EFFERENT PHRENIC NERVE ACTIVITY DURING INDUCED CHANGES IN ARTERIAL PRESSURE

E. M. GRUNDY, M. K. CHAKRABARTI AND J. G. WHITWAM

Stimulation of the systemic baroreceptors inhibits respiration (Heymans and Neil, 1958). In most of the studies in which this has been demonstrated, pressure has been applied to an isolated vascular area containing baroreceptors, or the carotid sinus and aortic nerves have been stimulated electrically. Hitherto there has not been a great deal of work on the effects of induced alterations in arterial pressure, in otherwise intact animals, on central respiratory activity. Garcia and Cherniack (1967) observed that increasing systemic arterial pressure with noradrenaline inhibited phrenic nerve activity in apnoeic dogs. Grunstein, Derenne and Milic-Emili (1975) showed that the transient inflation of an intra-aortic balloon caused an acute increase in systemic arterial pressure, a decrease in tidal volume and an increase in the inspiratory time in cats. However, both these studies were concerned with the effects of acutely induced hypertension and, hence, baroreceptor stimulation. Although Miserocchi and Quinn (1980) observed the effects of haemorrhage on respiration in spontaneously breathing cats, the absence of studies on the effects of hypotension induced with vasodilator drugs, and also during artificial ventilation, is surprising in view of the potential interest in this subject.

The present study was undertaken to quantify the changes in phrenic nerve activity (in intact animals under steady state conditions) in response to both increases and decreases in arterial pressure.

**SUMMARY**

Efferent activity in the phrenic nerve was recorded during induced hypotension and hypertension in anaesthetized, paralysed, artificially ventilated dogs. Changes in arterial pressure were induced with infusions of sodium nitroprusside, noradrenaline and angiotensin II, after which ventilation was adjusted to return the PaCO\textsubscript{2} near to control values. The PaO\textsubscript{2} was maintained above chemoreceptor threshold throughout. When a steady state was achieved quantitative measurements of phrenic nerve activity were made. In six dogs an increase in mean arterial pressure from 114 to 167 mm Hg caused a mean reduction of phrenic nerve activity of 28%. In six dogs a decrease in mean arterial pressure from 128 to 82 mm Hg caused an increase in phrenic nerve activity of 22%. This shows that the reduction in arterial pressure induced by vasodilator drugs causes a major sustained stimulus to respiration, while increase in arterial pressure causes marked respiratory depression.

**MATERIALS AND METHODS**

Investigations were performed on 10 unpremedicated mongrel dogs weighing between 13 and 20 kg. Anaesthesia was induced with methohexitone 15 mg kg\textsuperscript{-1} i.v. after which a cuffed tracheal tube was passed. The animal's lungs were ventilated with a Starling pump (intermittent positive pressure ventilation, IPPV) at a rate of 10 b.p.m. and a femoral artery and vein were cannulated.

Anaesthesia was maintained with a 1% solution of \(\alpha\)-chloralose, initially in a bolus dose of 30 mg kg\textsuperscript{-1} followed by a continuous i.v. infusion of 15–20 mg kg\textsuperscript{-1} h\textsuperscript{-1}. Suxamethonium 1–2 mg kg\textsuperscript{-1} h\textsuperscript{-1} was administered to provide
neuromuscular blockade. Airway and arterial pressures were recorded using resistance strain gauges (S.E. Laboratories) calibrated against water and mercury columns, respectively. The end-tidal carbon dioxide concentration was measured with an infra-red carbon dioxide analyser (Hartmann Braun, URAS 4) calibrated with preanalysed gas mixtures.

The phrenic nerve was exposed in the neck, dissected, desheathed and cut distally. It was placed on silver–silver chloride electrodes in a pool of mineral oil to record efferent activity. The signal from the recording electrode was passed through a preamplifier (Tektronix RM122) and a differential amplifier (Tektronix 2063) before being displayed. In addition, the electroneurogram was rectified and integrated (Neurolog 90-Digitimer). All signals were displayed on an ultra-violet recorder (S.E. Laboratories type 2112).

