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Respiratory autoresuscitation following severe acute hypoxemia in anesthetized adult rats

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ABSTRACT

In the present study we investigated the pattern and efficacy of respiratory autoresuscitation in spontaneously breathing adult male rats across three separate anesthetic backgrounds. Each animal was administered one of three injectable anesthetics to achieve a surgical plane of anesthesia: ketamine-xylazine (KET, n = 10), pentobarbital (PEN, n = 10), or urethane (URE, n = 10). Animals were tracheostomized and equipped with a femoral artery catheter to record airflow and arterial pressures. In response to a bout of breathing anoxic air, none of the 10 URE animals were able to mount a successful autoresuscitation response. In contrast, all KET and PEN animals survived all four consecutive anoxic exposures, restoring eupneic breathing in all cases. Moreover, only 4/10 URE animals expressed gasping breaths following the onset of respiratory arrest, and these were temporally delayed ($p < 0.001$) and much smaller in volume ($P \leq 0.012$) compared to KET and PEN animals. URE animals showed no clear aberrations in their cardiovascular responses to anoxia, with the exception of lower arterial pulse pressures compared to either KET or PEN animals at specific points following RA. Ketamine-xylazine and pentobarbital anesthesia can be reliably and effectively used to create models for the study of autoresuscitation in adult rats. In contrast, urethane causes catastrophic failure of respiratory autoresuscitation, by delaying or outright preventing the elaboration of gasping breaths following anoxia-induced respiratory arrest. The neuronal and synaptic alterations accompanying urethane anesthesia may therefore provide a means of understanding potential pathological alterations in rhythm generation that can predispose the respiratory control system to failed autoresuscitation following an episode of acute severe hypoxemia.

1. Introduction

Acute severe hypoxemia triggers a profound and complex cardiorespiratory response. The respiratory system initially responds with a powerful stimulation of breathing, which rapidly transitions into respiratory arrest (Cummings et al., 2013; Macefield and Nail, 1987). This cessation of eupneic (normal) breathing is followed by a process called "respiratory autoresuscitation" (Erickson and Sposato, 2009; Guntheroth, 1977; Lun et al., 2015). During respiratory autoresuscitation, respiratory arrest is interrupted by a series of gasping breaths which provide a final vital attempt to ventilate the lungs with oxygenated ambient air. If gasping does not occur, or if access to oxygenated ambient air is not possible, then death is inevitable (Fewell et al., 2000; Gershan et al., 1990). However, if the gasping process is successful in providing reoxygenation, then normal eupneic breathing rhythm can be restored (Fewell, 2005; Gershan et al., 1990).

Aberrations in the respiratory response to transient severe hypoxemia have been broadly implicated in the pathogenesis of unexplained sudden death (Nadkarni et al., 2012; Thach, 2005; Tolstykh and Cavazos, 2013). To date, animal research concerning the respiratory autoresuscitation process has primarily been performed in the content of furthering our understanding of sudden infant death syndrome (SIDS) (Fleming et al., 2015), and has therefore predominantly utilized neonatal or very young animals. However, sudden unexplained death is certainly not limited to infants under the age of 1 year, and occurs in children (sudden unexplained death in children, SUDC) (McGarvey et al., 2012), and adults (sudden unexplained death, SUD; sudden unexplained death in epilepsy, SUDEP) (Goldman, 2015; Puranik et al., 2014). We are therefore interested in furthering our understanding of the mechanisms regulating the respiratory autoresuscitation process in response to acute severe hypoxemia in adult animals.

The present study was performed in an effort to develop a reliable and reproducible model system in which to study respiratory

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autoresuscitation in adult rats. The purpose of this research project was specifically to determine whether the choice of anesthetic or composition of asphyxiate air have any influence over the pattern or success of respiratory autoresuscitation in adult rats.

2. Methods

2.1. Animals

All experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Central Michigan University. A total of 30 adult male Sprague-Dawley rats were studied. CD IGS strain animals (Charles River Laboratories, Wilmington, MA) were bred and maintained in the Central Michigan University Vivarium in a conventional housing system on a 12:12 h light-dark cycle, food and water ad libitum.

2.2. Protocol overview

Three experimental groups were defined by three injectable anesthetics used to instrument the animals for study. Each animal received one of 3 possible anesthetic regimens, which was randomized based upon their order of participation in the experiment: (1) ketamine-xylazine, (2) pentobarbital, or (3) urethane.

