Respiratory Physiology & Neurobiology 202 (2014) 35–43

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

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

Spontaneous swallowing occurs during autoresuscitation in the *in situ* brainstem preparation of rat

Tara G. Bautista[a,*], Angelina Y. Fong[b], Mathias Dutschmann[a]

[a] Florey Institute of Neuroscience and Mental Health, Gate 11, Royal Parade, University of Melbourne, Victoria 3010 Australia
[b] Department of Physiology, University of Melbourne, Victoria 3010, Australia

ARTICLE INFO

Article history:
Accepted 23 July 2014
Available online 30 July 2014

Keywords:
Respiratory
Upper airway
Autoresuscitation
Swallowing
Hypoxia
Hypothermia

ABSTRACT

Previous studies report that upper airway reflexes are operational during autoresuscitation from respiratory arrest. We investigated swallowing/breathing interactions, measured by recording of vagal (VNA) and phrenic nerve activities (PNA), during autoresuscitation in the *in situ* perfused brainstem preparation of juvenile rats. During the initial surgery, respiratory arrest was induced by exsanguination and cooling. Reperfusion (*i.e.* re-oxygenation and re-warming) of the brainstem circuits was associated with frequent spontaneous swallowing before resumption of respiration ($n = 6$, 'stage 1 autoresuscitation'). When recovered, the respiratory pattern was transiently apneustic-like ('stage 2 autoresuscitation'). Spontaneous swallowing often occurred at the end of the prolonged PNA ($n = 9/12$). Successful autoresuscitation was characterised by re-establishment of the 3 phase respiratory motor pattern and no spontaneous swallowing. Pharmacological inhibition (isoguvacine, 10 mM, 50–75 nl; $n = 10$) of the Kölliker-Fuse nucleus (KF) mimicked stage 2 autoresuscitation. However, the frequency of spontaneous swallowing after KF inhibition did not correlate with subsequent recovery of the eupneic respiratory motor pattern.

1. Introduction

Swallowing clears the upper airway of foreign material *e.g.* food, excess saliva, regurgitated gastric contents in infants, by directing such material into the oesophagus and away from the lower airways. Swallowing is tightly coordinated with respiration to maximise protection against aspiration, including its preferential initiation in the postinspiratory/expiratory phase in rats and humans (Bautista and Dutschmann, 2014; Hårdemark-Cedborg et al., 2009; Martin-Harris et al., 2003; Saito et al., 2002; Sun et al., 2011). Some evidence exists that the coordination of reflex swallowing with breathing is mediated *via* brainstem nuclei, including the pontine Kölliker-Fuse nucleus (KF) (Bonis et al., 2011; Sun et al., 2011). The post-inspiratory phase of respiration is gated by the KF. Recently, we and others (Bautista and Dutschmann, 2014; Bonis et al., 2013, 2011) have reported an increased incidence of spontaneous swallowing subsequent to inactivation of the dorsolateral pontine region, including the KF.

A poorly understood example of swallowing/breathing interaction is the presence of swallowing during autoresuscitation from respiratory arrest. Severe hypoxia (*e.g.* during cardiac arrest) leads to hypoxic coma, which describes a general depression of the central nervous system resulting in apnea and apparent lifelessness. Spontaneous recovery from this state involves the onset of gasping and eventual return to normal breathing ('autoresuscitation') if oxygen is made available to the lungs (Gershan et al., 1990; Gunteroth and Kawabori, 1975). Patients in both hypoxic coma and the gasping stage were initially thought areflexic. However, some studies in cats and mice demonstrate the preservation of upper airway protective reflexes in these states (Khurana and Thach, 1996; Tomori et al., 1991, 1993). The presence of the swallowing reflex may even facilitate the autoresuscitation process (Khurana and Thach, 1996; Tomori et al., 2013). Specifically, all mice that exhibited reflexive swallowing in response to oral administration of water were able to successfully autoresuscitate from hypoxic coma (Khurana and Thach, 1996). On the other hand, all non-swallowing mice failed to autoresuscitate. Similarly, in humans, the presence of swallowing is used as an early predictor of survival and/or recovery of consciousness following cardio-pulmonary resuscitation (Delooz and Lewi, 1989; Jørgensen, 1997).

In the current study, we investigated swallowing/breathing interaction during autoresuscitation from respiratory arrest in the *in situ* perfused brainstem preparation of juvenile rats. In obtaining

* Corresponding author at: Howard Florey Building, Gate 11, Royal Parade, University of Melbourne, Victoria 3010, Australia. Tel.: +61 03 8344 3775; fax: +61 09348 1707.
E-mail address: tara.bautista@florey.edu.au (T.G. Bautista).

http://dx.doi.org/10.1016/j.resp.2014.07.015
1569-9048/

the preparation, respiratory arrest is induced when the animal is exsanguinated and rapidly cooled. Autoresuscitation occurs following reperfusion with oxygenated and warmed (31 °C) artificial cerebrospinal fluid (Paton, 1996). We hypothesised that spontaneous swallowing would be frequently observed prior to the resumption of respiratory activity during autoresuscitation. In our experience, the pattern of the respiratory activity that recovers is apneustic, *i.e.* exhibiting prolonged inspiration and absent post-inspiration. Furthermore, we demonstrated earlier that chemical inhibition of KF results in an increase in spontaneous swallowing in addition to apneusis (Bautista and Dutschmann, 2014). Therefore, we also hypothesised that during autoresuscitation, frequent spontaneous swallowing would accompany the transient apneustic respiratory pattern. Lastly, we hypothesised that spontaneous swallowing facilitates recovery of the 3 phase respiratory pattern (defined as 'successful autoresuscitation'). Here we define the observation of spontaneous swallowing without respiratory activity during autoresuscitation as 'stage 1 autoresuscitation'. The presence of rhythmic respiratory activity that is transiently non-eupneic was termed 'stage 2 autoresuscitation'. To test our hypotheses, swallowing and breathing activities were monitored following reperfusion of the *in situ* preparation and were compared to inhibition of the KF.