In order to ensure the synchronization of bursts of phrenic nerve activity with the respiratory cycle, relatively large tidal volumes (approximately 20 ml kg⁻¹) were used. Oxygen was added to the inspired gas to maintain a PaO₂ greater than 23 kPa. Intermittent arterial blood analysis for PaO₂, PaCO₂, and pH was performed using a Radiometer ABL1 blood-gas analyser. Sodium bicarbonate was administered to maintain the base excess and pH within a narrow range. Oesophageal temperature was maintained in the range 37–38 °C.

In view of the large tidal volumes carbon dioxide was administered to maintain an FECO₂ of approximately 6% and the PaCO₂ was maintained around 5.8 kPa. Along with the large tidal volume, this ensured that the bursts of phrenic nerve activity (PNA) remained synchronized with the ventilator throughout each study. In addition, this technique allowed the PaCO₂ to be passed through each direction by altering the FiCO₂ without having to change the VT or respiratory rate.

After the completion of the surgical preparation, a period of 30 min was allowed for stabilization. Control measurements were obtained of blood-gas tensions, phrenic nerve activity, arterial pressure and heart rate.

An initial pilot study showed that a bolus dose of sodium nitroprusside (SNP) 10 mg decreased mean arterial pressure (MAP) and increased the PNA while a bolus of noradrenaline (NA) 0.5 mg produced the opposite result. However, when MAP increased, the FECO₂ and PaCO₂ decreased, whereas a decrease in MAP caused increases in FECO₂ and PaCO₂. Thus, the changes in PNA (figs 1 and 2) could have been the result of the changes in PaCO₂, which were the result of alterations in ventilation:perfusion ratios in the lungs, and not results of the changes in MAP per se. Angiotensin II (AII) caused effects similar to those of NA.

In subsequent studies "steady states" were achieved so that, by maintaining MAP constant, the PaCO₂ could be adjusted and maintained close to control values throughout each period of observation. The arterial pressure was either decreased with SNP in a dose sufficient to produce a decrease of approximately 45 mm Hg, or was increased with angiotensin II. The vasoactive drug was given as an initial bolus followed by an infusion to maintain a constant MAP. The carbon dioxide added to the inspired gas mixture was adjusted to maintain a constant FECO₂. It was necessary to add carbon dioxide to the gas mixture during hypertension and to reduce the FiCO₂ during hypotension. At each point where the MAP was related to the PNA, the MAP had been stable for 20–30 min and the PaCO₂ was close to control values. Relative to human requirements, the dog proved resistant to the effect of vasoactive drugs. After initial pilot observations, the range of change of pressure was to some extent determined by the preparation. After a substantial initial effect, very large doses of the drugs were required to produce further changes in pressure, and the preparation was unstable in terms of maintaining constant blood-gas tensions. To maintain the steady states outlined in the present study, the ranges of the rates of infusion of angiotensin II or SNP were 125–300 μg kg⁻¹ min⁻¹ and 100–350 μg kg⁻¹ min⁻¹, respectively. After the infusion of the vasoactive drug was stopped, MAP was allowed to return to control values. Again, alteration of the FiCO₂ was required to maintain PaCO₂ close to control values, and at least 15 min was allowed for stabilization before the PaCO₂, MAP and PNA were related and measured.

Measurements of PNA referred to in table I are in arbitrary units and are the sum of 10 consecutive peak heights of the integrated signal measured during the relevant period. Since the ventilator was set at the rate of 10 b.p.m. in all preparations, and the phrenic nerve signal remained locked to the ventilator cycle throughout, the measurement of PNA was numerically the ventilator rate–peak height product, that is PNA per min in arbitrary units.

