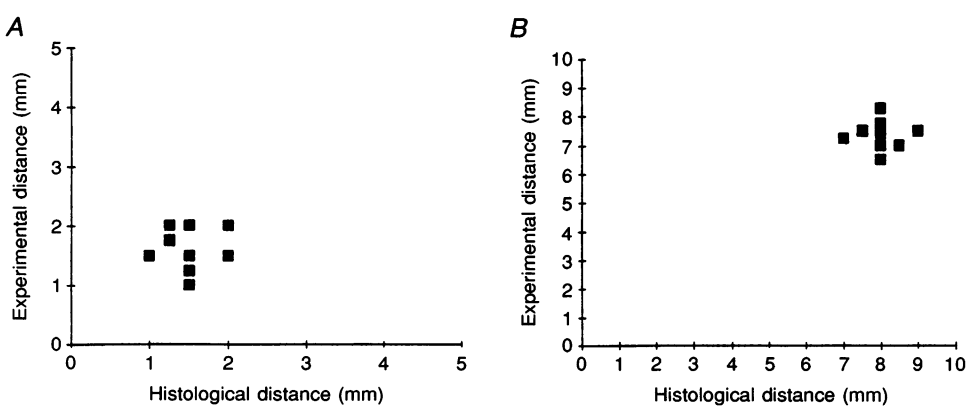

Figure 2. Stereotaxic localization in the RN of inhibitory stimulation sites

The relationship between the experimentally determined co-ordinates (ordinates) for sites at which electrical stimulation evoked complete inhibition of RO and the location of the RN measured histologically (abscissae) in 10 individual neonatal rabbits. *A*, distance from mid-line of superior colliculus; *B*, depth from surface of superior colliculus.

Table 2. Effect of electrolytic lesioning on breath frequency (f) and peak phrenic amplitude (PPA) in normoxia, before and after lesioning

Group		Before lesioning		After lesioning	
		Median	25th–75th centile	Median	25th–75th centile
RN	f (breaths min^{-1})	46·8	43·8–55·1	45·8	40·3–49·7
RN	PPA (arbitrary value)	8·4	6·0–13·1	7·2	6·2–12
Non-RN	f (breaths min^{-1})	42·0	39·9–51·6	39·4	35·4–52·9
Non-RN	PPA (arbitrary value)	10·7	8·8–12·8	11·2	9·0–12·9

RN, RN lesioned animals ($n = 6$); non-RN, lesions not in RN bilaterally ($n = 6$).

lesioned' animals electrical stimulation evoked total inhibition of RO on each side of the brainstem studied sequentially, and later histological examination showed that lesion sites were within the RN bilaterally (see Fig. 3). In non-RN lesioned animals, electrical stimulation either did not cause total inhibition of RO on either side or only evoked total inhibition of RO on one side. In these six animals lesions were placed at sites at which electrical stimulation produced less effective inhibition of RO, usually a decrease in PPA. Histology showed that lesion sites were either outside the RN or only within the RN unilaterally.

The effect on RO during normoxia

Neither bilateral electrolytic lesions in the RN nor lesions in areas adjacent to the RN had an effect on RO in normoxia. In control (normoxic) periods before and after lesioning, neither respiratory frequency nor peak phrenic activity changed (see Table 2).

The effect on the RO response to hypoxia

Figure 4 shows pre-control, control, minute 2 and minute 8 values measured during the response to hypoxia before and after lesioning. It can be seen that in the six RN lesioned animals, RO was greater than control at both 2 min and 8 min ($P < 0·05$), i.e. the decrease in RO during hypoxia was abolished. In the six non-RN lesioned animals, RO increased in five at 2 min of hypoxia and remained constant in one. In all of these, the biphasic response was abolished, since RO fell to be not significantly different from control values.

