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The role of excitatory amino acids and substance P in the mediation of the cough reflex within the nucleus tractus solitarii of the rabbit

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Abstract

We hypothesized that cough evoked by mechanical stimulation of the tracheobronchial tree in the rabbit is primarily mediated by glutamatergic neurotransmission at the level of the caudal portions of the medial subnucleus of the nucleus tractus solitarii (NTS) and the lateral commissural NTS where cough-related afferents terminate, and that this reflex is potentiated by local release of substance P. To test our hypothesis, we performed bilateral microinjections (30–50 nl) of ionotropic glutamate receptor antagonists or substance P into these locations in pentobarbitone anaesthetized, spontaneously breathing rabbits. Blockade of NMDA and non-NMDA receptors by 50 mM kynurenic acid abolished the cough reflex without affecting the Breuer–Hering inflation reflex or the pulmonary chemoreflex. Blockade of non-NMDA receptors using 10 mM CNQX or 5 mM NBQX caused identical effects. Blockade of NMDA receptors by 10 mM D-AP5 strongly reduced, but did not abolish cough responses. Microinjections of 1 mM substance P increased peak and rate of rise of abdominal muscle activity as well as cough number. These results are the first to provide evidence that ionotropic glutamate receptors, especially non-NMDA receptors, located within specific regions of NTS are primarily involved in the mediation of cough evoked by mechanical stimulation of the tracheobronchial tree in the rabbit. Present findings on substance P cough-enhancing effects extend previous observations and are relevant to the tachykinin-mediated central sensitization of the cough reflex. They also may provide hints for further studies on centrally acting antitussive drugs.

Keywords: Respiratory reflexes; Ionotropic glutamate receptors; Control of breathing; Abdominal muscles; Central sensitization

1. Introduction

Cough is an important airway defensive reflex regulated by complex central mechanisms [6–8,17,35,40] that are subserved by several brainstem structures [8,12,25,26] including, in particular, the first and the last relay medullary stations of the reflex pathway, i.e., the second order sensory neurons located in the nucleus tractus solitarii (NTS) and the expiratory premotor neurons of the caudal ventral respiratory group (cVRG).

Whilst it is widely agreed that tracheobronchial rapidly adapting receptors (RARs) are involved in cough mediation, the role of bronchopulmonary C-fibers and Aδ-nociceptive pulmonary afferent fibers in this reflex remains controversial [14,22,27,47,48]. Central terminations of lung RARs, slowly adapting stretch receptors (SARs) and bronchopulmonary C-fibers have been found in largely nonoverlapping regions of the caudal half of the NTS both in the cat and the rat (for review see Refs. [25,26]). In more detail, electrophysiological studies have identified the caudal portion of the medial subnucleus of the NTS (mNTS) and, especially, the lateral aspect of the commissural subnucleus of the NTS (comNTS) as the predominant sites of RAR central projections and RAR second order neurons, termed RAR cells [25,26]. Bonham et al. [9] were the first to suggest that the excitatory input from RARs is mediated by glutamate in the rat. Their suggestion was based on the observation that the discharge of two neurons which exhibited discharge patterns characterized by a small and variable number of spikes per burst, and therefore assumed to be RAR cells, was facilitated or inhibited by applications of DL-homocysteic acid and kynurenic acid, respectively. More recently, it has been proposed that glutamate

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is the primary neurotransmitter at the synapse between RAR afferents and RAR cells [16]. In fact, ionophoretic application of non-*N*-methyl-D-aspartate (non-NMDA) receptor antagonists to nine putative RAR cells within the comNTS of the rat abolished cell firing in response to vagus nerve electrical stimulation and to lung inflation or deflation. Noticeably, despite evidence implicating glutamate as a principal neurotransmitter at the level of the synapse between RAR afferents and RAR second order neurons, the effects induced by ionotropic glutamate receptor blockade on the cough reflex and RAR cell responses to tussigenic stimuli have not been investigated. In particular, the specific role played by NMDA receptors also remains to be elucidated.

