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INNER EAR INSULT ABLATES THE AROUSAL RESPONSE TO HYPOXIA AND HYPERCARBIA

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Abstract—*Introduction:* Sudden Infant Death Syndrome (SIDS) remains the leading cause of infant mortality in Western societies. A prior study identified an association between hearing suppression on the newborn hearing test and subsequent death from SIDS. This is the first finding of an abnormality in SIDS cases prior to death. A following study identified that inner ear dysfunction precipitates a marked suppression of the hypercapnic ventilatory response (HCVR). Failure of arousal has been proposed to be a key component in SIDS. The objective of the present study was to assess whether inner ear dysfunction not only weakens the hypercapnic response, but also plays a role in suppressing the arousal response to suffocating gas mixtures.

***Methods:* Wild-type mice ($n = 28$) received intra-tympanic gentamicin (IT-Gent) injections bilaterally or unilaterally to precipitate inner ear hair cell dysfunction. Three control groups ($n = 22$) received intra-tympanic saline (IT-Saline) bilaterally or unilaterally (right or left), or intra-peritoneal gentamicin (IP-Gent). The body movement arousal responses to severe hypoxia–hypercarbia combined (5% CO_2 in nitrogen) were tested under light anesthesia 8 days following the administration of gentamicin or saline.**

***Results:* After injections, the bilateral and unilateral IT-Gent-treated animals behaved similarly to controls, however the HCVR as well as the arousal movements in response to severe hypoxia–hypercarbia were suppressed in IT-Gent-treated animals compared to control animals ($P < 0.05$). Thus the HCVR was significantly decreased in the bilateral ($n = 9$) and unilateral IT-Gent-treated mice ($n = 19$) compared to bilateral ($n = 7$) and unilateral IT-Saline ($n = 9$) control groups ($p < 0.05$). Arousal movements were suppressed in the bilateral IT-Gent group ($n = 9$) compared to bilateral IT-Saline controls ($n = 7$, $P < 0.0001$) and in the unilateral IT-Gent group ($n = 19$) compared to unilateral IT-Saline controls ($n = 10$, $P < 0.0001$).**

***Discussion:* The findings support the theory that inner ear dysfunction could be relevant in the pathophysiology of SIDS. The inner ear appears to play a key role in arousal from suffocating gas mixtures that has not been previously identified.**

Key words: inner-ear, gentamicin, vestibular, arousal, severe hypoxia–hypercarbia, SIDS.

INTRODUCTION

Sudden Infant Death Syndrome (SIDS) remains the leading cause of postnatal infant mortality in Western societies. It is defined as the unexpected sudden death of a child less than 1 year of age in which an autopsy does not show an explainable cause of death (Hauck and Tanabe, 2008). Although the underlying mechanism has remained elusive; several theories propose failure of respiratory and/or cardiac pathways (Guntheroth, 1995; Horne et al., 2005; Goldwater, 2011; Garcia et al., 2013). Petechiae on autopsy of SIDS cases as well as histological evidence of mild respiratory infection in a high percentage of SIDS victims favor a respiratory-related related mechanism (Guntheroth, 1995; Trachtenberg et al., 2012). Moreover, respiratory tracings and abnormalities in brainstem areas involved with cardio-respiratory control and arousal further support the view that a failure of the infant to arouse from a suffocating hypoxic environment is part of the terminal event in SIDS (Kinney et al., 1995; McNamara et al., 1998; Panigrahy et al., 2000; Rand et al., 2007; Kinney, 2009; Darnall et al., 2010.

The arousal response includes two parts: (a) a respiratory component that increases ventilation (rate and depth) and (b) a defensive body movement component that allows an animal or human infant to physically move away from an environment containing suffocating gas mixtures (Phillipson et al., 1979; Mograss et al., 1994; Heydt et al., 2004). Both components are integral for eliciting an effective arousal to the threat of asphyxia during sleep and can occur without waking the animal or infant (Mograss et al.,

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Abbreviations: f_{inst}, instantaneous respiratory frequency; HCVR, hypercapnic ventilatory response; IP-Gent, intra-peritoneal gentamicin; IP-Saline, intra-peritoneal saline; IT-Gent, intra-tympanic gentamicin; IT-Saline, intra-tympanic saline; nTV, normalized tidal volume; SIDS, Sudden Infant Death Syndrome; TV, tidal volume; V_{min}, minute ventilation.

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1994). Thus, if the airway were to become obstructed (e.g. by soft bedding or a pillow cushion) the body movement component would actively move the head away to access fresh air.

Previous human and animal research supports an integral relationship between inner ear function and respiration (Monahan et al., 2002; Thurrell et al., 2003; Miyamura et al., 2004; Jauregui-Renaud et al., 2005; Allen et al., 2011) and a recent report identified suppressed right sided hearing on the newborn hearing screen test in infants that later died of SIDS when compared to control infants (Rubens et al., 2008). This implies that the involvement of inner ear dysfunction could be a potential contributor to the occurrence of SIDS.

