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Respiratory recovery following organophosphate poisoning in a rat model is suppressed by isolated hypoxia at the point of apnea

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Abstract

Normal respiratory activity (eupnea) and gasping represent different types of respiratory activity, one of which is supported by oxygen (eupnea) and the other suppressed by oxygen (gasping). There is a loss of respiratory activity post-organophosphate (OP) poisoning that returns following treatment. It is not clear if post-OP respiratory activity represents eupnea or gasping. Depending on the type of respiratory activity, oxygenation during recovery from OP poisoning may have the potential to either support or suppress respiratory activity. We hypothesize that respiratory recovery following OP-induced central apnea represents a resumption of eupnea and is supported by oxygenation. We used an animal model of acute OP poisoning with detailed physiologic recordings. Animals were poisoned with dichlorvos and allowed to recover during a period of mechanical ventilation. Two experimental models were analyzed, (1) animals supported with 100% oxygen and (2) animals supported with a normoxic gas mixture titrated to a PaO₂ of 115mmHg. Rats in this study demonstrated breathing that resumes spontaneously following OP-induced apnea with characteristics of both eupnea and gasping. The post-OP respiratory activity was suppressed by hypoxia, a characteristic of eupneic respiration and not gasping respiration. However, the respiratory rate during post-apneic breathing corresponded more closely to gasping. Analysis of phrenic nerve discharge activity was distinct from both eupnea and gasping, with peak inspiratory and post-inspiratory discharge activities significantly reduced compared to both eupnea and gasping. In summary, in this animal model post-apneic breathing distinct from eupnea and gasping that emerges following prolonged OP-induced central apnea is suppressed by hypoxia.

Keywords

Organophosphate; gasping; respiratory control; apnea

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1.0 Introduction

Acute organophosphate poisoning (OP) causes progressive respiratory failure that is typically treated with medications with or without mechanical ventilation. Animal studies from our lab demonstrate that lethal doses of OP cause progressive central hypoventilation followed by apnea. In some animals this is followed much later by a spontaneous recovery of respiratory activity (Gaspari and Paydarfar, 2007a). The goal of mechanical ventilation in the clinical scenario of acute OP poisoning is to support tissue oxygenation until respiratory activity and pulmonary function normalize. Depending on the type of respiratory activity, different levels of oxygenation have the potential to either support or suppress respiratory activity. The effect of oxygenation on recovery following OP-induced central respiratory failure has not been well studied and it is possible that increased oxygenation may be counterproductive to recovery of central respiratory control after OP exposure.

The two forms of respiration and the different effect of oxygenation is demonstrated by the respiratory activity that follows cardiac arrest. Cardiac arrest from a myocardial infarction causes progressive hypoxic-ischemic depression of neural activity, culminating in central apnea due to depression of brainstem neurons that generate eupneic respiratory rhythm (Nava and Bellemare, 1989; St John *et al.*, 1989). As hypoxia progresses to anoxia, breathing activity can resume in the form of gasping induced by anoxic excitation of brainstem circuits (Guntheroth and Kawabori, 1975; St John *et al.*, 1989). The neural circuitry that produces eupneic respiration is supported by oxygen and suppressed by anoxia, but the neurons that produce gasping require anoxia and are suppressed with increased oxygen levels.

It is not clear if the recovery of respiration following acute OP-induced apnea represents resumption of eupneic respiratory activity or emergence of gasping respiratory activity. In the present study, we analyze OP associated post-apneic breathing by characterizing its response to oxygen levels and by comparing its phrenic nerve activity to that of eupneic breaths. We hypothesize that the post-apneic respiratory activity represents recovery of eupneic respiratory activity and therefore increasing oxygenation following OP-induced apnea will support the resumption of respiratory activity.

2.0 MATERIALS AND METHODS

The University of Massachusetts Medical School Institutional Animal Care and Utilization Committee approved all experimental procedures and protocols.

2.1 General Preparation

The animal model and experimental methodology used in this study have been published previously (Gaspari and Paydarfar, 2007b). Briefly, 17 adult male Wistar rats (280–350 gms) were anesthetized using isoflurane (Abbott Labs, North Chicago, IL) and tracheostomized prior to receiving 100 mg/kg of dichlorvos subcutaneously (Sigma-Aldrich, St Louis MO). Recordings included tracheal airflow, esophageal pressure, tracheal end-tidal CO₂, femoral arterial pressure and oxyhemoglobin saturation. Figure 1 is an example of some of the recordings. Inspiratory effort of the animal was monitored during mechanical ventilation through an analysis of the esophageal pressure tracing. Inspiratory activity was visualized during positive pressure ventilation as negative deflections of the tracing (Figure 1).