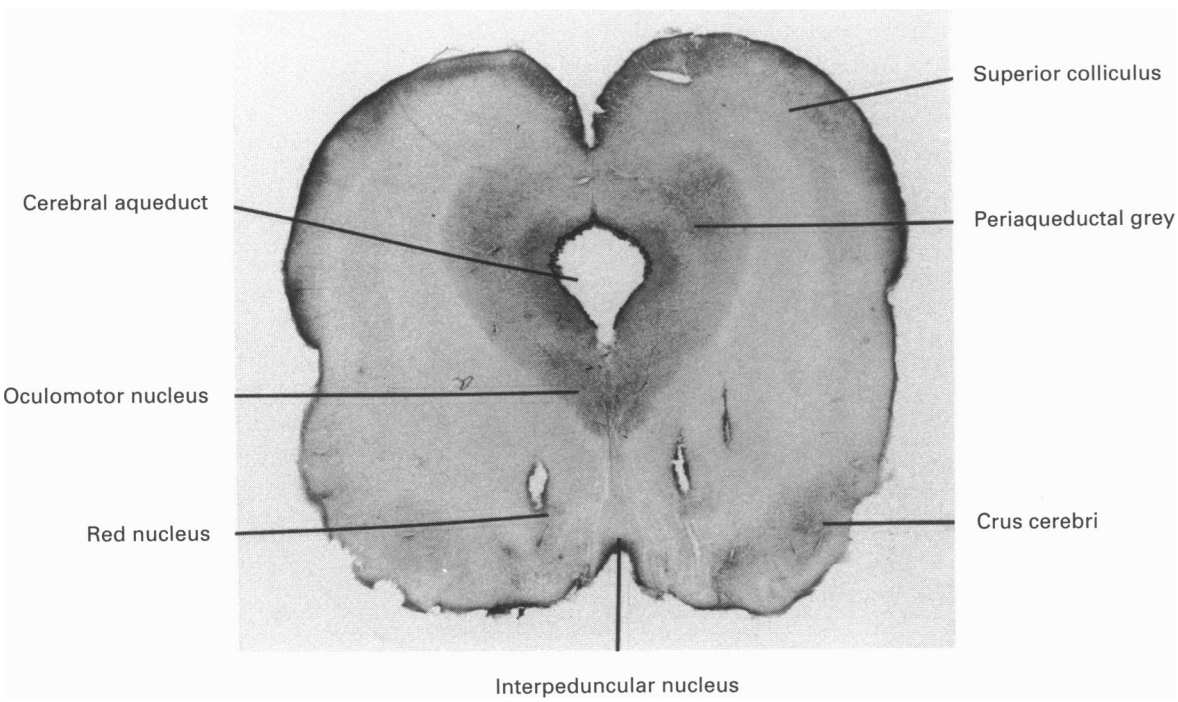

Figure 3. Histological location of lesions bilaterally in red nucleus

Photomicrograph of 150 μm thick transverse section of the midbrain, showing bilateral electrolytic lesions made in red nucleus at sites at which electrical stimulation inhibited RO. After lesioning, biphasic respiratory response to hypoxia was abolished.

The cardiovascular response to hypoxia before and after lesioning

Figure 5 shows the arterial blood pressure response to 8 min of hypoxia in six rabbits before and after bilateral RN lesions. Pre- and post-lesioning the arterial blood pressure increased to a peak (*ca* 3 min) followed by a decrease back to, or below, pre-hypoxia control values. There was no change in heart rate between control (300 beats min^{-1} (range, 288–345), median (25th–75th centiles)) and hypoxic periods (327 beats min^{-1} (range, 300–360)) before lesioning. Nor were changes seen after lesioning (345 beats min^{-1} (range, 330–360) and 338 beats min^{-1} (range, 300–360), control and hypoxia, respectively).

DISCUSSION

This study provides two pieces of evidence which clearly show that the RN is involved in the inhibition of RO during hypoxia in the neonate. The first is that phrenic nerve activity in young decerebrate rabbits was inhibited by electrical stimulation in the RN during normoxia, and that the sites at which stimulation caused apnoea differed markedly from those at which it increased breath frequency and/or peak phrenic activity. Second, electrolytic lesions made at sites where electrical stimulation had its maximum inhibitory effect on RO abolished the biphasic respiratory response to hypoxia. Histological examination of the location of lesion sites indicated that areas within the RN are essential to the mechanism decreasing RO during hypoxia.

