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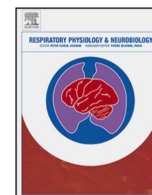

The role of the Kölliker–Fuse nuclei in the determination of abdominal motor output in a perfused brainstem preparation of juvenile rat

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ABSTRACT

The abdominal muscles are largely quiescent during normal breathing but may exhibit tonic activity or subtle respiratory modulation. The origin of baseline abdominal motor nerve activity (AbNA) if present remains uncharacterised. The contribution of the Kölliker–Fuse nucleus (KF) in the dorsolateral pons in the patterning and amplitude of AbNA was investigated using in situ perfused brainstem preparations of juvenile rats ($n = 12$). Two types of AbNA were observed: Type I – expiratory-modulated ($n = 7$), and Type II – weakly inspiratory/post-inspiratory-modulated ($n = 5$). Despite this, all preparations exhibited the same bi-phasic late expiratory/postinspiratory bursts upon elicitation of the peripheral chemoreflex. Interestingly, the type of AbNA exhibited correlated with postinspiratory duration. Targeted microinjections of GABA-A receptor agonist isoguvacine (10 mM; 70 nl) into KF however did not significantly modify pattern or amplitude of baseline AbNA in either Type besides the selective abolition of the postinspiratory phase and, consequently, postinspiratory modulation in AbNAwhen present. In sum, the KF is not a major contributorin setting baseline abdominal motor output.

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1. Introduction

At rest, ventilation of the lungs is primarily executed *via* the contraction of the diaphragm in humans and rats. Airflow to and from the lungs is valved by the upper airway resistance structures including the larynx and the tongue (Dutschmann et al., 2014). On the other hand, abdominal muscles exhibit absent or minimal activity under normal chemical drive. If present, resting abdominal activity is predominantly tonic during the expiratory period but may exhibit a weak postinspiratory burst in juvenile rats (Abdala et al., 2009), decrementing in expiration in adult rats under anaesthesia (Janczewski and Feldman, 2006), or exhibiting a slightly augmenting pattern as seen in adult rats in NREM sleep (Sherrey et al., 1988). In adult humans, resting tonic abdominal activity is mostly related to posture (Iscoe, 1998). In contrast, abdominal muscles are strongly recruited in response to high chemical drive in both humans and animals to facilitate deeper respiratory efforts (termed 'active expiration') *e.g.* during exercise (Abraham et al., 2002; Fregosi, 1994; Iizuka and Fregosi, 2007). Abdominal muscle contraction is also integral in a number of airway protective reflexes (*e.g.* cough) that require the generation of high intrathoracic pressures for the expulsion of airway irritants (Bolser and DeGennaro, 1994; Poliacek et al., 2008).

Few studies have examined the origin of abdominal motor activity at rest. Abdominal motoneurons have cell bodies within the ventral horn of the lumbar and thoracic spinal cord (Holstege et al., 1987; Smith and Hollyday, 1983) while pre-motoneurons are localised to the caudal ventral respiratory group/nucleus retroambiguus in the medulla (Boers et al., 2006; Saji and Miura, 1990; Vanderhorst et al., 2000). Under conditions of high chemical drive, the recruitment of late expiratory abdominal nerve activity depends on the conditional active expiratory oscillator of the retrotrapezoid nucleus (RTN)/ventrolateral parafacial respiratory group (pFRG) (Abdala et al., 2009; Huckstepp et al., 2015; Moraes et al., 2012). Neurons within the pontine Kölliker–Fuse nucleus (KF) are reciprocally connected with both the nucleus retroambiguus and RTN/pFRG (Rosin et al., 2006; Song et al., 2012). While the KF was shown to primarily gate respiratory motor activity of cranial nerves innervating laryngeal adductor and tongue muscles (Bautista and Dutschmann, 2014a,b; Dutschmann and Herbert, 2006), the role of the KF in the control of abdominal motor activity remains largely undetermined.

In the current study, we used reversible chemical blockade of the KF in *in situ* perfused brainstem preparations of juvenile rats to investigate the specific contribution of the KF in determining resting abdominal nerve activity.

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2. Methods

2.1. Ethical approval

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

2.2. Perfused-brainstem preparation

Experiments were performed using the arterially perfused, in situ brainstem preparation (Paton, 1996) of juvenile Sprague-Dawley rats of either gender (postnatal days 16–21, 32–45 g). All basic procedures performed to obtain the perfused brainstem preparations in the present study follow the exact protocol as described previously (Dutschmann et al., 2009) with the lungs and heart being additionally removed.