2.2 Phrenic Nerve Isolation and Recording

The right phrenic nerve was isolated in one animal at the mid-cervical level using a dorsal approach. Recordings were performed on an uncut phrenic nerve draped over a bipolar

electrode and covered with petroleum jelly. The signal was amplified (Molecular Devices, Sunnyvale, CA), filtered (lowpass and highpass cutoff 2000 Hz and 400 Hz respectively) and conditioned using a noise reduction device (HumBug noise eliminator, Quest Scientific, Vancouver Canada). Phrenic nerve activity was whole-wave rectified and step-wise integrated over 50 ms intervals. The onset of the inspiratory phase of activity was defined as an integrated signal that exceeded 3 standard deviations above the mean of the signal during the mid- to late-expiratory phase. In order to quantify the statistical properties of phrenic nerve activity for a full respiratory cycle during baseline and experimental conditions, we averaged integrated phrenic nerve activity with respect to time of inspiratory onset, i.e., event-triggered averaging.

2.3 Experimental Groups and Protocols

Following all surgical procedures, a baseline period of at least 20 minutes was recorded before the animals received dichlorvos (Sigma-Aldrich, St Louis MO) 100mg/kg subcutaneously. This dose of dichlorvos is based on previous work in our lab (Gaspari and Paydarfar, 2007a) and was chosen as it represents 3xLD50 for dichlorvos and reliably induced a central apnea in this animal model. Recordings were continued until the animal reached the study endpoint (death or 90 minutes, whichever came first). Animals were grouped into two cohorts that differed in terms of oxygenation. Animals in Group I (n=10) received oxygen supplementation that was titrated to achieve a P_aO_2 of ~115 mmHg during the baseline (pre-poisoning) period; once this level was achieved the fraction of inspired oxygen (F_iO_2) was kept at the same level throughout the experiment. Animals in Group II (n=7) received 100% inspired oxygen throughout the experiment. Both groups received mechanical ventilation initiated at the point of apnea that continued until the study endpoint. In spontaneously breathing animals, apnea was defined as no respiratory activity for 20 seconds. Arterial blood was sampled for determination of P_aO_2 during the baseline period.

2.4 Data Recording and Analyses

Data were recorded using an A–D converter and PC data acquisition system (ADI instruments, Colorado Springs CO). Cardio-respiratory parameters were expressed as mean $\pm$ standard deviation. Normality of data was determined using Lilliefors test for normality. For groups with normal distributions, between-group comparisons were performed using an unpaired Student's *t*-test, repeated measure ANOVA or Fisher's Exact test where appropriate (Armitage and Berry, 2001). For data with non-normal distributions comparisons were performed using Wilcoxon signed-rank test.

3.0 RESULTS

3.1 Post-apneic Breathing Following Organophosphate Poisoning

Animals in both experimental groups demonstrated a central apnea with declining airflow and respiratory effort prior to the point of apnea (see table 1 for baseline characteristics). In all animals apnea occurred an average of 4min 32sec ($\pm 1:57$) after poisoning, with no differences between groups (table 2, $p=0.29$, Wilcoxon signed-rank test.). The majority of animals with mechanical ventilation at the point of apnea survived to the end of the study. Some animals did not survive (5 of 17) despite mechanical ventilation with no differences between groups (2 of 7 in Group I (normoxic) vs 3 of 10 in Group II (hyperoxic), $p=1.00$, Fisher's Exact test). Animals that did not survive despite mechanical ventilation had a large amount of secretions filling the tracheal tube and an inability to ventilate. These animals were characterized by decreasing tidal volumes despite steady mechanical ventilation. In all animals the cardiovascular parameters (blood pressure and pulse rate) demonstrated no change from baseline at the point of apnea.

