Toxicology 302 (2012) 242–247

Contents lists available at SciVerse ScienceDirect

Toxicology

journal homepage: www.elsevier.com/locate/toxicol

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

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ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form 13 July 2012
Accepted 9 August 2012
Available online 18 August 2012

Keywords:
Organophosphate
Gasping
Respiratory control
Apnea

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 PaO_2 of 115 mmHg. 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.

1. 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, 2007). 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 are 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

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http://dx.doi.org/10.1016/j.tox.2012.08.005

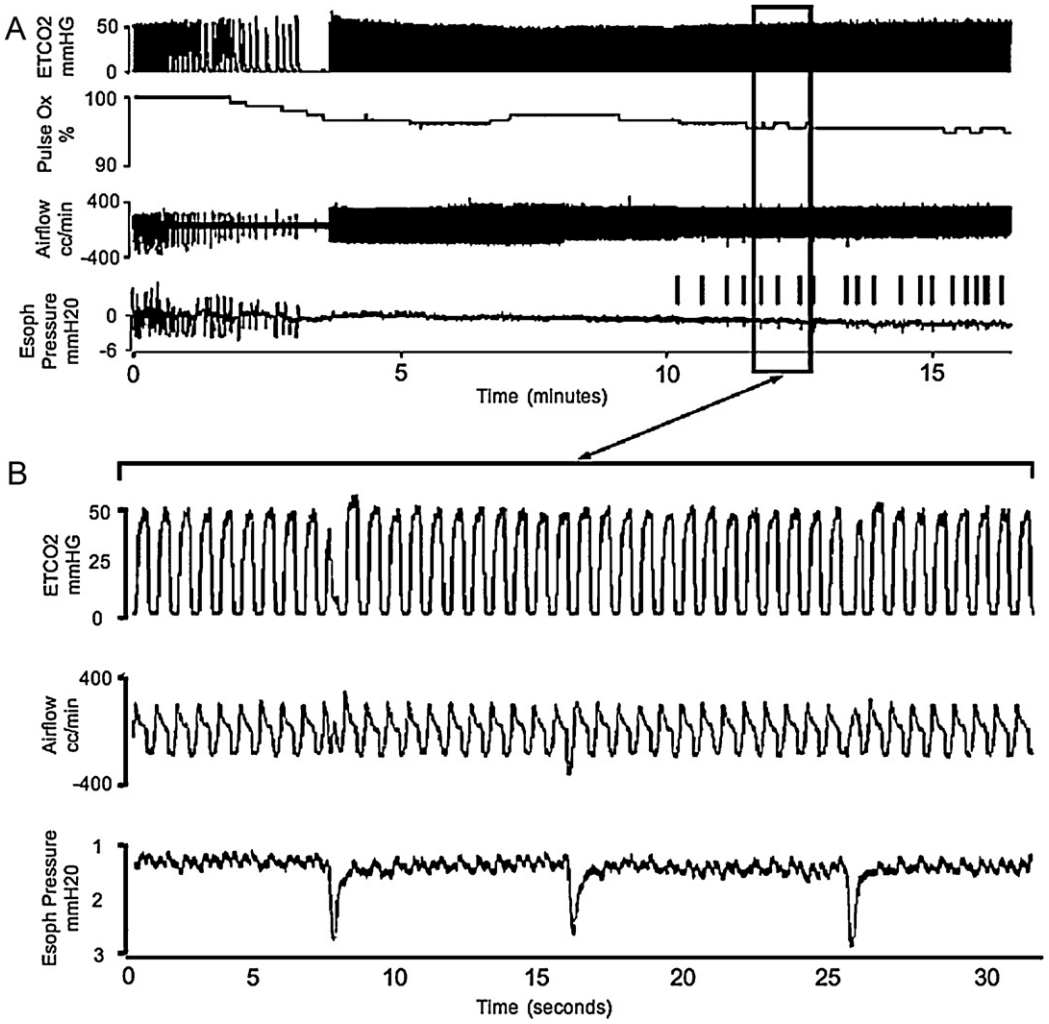

Fig. 1. (A and B) Physiologic tracings of a single animal treated with dichlorvos poisoning and receiving mechanical ventilation initiated at the point of apnea. The animal received dichlorvos at time 0 (A). Apnea occurred at ~3.5 min and mechanical ventilation was initiated at ~4 min. Note the negative 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 (B). (B) A recording during mechanical ventilation roughly 12 min post poisoning.

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. 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, 2007). Briefly, 17 adult male Wistar rats (280–350 g) 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 CO_2, femoral arterial pressure and oxyhemoglobin saturation. Fig. 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 (Fig. 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 min was recorded before the animals received dichlorvos (Sigma–Aldrich, St. Louis, MO) 100 mg/kg subcutaneously. This dose of dichlorvos is based on previous work in our lab (Gaspari and Paydarfar, 2007) 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 min, whichever came first). Animals were grouped into two cohorts that differed in terms