Although peripheral chemoreceptor inputs to the CNS are maintained throughout hypoxia in the neonate (Schweiler, 1968; Blanco *et al.* 1984), the stimulatory effect of these inputs on breathing is 'gated out' after a few minutes of hypoxia and breathing declines despite sustained chemoreceptor discharge (Ackland, Moore & Hanson, 1993). The mechanism underlying this decrease in CNS sensitivity to peripheral chemoreceptor stimulation remains controversial. However, there is evidence that the decrease in peripheral chemoreflex during hypoxia involves the operation of a CNS mechanism which inhibits RO, as opposed to a reduction in arterial chemoreceptor discharge or a change in respiratory

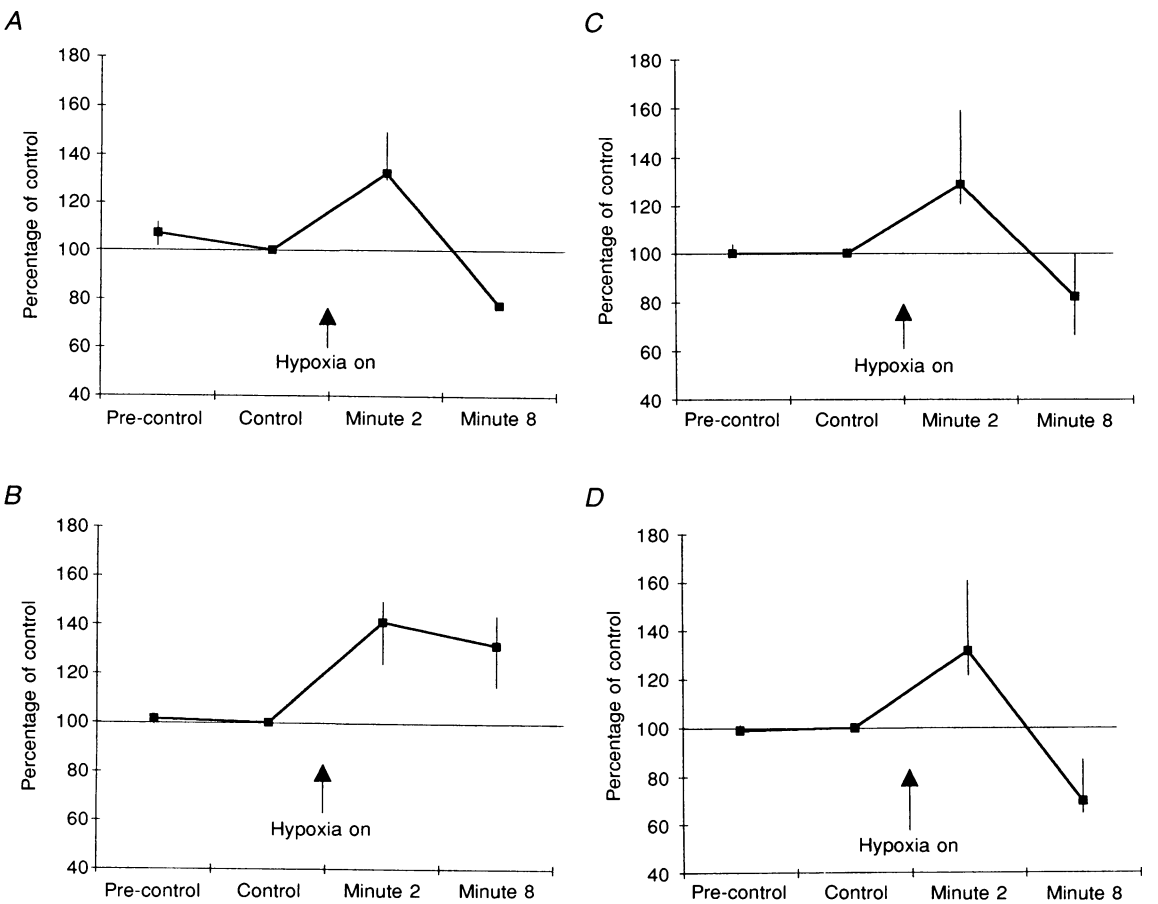

Figure 4. Effect of mesencephalic lesions on biphasic respiratory response

Respiratory output expressed as a percentage of control during pre-control periods and 8 min exposures to hypoxia before and after bilateral lesions in the RN (*A* and *B*, respectively), and before and after bilateral lesions outside the RN (*C* and *D*, respectively). $n = 6$ for each group. Points show median and IQRs. Where not shown, the IQR lies within the symbol. Note that the fall in RO after 8 min of hypoxia in both groups is abolished by lesions of the RN, but not by lesioning outside it.

mechanics (see Noble, 1991; Moore & Hanson, 1994 for reviews).

