Time domains of the hypoxic ventilatory response in awake ducks: episodic and continuous hypoxia

G.S. Mitchell b,*, F.L. Powell c, S.R. Hopkins c, W.K. Milsom a

a Department of Zoology, University of British Columbia, Vancouver, BC, Canada
b Department of Comparative Biosciences, University of Wisconsin, 2015 Linden Drive West, Madison, WI 53706, USA
c Division of Physiology, Department of Medicine, University of California at San Diego, La Jolla, CA, USA

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Abstract

Time-dependent ventilatory responses to episodic and continuous isocapnic hypoxia were measured in unidirectionally ventilated, awake ducks. Three protocols were used: (1) ten 3-min episodes of moderate hypoxia (10% O2) with 5-min normoxic intervals; (2) three 3-min episodes of severe hypoxia (8% O2) with 5-min normoxic intervals; and (3) 30-min of continuous moderate hypoxia. Ventilation (VT) increased immediately within a hypoxic episode (acute response), followed by a further slow rise in VT (short-term potentiation). The peak VT response increased from the first to second moderate hypoxic episode (progressive augmentation), but was unchanged thereafter. During normoxic intervals, VT increased progressively (56% following the tenth episode; long term facilitation). Time-dependent changes were not observed during or following 30-min of continuous hypoxia. Although several time-dependent ventilatory responses to episodic hypoxia are observed in awake ducks, they are relatively small and biased towards facilitation versus inhibitory mechanisms. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Birds; Duck; Chemoreceptors; Hypoxic response; Control of breathing; Hypoxia; Plasticity; Episodic versus continuous hypoxia

1. Introduction

Ventilatory responses to hypoxemia are of considerable interest in birds, particularly given their ability to ascend rapidly to high altitudes during flight (Bouverot, 1985). In mammals, ventilatory responses during or following hypoxia are highly complex, and result from an interplay between several time-dependent facilitatory and inhibitory mechanisms (cf. Bisgard and Neubauer, 1995; Powell et al., 1998).

Mechanisms contributing to ventilatory responses during or following hypoxemia in mammals can be identified by: (i) the time domain of their effects (milliseconds to years), (ii) the direction of their effects (facilitation vs. depression), and (iii) their differential effects on ventilatory pattern (i.e. tidal volume vs. breathing frequency). In many instances, the mechanisms are also spe-
specific to (iv) stimulus paradigm (e.g. intensity, duration and pattern), (v) the nature of the stimulus (e.g. hypoxia versus carotid sinus nerve stimulation) (cf. Eldridge and Millhorn, 1986), and (vi) the animal model (species, conditioning or experience, and the state of consciousness). Finally (vii), mechanisms can be distinguished by the requirement for certain neurotransmitters or modulators.

Based on these criteria, several unique facilitatory mechanisms can be identified in mammals during or following short to intermediate term hypoxic exposures (cf. Eldridge and Millhorn, 1986; Bisgard and Neubauer, 1995; Powell et al., 1998). The Acute Hypoxic Ventilatory Response is manifest as an immediate (within breath) augmentation of breathing. Short Term Potentiation, formerly referred to as ‘afterdischarge’ (Eldridge and Millhorn, 1986; Wagner and Eldridge, 1991), is a further progressive increase in breathing (mainly in VT) occurring over seconds to minutes. Short-term potentiation is also manifest as a slow, post-hypoxic decline in breathing with a time constant of 1–2 min. Progressive Augmentation is a sequential increase in the hypoxic ventilatory response (particularly in VT) with successive hypoxic exposures. Thus far, progressive augmentation has been identified only in anesthetized cats during episodic electrical stimulation of the carotid sinus nerve (Fregosi and Mitchell, 1994) and in awake goats during successive episodes of hypoxia (Turner and Mitchell, 1997). Long Term Facilitation is a persistent, serotonin-dependent augmentation of breathing that lasts for many minutes to hours following episodic hypoxia (cf. McCrimmon et al., 1995).

