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Research Report

Ionotropic glutamate receptors mediate excitatory drive to caudal medullary expiratory neurons in the rabbit

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Abstract

Most of the neurons of the caudal ventral respiratory group (cVRG) are bulbospinal expiratory neurons that receive their main excitatory drive from more rostral, but not yet defined regions. This study was devoted to investigate the functional role of ionotropic excitatory amino acid (EAA) receptors in the excitatory drive transmission to cVRG expiratory neurons during eupnoeic breathing and some respiratory reflexes including cough induced by mechanical stimulation of the tracheobronchial tree. The experiments were performed on spontaneously breathing rabbits under pentobarbitone anesthesia making use of microinjections (30–50 nl) of EAA receptor antagonists into the cVRG. Phrenic nerve and abdominal muscle activities were recorded. Bilateral microinjections of 50 mM kynurenic acid (KYN), a broad-spectrum EAA antagonist, and 10 mM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA antagonist, or 5 mM 6-nitro-7-sulphamoyl-benzo(f)quinoxaline-2,3-dione (NBQX), a more specific non-NMDA antagonist, completely suppressed spontaneous rhythmic abdominal activity and reflex expiratory responses either to tracheal occlusion at end-inspiration (Breuer–Hering inflation reflex) or to expiratory threshold loading (5 cm H_2O); they also suppressed both the inspiratory and expiratory components of the cough reflex. Spontaneous rhythmic abdominal activity and the reflex respiratory responses were strongly reduced, but not completely abolished by microinjections of 10 mM D(−)-2-amino-5-phosphonopentanoic acid (D-AP5), an NMDA antagonist. The results provide evidence that the excitatory drive to cVRG bulbospinal expiratory neurons during eupnoeic breathing and the investigated respiratory reflexes is mediated by EAA receptors. They also support the view that neurons located in the cVRG are not merely elements of the expiratory output system.

Theme: Endocrine and autonomic regulation
Topic: Respiratory regulation

Keywords: Cough; Ionotropic glutamate receptor; Control of breathing; Caudal ventral respiratory group; Expiratory activity; Rabbit

1. Introduction

Expiratory neurons are mainly concentrated in two locales, i.e., in the caudal part of the ventral respiratory group (cVRG) and at the rostral pole of the VRG, in the so-called Bötzinger complex (BötC); most of the expiratory neurons of the cVRG are bulbospinal neurons that receive their main excitatory drive input from more rostral but not yet defined regions (reviewed in Refs. [2,19,43]).

Most of morphological and electrophysiological lines of evidence indicate that caudal expiratory neurons are probably not involved in the respiratory rhythmogenesis, since they do not have medullary axon collaterals and therefore connections with other medullary respiratory neurons; studies performed by using transections, lesions or excitatory amino acid (EAA) receptor antagonists have led to similar conclusions [2,9,19,26,43]. However, chemical activation of cVRG neurons via microinjections of EAA receptor agonists has been proved to cause transient inhibition of inspiratory activity both in rats [10,12] and cats [6]. In more detail, our experiments in the cat [6] have shown that microinjections of the broad-spectrum EAA

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agonist D,L-homocysteic acid (DLH), into the cVRG cause the activation of the expiratory motor output, associated with a corresponding period of silence in phrenic nerve activity, thus suggesting that caudal expiratory neurons have ionotropic EAA receptors and may affect the pattern of breathing possibly through axon collaterals when strongly activated. Injections of DLH probably activate [6] different types of cVRG neurons (either with respiratory or non-respiratory discharge patterns) including expiratory neurons usually quiescent but recruited only when forceful contractions of the abdominal muscles are required; these latter neurons are possibly characterized by properties different from neurons engaged under normal conditions and may have axon collaterals [19]. This hypothesis is corroborated by the fact that neuroanatomical studies have shown that cVRG neurons project to different brainstem regions, such as the rostral VRG, the parabrachialis medialis/Kölliker Fuse nuclei and the nucleus tractus solitarii ([16,42,49]; for further reference, see also [19]). These findings have been interpreted as relevant to some physiological conditions, such as cough and other reflexes, which require the activation of expiratory motoneurons and the concomitant inhibition of inspiratory activity [23,36,38].