We used supracollicular decerebrate young rabbits so that, after decerebration, anaesthesia could be withdrawn. This allowed us to avoid the cardiovascular and respiratory effects of anaesthesia when investigating the mechanism of the descending inhibition. In this preparation, unilateral electrical stimulation in the RN produced cessation of phrenic nerve activity during normoxia. Our results in young rabbits thus follow a series of previous studies in both decerebrate and anaesthetized adult animals. In 1936, Kabat reported that electrical stimulation within the medial part of the mesencephalic tegmentum elicited a decrease in ventilation. Later, others found that electrical stimulation in the caudal, ventromedial mesencephalon including the RN decreased RO (Baxter & Olszewski, 1955; Evans & Pepler, 1974; Coles, 1987; Schmid *et al.* 1988; Gallman *et al.* 1991). Bassal & Bianchi (1982) concluded that the only areas in the mesencephalon where electrical stimulation produced inhibition of RO were the RN and the rubrospinal tract (RST). Most recently, microinjections of glutamate indicate that cell bodies in the vicinity of the RN produce phrenic inhibition in adult cats (Gallman *et al.* 1991) and young rabbits (Waites, Ackland, Noble & Hanson, 1995). Clearly therefore the area can evoke powerful descending inhibitory effects on RO. In our studies extensive tracking in this area showed that electrical stimulation of areas adjacent to the RN either produce a stimulation of RO or no effect. Stimulation of the superior colliculus and periaqueductal grey produced a stimulation of phrenic nerve activity, as previously reported in the adult cat (Bassal & Bianchi, 1982; Gauthier & Monteau, 1984).

Electrolytic lesions placed bilaterally at RN inhibitory sites had no effect on RO in normoxia, but in six animals abolished the BRR in hypoxia, making it clear that they had removed an integral component of the response but had not affected the brainstem processes producing eupnoea. In six animals in which lesions were not in the RN bilaterally, the respiratory response to hypoxia remained biphasic. We therefore believe that the RN plays a crucial role in the neonatal BRR to hypoxia. Whilst the RN is not responsible for the genesis of respiratory drive it is clear from our results that the RN has a modulatory role on RO in the neonatal respiratory response to hypoxia. This accords with its other motor functions, in which it does not initiate movement but modulates motoneurone discharge and sensory input to the spinal cord via descending influences (see Keifer & Houk, 1994).

The RN is composed of a caudal magnocellular division and a rostral parvicellular division (see Massion, 1967; Keifer & Houk, 1994 for reviews). The parvicellular division receives input predominantly from the cerebral cortex and its sole efferent pathway is to the ipsilateral inferior olive; very little is known about its functional role. The magnocellular part of the RN receives input mainly from the interpositus nucleus of the cerebellum (via the superior cerebellar peduncle). Efferent fibres from the magnocellular division decussate near to the RN and descend contralaterally as the RST, projecting to the caudal ventrolateral brainstem and to neurones in all segments of the spinal cord. Whilst there

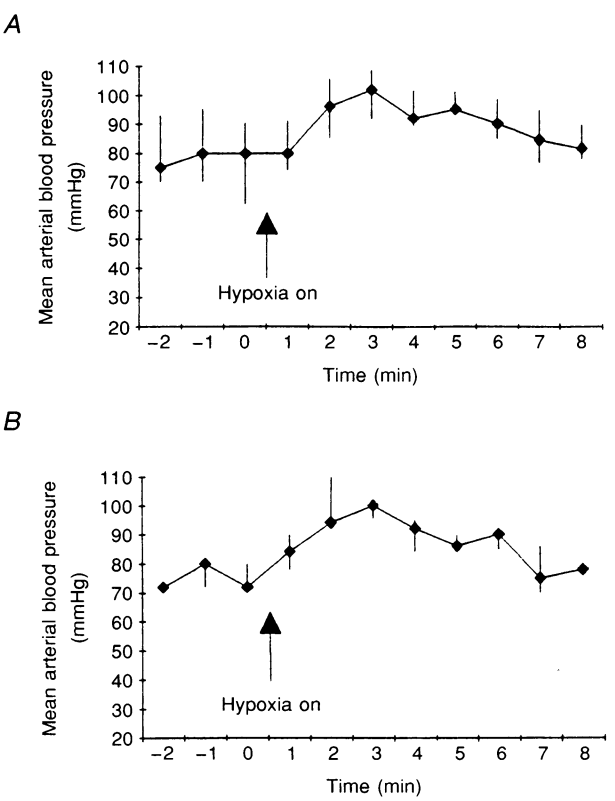

Figure 5. Effect of hypoxia on arterial blood pressure before and after RN lesions