2. Methods

All experimental procedures were performed in accordance with the Australian code of practice for the care and use of animals for scientific purposes. Approval for the study was obtained from the animal ethics committee of Florey Institutes of Neuroscience and Mental Health (AEC 12-084).

All chemicals were purchased from Sigma–Aldrich, Australia unless otherwise stated.

2.1. Perfused-brainstem preparation

Experiments were performed using the *in situ* arterially perfused brainstem preparation (Paton, 1996) of juvenile Sprague-Dawley rats (either gender; postnatal days 17–21). A total of $n = 25$ rats were used in this study. All basic procedures performed to obtain the perfused brainstem preparations in the present study follow the protocol as described previously (Paton, 1996). Additionally, the lungs and heart were removed (Dutschmann et al., 2009). These initial surgical procedures took approximately 15–25 min before reperfusion with artificial cerebrospinal fluid commenced. Rhythmic contractions of respiratory muscles usually returned within 5–10 min after the onset of reperfusion (stage 2 autoresuscitation). Respiratory-related movements were abolished by vecuronium bromide (0.3 μg ml^{-1}).

2.2. Nerve recording

Activity from the cut peripheral ends of isolated nerves was recorded using suction electrodes. In all experiments, phrenic nerve activity (PNA) was recorded to monitor inspiratory respiratory activity. Cervical vagal nerve activity (VNA) served as an index for both inspiratory and post-inspiratory respiratory activity as well as swallowing. Inspiratory and post-inspiratory bursts in VNA are reflective of laryngeal abductor and adductor muscle activation, respectively (Dutschmann and Herbert, 2006). In our previous study, we described how a swallow can be identified by a short (300–500 ms), spindle-shaped burst in VNA that is associated with a small, decrementing burst in hypoglossal nerve activity (Bautista and Dutschmann, 2014). The swallow-related VNA burst corresponds with laryngeal adduction of the vocal folds during each swallow, whereas the hypoglossal nerve burst corresponds with the activation of tongue retractor muscles. Hypoglossal nerve activity was used to confirm swallows in a subset of animals (data not shown).

During investigations of central respiratory control using the *in situ* preparation, PNA is routinely recorded within 1 min of reperfusion of preparations with ACSF. Here, in a subset of preparations ($n = 6$), VNA was also recorded shortly after PNA recording commenced to monitor whether swallows occurred before resumption of inspiratory bursts in PNA.

Nerve signals were amplified (differential amplifier DP-311, Warner Instruments, Hamden, USA), band-pass filtered (100 Hz to 5 kHz), digitised (PowerLab/16SP ADInstruments, Sydney, Australia) and stored on a computer using LabChart v7.0/s software (ADInstruments). During *post hoc* analysis, additional digital filtering in the Chart program (high pass > 10 Hz) was applied when necessary to remove movement artefacts.

2.3. Transition from stage 2 autoresuscitation to baseline eupneic 3 phase respiratory patterning

In each preparation, the respiratory motor pattern following reperfusion was 'fine-tuned' by varying the perfusion flow rate in order to obtain a ramping envelope of the integrated PNA with discharge duration of approximately 1 s or shorter. The presence of a sharp onset of post-inspiratory activity in VNA was another criterion for an optimised respiratory motor pattern. Flow rates (18–22 ml min^{-1}) and perfusion pressures (40–70 mm Hg) required to obtain consistent respiratory patterning in recorded nerves varied between animals. In addition to changing perfusion flow rate, adjustments to the respiratory motor pattern were achieved by bolus systemic administration of sodium cyanide (NaCN; 0.1–0.2 ml, 0.1%, w/v in saline) into the perfusate to stimulate peripheral chemoreceptors. Usually 1–3 bolus doses of NaCN were required to achieve a stable, eupneic 3 phase respiratory pattern *i.e.* a pattern exhibiting inspiration, post-inspiration, expiration.

2.4. Microinjections into the Kölliker-Fuse nucleus

We compared stage 2 autoresuscitation to our previous experiments (Bautista and Dutschmann, 2014), in which the KF was inhibited by bilateral microinjection (50–70 nl) of the GABA-A receptor agonist isoguvacine (10 mM). The procedures used to identify the KF and perform the microinjections are detailed in earlier publications (Bautista and Dutschmann, 2014; Dutschmann et al., 2009). Afterwards, microinjection sites were marked by microinjection of either rhodamine beads or pontamine sky blue (100 nl) to enable histological verification of their location. At the end of the experiments, brains were removed, fixed with 4% paraformaldehyde solution before being sectioned at 50 μm to verify marked microinjection sites. All microinjection sites were located within the KF (Bautista and Dutschmann, 2014).

2.5. Elicitation of sequential pharyngeal swallowing by oral water injection

Swallowing was elicited by oral water injection in a subset of preparations to verify if spontaneous bursts observed in VNA during autoresuscitation, tuning and KF inhibition were in fact swallow-related (Bautista and Dutschmann, 2014). Distilled water (0.2–0.6 ml, room temperature) was manually injected into an oral cannula whose caudal end was 0.75 cm above the laryngeal apparatus. This resulted in sequential pharyngeal swallows, observed as bursts in VNA that were spindle-shaped (occasionally gently decrementing) and of 300–500 ms duration (see Suppl. Fig. 1).