A test of the oxidative damage hypothesis for discontinuous gas exchange in the locust Locusta migratoria

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The discontinuous gas exchange cycle (DGC) is a breathing pattern displayed by many insects, characterized by periodic breath-holding and intermittently low tracheal O2 levels. It has been hypothesized that the adaptive value of DGCs is to reduce oxidative damage, with low tracheal O2 partial pressures (PO2 ~2–5 kPa) occurring to reduce the production of oxygen free radicals. If this is so, insects displaying DGCs should continue to actively defend a low tracheal PO2 even when breathing higher than atmospheric levels of oxygen (hyperoxia). This behaviour has been observed in moth pupae exposed to ambient PO2 up to 50 kPa. To test this observation in adult insects, we implanted fibre-optic oxygen optodes within the tracheal systems of adult migratory locusts Locusta migratoria exposed to normoxia, hypoxia and hyperoxia. In normoxic and hypoxic atmospheres, the minimum tracheal PO2 that occurred during DGCs varied between 3.4 and 1.2 kPa. In hyperoxia up to 40.5 kPa, the minimum tracheal PO2 achieved during a DGC exceeded 30 kPa, increasing with ambient levels. These results are consistent with a respiratory control mechanism that functions to satisfy O2 requirements by maintaining PO2 above a critical level, not defend against high levels of O2.

Keywords: discontinuous gas exchange; insect; hyperoxia

1. INTRODUCTION

Many insects at rest breathe using the discontinuous gas exchange cycle (DGC): an alternating pattern of gas exchange and breath-holding. This behaviour is known to occur among at least five [1], and possibly as many as seven [2], insect orders. It is characterized by the sequential repetition of three phases: a ‘closed phase’ when the spiracles are shut and tracheal PO2 drops continuously while PCO2 rises; a ‘flutter phase’ when the spiracles rapidly open and close releasing minimal CO2 while admitting some O2 to the tracheal system; and an ‘open phase’ when both CO2 and O2 move freely between the atmosphere and tracheal system. Large fluctuations in tracheal PO2, varying from near atmospheric (approx. 20 kPa) to severely hypoxic (2–5 kPa), are a peculiar characteristic of this pattern. The potential adaptive significance of the DGC has led to much debate, with suggestions that it is adaptive to reduce oxidative damage (oxidative damage hypothesis [3]), to reduce respiratory water loss (hygric hypothesis, [4]), or as a consequence of neural downregulation affecting respiratory control (neural hypothesis, [5]). The hygric and neural hypotheses both assume that the low tracheal PO2 during the closed and flutter phases occurs inadvertently, either as a consequence of protracted spiracle closure reducing respiratory water loss, or due to a change in the behaviour of the insect’s respiratory control system. The oxidative damage hypothesis is based on the assumption that the DGCs primary function is the production of a low tracheal PO2 during the closed phase that is then maintained during the flutter phase [3,6–8].

Oxygen is a toxic molecule, capable of damaging tissues through the production of reactive oxygen species (ROS) [9]. According to the oxidative damage hypothesis, DGCs evolved as a mechanism to reduce tracheal PO2 periodically when the insect’s metabolic rate is low, thereby lowering ROS production. The respiratory behaviour of moth pupae supports this idea, as they regulate their tracheal PO2 at approximately 5 kPa during the flutter phase of their DGC, even in hyperoxic atmospheres up to 50 kPa [3]. While the hygric and neural hypotheses do not require the maintenance of a low PO2 during DGCs, the question remains: is a low and constant tracheal PO2 during the flutter phase a universal feature of DGCs displayed by adult insects? Pupae are physiologically atypical, as they represent a life stage characterized not only by a very low metabolic rate [10], but also reduced neurological functions [11].

To determine whether tracheal PO2 during the flutter phase of a DGC is independent of ambient PO2 in adult insects, we implanted fibre-optic oxygen optodes into the tracheal systems of adult locusts exposed to hyperoxic, normoxic and hypoxic atmospheres.

2. MATERIAL AND METHODS

Adult migratory locusts Locusta migratoria (810 ± 72 mg) were reared at the University of Adelaide. Locusts were maintained in plastic terraria, at 33 ± 1°C, approximately 30 per cent RH, 12 L:12 D cycle and ad libitum food. They were fasted 24 h prior to gas exchange measurements and weighed to 0.1 mg (AE163, Mettler, Greifensee, Switzerland) immediately before each experiment. Flow-through respirometry was performed at 22–23°C using a LI-820 CO2 analyser (Li-COR Biosciences, Lincoln, NE, USA) and gas mixes produced by mass flow controllers (Aalborg q¼ n× 4) connected to cylinders of compressed O2 and N2. Gas mix composition was verified using a calibrated Oxzilla II oxygen analyser (Sable Systems, Nevada, USA).