In addition to mechanisms of facilitation, physiological levels of hypoxia can also exert inhibitory influences on respiratory motor output. Short Term Depression is a rapid decline in frequency during the first 30–60 sec of carotid chemoafferent activation or an undershoot in frequency following chemoafferent activation in anesthetized rats (Hayashi et al., 1993). A similar, and presumably related mechanism, is the Post-Hypoxia Frequency Decline observed at the end of a hypoxic exposure in rats (Coles and Dick, 1996; Bach et al., 1999). Hypoxic Ventilatory Decline is a progressive decrease in breathing that occurs during 2–15 min of continuous hypoxia, even when PaCO₂ is maintained constant. Hypoxic ventilatory decline, also termed ‘roll off’, has been identified in a number of mammalian species, and typically results in diminished tidal volumes (cf. Bisgard and Neubauer, 1995).

Each of these unique mechanisms presumably makes a contribution to the overall ventilatory response during and following hypoxia. However, literature on the avian hypoxic ventilatory response (Bouverot, 1978; Scheid and Piiper, 1986) has not addressed the relative roles of these different time-dependent mechanisms. Thus, one important goal of this study was to determine which, if any, of these short to intermediate time scale mechanisms occur in awake ducks. An awake, unidirectionally ventilated duck model removes several complicating factors that confound interpretation of data obtained in most mammalian studies: (1) unidirectional ventilation produces rapid and precise control of arterial blood gases, thus allowing assessment of ventilatory responses to rapid changes in PaO₂ at constant PaCO₂; and (2) the use of awake animals helps to elucidate the physiological significance of mechanisms most commonly identified in anesthetized preparations.

2. Methods

2.1. Animal preparation and ventilatory measurements

Experiments were performed on 11 unanesthetized, male white Pekin ducks (Anas platyrhynchos) weighing 2.5 ± 0.3 (S.D.) kg. The trachea, interclavicular air sac and the branchial artery in one wing of each animal were cannulated under local anesthesia (2% Xylocaine Hydrochloride). Animals were then lightly restrained in a supine position and unidirectionally ventilated with gas entering the tracheal cannula, passing through the lungs, and exiting via the interclavicular air sac cannula. Birds were unidirectionally ventilated with humidified gas containing 4% CO₂ at 3 L/min. This CO₂ level produced normal PaCO₂ values and a spontaneous breathing pattern similar to spontaneously breathing ducks (Bouverot
et al., 1974; Powell et al., 1978). The flow rate was chosen to maintain continuous unidirectional flow through the lungs during the maximum inspiratory efforts produced by the hypoxic and hypercapnic exposures. With such flows, ventilatory efforts could not alter lung gas composition in this preparation, so parabronchial and arterial $P_{O_2}$ and $P_{CO_2}$ were rigorously maintained. Unidirectional gas flow exiting the bird through the interclavicular airsac cannula was continuously sampled for $O_2$ and $CO_2$ (OM-11 and LB-2, respectively, Beckman Instruments). Cloacal temperature was measured and maintained at 40–41°C with a servo-controlled heating pad and lamp.

Spontaneous ventilation was measured using a pneumotachograph (Fleisch #0) connected to a differential pressure transducer (MP45, Validyne) situated between the inspired gas line and the tracheal cannula. The pressure signal was amplified (Gould DC Amplifier), the bias flow was offset electronically, and the resulting signal was integrated (Gould Integrating Amplifier) to yield a signal proportional to tidal volume ($VT$). Ventilatory flow rate, $VT$, $F_{O_2}$ and $F_{CO_2}$ of the gas exiting the interclavicular airsac, and arterial pressure were recorded continuously on a chart recorder (Gould Instruments).

2.2. Experimental protocols

Four different protocols were used in these experiments: three experimental protocols and one sham protocol.

**Episodic moderate hypoxia** was studied in eight birds exposed to ten 3-min episodes of hypoxia ($F_{O_2} = 0.10$) separated by 5-min of normoxia. Inspired gases were changed by switching between pressure balanced gas lines delivering either air or the hypoxic gas; $CO_2$ was added to the inspired gas line downstream of the switching valve such that $F_{CO_2}$ was unaffected by inspired gas changes. With this setup, $F_{O_2}$ changed within a single breath and $F_{CO_2}$ remained constant. Ventilation and expired gases were measured continuously throughout this 80-min protocol, and for another hour following the last hypoxic episode. A series of sham experiments was performed on three birds, using the identical procedure except that the switch was made between pressure balanced gas lines, both of which delivered air (i.e. normoxia).