The different F_iO_2 between groups resulted in a difference in tissue oxygenation (PaO_2) at baseline, at the point of apnea and during the period of mechanical ventilation; but a clinical difference in oxyhemoglobin saturation (as measured by pulse oximetry) between groups only occurred at the point of apnea (table 2). Animals receiving a normoxic gas mixture (Group I, normoxic) demonstrated no hypoxia for the first five minutes but dropped their oxyhemoglobin saturation to 24% by 10 minutes post-poisoning before rebounding to close to 100% (fig 2a). Animals inspiring 100% oxygen (Group II, hyperoxic) showed virtually no change in oxyhemoglobin saturation despite the decreasing respiratory rate (fig 2b). Arterial blood gas analysis during the baseline period and during the period of mechanical ventilation confirmed the oxyhemoglobin saturation data. The decreasing respiratory rate was unrelated to level of tissue oxygenation as respiratory rate decreased in both groups despite differing oxygenation levels (Figure 3).

There was a significant difference between the two groups in post-apneic breathing. No animals that survived to mechanical ventilation in Group I (normoxic) exhibited post-apneic breathing (0 of 7) whereas 4 out of 5 animals in Group II (hyperoxic) exhibited resumption of sustained (greater than 3 min) respiratory activity (Fig 4). The post-apneic breathing in these animals was characterized by an irregular, slow respiratory rate (8.6 ± 4.2 bpm) lasting an average of 25.4 ± 21.7 min. Inspiratory effort (i.e., peak negative esophageal pressure) during post-apneic breathing decreased $52 \pm 40.6\%$ from baseline. The difference in post-apneic breathing was not related to differences in mean arterial pressure as the drop in blood pressure 30 minutes post dichlorvos did not differ significantly between groups (50% vs 56%, $p = 0.77$, student's t-test).

3.2 Characterization of Phrenic Nerve Activity

In order to characterize the neural inspiratory activity during post-apneic breathing following OP poisoning, we analyzed phrenic nerve activity in one of the animals receiving 100% oxygen (Group II hyperoxic, Figure 5). Figure 6 shows the event-triggered average of integrated phrenic nerve activity for 100 consecutive breaths recorded during the baseline period and for 100 consecutive post-apneic breaths that resumed 19 min 42 sec after onset of apnea. Compared to the baseline breaths peak phrenic amplitude of post-apneic breaths decreased by 60%. Normalizing the peak amplitude allows a comparison of the morphology of integrated phrenic activity. There was no difference between baseline and post-poisoning in the normalized phrenic activity during the inspiratory period. However, compared to the baseline phrenic activity, the post-apneic breaths had reduced phrenic activity during the post-inspiratory, inspiratory period (repeated measure ANOVA, $p < 0.004$).

4.0 DISCUSSION

Animals exposed to subcutaneous dichlorvos (100mg/kg) exhibit a rapidly progressive decline in respiratory activity culminating in apnea within minutes of exposure. Our lab has shown previously that OP-induced central apnea in this preparation is fatal; survival is possible only if the animal is supported by artificial mechanical ventilation. As demonstrated in previous research from our lab, regardless of oxygenation a percentage of animals die despite mechanical ventilation and are characterized by increased pulmonary secretions relative to animals that recover with ventilation. (Gaspari, 2007) This variation in pulmonary response to OP emphasizes the heterogeneity of OP poisoning. In the present study we found that respiratory rhythm can recover during ventilator support following OP-induced central apnea but only in animals that were not hypoxic at the point of apnea.

The most important difference between groups related to the tissue oxygenation at the point of apnea. Group I (normoxic) were significantly more hypoxic at this point compared to animals in Group II (hyperoxic). Animals in Group I demonstrated post-hypoxic respiratory

depression and a lack of recovery post-apnea. It is well known that profound hypoxia causes respiratory depression in animals without intact carotid body afferent signaling(Watt *et al.*, 1942). Animals in this study had an intact glossopharyngeal nerve but the OP poisoning may have interfered with afferent signaling from the carotid body. Existing pontine neural mechanisms involved in post-hypoxic respiratory depression may also have been involved(Coles and Dick, 1996) but further study is needed. Severe hypoxia at the point of apnea in Group I may have contributed to the acute loss of respiratory activity but the respiratory depressant effects of severe hypoxia should have been eliminated with the increased oxygenation from mechanical ventilation. Increasing tissue oxygenation eliminates the suppressive effects of hypoxia on eupnea(Fung *et al.*, 1994).