Two groups of locusts were measured: a control group and a treatment group. Individuals in the control group were placed within 10 ml syringe barrels and gas exchange was measured over a 2–8 h period following 1 h of acclimation. They were measured in normoxia (n = 9) using outside air scrubbed of CO2 and water vapour, as well as hypoxia (7 kPa; n = 4) and hyperoxia (40 kPa; n = 4). Briefly, the gas mix was regulated at 350 ml min–1 using a mass flow controller, passed through the syringe containing the resting locust, through a small column of desiccant, and into the CO2 analyser. The analogue outputs of the mass flow controller and CO2 analyser were recorded at 1 s intervals to a computer with a

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for all treatments was 29.8 min. The only significant effect of ambient PO$_2$ on mean DGC duration was found between 21.3 kPa (33.2 ± 4.1 min) and 40.5 kPa PO$_2$ (18.3 ± 2.4 min; $p < 0.05$). The reduced duration in 40.5 kPa is attributable to a decrease in the closed and open durations and the virtual elimination of the flutter phase (figure 1). The lowest tracheal PO$_2$ during a DGC occurred at the transition from the closed to the flutter phase (figure 2). This minimum PO$_2$ varied between 3.41 ± 0.43 kPa in normoxia, dropping slightly to 3.13 ± 1.52 and 1.21 ± 0.22 kPa in atmospheres between 15.2 and 5.1 kPa, respectively (figure 1). In hyperoxia, however, the minimum tracheal PO$_2$ increased with ambient PO$_2$ with a slope of 1.38, rising to 17.66 ± 1.56 kPa in 30.4 kPa O$_2$, and 30.10 ± 1.21 kPa in 40.5 kPa O$_2$ (figure 1).

4. DISCUSSION

The minimum tracheal PO$_2$ in adult locusts displaying DGCs is dependent on ambient PO$_2$ in hyperoxia (figure 1). This is in contrast to a tracheal PO$_2$ maintained at low levels in hyperoxia, as expected by the oxidative damage hypothesis [3]. Why then do moth pupae display a consistently low tracheal PO$_2$ in hyperoxia during their flutter phase, when locusts do not? The answer lies in the relative tracheal and body fluid volumes of the two insects. Current research indicates that spiracles flutter open and closed in response to internal hypoxia and open fully in response to high CO$_2$ [12,13]. Owing to its high solubility, most of the CO$_2$ produced during the closed and flutter phases of the DGC is sequestered in the insect’s body fluids, while the bulk of the O$_2$ used during the closed phase is obtained from air in the tracheal system [14,15]. Therefore, a flutter phase and its associated low and stable PO$_2$ can occur only if the tracheal O$_2$ store is depleted to a hypoxic threshold before CO$_2$ accumulation in the body fluids initiates an open phase. This is determined by the ratio of the insect’s O$_2$ store (tracheal system volume) to its CO$_2$ sink (body fluid volume and buffer capacity).

Moth pupae possess a tracheal system that contains approximately 140 μl of air per gram of body mass, while a locust’s tracheal system holds approximately 310 μl. g$^{-1}$ (for references, see the electronic supplementary material). As such, moth pupae have approximately half the volume of O$_2$ on which to draw during the closed phase of their DGC relative to their CO$_2$ sink (body fluid volume; proportional to mass) compared with a locust. Additionally, moth pupae have higher whole body buffer capacity (75 mmol HCO$_3^-$ pH$^{-1}$ kg$^{-1}$) than those of a locust (28–43 mmol HCO$_3^-$ pH$^{-1}$ kg$^{-1}$; electronic supplementary material) and, therefore, have a greater capacity to store CO$_2$ relative to pH change. These differences mean that a pupa in hyperoxia can deplete its tracheal O$_2$ reserve to approximately 5 kPa during the closed phase of its DGC and transition to the hypoxia-initiated flutter phase before it has accumulated sufficient CO$_2$ to initiate an open phase. This accounts for the consistently low tracheal PO$_2$ displayed by moth pupae in hyperoxic atmospheres. In contrast, a locust's substantial O$_2$ reserve in hyperoxia
Table 1. Effect of ambient $P_O_2$ on the mean closed, flutter and open-phase durations (± 95% CI) of DGCs displayed by adult locusts.

<table>
<thead>
<tr>
<th>ambient $P_O_2$ (kPa)</th>
<th>closed (min)</th>
<th>flutter (min)</th>
<th>open (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypoxia</td>
<td>5.5</td>
<td>5.4</td>
<td>10.8</td>
</tr>
<tr>
<td>7</td>
<td>6.5 ± 0.9</td>
<td>13.2 ± 3.6</td>
<td>15.7 ± 4.6</td>
</tr>
<tr>
<td>10</td>
<td>12.1 ± 0.5</td>
<td>4.5 ± 5.3</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>15</td>
<td>18.8 ± 0.2</td>
<td>5.7 ± 0.2</td>
<td>8.7 ± 1.7</td>
</tr>
<tr>
<td>normoxia</td>
<td>14.5 ± 1.5</td>
<td>3.5 ± 0.9</td>
<td>10.7 ± 2.1</td>
</tr>
<tr>
<td>hyperoxia</td>
<td>15.8 ± 0.6</td>
<td>1.6 ± 1.6</td>
<td>6.1 ± 2.5</td>
</tr>
<tr>
<td>30</td>
<td>11.7 ± 1.4</td>
<td>1.4 ± 0.6</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

means that its tracheal $P_O_2$ cannot drop to the hypoxic flutter threshold during the closed phase before excessive accumulation of CO$_2$ initiates an open phase. This ensures that the locust’s tracheal $P_O_2$ remains close to ambient levels (figures 1 and 2). We conclude that the apparent independence of tracheal $P_O_2$ in moth pupae can be explained as the by-product of a comparatively small tracheal volume coupled with a large CO$_2$ buffer, and the interaction between a hypoxic threshold initiating the flutter phase and a high CO$_2$ threshold initiating the open phase.

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