

Hypothalamic and mesencephalic regions involved in the control of laryngeal activity and subglottic pressure in spontaneously breathing anaesthetized rats

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ABSTRACT

Background

Abduction and adduction of the larynx allow changes in airflow necessary for the vibration of the vocal folds and emission of voice. It is known that stimulation of the Periaqueductal Gray matter (PAG) and nucleus retroambiguus (nRA) produces vocalization (1) and lesions in PAG cause mutism in animals and humans (2). The nRA is the perfect target to turn passive into active expiration modifying the activity of laryngeal motoneurons located in the nucleus ambiguus (3). We have shown that rostral and ventral pontine structures are involved in changes of laryngeal caliber (4). It has been also demonstrated a high expression of FOXP2 protein at mesencephalic and pontine regions (PAG, Parabrachial complex and A5 Region) involved in cardiorespiratory control. FOXP2 is a transcription factor required for brain and lung development and it is closely related to vocalization (5).

Objectives

The aim of this study was to characterize the relations between hypothalamic and mesencephalic regions involved in cardiorespiratory control and their possible role in modulating laryngeal activity and their effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=7), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2 mg/kg, i.v., as necessary). A double tracheal cannulation to develop the classical technique of the “glottis isolated in situ” and for the recording of respiratory airflow was carried out. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, $\pm 0,1$ psi) by passing a stream of humidified warm medical air upwards through the larynx at a constant rate of 30-70 ml/min with a thermal mass digital air flow meter controller (Bronkhorst Hi-Tec F-201CV-AGD-22-V). Thus, at constant air flow, changes in pressure indicate changes in laryngeal resistance.

Bilateral parietostomy allowed access to the Dorsomedial Hypothalamic Nucleus and Perifornical area (DMH-PeF), dIPAG and Cuneiform Nucleus (CnF). Electrical stimulations of these regions using concentric bipolar electrodes (1 ms pulses, 20-40 μ A, 100 Hz for 5 s) was performed. Respiratory flow, pleural pressure, blood pressure, heart rate and ECG activity were also recorded.

Results

DMH-PeF, dIPAG, and CnF stimulations evoked a decrease of laryngeal resistance (subglottal pressure) ($p < 0,001$) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p < 0,001$), together with a pressor ($p < 0,001$) and tachycardic response ($p < 0,001$).

Conclusions

The results of our study contribute with new data on the role of the hypothalamic-mesencephalic neuronal circuits in the control mechanisms of subglottic pressure and laryngeal activity.

Subglottic Pressure, Laryngeal Motoneurons, DMH-PeF, dIPAG, Nucleus Ambiguus

1. Holstege G. Anatomical study of the final common pathway for vocalization in the cat. *J Comp Neurol.* 1989; 284, 242-252.
2. Esposito A, Demeurisse G, Alberti B, Fabbro F. Complete mutism after midbrain periaqueductal gray lesion, *Neuroreport.* 1999; 10: 681– 685.
3. Subramanian HH, Holstege G. The nucleus retroambiguus control of respiration, *J Neurosci.* 2009; 29:3824-3832.
4. Lara JP, Dawid-Milner MS, Lopez MV, Montes C, Spyer KM, Gonzalez-Baron S. Laryngeal effects of stimulation of rostral and ventral pons in the anaesthetized rat, *Brain Res.* 2002; 934(2): 97-106.
5. Stanic D, Dhingra R and Dutchmann M. Expression of the transcription factor FOXP2 in brainstem respiratory circuits of adult rat is restricted to upper airway premotor areas, *Respir Physiol Neurobiol.* 2018; 250: 14-18.