Laryngeal effects of stimulation of the dorsolateral Periaqueductal Grey Matter in spontaneously breathing anaesthetized rats.

M. González-García[1,2,3], L. Carrillo-Franco[1,2,3], C. Morales-Luque[1], A. Díaz-Casares[1,3], M. Ponce-Velasco[3,4], B. Gago[1,3], M.S. Dawid-Milner[1,2,3] and M.V. López-González[1,2,3]

(1) Department of Human Physiology, Faculty of Medicine, University of Malaga, Malaga (Spain)
(2) Unit of Neurophysiology of the Autonomic Nervous System (CIMES), University of Malaga, Málaga (Spain)
(3) Biomedical Research Institute of Malaga (IBIMA Plataforma BIONAND), Malaga, Spain
(4) Department of cell biology, University of Málaga, Málaga, Spain

ABSTRACT

Background

The stimulation of the Periaqueductal Gray matter (PAG) and nucleus retroambiguus (nRA) produces vocalization (1). The nRA is the perfect target to turn passive into active expiration modifying the activity of laryngeal motoneurons located in the nucleus ambiguous (nA) (2).We have shown that rostral and ventral pontine structures are involved in changes of laryngeal caliber (3). A high expression of FOXP2 protein (transcription factor closely related to vocalization) at mesencephalic (PAG) and pontine regions (Parabrachial complex and A5 Region) involved in cardiorespiratory control has been described (4).

Objectives

The aim of this study was to characterize the possible role of the dlPAG in modulating laryngeal activity and their effects on vocalization.

Methods

Experimental studies were carried out with non-inbred male rats (n=27), SPF, Sprague-Dawley (250-300 g) housed under standard conditions. Animals were anesthetized with sodium pentobarbitone (60 mg/kg i.p., initial dose, supplemented 2mg/ kg, i.v., as necessary).

Neuromorphological study (n=6)

The pattern of staining for c-Fos and FOXP2 protein immunoreactivity (c-Fos-ir) were examinated throughout the rostrocaudal extent of the nRa/nA region during electrical stimulation of the dlPAG.

Neuropharmacological study (n=21)

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, ±0,1 psi) 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 dlPAG. Electrical stimulations (n=7) of this region using concentric bipolar electrodes (1ms pulses, 20-40μA, 100Hz for 5s) were performed. Microinjections of PBS-Evans Blue (250nl, pH 7.4±0.1, 5-s duration) (n=7) or glutamate (0,25M, 250nl) (n=7) were performed. 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

Activation of the dlPAG elicited a selective increase in c-Fos-ir with an ipsilateral predominance in nRA/nA somatas ($p<0.01$) and confirm the expression of FOXP2 bilaterally in both nuclei. dlPAG PBS-Evans Blue microinjections did not produce any significant changes in any of the cardiorespiratory variables recorded. dlPAG electrical and chemical (glutamate) 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

Our study contributes with new data on the role of the 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, dlPAG, Nucleus Ambiguus

References:

1. González-García M, Carrillo-Franco L, Peinado-Aragonés CA, Díaz-Casares A, Gago B, López-González MV, Dawid-Milner MS. Impact of the glutamatergic neurotransmission within the A5 region on the cardiorespiratory response evoked from the midbrain dlPAG. Pflugers Arch. 2023 475(4):505-516.
2. Holstege G. Anatomical study of the final common pathway for vocalization in the cat. J Comp Neurol. 1989; 284, 242-252.
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.

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