The effect of 8 min of hypoxia on mean arterial blood pressure in 6 rabbits before (*A*) and after (*B*) placement of lesions bilaterally in the RN. Note that, unlike the effect on the biphasic respiratory response seen in Fig. 4, lesions did not affect the blood pressure response.

is no evidence to indicate a pathway from the RN to the dorsal respiratory group (Edwards, 1972; Horst, Luiten, Kuipers, 1984) there is evidence for projections to the ventral respiratory group (Horst *et al.* 1984), in particular to bulbospinal neurones in the region of the ambigual complex (Schmid *et al.* 1988). Interestingly, Vibert, Caille, Bertrand, Gromysz & Hugelin (1979) observed that respiratory-modulated units could be recorded from the most ventral part of the RN in adult cats.

Electrical stimulation of either the RN or RST inhibits RO in the adult (Schmid *et al.* 1988) and neonate. Thus, it seems likely that the RN acts via the RST to inhibit RO during hypoxia in the fetus. The findings of Gluckman & Johnston (1987) appear to support this hypothesis. They found that the inhibition of fetal breathing movements during hypoxaemia was abolished by placement of bilateral lesions in the ventrolateral pons. The lesions produced were relatively large; thus in order to define a locus for the inhibitory mechanism, a compilation was made from the histology of the brainstems of all fetuses in which lesions prevented an inhibition of FBM during hypoxia. The small site where all the successful lesions overlapped was located in the ventrolateral pons and appears to overlay the RST, although not identified as such by the authors (but see Gallman *et al.* 1991 for discussion).

It appears that the inhibition of RO mediated by the RN does not occur solely at the level of the phrenic motor neurones as in adult, peripherally chemodenervated cats, exposure to hypoxia results in the inhibition of bulbospinal inspiratory neurones coincident with phrenic inhibition (England, Melton, Douse & Duffin, 1995). Moreover, latency calculations made by Schmid *et al.* (1988) indicate that electrical stimulation of the RST inhibits RO by a polysynaptic pathway involving the medulla and/or pons. However, it remains possible that there is also a direct monosynaptic inhibitory pathway between the RN and phrenic motor neurones at the spinal level. Direct support for the involvement of the pons comes from the finding that cooling of the dorsal pons in the region of the locus coeruleus (LC), at the level of the middle cerebellar peduncle, reversibly abolishes the BRR in lambs (Moore *et al.* 1991) and that in the neonatal rabbit, electrical stimulation in the region of the LC inhibits RO (Ackland, 1995). Thus hypoxia may act via the LC to initiate the inhibitory mechanism involving the RN. Alternatively the effect of the LC on RO may represent a parallel pathway. Further work is needed to discriminate between these possibilities.

Interestingly, we also found that during hypoxia, mean arterial blood pressure showed an initial transient increase (after 2–3 min) followed by a decrease to pre-hypoxia control levels, both in animals with a BRR and those showing a sustained increase in RO after bilateral RN lesions. Thus, our results suggest that during hypoxia the RN has a differential effect on respiratory and cardiovascular responses. During hypoxia in fetal sheep a similar differential effect on cardiorespiratory output has been reported: hypoxia prevents the occurrence of FBM and thus inhibits respiratory chemoreflexes, but does not affect the operation of fetal cardiovascular chemoreflexes (Moore, 1988; Giussani, Spencer, Moore, Bennet & Hanson, 1993). This differential chemoreflex response ensures that appropriate cardiorespiratory responses are evoked, even in immature mammals. The peripheral arterial chemoreceptor inputs underlying these responses are integrated and modified centrally (for review see Spyer, 1994). However, the role of the RN in these mechanisms is not yet understood.

Our study indicates that the mechanism of the inhibition of RO during hypoxia involves a specific site within the mesencephalon and that it is not merely a reflection of widespread depression of neurones by hypoxia or accumulation of inhibitory neuroactive agents in the medulla. Furthermore, our results substantiate the circumstantial evidence for the involvement of a mesencephalic mechanism in the ventilatory response to hypoxia in the neonate. Possibly the mechanism is a persistence of that which inhibits FBM *in utero*, whilst in the adult the inhibitory response appears to be overridden. Further studies are needed to elucidate the nature of the involvement of the RN in this mechanism.