The technique of altering the MAP while
maintaining normal blood-gas tensions proved difficult and, after pilot experiments, each study was restricted to a single increase or decrease in MAP, with a maximum change which could be readily maintained while the \( P_{\text{aCO2}} \), was re-adjusted to around its control value.

Statistical analysis was performed using paired \( t \) tests; \( P < 0.05 \) was regarded as significant.

**RESULTS**

The effects of bolus injections of NA 500 \( \mu \)g and SNP 10 mg on MAP are illustrated in figures 1 and 2. In response to the injection of NA, there was a rapid increase in MAP (fig. 1) from approximately 120 mm Hg to a maximum of 240 mm Hg within 45 s. However, there was no observable change in PNA until MAP was greater than 200 mm Hg. When MAP reached 220 mm Hg, PNA was virtually abolished and remained so until MAP started to decrease — at which point a change from 240 mm Hg to 220 mm Hg was associated with an immediate increase in PNA to near control values. MAP started to decrease within 10 s of starting the injection of SNP (fig. 2), and the minimum MAP was reached within 25 s. The effect persisted for more than 2 min and was associated with a large increase in PNA. The induced increases and decreases in MAP were associated with alterations

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**Fig. 1.** The effect of a bolus of noradrenaline 500 \( \mu \)g i.v. on mean arterial pressure and phrenic nerve activity.

**Fig. 2.** The effect of a bolus dose of sodium nitroprusside 10 mg i.v. on mean arterial pressure and phrenic nerve activity.
in \( F_{\text{ECO}_2} \) and \( P_{\text{aCO}_2} \) which made interpretation of the results of these dynamic studies extremely difficult. Because of the rapid nature of these changes, the \( F_{\text{ECO}_2} \) did not stabilize before the direction of the change in MAP was reversed. \( F_{\text{ECO}_2} \) varied by a maximum of 2% in response to changes in MAP; following bolus doses of SNP, \( P_{\text{aCO}_2} \) increased by values varying from 0.5 kPa to 0.9 kPa in different preparations. However, it is impossible to say whether these represent the maximum changes which would have occurred if a steady state had been induced and maintained.

The apparent delay of 5–15 s before a change in MAP caused a change in PNA also presents a problem in interpretation, since accurate measurement of this time was impossible because of the duration of the respiratory cycle which, in these studies, was 6 s so that the observed effect on PNA was always retrospective by at least this period. Thus, the relationship between MAP and PNA cannot be determined from simple studies of this type.

Examples of studies in which the MAP was either increased by AII or decreased by SNP, and in which the blood-gas tensions were maintained constant (by adjusting the \( F_{\text{tCO}_2} \)), are shown in figures 3 and 4, respectively. The data obtained from six dogs are summarized in table I and the relevant blood-gas analyses are shown in table II.
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**Table I. Changes in phrenic nerve activity (arbitrary units) associated with changes in mean arterial pressure in six dogs (mean ± SEM)**

<table>
<thead>
<tr>
<th></th>
<th>Control (pre)</th>
<th>During changed AP</th>
<th>P</th>
<th>Control (post)</th>
<th>P</th>
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<tbody>
<tr>
<td>Induced hypertension</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>114 ± 9</td>
<td>167 ± 10</td>
<td>&lt; 0.02</td>
<td>113 ± 9</td>
<td>&lt; 0.03</td>
</tr>
<tr>
<td>PNA</td>
<td>31.0 ± 6.2</td>
<td>22.3 ± 4.8</td>
<td>&lt; 0.01</td>
<td>31.0 ± 6.3</td>
<td>&lt; 0.04</td>
</tr>
<tr>
<td>Induced hypotension</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>128 ± 8.0</td>
<td>82 ± 6.0</td>
<td>&lt; 0.005</td>
<td>124 ± 7.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>PNA</td>
<td>17.2 ± 4.0</td>
<td>21.0 ± 4.2</td>
<td>&lt; 0.01</td>
<td>15.5 ± 3.2</td>
<td>&lt; 0.04</td>
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</tbody>
</table>