Cough is an airway defensive reflex aimed at removing mucus and foreign particles from the respiratory tract by the generation of large expiratory airflows [23]. Previous studies in cats and rabbits have shown that the same neuronal network involved in the generation of the normal pattern of breathing also participates in the production of the cough motor pattern ([8,20,27,33,39]; reviewed in Refs. [36,40]). In particular, our data and those from other investigations support the view that at least part of BötC augmenting expiratory neurons conveys an excitatory drive to cVRG expiratory neurons and hence to expiratory motoneurons both during eucapnic breathing and the expiratory thrusts of coughing evoked by mechanical stimulation of the tracheobronchial tree ([4,5,8,15,21,27,39]; reviewed in Refs. [19,36,40]).

This study was undertaken to investigate the functional role of ionotropic EAA receptors in the transmission of the excitatory drive to cVRG expiratory neurons primarily during eupnoeic breathing and cough induced by mechanical stimulation of the tracheobronchial tree. The investigation was also extended to the activation of abdominal muscles evoked by the Breuer–Hering (B-H) inflation reflex and expiratory threshold loading. The experiments were carried out on spontaneously breathing rabbits under pentobarbitone anesthesia making use of microinjections of broad-spectrum and selective EAA receptor antagonists.

2. Materials and methods

2.1. Animal preparation

Experiments were carried out on 29 male New Zealand white rabbits (2.8–3.3 kg) anesthetized with sodium pentobarbitone (40 mg/kg i.v., supplemented by 2–4 mg/kg every 30 min; Sigma Chemicals, St. Louis, MO, USA). Atropine (0.15 mg/kg i.m.) and dexamethasone (2 mg/kg i.m.) were administered to reduce mucosal secretion in the airways and to minimize brain oedema, respectively. The adequacy of anesthesia was assessed by the absence of reflex withdrawal of the hindlimb in response to noxious pinching of the hindpaw; additional criteria were the presence of a stable and regular pattern of phrenic bursts and the absence of fluctuations in arterial blood pressure or phrenic nerve activity, whether spontaneous or in response to somatic nociceptive stimulation. All animal care and experimental procedures were conducted in accordance with the Italian legislation and the official regulations of the European Community Council on the use of laboratory animals (Directive 86/609/EEC). The study was approved by the Animal Care and Use Committee of the University of Florence.

Experimental procedures and details about the methods employed have previously been described [4,6,7,8,9,26–28]. After cannulation of the trachea, polyethylene catheters were inserted on one side into a femoral artery for the measurement of arterial blood pressure and into a femoral vein for systemic administration of drugs, respectively. C_3 or C_5 phrenic roots on both sides were dissected free, cut distally and prepared for recordings. The animal was placed in a prone position and fixed in a stereotaxic instrument by a stereotaxic head holder and vertebral clamps; the head was ventroflexed to facilitate recordings from the medulla. The dorsal surface of the medulla was widely exposed by occipital craniotomy. The posterior part of the cerebellum was removed by gentle suction to provide access to the rostral medulla. Body temperature was maintained at 38.5–39 °C by a heating blanket controlled by a rectal thermistor probe.

2.2. Recording procedures

Efferent phrenic nerve activity was recorded using bipolar platinum electrodes from desheathed C_3 or C_5 phrenic roots. The electromyographic (EMG) activity of abdominal muscles was recorded by wire electrodes (Nichrome wires, insulated except for 1 mm at the tips, diameter 0.1 mm) inserted into the external or the internal oblique abdominal muscles. Phrenic and abdominal discharges were amplified, full-wave rectified and "integrated" (low-pass RC filter, time constant 100 ms). Extracellular recordings from medullary neurons were made with tungsten microelectrodes (5–10 MΩ impedance at 1 kHz). The most rostral extent of the area postrema on the midline was defined as the obex and used as a standard point of anatomic reference. Neuronal activity was recorded from expiratory neurons of the cVRG (1.6–3.0 mm caudal to the obex, 2.0–2.5 mm lateral to the midline and 2.0–2.6 mm below the dorsal medullary surface). To determine the rostral extent of the cVRG, extracellular recordings were