Ackland, G. L. (1995). Effects of hypoxia on the control of breathing in the newborn. PhD Thesis, University College London, UK.

Ackland, G. L., Moore, P. J. & Hanson, M. A. (1993). Hypoxia inhibits the effect on breathing of transient chemoreceptor stimulation in anaesthetized newborn rabbits. *Journal of Physiology* **467**, 280*P*.

Ackland, G. L., Waites, B. A., Noble, R. & Hanson, M. A. (1995). Bilateral lesions in the red nucleus abolish the biphasic respiratory response to hypoxia in decerebrate newborn rabbits. *Journal of Physiology* **483.P**, 89*P*.

Bassal, M. & Bianchi, A. L. (1982). Inspiratory onset or termination induced by electrical stimulation of the brain. *Respiration Physiology* **50**, 23–40.

Baxter, D. W. & Olszewski, J. (1955). Respiratory responses evoked by electrical stimulation of pons and mesencephalon. *Journal of Neurophysiology* **18**, 276–287.

Blanco, C. E., Hanson, M. A., Johnson, P. & Rigatto, H. (1984). Breathing pattern of kittens during hypoxia. *Journal of Applied Physiology* **56**, 12–17.

Boddy, K., Dawes, G. S., Fisher, R., Pinter, S. & Robinson, J. S. (1974). Foetal respiratory movements, electrocortical and cardiovascular responses to hypoxaemia and hypercapnia in sheep. *Journal of Physiology* **243**, 599–618.

Bureau, M. A., Lamarche, J., Foulon, P. & Dalle, D. (1985). The ventilatory response to hypoxia in the newborn lamb after carotid body denervation. *Respiratory Physiology* **60**, 109–119.

Coles, S. K. (1987). Mesencephalic apnoeic regions in the rat. *Journal of Physiology* **382**, 176*P*.

Dawes, G. S., Gardner, W. N., Johnston, B. M. & Walker, D. W. (1983). Breathing in fetal lambs: the effect of brain stem section. *Journal of Physiology* **335**, 535–553.

EASTON, P. A., SLYKERMAN, L. J. & ANTHONISEN, N. R. (1986). Ventilatory response to sustained hypoxia in normal adults. *Journal of Applied Physiology* **61**, 906–911.

EDEN, G. J. & HANSON, M. A. (1987). Maturation of the respiratory response to acute hypoxia in the newborn rat. *Journal of Physiology* **392**, 1–9.

EDWARDS, S. B. (1972). The ascending and descending projections of the red nucleus in the cat: an experimental study using an autoradiographic tracing method. *Brain Research* **48**, 45–63.

ENGLAND, S. J., MELTON, J. E., DOUSE, M. A. & DUFFIN, J. (1995). Activity of respiratory neurons during hypoxia in the chemodenervated cat. *Journal of Applied Physiology* **78**, 856–861.

EVANS, M. H. & PEPLER, P. A. (1974). Respiratory effects mapped by focal stimulation in the rostral brain stem of the anaesthetised rabbit. *Brain Research* **75**, 41–57.

GALLMAN, E. A., LAWING, W. L. & MILLHORN, D. E. (1991). Mesencephalic stimulation elicits inhibition of phrenic nerve activity in cat. *Journal of Physiology* **436**, 405–420.

GAUTHIER, P. & MONTEAU, R. (1984). Inspiratory on-switch evoked by mesencephalic stimulation: activity of medullary respiratory neurones. *Experimental Brain Research* **56**, 475–487.

GIUSSANI, D. A., SPENCER, J. A. D., MOORE, P. J., BENNET, L. & HANSON, M. A. (1993). Afferent and efferent components of the cardiovascular reflex responses to acute hypoxia in term fetal sheep. *Journal of Physiology* **461**, 431–449.

GLUCKMAN, P. D. & JOHNSTON, B. M. (1987). Lesions in the upper lateral pons abolish the hypoxic depression of breathing in unanaesthetized fetal lambs *in utero*. *Journal of Physiology* **382**, 373–383.

HANSON, M. A. & WILLIAMS, B. A. (1989). The effect of decerebration and brain stem transection on the ventilatory response to acute hypoxia in normoxic and chronically hypoxic newborn rats. *Journal of Physiology* **414**, 25*P*.