**Episodic severe hypoxia** was studied in seven birds after they had completed either the moderate hypoxia or sham protocol. Ducks were exposed to three 3-min hypoxic episodes ($F_{O_2} = 0.08$) separated by 5-min normoxic intervals. After the final hypoxic episode, ventilation was measured for 1 h.

**Sustained moderate hypoxia** was studied in a third series of four ducks exposed to 30-min of hypoxia ($F_{O_2} = 0.10$). This level and duration of hypoxia was chosen to be comparable to the total hypoxic exposure in the episodic moderate hypoxia series. Measurements continued for 20-min following the return to normoxia.

At the end of each experiment, **maximum tidal volumes** were assessed in each bird by administering 10% $CO_2$ in air for 10-min.

2.3. Arterial blood gases

An arterial cannula was used to measure arterial pressure (R1000, Biotech) and withdraw 1-ml blood samples for $P_{O_2}$, $P_{CO_2}$ and pH analysis (Model PHM 71, Radiometer). Residual blood left after the analysis (ca. 0.4-ml) was returned to the animals.

In the episodic moderate hypoxia protocols, arterial blood samples were taken immediately before the first and fifth hypoxic episodes, during the last 30 sec of the first, fifth and tenth hypoxic episodes, immediately after the tenth hypoxic episode, and at 20, 40 and 60 min following the last hypoxic episode. Arterial samples were taken at the corresponding times in sham experiments. During episodic severe hypoxia protocols, arterial samples were drawn immediately before the first hypoxic episode, during the last 30-sec of the second hypoxic episode, and at 10 and 30-min following the last hypoxic episode. In the sustained moderate hypoxia protocol, arterial samples were taken, on average at: 30 and 90 sec, 4, 10, 20 and 30 min after the switch to hypoxia, and 30 and 90 sec, and 4, 10 and 20 min after the return to normoxia.
2.4. Data analysis

For episodic and sham protocols, ventilation and its components (VT, fR and VI) were averaged over 30-sec intervals for a period beginning 5-min before the first hypoxic episode (or sham hypoxic episode), and extending until 5-min after the last hypoxic episode. A more detailed analysis was also performed on data obtained during the first and last hypoxic episodes, with averages calculated for the first 10-sec, and 11–30 sec after the onset of hypoxia. Breathing pattern was analyzed over 3-min intervals centered at 20, 40 and 60 min after the last hypoxic episode in the episodic moderate hypoxia protocol, and at 10 and 30 min post-hypoxia in the episodic severe hypoxia protocol. In the sustained moderate hypoxia experiments, breathing pattern was averaged over 30 sec intervals centered 30 sec, 2.5, 5, 10, 20 and 30 min after the onset of hypoxia, and 6 and 15 min after the return to normoxia.

The statistical significance of time-dependent changes in variables was assessed via repeated measures ANOVA. Individual comparisons were made using T-tests with the Bonferroni correction for multiple comparisons. *P* < 0.05 was considered significant. Reported values are means ± S.E.

3. Results

Fig. 1 shows the tidal volume traces for the first and fifth hypoxic episodes in a single bird during the episodic moderate hypoxia protocol; a similar trace is shown at 30-min following the tenth and final hypoxic exposure. Also shown are the rapid change in PO2 and constant level of PCO2 in the gas leaving the lung. Fig. 1 illustrates that: (i) the magnitude of the ventilatory response increased throughout the hypoxic episode; (ii) ventilation did not return to control conditions during the normoxic intervals between hypoxic episodes; (iii) the ventilatory response increased during successive hypoxic episodes; and (iv) ventilation still had not returned to control conditions 30-min following the tenth hypoxic episode. These trends from a single bird are analyzed quantitatively below.

3.1. Arterial blood gases

Table 1 summarizes the arterial blood gases and pHa for episodic hypoxia and sham experiments. Average PaO2 levels for all measurements in all protocols were 98.1 ± 0.8 Torr in normoxia, 59.5 ± 0.7 Torr in moderate hypoxia and 43.5 ± 2.1 Torr in severe hypoxia. Average PaCO2 and pHa levels were 30.5 ± 0.2 Torr and 7.445 ± 0.007, respectively.