**Table II. Changes in mean Pa<sub>CO<sub>2</sub> (kPa), pH<sub>a</sub> and Pa<sub>O<sub>2</sub> (kPa) during alterations in mean arterial pressure in six dogs (mean ± SEM)**

<table>
<thead>
<tr>
<th></th>
<th>Control (pre)</th>
<th>During changed AP</th>
<th>P</th>
<th>Control (post)</th>
<th>P</th>
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<tbody>
<tr>
<td>Induced hypertension</td>
<td></td>
<td></td>
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<tr>
<td>Pa&lt;sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.0 ± 0.2</td>
<td>6.1 ± 0.15</td>
<td>ns</td>
<td>6.09 ± 0.19</td>
<td>ns</td>
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<tr>
<td>pH&lt;sub&gt;a&lt;/sub&gt;</td>
<td>7.27 ± 0.02</td>
<td>7.25 ± 0.02</td>
<td>ns</td>
<td>7.21 ± 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>Pa&lt;sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>26.7 ± 1.4</td>
<td>30.9 ± 1.9</td>
<td>ns</td>
<td>31.9 ± 1.38</td>
<td>ns</td>
</tr>
<tr>
<td>Induced hypotension</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pa&lt;sub&gt;CO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6.1 ± 0.23</td>
<td>6.2 ± 0.25</td>
<td>ns</td>
<td>6.3 ± 0.29</td>
<td>ns</td>
</tr>
<tr>
<td>pH&lt;sub&gt;a&lt;/sub&gt;</td>
<td>7.22 ± 0.02</td>
<td>7.212 ± 0.02</td>
<td>ns</td>
<td>7.18 ± 0.02</td>
<td>ns</td>
</tr>
<tr>
<td>Pa&lt;sub&gt;O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>29.5 ± 1.4</td>
<td>29.7 ± 1.6</td>
<td>ns</td>
<td>30.1 ± 1.9</td>
<td>ns</td>
</tr>
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</table>

During induced hypertension a mean increase in MAP of 53 mm Hg, from 114 mm Hg to 167 mm Hg, caused a mean reduction in PNA of 28%. Conversely, a mean reduction in MAP from 128 mm Hg to 82 mm Hg caused a mean increase in PNA of 22% (table I). There were no statistically significant differences in Pa<sub>CO<sub>2</sub>, Pa<sub>O<sub>2</sub> or pH at the times when measurements of MAP and PNA were made.

**DISCUSSION**

This study shows that, in an intact animal with a constant Pa<sub>CO<sub>2</sub>, minimal peripheral chemoreceptor drive and constant inflation of the lungs, an increase in arterial pressure causes marked respiratory depression, whereas a reduction in pressure stimulates respiration as judged by phrenic nerve activity.

The principal factors which influence central respiratory activity in anaesthetized animals are chemical, for example Pa<sub>CO<sub>2</sub> and Pa<sub>O<sub>2</sub> (Duffin, 1971); impulses in afferent nerves from the lungs (Adrian, 1933; Bartoli et al., 1973; Feldman, Cohen and Wolotsky, 1976), the intercostal muscles (Remmers, 1970) and the baroreceptors (Aviado and Schmidt, 1955; Heymans and Neil, 1958; Sampson, Biscoe and Campion, 1968); and other factors such as temperature (Trippenbach and Milic-Emili, 1977) and the depth of anaesthesia. Thus the respiratory neurones in the brain stem receive a variety of interacting inputs and then transmit impulses down at least two descending pathways to the spinal motor neurones which innervate the respiratory muscles (Newsom Davis and Plum, 1972; Mitchell and Berger, 1981; Feldman, McCrimmon and Speck, 1984).