During the post-apneic period, animals in Group II (hyperoxic) demonstrated significantly higher PaO₂ (table 2) compared to Group I (normoxic) animals. It is possible that hyperoxic conditions post-apnea in Group II stimulated post-apneic breathing over normoxia (Group I) but this seems unlikely. Hyperoxia has been shown to mildly decrease minute ventilation secondary to a drop in respiratory rate and an increase in tidal volume (Arieli *et al.*, 1988; Arieli, 1994). In contrast, a review by Dean et al provides some evidence that hyperoxia can have excitatory effects on the respiratory control system. Hyperoxic hyperventilation has been described where hyperoxia produces an increase in respiratory rate(Dean *et al.*, 2004). It is possible that stimulatory affects of hyperoxic conditions in Group II may be responsible for the increased post-apneic respiratory activity. Our data would support this as one of the major differences between our study groups was the hyperoxic conditions during the time when the OP exposure was inducing apnea but alternative explanations remain possible.

Respiratory activity post OP-induced apnea was distinct from both eupnea and gasping in a number of respects. Aspects of the respiratory activity such as tissue oxygenation and respiratory effort are supportive of eupnea. Post-apneic breathing occurred during a period of hyperoxia, the reverse of what would be expected for gasping respirations(St-John, 1998). Additionally, post-apneic respiratory effort, as measured by both esophageal pressure and phrenic activity, was less than half of baseline effort. This is in contrast to gasping, which generates a significantly increased inspiratory effort compared to eupneic breaths. However, the inter-breath intervals corresponded more closely to gasping (18bpm) than eupnea (80 bpm).

The clinical implications of this research should be interpreted with some caution as our findings relate to a single organophosphate (dichlorvos) in an animal model. The data on phrenic nerve activity is from a single animal and more research is needed to support any research hypotheses regarding neural output, but the characteristic of the neural output that supported respiratory activity post OP-induced apnea is neither eupnea nor gasping, as evidenced by analysis of the phrenic activity pattern of this single animal. Our overall findings demonstrated that the recovery of respiratory activity post OP exposure is decreased by hypoxia at the point of apnea and increased by hyperoxia during the recovery period. This may have some clinical relevance for emergency medical services responding to mass casualty events where patients exposed to OP agents are involved. Our results suggest that early treatment with oxygen may have significant impact on respiratory recovery for patients exposed to acute OP agents.

5.0 Conclusion

In summary, apnea following acute OP poisoning is not absolute as respiratory activity resumes following a supportive therapy. Post-hypoxic respiratory depression occurs in animals that were hypoxic at the point of apnea even when oxygenation was maintained post

apnea. The resumption of respiratory activity represents an emergence of a respiratory activity distinct from eupnea and gasping.

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Highlights

- Respiratory recovery returns following organophosphate induced central apnea
- Return of respiratory activity post organophosphate-induced apnea is distinct from both normal respiration and gasping
- Respiratory activity that emerges post organophosphate-induced apnea is suppressed by hypoxia at the point of apnea
- Respiratory activity that emerges post organophosphate-induced apnea is supported by oxygenation during the recovery period

Figure 1.

A,B – Physiologic tracings of a single animal treated with dichlorvos poisoning and receiving mechanical ventilation initiated at the point of apnea.

The animal received dichlorvos at time zero (fig 1A). Apnea occurred at ~3.5 minutes and mechanical ventilation was initiated at ~4 minutes. Note the negative deflections in the esophageal tracing (black bars) that represent the animal's spontaneous inspiratory effort distinct from the positive esophageal pressure deflections due to artificial mechanical ventilation. A magnified view of the tracings is provided in fig 1B. Fig 1B represents a recording during mechanical ventilation roughly 16 minutes post poisoning.

Figure 2.

A,B – Oxyhemoglobin saturation and respiratory rate following injection of dichlorvos in normoxic and hyperoxic rats with mechanical ventilation at the point of apnea. Grouped data showing oxyhemoglobin saturation (solid line, left y-axis) and respiratory rate (dashed line, right y-axis) for spontaneously breathing animals with acute OP breathing either titrated oxygen mixture with a P_aO_2 of roughly 115 mmHg (A) or 100% oxygen (B). Dichlorvos was administered at time zero and animals were placed on mechanical ventilation at the point of apnea (this time was not significantly different between groups – see Table 2). Results are shown as mean value $\pm$ standard deviation.

Figure 3.

Correlation of respiratory rate and oxyhemoglobin saturation

Data are plotted as a scatter plot of respiratory rate and oxyhemoglobin saturation for 4 minutes immediately post-poisoning. During a time of decreasing oxygen saturation (see fig 2) normoxic animals (Group 1, circles) demonstrate lower respiratory rates with increasing hypoxia. Hyperoxic animals (Group II, crosses) demonstrate no correlation between oxygenation and respiratory rate.