3.2. Ventilatory responses within an episode of moderate hypoxia/normoxia

Fig. 2 (left) shows the changes in VI, VT and fR during the first episode of moderate hypoxia. Each of these variables increased during the first 10-sec of hypoxia, and tended to increase further...
throughout this short hypoxic exposure. However, the only significant difference within the first hypoxic episode was in $V_{I}$ and $f_R$ between the first 10-sec and last 30-sec of the episode. Ventilatory variables decreased within 10-sec on return to normoxia after the first hypoxic episode. However, the values measured during the first 10-sec of normoxia remained significantly greater than those measured between 30-sec and 5-min of normoxia. This decay towards pre-hypoxic control values beyond 10-sec may reflect short-term potentiation. There was no detectable undershoot in $f_R$ suggesting an absence of short-term depression or post-hypoxia frequency decline.

3.3. Ventilatory responses in successive episodes of moderate hypoxia/normoxia

Fig. 2 (right) shows the changes in $V_{I}$, $V_T$ and $f_R$ during the tenth episode of moderate hypoxia. While the average response was similar to the first hypoxic episode, there were subtle differences. The acute hypoxic response approached a plateau more rapidly during the tenth versus the first episode. Also, $V_T$ and $V_{I}$ near the end of the tenth hypoxic episode were significantly elevated relative to the 10 and 30-sec points, whereas $V_T$ was not elevated relative to the 10 and 30-sec point in the first episode.

Fig. 3 shows mean values of ventilatory variables recorded during the last 30-sec of each hypoxic episode, and during successive normoxic intervals. There was a significant and progressive increase in $V_T$ during successive hypoxic episodes (i.e. progressive augmentation), with a nonsignificant trend towards similar progressive increases in $V_{I}$ ($P = 0.07$). However, the increase in hypoxic $V_T$ occurred mainly between the first and second hypoxic episodes; with little further change between the second and tenth episodes.

Tidal volume increased progressively during normoxic intervals between hypoxic episodes, evidence for the development of long term facilitation (Fig. 3). Because the mean level of $V_T$ continued to increase during successive normoxic intervals, and the response during hypoxia attained a stable value after the second episode, the overall hypoxic $V_T$ response (hypoxic-normoxic values) diminished with successive hypoxic episodes.

Fig. 3 also illustrates the time-course of recovery of long term facilitation after the final hypoxic episode. After the last hypoxic episode, frequency returned to baseline levels almost immediately, but there were strong trends for $V_{I}$ ($P = 0.07$) and $V_T$ ($P = 0.08$) to remain elevated above baseline levels. $V_T$ was significantly greater than baseline values 5-min after the last hypoxic bout with a

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**Table 1**

Average ($\pm$ S.E.M.) arterial blood gases and pH measured in normoxic baseline conditions before hypoxia (baseline)*

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Condition</th>
<th>$F_{I,O_2}$</th>
<th>$P_{O_2}$ (Torr)</th>
<th>$P_{CO_2}$ (Torr)</th>
<th>pHa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Episodic moderate hypoxia</td>
<td>Baseline</td>
<td>0.21</td>
<td>100.0 ± 1.2</td>
<td>30.1 ± 0.4</td>
<td>7.46 ± 0.01</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>hpx 1st</td>
<td>0.10</td>
<td>60.7 ± 1.2</td>
<td>30.4 ± 0.4</td>
<td>7.46 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Air 4th</td>
<td>0.21</td>
<td>97.6 ± 1.4</td>
<td>30.9 ± 0.6</td>
<td>7.47 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>hpx 5th</td>
<td>0.10</td>
<td>60.3 ± 1.2</td>
<td>30.7 ± 0.7</td>
<td>7.47 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>hpx 10th</td>
<td>0.10</td>
<td>57.4 ± 0.8</td>
<td>31.1 ± 0.7</td>
<td>7.44 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>Air 10 min</td>
<td>0.21</td>
<td>99.0 ± 1.8</td>
<td>30.4 ± 0.8</td>
<td>7.44 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Air 30 min</td>
<td>0.21</td>
<td>101.0 ± 2.4</td>
<td>30.6 ± 0.6</td>
<td>7.44 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Air 60 min</td>
<td>0.21</td>
<td>97.0 ± 0.6</td>
<td>30.4 ± 0.6</td>
<td>7.45 ± 0.02</td>
</tr>
<tr>
<td>Episodic severe hypoxia</td>
<td>Baseline</td>
<td>0.21</td>
<td>96.8 ± 1.3</td>
<td>30.5 ± 1.5</td>
<td>7.46 ± 0.03</td>
</tr>
<tr>
<td>(n = 3)</td>
<td>hpx 1st</td>
<td>0.10</td>
<td>41.1 ± 1.3</td>
<td>30.4 ± 0.5</td>
<td>7.38 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>air 30 min</td>
<td>0.21</td>
<td>96.5 ± 1.0</td>
<td>29.7 ± 1.0</td>
<td>7.48 ± 0.02</td>
</tr>
</tbody>
</table>