The design of the present study eliminated, as far as possible, all the variables except arterial pressure. It shows that large sustained changes in mean arterial pressure from the set point of the animal will cause substantial changes in efferent phrenic nerve activity which may be taken as a measure of the central respiratory drive to ventilation. Although it is known that the response of the baroreceptors is approximately linear between MAP values of 80 and 180 mm Hg (Pelletier, Clement and Shepherd, 1972) the precise relationship between changes in MAP and PNA would require much more detailed studies than have been described here.

A major problem in the current study was the
maintenance of a constant $P_{a\text{CO}_2}$, while mean arterial pressure was varied. It is known that, during induced hypotension, deadspace ventilation increases (Eckenhoff et al., 1963). Thus in the presence of constant ventilation of the lungs an increase in $P_{a\text{CO}_2}$ of the order 0.5–0.9 kPa (as was seen in the present study) could be anticipated. Moreover, the time course of an increase in $P_{a\text{CO}_2}$ is known to be relatively prolonged (Ivanov and Nunn, 1968). Any adjustment of ventilation causes a change in afferent activity from the lungs and, hence, would also be expected to modify the activity of the respiratory centres. These problems were avoided by maintaining tidal volume and respiratory rate constant and producing a high normal $P_{a\text{CO}_2}$ in the control situation by ventilating with carbon dioxide. Thus a constant $P_{a\text{CO}_2}$ could be maintained by the removal of or the addition of carbon dioxide to the inspired gas mixture (without changes in $V_t$ or respiratory rate).

The $P_{a\text{O}_2}$ was maintained above 23 kPa and variations in $P_{a\text{O}_2}$ above this value would not be expected to cause significant changes in central respiratory activity (Duffin, 1971). Cyanide is known to stimulate chemoreceptors; however, recent work has shown that SNP releases very little, if any, cyanide provided it is not exposed to light before infusion (Bisset et al., 1981).

During artificial ventilation, the induction of hypotension will cause an increase in respiratory drive not only as a result of the increase in $P_{a\text{CO}_2}$, with changes in ventilation: perfusion ratios in the lungs, but as a direct result of the decrease in arterial baroreceptor activity, and this appears to be the first work on this aspect of hypotension induced with vasodilators. In a previous study on the effect of noradrenaline on phrenic nerve activity Garcia and Cherniak (1967) avoided the effects of changes in mechanical ventilation on PNA by making observations during apnoeic oxygenation and they counteracted the effect of the increase in $P_{a\text{CO}_2}$ with Tris buffer. They noted a decrease in PNA in response to large increases in arterial pressure. In another study (Grunstein, Derenne and Milic-Emili, 1975) the effect of transient hypertension lasting only 15–30 s (that is, before any changes in $P_{a\text{CO}_2}$ could occur) was observed. Apart from these studies very little has been done on the potential interactions of, for example, the effects of hypoxia and hypotension or PEEP on central respiratory drive in the intact animal. Physiological studies have concentrated on electrical stimulation of the nerves from the various afferent sources or stimulation of isolated groups of physiological receptors (for example by perfusion) and such studies give little idea of the potential magnitude of changes in the intact animal subject to a normal physiological stimulus.

For example, a carotid sinus nerve contains both baroreceptor and chemoreceptor fibres. Low intensity electrical stimulation of the nerve has been shown to inhibit respiratory activity and is, therefore, assumed to cause selective activation of baroreceptor afferents. High intensity stimulation can be stimulatory and is presumed to have activated afferent fibres from the chemoreceptors, since the latter are smaller in size (Biscoe and Millar, 1968; Sampson, Biscoe and Campion, 1968; Biscoe and Sampson, 1970). However, since the thresholds for these fibre groups are not sharply delineated and considerable overlap must exist, it is doubtful if one can stimulate baroreceptor fibres without recruiting at least some chemoreceptor fibres — particularly if an attempt is made to stimulate all the baroreceptor fibres with trains of electrical stimuli. Thus their inhibitory effects may be, at least in part, offset by excitation from chemoreceptor fibres. Moreover, more recent work has suggested just the opposite: namely, that low intensity stimulation of the carotid sinus nerve selectively stimulates chemoreceptor afferents while having a minimal effect on baroreceptor afferents (Eldridge, Millhorn and Waldrop, 1982), and it is difficult to reconcile the findings of these two groups of workers. Thus the effects of electrical stimulation of the carotid sinus nerve may be difficult to interpret and, whatever the relative fibre sizes and stimulus thresholds of baroreceptor and chemoreceptor fibres, it seems unlikely that one group of afferents can be maximally stimulated without involving the other group. Hence, when a physiological stimulus is required, hypertension or hypotension would seem to be more appropriate.

