Effects of stimulation of cuneiform nucleus and the dorsomedial Hypothalamic nucleus and Perifornical area on the mechanisms involved in the control of laryngeal activity and subglottic pressure in spontaneously breathing anaesthetized rats.

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ABSTRACT

Background:

The dorsomedial Hypothalamic nucleus and Perifornical area (DMH-PeF) and the mesencephalic Cuneiform nucleus (CnF) have been involved in sympathetic activity due their connectivity with several nuclei involved in cardiorespiratory control, e.g. dorsolateral Periaqueductal Gray Matter (dlPAG), the Parabrachial/Kölliker-Fuse complex (PBc/KF), the Solitary Tract Nucleus (NTS) and the Rostral Ventrolateral Medulla (RVLM) (1). In previous studies we have demonstrated a functional interaction between hypothalamic and mesencephalic structures (DMH-PeF, dlPAG) with several pontine regions (PBc, A5) (2, 3). We have also shown that rostral and ventral pontine structures are involved in the changes of laryngeal caliber (4).

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 possible effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=42), SPF, Sprague-Dawley (250-300g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/kg, i.v., as necessary). A double tracheal cannulation was used to obtain an "isolated glottis in situ" and to record respiratory airflow. Subglottic pressure was recorded with an aneroid transducer (Hugo Sachs Elektronik D-7801, ± 0,1psi) by passing a stream of humidified warm medical air upwards through the larynx at a constant rate of 30-70ml/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 DMH-PeF and CnF. Electrical (n=14) and chemical (n=14) stimulations of these regions using concentric bipolar electrodes (1ms pulses, 20-40µA, 100Hz for 5s) or glutamate (0,25M, 250nl) was performed. Microinjections (n=14) of PBS-Evans Blue (250nl, pH 7.4±0.1, 5-s duration) served as control purpose. Respiratory flow, pleural pressure, blood pressure and heart rate were also recorded.

Only data from animals in which the histology showed that the microelectrodes were positioned within the dlPAG and the A5 region were used for statistical procedures.

Results

DMH-PeF and CnF PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. However, electrical stimulations in both regions 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 ($p<0,001$).

Glutamate microinjections within the DMH-PeF and CnF evoked a decrease of laryngeal resistance (subglottal pressure) ($p<0,01$ and $p<0,001$ respectively) accompanied with an inspiratory facilitatory response consisted of an increase in respiratory rate ($p<0,001$ in both cases), together with a pressor ($p<0,001$ and $p<0,01$ respectively) and tachycardic response ($p<0,001$in both cases).

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.

Ethical approval

All experimental protocols were performed in accordance with the recommendations of the European Union directive (2010/63/EU) for animal care and experimental procedures. The experiments were approved by the Ethical Committee for Animal Research of the University of Malaga and the Junta de Andalucía.

Keywords

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

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Funding: Universidad Málaga/CBUA The study was supported by a program grant Junta de Andalucía, Group nº CTS-156, Spain.