Once anesthetized and surgically prepared for cardiorespiratory monitoring, all animals were subjected to an identical protocol, regardless of experimental group (Fig. 1). The protocol began with 5 min of baseline monitoring, followed by a 5 min exposure to mild hypoxia (10% inspired O_2 balance N_2) to evaluate responsiveness to peripheral chemoreflex activation. Following the mild hypoxia exposure, a series of exposures to anoxic inspired air were performed to study the cardiorespiratory response to severe hypoxemia and the related respiratory autoresuscitation process. Anoxic exposures were repeated for a total of 4 iterations, unless the animal failed to autoresuscitate from one of the respiratory arrests. Since no interventions were used to rescue animals who failed to autoresuscitate, such failure led to certain death and a terminal event in the study. Arterial blood samples were drawn for analysis during the baseline recording immediately prior to mild hypoxia test, and again at the point of onset of primary apnea in the first two exposures to anoxia.

2.3. Anesthesia

Anesthetics were administered via intraperitoneal injections performed under brief isoflouorane sedation (3% isofluorane, balance O_2). Dosages were based upon broad usage in literature, current guidelines of major research institutions, and verified previously through our own laboratory experience. Ketamine-xylazine (KET condition) was administered as 90 mg/kg ketamine (Ketasthesia, Henry Schein), and 10 mg/kg xylazine (Anased, Henry Schein), supplemented as needed using 50% of the loading dose of ketamine alone. Pentobarbital (PEN condition; P3761, Sigma-Aldrich) was administered using 70 mg/kg, supplemented as needed using 50% of the loading dose. Urethane (URE condition) was administered at a dosage of 1.5 g/kg. Since urethane (U2500, Sigma-Aldrich) anesthesia persists for 12 h or more in duration, no supplemental dosing was required. Urethane and pentobarbital were prepared within 12 h of experimental usage. Ketamine-xylazine was administered from multiple dose vials, and all drugs were stored in light proof settings.

2.4. Surgical instrumentation

A tracheostomy tube (14 gauge catheter) was placed to achieve direct measurement of respiratory flow. An arterial catheter (BPE-T50, Instech Laboratories) was placed in the right femoral artery to allow direct monitoring of the arterial blood pressure waveform. Patency of the catheter was maintained using sterile saline flush containing heparin (20 u/mL).

2.5. Monitoring

Cardiorespiratory variables of interest were continuously monitored following surgical instrumentation throughout the remainder of experiments. A pneumotachograph (8421, Hans Rudolph) connected to a pressure transducer (PA1, Hans Rudolph) provided continuous measurement of instantaneous respiratory flow of the animal. Inspired air mixture could be rapidly switched between room air and pre-mixed gases using a mechanical valve system.

Gas monitors were used to sample end tidal CO_2 (Micro CapStar, CWE Inc) and inspired O_2 fractions (OxyStar-100, CWE Inc). Arterial blood gas analysis was performed using a Vetscan iStat 1 and CG4+ cartridges (Abaxis). Animal temperature was maintained using a temperature controller equipped with a warming pad and a YSI-402 thermistor probe sensing rectal temperature (TC-1000, CWE).

Analog signals were sampled at 1000 Hz and digitized (USB-6218, National Instruments) for real-time visualization and storage of raw signals using custom written software (Labview, National Instruments). Chart software (v5, ADInstruments) was used for subsequent offline analysis. Cardiorespiratory variables extracted from the raw traces were: tidal volume (VT), breathing frequency (fB), minute ventilation ($\dot{V}E$), heart rate (HR), systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, and arterial pulse pressure.

2.6. Response to breathing 10% inspired O_2

Animals breathed room air for a period of 5 min before breathing a mildly hypoxic inspired gas mixture of (10% O_2, balance N_2) for 5 min. To calculate a 'delta' for cardiorespiratory variables, data

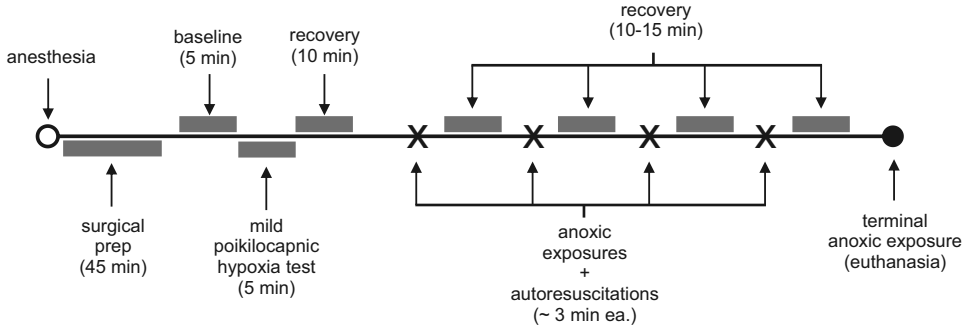

Fig. 1. Schematic timeline of the experimental protocol.