To better understand the potential relationship between SIDS and inner ear dysfunction, we developed an animal model with induced inner ear dysfunction (Allen et al., 2011). Intra-tympanic gentamicin (IT-Gent) injection is an established method for damaging inner ear cochlear and vestibular hair cells (Husmann et al., 1998; Wanamaker et al., 1999; Hoffer et al., 2001; Heydt et al., 2004; Hirvonen et al., 2005; Allen et al., 2011). In a previous study, animals with histologically confirmed inner ear damage following IT-Gent injection displayed a suppressed hypercapnic ventilatory response (HCVR; Allen et al., 2011). The objective of the present study was to verify these findings and to assess whether inner ear injury also decreases the body movement arousal response to suffocating gas mixtures. If this were the case it could be pertinent to the mechanism of SIDS. The hypothesis was that inner ear injury would have no effect on the body movement arousal response.

EXPERIMENTAL PROCEDURES

Wild-type (CD-1) mice of both sexes were studied. The use of the animals and the associated procedures of the study were approved by the Animal Care and Use Committee at Seattle Children's Research Institute.

Injection procedures

At postnatal day 18, animals underwent a hearing test as outlined in our previous study (Allen et al., 2011). Mice were accepted for the study only if they displayed an adequate response to the acoustic stimulus. Following the hearing test, all accepted animals were anesthetized with 2% isoflurane in room air while breathing spontaneously. Either intra-tympanic (IT) or intra-peritoneal (IP) injections were administered once the animal was unresponsive. The procedure for injections was the same as outlined in our previous project (Allen et al., 2011). For the current project we added unilateral IT-Gentin addition to bilateral IT-Gent. The effect of inner ear injury on the respiratory response to hypercarbia and the body movement arousal response to hypoxia–hypercarbia combined was studied 8 days after initial injection (i.e., postnatal day 26).

Gas composition and anesthesia used during experiments

All experiments were conducted in a small animal plethysmography test chamber (Buxco Halcyon plethysmograph; Wilmington, NC, USA). Chamber temperature was maintained between 26 and 28 °C. Air was defined as gas composition 21% O_2 balanced N_2. The hypercarbia had a gas composition of 21% O_2 5% CO_2 balanced N_2. The severe hypoxia–hypercarbia gas mixture had a composition of 95% N_2 5% CO_2.

Both the HCVR and arousal tests were performed with the subject under light anesthesia. To anesthetize the subject, sevoflurane in air was delivered into the chamber via a vaporizer connected to the inflow chamber. The test gas independent of composition passed through the sevoflurane vaporizer and plethysmography chamber throughout the experiment at a continuous rate of 300 ml/min. Sevoflurane concentration was maintained with a final concentration between 1.25% and 1.5% so that the subject was just immobile.

Inspired 1.25–1.5% sevoflurane is substantially less than the 3% needed for surgical procedures (Liao et al., 2006; Koyama et al., 2009; De Segura et al., 2009). Animals were thus only lightly anesthetized and would become conscious within seconds of discontinuing the anesthesia.

HVCR test

Once respiration had stabilized and the animal was still, the plethysmograph was calibrated by injection of known volumes (0.05 and 0.1 ml) of air into the chamber (Fig. 1). Ventilatory measures were then obtained while the animal was breathing air and then while exposed to the hypercarbia gas mixture for 5 min. Instantaneous respiratory frequency (f_{inst}, Hz) and tidal volume (TV, μl) were recorded continuously. Mean minute ventilation (V_{min}, ml/min) was calculated continuously as $= TV \times f_{inst} \times 60/1000$. The HCVR response was reported as a normalized V_{min} ratio (nV_{min}) and was calculated by comparing the peak V_{min} recorded during CO_2 exposure to V_{min} in air. Similarly, f_{inst} and TV values at peak V_{min} during hypercarbia were compared to the respective metrics in air. These comparisons were also reported as a normalized frequency ratio (nf_{inst}) and normalized TV ratio (nTV).

For the HCVR assessments, unilateral and bilateral IT-Gent groups were compared to unilateral and bilateral intra-tympanic saline (IT-Saline) control groups. For the test animals, nine mice were given IT-Gent bilaterally, 19 received unilateral IT-Gent (right 14, left 5). Two control groups were utilized. The first control group ($n = 7$) received bilateral saline injections intra-tympanically (bilateral IT-Saline). The second control group ($n = 10$) received a unilateral intra-tympanic injection (unilateral IT-Saline, right 5, left, 5).

Assessment of the body movement arousal responses to severe hypoxia–hypercarbia

Immediately after testing the HCVR response, the hypercarbia gas mixture was replaced with the severe hypoxia–hypercarbia gas mixture. The response from this point until the end of the experiment was recorded on high definition video using a Lumix DMC-G3 Camera