* Selected bouts of episodic hypoxia (hpx 1st, etc.) and normoxia (air 4th, etc.), and normoxic recovery at selected times after the last bout of hypoxia (air 10 min, etc.).
paired $t$-test, but individual variability between ducks prevented this effect from being significant by a repeated measures ANOVA of normoxic values before, and 5, 10, 40 and 60 min after hypoxia. Results were the same when ventilatory variables were expressed in absolute terms, or normalized to the maximum values obtained during the CO$_2$ challenge.

Fig. 2. Time course of ventilatory response to first (left panel) and tenth (right panel) episodes of episodic moderate hypoxia. First point (0 min) is normoxic average prior to hypoxia, next point is average during first 10 sec of hypoxia, followed by average values for every 30-sec of hypoxia to 3 min. Similarly, the first point after 3 min is the 10 sec average after restoring normoxia, followed by 30-sec averages for 3–5 min of normoxia. Significant differences between the 10 sec point and subsequent points, in either hypoxia or normoxia, are indicated by filled symbols ($P < 0.05$). Asterisks ($P < 0.05$) indicate significant differences between the 30-sec point and later points. Data were not available for all ducks after 3 min of normoxic recovery from the first hypoxic bout (left panel, 6–8 min), so these points were not tested statistically.
Fig. 3. Average ventilatory values measured during the last 30 sec of ten moderate hypoxia episodes and normoxia before, between and 60 min after hypoxic bouts. Filled symbols are significantly different from the first value measured at same FIO2 (P < 0.05). Progressive augmentation is demonstrated by significantly greater VT responses in later hypoxic bouts. Long term facilitation of VT and VI during normoxia was not significant.

3.4. Ventilatory responses to episodic severe hypoxia

All but one of the ducks in this series had previously experienced the episodic moderate hypoxia or sham protocol. There were no differences based on which protocol the birds had experienced and, therefore, the results have been pooled. Fig. 4 shows ventilatory changes during the third and final hypoxic episodes. Compared to responses during moderate hypoxia (Fig. 2), short-term potentiation of VT was both greater in
magnitude and longer in duration. Ventilation increased significantly following the acute response because of a significant increase in frequency after the 30-sec point (Fig. 4). All variables remained significantly elevated from baseline at 30-sec post-hypoxia, and VT was still significantly elevated 60-sec after return to normoxia.

Progressive augmentation was not apparent in ventilatory responses to successive, severe hypoxic episodes, but it should be noted that the VT attained in the first episode of severe hypoxia was very near the maximum measured during the CO₂ challenge (94% maximum). Long term facilitation was evident in the normoxic intervals between hypoxic episodes for VT, but not V̇₁ (P = 0.11) (Fig. 5). Long term facilitation was significant 10-min after the final hypoxic episode, but was no longer different from baseline at 30-min (Fig. 5).

3.5. Ventilatory responses during sustained moderate hypoxia

There was no evidence for short-term depression or post-hypoxia frequency decline at any time during or following a severe hypoxic episode. During 30 min of sustained hypoxia, there was a nonsignificant trend for a progressive increase in V̇₁ (Fig. 6). When normoxia was restored after sustained hypoxia, VT and V̇₁ returned immediately to control values. Therefore, in contrast to some mammals, there was no clear evidence for hypoxic ventilatory decline or post-hypoxia frequency decline during or following 30 min of continuous moderate hypoxia. Furthermore, as in mammals, there was no evidence for long term facilitation following 30 min of continuous hypoxia.