In the present study the measurements of the changes in respiratory activity used the peak height of the rectified integrated signal from the phrenic nerve. In the spontaneously breathing intact animal, PNA occurs during inspiration and the peak height of the integrated PNA signal correlates well with both muscle force output and ventilation (Eldridge, 1971, 1975, 1976). However, in the artificially ventilated animal, with intact vagus nerves, PNA occurs during the expiratory phase (Tang, Maire and Amassian,
1957; Okada and Fox, 1967), being inhibited by the onset of inspiration occasioned by the inflation of the lungs. Whether the PNA represents an "intended tidal volume" is conjectural. The measurements utilized in this study are a representation of central respiratory activity and, during spontaneous and controlled ventilation, are known to depend on arterial pressure in addition to blood-gas tensions (Garcia and Cherniack, 1967; Cohen and Gootman, 1970; Trippenbach and Milic Emili, 1977; Ledlie et al., 1981).

Various factors may interact to affect activity of the peripheral chemoreceptors which may complicate this simple view of the effects of sodium nitroprusside. If the arterial pressure decreases to very low values, afferent activity from the peripheral chemoreceptors will increase (because of the poor perfusion) (Biscoe and Millar, 1968; Duffin, Triscott and Whitwam, 1976). However, since SNP will cause local dilatation of the vessels of the peripheral chemoreceptors, it could, under conditions of normoxia, remove the background chemoreceptor drive (Biscoe, 1971) which normally accounts for 10–15% of resting ventilation in man and much more during hypoxia (Duffin, 1971). Unlike the condition of hyperoxia in the present study, its local effects in the peripheral chemoreceptors during hypoxia could reduce the drive to ventilation and offset at least in part the effect of the reduced input from the baroreceptors. However, since the $P_{aCO_2}$ increases during hypotension induced with SNP during fixed mechanical ventilation, it seems likely that the balance of effect will be an increased drive to ventilation. When hypotension occurs during haemorrhage these considerations may not apply, since constriction of the vessels in the peripheral chemoreceptors will occurs and both chemoreceptors and baroreceptors will contribute to stimulate ventilation. Miserochi and Quinn (1980) showed that haemorrhage in spontaneously breathing cats anaesthetized with pentobarbitone caused a strong stimulus to ventilation which required the administration of carbon dioxide to maintain a normal $P_{aCO_2}$ in the presence of hyperventilation — findings which would be in keeping with those of the present study. They stated that a reduction in baroreceptor, and an increase in chemoreceptor, activity were responsible for the observed stimulation of respiration. However, the lowest arterial pressures obtained in their preparations were 60 mm Hg — a value greater than that required to modify chemoreceptor drive resulting from decreased blood flow in cats anaesthetized with inhalation agents (Biscoe and Millar, 1968). Their preparation received 50% oxygen in nitrogen and in the four out of 30 in which they measured $PaO_2$, it was "in the range 250 torr". It is possible that local vasoconstriction in the chemoreceptor areas, as suggested above, may have contributed to the perceived increase in ventilation.

In conclusion, the effect of the baroreceptors during acute severe hypertension will be to cause respiratory depression which could make the underlying cause worse. A loss of baroreceptor activity during hypotension (induced hypotension or after blood loss) will tend to increase the drive to respiration and contribute to the observed changes in ventilation in such situations.

REFERENCES

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