2.3. Nerve recording

Activity from the cut proximal ends of isolated nerves was recorded using monopolar suction electrodes. In all experiments, phrenic nerve activity (PNA) was used to monitor inspiratory respiratory activity. Cervical vagal nerve activity (VNA) served as an index for both inspiratory and postinspiratory respiratory activity. In addition, activity of the iliohypogastric branch of abdominal nerve (AbNA) was also recorded. This was identified as the first large nerve to emerge from the spinal cord caudal to the thoracic cage. Nerve signals were amplified (×100, 000, differential amplifier DP-311, Warner Instruments, Hamden, USA), band-pass filtered (100 Hz–5 kHz), digitised (PowerLab/16SP ADInstruments, Sydney, Australia) and stored on a computer using Chart v7.0/s software (ADInstruments). During *post-hoc* analysis, additional digital filtering in the Chart program (high pass >10–100 Hz) was applied when necessary to remove movement and residual mains electricity artefacts.

In each preparation, PNA was used to fine-tune the perfusion in order to obtain a ramping envelope of the integrated PNA with discharge duration of approximately 1 s or shorter. Optimal perfusion also corresponded with the presence of postinspiratory activity in VNA. Flow rates (18–22 ml min^{-1}) and perfusion pressures (40–70 mmHg) required to obtain consistent respiratory patterning in recorded nerves varied between animals.

2.4. Experimental protocol

Following tuning of the respiratory pattern, no interventions were made for approximately 5 min to allow the respiratory parameters to stabilize. Stimulation of peripheral chemoreceptors by a bolus injection of sodium cyanide (NaCN; 0.1–0.2 ml, 0.1% w/v in saline) was then performed. The baseline respiratory pattern and frequency were allowed to recover before a second NaCN injection was administered to test the reproducibility of the response. Again, respiratory pattern and frequency were allowed to recover before microinjections of drugs into the KF region commenced.

Local microinjections of drugs (50–70 nl) into the KF region were performed using multi-barrel borosilicate glass pipettes. The individual barrels were filled with L-glutamate (10 mM), GABA-A receptor agonist isoguvacine (10 mM) and either rhodamine beads (2%) or Chicago sky blue (2%). Volumes of microinjections were monitored by movement of the liquid meniscus in relation to a calibrated scale, as examined through a microscope at high magnification.

Landmarks on the dorsal surface of the brainstem were used to approximate the rostro-caudal and medio-lateral coordinates of the KF (0.2–0.5 mm caudal to the caudal end of the inferior colliculus, 2.0–2.5 mm lateral to midline and 1 mm ventral to the dorsal surface) The KF was more precisely identified by a postinspiratory breath-hold or tachypnea elicited upon unilateral microinjection of glutamate (10 mM, 40 nl). After identification of the KF, microinjection of isoguvacine (70 nl) was performed at the same site before moving the pipette to the same coordinates on the contralateral site for the second microinjection of isoguvacine. The contralateral KF was not mapped with glutamate to prevent dilution of the effect of isoguvacine. Microinjection of either rhodamine beads or Chicago sky blue (100 nl) were performed into the same sites for later histological verification.

2.5. Histological verification of microinjection sites

Brainstems were removed and fixed for several days in 4% paraformaldehyde. They were then transferred to 30% sucrose for at least a day before being serially sectioned at 50–60 μm using a cryostat. The locations of microinjections were documented on schematic drawings of coronal sections showing the parabrachial complex of the dorsolateral pons.

2.6. Quantification of respiratory parameters and abdominal nerve activity

All analyses of nerve activity were performed offline using Chart software. To quantify the respiratory parameters and AbNA, 20 representative, sequential respiratory cycles during baseline and similarly after KF inactivation were analysed. The baseline period was chosen just prior to the first microinjection of glutamate into the KF region. The treatment period, *i.e.* after bilateral microinjection of isoguvacine, was taken at least 30 s after the second microinjection to exclude non-specific volume effects. Recovery from drug effects was not analysed (although present in most) as in some rats, the long-lasting excitotoxic effects of the Chicago blue dye interfered with normal recovery.

The total length of a respiratory cycle (Ttot) was defined from the onset of the inspiratory burst in PNA to the onset of the subsequent burst. The inspiratory period (Ti) was measured from the onset of the PNA burst to its off-switch, which coincided with the high amplitude, decrementing burst in VNA. The post-inspiratory period (Tpi) was defined from the onset of the high amplitude, decrementing burst in VNA to its offset. The late expiratory period (Te_2) was defined by the silent period in VNA. The expiratory period (Te) was defined as the including both the postinspiratory and late expiratory phases, *i.e.* $Te = Tpi + Te_2$.

Raw nerve signals were integrated $\tau = 0.05$ during analysis. However, for illustrative purposes only, weaker signals were integrated at a higher time constant to for *e.g.* better illustrate respiratory modulation of nerve activity ($\tau = 0.05–0.15$ s).

For each respiratory cycle, mean AbNA amplitude in each respiratory phase was quantified by dividing the area under the curve of the integrated AbNA trace during the phase by the duration of the phase. Mean AbNA in each respiratory phase was reported as the average of the 20 representative cycles at baseline or after KF inactivation. It is important to note that since bilateral KF inhibition virtually abolished the postinspiratory phase in almost preparations, the remaining expiratory AbNA (*i.e.* that occurring between apneustic PNA bursts) is referred to as simply “post-KF inhibition expiratory” AbNA.

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