4. Discussion

This study is the first to demonstrate the existence of facilitatory, time-dependent responses to episodic hypoxia in birds. However, short-term potentiation, progressive augmentation and long term facilitation are all less robust in awake ducks versus anesthetized mammalian species with episodic hypoxemia or carotid sinus nerve stimulation (cf. Eldridge and Millhorn, 1986; Hayashi et al., 1993; Fregosi and Mitchell, 1994; Bach and Mitchell, 1996). The expression of these mechanisms in awake ducks is more similar to awake humans or goats exposed to episodic hypoxia (Engwall and Bisgard, 1990; Engwall et al., 1991;
Fregosi, 1991; Dwinell et al., 1997; Turner and Mitchell, 1997), although long term facilitation was less robust than reported in awake dogs (Cao et al., 1992). One cannot be certain that these time-dependent ventilatory responses in ducks result from mechanisms identical to those described in mammals since they were identified based solely on the stimulus protocol, their respective time domains and the ventilatory pattern changes involved. No effort was made to identify specific neurochemical involvement (cf. Powell et al., 1998).

No evidence was found for inhibitory ‘memories’ analogous to short term depression (and post-hypoxia frequency decline) or hypoxic ventilatory decline, even when hypoxemia was prolonged for 30 min. These results indicate that awake ducks, like many mammalian species, exhibit a complex array of ventilatory responses during and following hypoxia. These responses may impart a degree of plasticity or adaptability to the avian respiratory control system, although the effects do not appear to be large.

4.1. Experimental model

The study of time-dependent ventilatory responses in awake, unidirectionally ventilated ducks has several powerful experimental advantages. First, unidirectional ventilation allows rapid and precise control of arterial blood gas composition such that ventilatory responses to hypoxemia are not obscured by coincident variations in intrapulmonary or arterial PCO2. Rapid changes in oxygen levels without simultaneous changes in arterial PCO2 are difficult to achieve in a spontaneously breathing mammal. Thus, most studies resort to anesthetized, paralyzed and vagotomized mammals to overcome this limitation (cf. Eldridge and Millhorn, 1986). It should be emphasized that ducks investigated here were not anesthetized, paralyzed or vagotomized. Second, it is extremely difficult to change arterial PO2 with kinetics sufficient rapid to detect short time-scale mechanisms such as short-term depression. Unidirectional ventilation allows experimental changes in arterial PO2 within one to two breaths, providing superior resolution of short time scale mechanisms. In the present study, it was documented that changes in arterial PO2 were complete within 30-sec, with the majority of the change completed in 10-sec.

An additional feature inherent to using birds as an experimental model is that the distribution of

![Graph of ventilatory responses to 30 min of sustained moderate hypoxia.](image-url)
motor outflow during or following hypoxemia is different from mammals. In most studies using mammals, time domains of the ventilatory response to hypoxia or carotid sinus nerve stimulation are monitored in phrenic motor output. However, there is heterogeneity between different inspiratory motor outputs in mammals. For example, long term facilitation is larger in inspiratory intercostal versus phrenic nerve activities in cats (Fregosi and Mitchell, 1994). Since birds do not have a diaphragm, time dependent responses must result from mechanisms that invoke other (primarily intercostal) motor outputs.

4.2. Facilitatory time dependent ventilatory responses

4.2.1. Acute response and short term potentiation

The acute response to hypoxia in ducks is similar to that seen in other avian species and mammals (Bouverot, 1978). The acute response represents the immediate response of the carotid body chemoreceptors to hypoxia. Neural pathways mediating this response may underlie mechanisms operational in longer time domains. For example, neuromodulators co-released with, or neuromodulatory influences mediated by different receptors for the same transmitter substance could translate an acute response into longer actions, such as short-term potentiation.

Awake ducks exhibit short-term potentiation during and following hypoxia with a time course that is relatively short when compared with anesthetized or decerebrate mammals (cf. Eldridge and Millhorn, 1986). However, short-term potentiation in awake ducks is similar to that seen in awake humans (cf. Fregosi, 1991) and goats (Engwall et al., 1991). Although the mechanism of short-term potentiation is unknown in any species, its magnitude depends on stimulus intensity (Wagner and Eldridge, 1991). Similarly, short-term potentiation in ducks exhibited some dependence on (hypoxic) stimulus intensity. Short-term potentiation may play a smoothing role in the control of breathing, prompting system stability during reflex activation of the respiratory system (Eldridge and Millhorn, 1986). A similar function might be expected in birds, although the short time constant may limit its effectiveness in the absence of powerful sensory stimuli.