Figure 4.

Post-apneic breathing in animals with hyperoxia and normoxia

A comparison of post-apneic breathing between animals with 100% oxygen (Group II) and those with oxygen titrated to a PaO₂ of ~115 mmHg (Group I). Animals with titrated oxygen (Group I) experienced hypoxia at the point of apnea and animals with 100% oxygen did not. The percent of animals that demonstrated respiratory activity post-apnea is depicted as a bar and is referenced to the y axis on the left. The number of actual breaths post apnea (in all animals) is depicted as a dot and is referenced to the y axis on the right. The p-value corresponds to a comparison of the percent of animals in each group with respiratory activity post-apnea.

Figure 5.

Individual phrenic nerve activity pre and post apnea

A single animal phrenic nerve activity pre and post apnea is presented as both raw phrenic activity and integrated and rectified phrenic activity. When compared to baseline, phrenic activity post apnea is slower and has a lower peak amplitude. The time scale for both baseline and post-apnea are identical and represented by a 5 sec bar below the x-axis.

Figure 6.

A,B,C,D – Grouped phrenic nerve activity pre and post apnea

Plots represent phrenic nerve activity pre- and post-apnea. Fig 6A and 6B include individual phrenic nerve discharge activity pre- and post-apnea (respectively) synchronized to the initiation of inspiration. Fig 6C includes event-triggered averaging of phrenic activity during the baseline period and post-apnea. Data are an average of 100 breaths for both the baseline period and post-apneic period and is binned into 50 msec time intervals. Activity occurring during the inspiratory period and post-inspiratory period of the respiratory cycle are labeled with black bars at the top of the graph. 6D provides a close up view of the post-inspiratory activity from the data presented in 6C. Note the loss of post-inspiratory activity during post-apneic breathing.

Table 1

Characteristics of study groups

	Normoxic group I (n=10)	Hyperoxic group II (n=7)	
Baseline			
Respiratory Rate (bpm)	58.6 (7.9)	54.2 (4.7)	P=0.1
End Tidal CO ₂ (mmHg)	56.6 (5.2)	51.4 (1.1)	P=0.05
Mean Arterial Pressure (mmHg)	88.8 (10.3)	106.8 (14.5)	P=0.02
Oxyhemoglobin Saturation (%)	97 (1.5)	98.5 (1.7)	P=0.1
Respiratory Effort (mmHg)	– 3.54 (2.2)	– 4.04 (2.5)	P=0.7

Normoxic animals received titrated oxygen (PaO₂ ~115 mmHg) and hyperoxic animals received 100% oxygen throughout the experiment. Mean (SD) values are for the last 5-minutes of the baseline period, prior to injection of dichlorvos. Respiratory effort is the peak negative inspiratory pressure. There was no statistical difference between groups for any of the baseline variables except for mean arterial pressure.

Comparisons between groups were performed using student's T-test.

Table 2

F_iO_2 , time to apnea, and oxyhemoglobin saturation (O_2 sat) and partial pressure of oxygen in arterial blood (PaO_2) for normoxic and hyperoxic groups.

	Normoxic group I (n=10)	Hyperoxic group II (n=7)	
Time to Apnea	3:38 (0:49)	5:39 (2:23)	$P=0.29^B$
O_2 sat (baseline)	96.8% (1.48)	98.5% (1.73)	$P=0.1^A$
O_2 sat (apnea)	24.1% (39.1)	98.25% (2.36)	$P=0.01^B$
O_2 sat (post-apnea)	95.5% (3.37)	98% (2.83)	$P=0.2^A$
PaO_2 (baseline) *	115.6 (7.0)	238.0 (14.1)	$P=0.0001^A$
PaO_2 (post-apnea) *	123.1 (22.3)	298 (22.6)	$P=0.0002^A$

Data are presented as mean (standard deviation). Baseline oxyhemoglobin saturation (O_2 sat) data represent an average of 3 minutes preceding dichlorvos injection. Apnea oxyhemoglobin saturation data represents a single point of data at the time point immediately prior to initiation of mechanical ventilation. The post-apnea period represents an average of 3 minutes of oxyhemoglobin saturation data starting 5 minutes after the point of apnea. Comparisons between groups were performed using student's t-test^A or Wilcoxon signed-rank test^B

* PaO_2 was obtained using arterial blood gas analysis for a selection of animals in each group (n=5 normoxic group, n=2 hyperoxic group) and is presented in mmHg.