HORST, G. J., LUITEN, P. G. M. & KUIPERS, F. (1984). Descending pathways from the hypothalamus to dorsal motor vagus and ambiguous nuclei in the rat. *Journal of the Autonomic Nervous System* **11**, 59–75.

JOHNSTON, B. M. & GLUCKMAN, P. D. (1992). Peripheral chemoreceptors respond to hypoxia in pontine-lesioned fetal lambs in utero. *Journal of Applied Physiology* **75**, 1027–1034.

KABAT, H. (1936). Electrical stimulation of points in the forebrain and mid-brain: the resultant alterations in respiration. *Journal of Comparative Neurology* **64**, 187–208.

KEIFER, J. & HOUK, J. C. (1994). Motor function of the cerebellorubrospinal system. *Physiological Reviews* **74**, 509–542.

LAHIRI, S., MULLIGAN, E., NISHINO, T., MOKASHI, A. & DAVIES, R. O. (1981). Relative responses of aortic body and carotid body chemoreceptors to carboxyhemoglobinemia. *Journal of Applied Physiology* **50**, 580–586.

MARTIN-BODY, R. L. (1988). Brain transections demonstrate the central origin of hypoxic ventilatory depression in carotid body-denervated rats. *Journal of Physiology* **407**, 41–52.

MARTIN-BODY, R. L. & JOHNSTON, B. M. (1988). Central origin of the hypoxic depression of breathing in the newborn. *Respiration Physiology* **71**, 25–32.

MARTIN-BODY, R. L., ROBSON, G. J. & SINCLAIR, J. D. (1985). Respiratory effects of sectioning the carotid sinus, glossopharyngeal and abdominal vagus nerves in the awake rat. *Journal of Physiology* **361**, 35–45.

MASSION, J. (1967). The mammalian red nucleus. *Physiological Reviews* **47**, 383–436.

MOORE, P. J. (1988). The involvement of the peripheral chemoreceptors in the control of fetal breathing movements. PhD Thesis, University of Reading, UK.

MOORE, P. J. & HANSON, M. A. (1994). Control of breathing: central influences. In *Fetus and Neonate – Physiology and Clinical Applications*, vol. II, *Breathing*, ed. HANSON, M. A., SPENCER, J. A. D., RODECK, C. H. & WALTERS, D. V., pp. 109–136. Cambridge University Press, Cambridge, UK.

MOORE, P. J., PARKES, M. J., NOBLE, R. & HANSON, M. A. (1991). Reversible blockade of the secondary fall of ventilation during hypoxia in anaesthetized newborn sheep by focal cooling in the brain stem. *Journal of Physiology* **438**, 242*P*.

NOBLE, R. (1991). Concepts of neural control applied to the developing brainstem. In *The Fetal and Neonatal Brainstem*, ed. HANSON, M. A., pp. 48–58. Cambridge University Press, Cambridge, UK.

SCHMID, K., BÖHMER, G. & FALLERT, M. (1988). Medullary respiratory-related neurons with axonal connections to rostral pons and their function in termination of inspiration. *Pflügers Archiv* **403**, 58–65.

SCHWEILER, G. H. (1968). Respiratory regulation during postnatal development in cats and rabbits and some of its morphological substrate. *Acta Physiologica Scandinavica* **72**, suppl. 304, 1–123.

SPYER, K. M. (1994). Central nervous mechanisms contributing to cardiovascular control. *Journal of Physiology* **474**, 1–19.

VAN BEEK, J. H. G. M., BERKENBOSCH, A., DE GOEDE, J. & OLIEVIER, C. N. (1984). Effects of brainstem hypoxaemia on the regulation of breathing. *Respiration Physiology* **57**, 171–188.

VIBERT, J. P., CAILLE, D., BERTRAND, F., GROMYSZ, H. & HUGELIN, A. (1979). Ascending projection from the respiratory centre to mesencephalon and diencephalon. *Neuroscience Letters* **11**, 29–33.

WAITES, B. A., ACKLAND, G. L, NOBLE, R. & HANSON, M. A. (1995). The effects of hypoxia in the control of breathing by the brain stem in the neonate. *Japanese Journal of Physiology* (in the Press).

WOODRUM, D. E., STANDAERT, T. A., MAYOCK, D. E. & GUTHRIE, R. D. (1981). Hypoxic ventilatory responses in the newborn monkey. *Pediatric Research* **15**, 367–370.

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