4.2.2. Progressive augmentation

Progressive augmentation has been demonstrated previously only in anesthetized cats during carotid sinus nerve stimulation (Fregosi and Mitchell, 1994) and awake goats during episodic hypoxia (Turner and Mitchell, 1997). In anesthetized cats, progressive augmentation was evident only in the inspiratory intercostal nerve activity, but not in the integrated phrenic neurogram. This difference may arise because inspiratory intercostal nerve activity is only 60–70% of maximal activation during the first stimulus episode whereas the phrenic neurogram is already more than 90% of maximal activity during the same stimulus episode (Fregosi and Mitchell, 1994). Thus, neural saturation may mask progressive augmentation in the phrenic nerve under normal circumstances since progressive augmentation of this motor output was revealed only after the animal was pretreated with the serotonin receptor antagonist methysergide. Methysergide decreased phrenic activation during the first stimulus episode as a percentage of maximal activity, thus allowing the manifestation of progressive augmentation. It appears that a similar mechanism may have masked progressive augmentation during severe hypoxic episodes in the current study since tidal volume was in excess of 90% maximum during the first hypoxic episode, leaving little opportunity for further increase. However, during the moderate episodic hypoxia protocol, progressive augmentation was observed. Although the mechanism underlying progressive augmentation is unknown, it does not require serotonin receptor activation, at least in mammals, and therefore is distinct from long term facilitation.

4.2.3. Long term facilitation

The progressive increase in ventilatory activity observed during normoxic intervals between hypoxic episodes is indicative of long term facilitation (Millhorn et al., 1980a,b; Eldridge and-
Millhorn, 1986; McCrimmon et al., 1995). However, by 20-min post-hypoxia, long term facilitation was no longer significant. Four ducks showed a very robust long-term facilitation at this time point whereas the other four did not. Thus, it appears that long term facilitation is relatively weak, or inconsistent, in awake ducks relative to anesthetized cats following episodic carotid sinus nerve stimulation (Millhorn et al., 1980a) or anesthetized rats after episodic hypoxia (Hayashi et al., 1993; Bach and Mitchell, 1996). The observation that facilitatory time-dependent mechanisms are less robust in awake ducks and mammals relative to anesthetized, vagotomized mammals may be explained either by the properties of serotonergic raphe neurons in awake animals or the influence of intact vagus nerves. Mateika and Fregosi (1997) reported that intact vagus nerves suppress long term facilitation in anesthetized, spontaneously breathing cats, possibly due to inhibitory vagal memories (cf. Eldridge and Millhorn, 1986) that offset long term facilitation. Alternately, raphe neurons discharge at near maximal rates in awake cats (Jacobs and Azmitia, 1992). Thus, although respiratory stimulation can increase the discharge of at least some raphe neurons in awake mammals (Veasey et al., 1995), the proportionate increase may be small relative to that in sleeping or anesthetized animals during chemoreceptor activation. It is unclear to what extent either effect influences time dependent hypoxic ventilatory responses in awake, unidirectionally ventilated ducks.

In mammals, long term facilitation requires the neuromodulator serotonin (Millhorn et al., 1980b; Fregosi and Mitchell, 1994; McCrimmon et al., 1995; Bach and Mitchell, 1996). When a serotonin receptor antagonist (methysergide) is applied prior to episodic hypoxia, the progressive build up of respiratory activity between episodes of carotid sinus nerve stimulation or hypoxemia is abolished, and post-stimulation or post-hypoxia facilitation is eliminated. Although it is likely that similar mechanisms are operational during long-term facilitation in ducks, this was not verified in the current study since serotonin receptor antagonists were not applied.

4.3. Physiological significance

Different time domains in hypoxic ventilatory responsiveness may impart a degree of flexibility or plasticity to the avian respiratory control system. The presence of facilitatory mechanisms, with an apparent lack of inhibitory mechanisms such as hypoxic ventilatory decline may be of some adaptive advantage to an animal class that commonly experiences hypoxemia. It may be highly beneficial to release such ‘brakes’ so that an optimal level of ventilation is achieved during severe or sustained hypoxia. On the other hand, it may be that the normal physiological role of facilitatory time-dependent mechanisms is to offset or ‘neutralize’ inhibitory mechanisms that are an unavoidable consequence of hypoxia in mammals. In an avian species lacking these inhibitory mechanisms, well-developed facilitatory mechanisms may be less important, thus explaining the relatively weak progressive augmentation and long term facilitation observed in these awake ducks. These hypotheses await experimental verification.

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