Recent lines of evidence suggest that a receptor subtype found in the larynx and rostral trachea, quite distinct from the well-defined SARs and RARs, is primarily involved in the mediation of cough in guinea-pigs [13]. These receptors, termed "cough receptors", are innervated by slow-conducting Aδ-fibers. They are activated by punctate mechanical stimulation and acid, but are unresponsive to capsaicin, bradykinin, smooth muscle contraction, longitudinal or transverse stretching of the airways, or lung distension. Their central projections to the NTS have not been determined, but neuronal tracing studies have shown that the comNTS is a primary site of vagal afferent termination also in the guinea-pig [28,29].

Central interactions between vagal afferents possibly involved in the genesis of the cough reflex have been shown. Interactions within the comNTS between airway C-fibers and RAR afferents may act synergistically to regulate airway tone in the anaesthetized guinea-pig [28]. Similarly, C-fiber activation by capsaicin or bradykinin applied to the trachea does not evoke cough, but sensitizes the cough reflex evoked by "cough receptors" in anaesthetized guinea-pigs. These effects were mimicked by substance P microinjections into the comNTS and consisted of reductions in the threshold for evoking cough with electrical stimulation of the tracheal mucosa and increases in the peak amplitude of expiratory pressures associated with coughing [29].

In this scenario, we advanced the following hypotheses. First, in the rabbit the caudal portion of the mNTS and, especially, the lateral aspect of the comNTS contain most of the second order sensory neurons in the afferent pathway of the cough reflex activated by the mechanical stimulation of the tracheobronchial tree. Second, the primary synaptic neurotransmitter between cough afferents (either from the RARs or recently described "cough receptors") and second order neurons is glutamate. Therefore, ionotropic glutamate receptor blockade within the region where cough-related second order neurons are located should selectively abolish the cough reflex evoked by the mechanical stimulation of the tracheobronchial tree without affecting the Breuer–Hering (B–H) inflation reflex [8,22,36,46,48] and the pulmonary chemoreflex [21,23,27,44] due to SAR and C-fiber afferent stimulation, respectively. These reflexes require glutamatergic neurotransmission at the first central synapse [9,49], but their second order neurons are located in different albeit neighbouring subnuclei of the NTS [25,26]. Third, substance P application to the same caudal NTS regions investigated by means of excitatory amino acid (EAA) receptor blockades potentiates the cough reflex induced by the mechanical stimulation of the tracheobronchial tree.

In the present study we addressed these issues by using bilateral microinjections of ionotropic glutamate receptor antagonists or substance P into the caudal mNTS and the lateral comNTS of pentobarbitone anaesthetized, spontaneously breathing rabbits.

Preliminary accounts of present results have already been published in abstract form [34].

2. Methods

2.1. Animal preparation

Experiments were performed on 29 male New Zealand white rabbits (2.7–3.5 kg) anaesthetized with sodium pentobarbitone (40 mg kg^{-1} i.v., supplemented by 2–4 mg kg^{-1} every 30 min; Sigma–Aldrich, St. Louis, MO, USA). Atropine (0.15 mg kg^{-1} i.m.) and dexamethasone (2 mg kg^{-1} i.m.) were administered to reduce mucosal secretion in the airways and to minimize brain oedema, respectively. The adequacy of anaesthesia 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 [7,8,32,33,35]. After cannulation of the trachea, polyethylene catheters were inserted into a femoral artery and vein for monitoring arterial blood pressure and for drug delivery, respectively. The C_3 or C_5 phrenic root on one side was dissected free, cut distally and prepared for recordings. The animal was placed in a prone position and fixed by a stereotaxic head holder and vertebral clamps; the head was ventroflexed for optimal exposure of the dorsal surface of the medulla by occipital craniotomy. 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 the central stump of the cut and desheathed C_3 or C_5 phrenic root. 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 activities were amplified, full-wave rectified, and "integrated" (low-pass RC filter, time constant 100 ms). Arterial blood pressure was recorded by a strain-gauge manometer. End-tidal CO_2 partial pressure was measured by an infrared CO_2 analyzer (Datex, CD-102; Normocap, Helsinki, Finland). Integrated phrenic and abdominal activities as well as the signals of the other variables studied were recorded on an eight-channel rectilinearly writing chart recorder (model 8K20; NEC San-ei, Tokyo, Japan). Cardiorespiratory variables were also acquired and analyzed using a personal computer, equipped with an analog-to-digital interface (Digidata 1200, Axon Instruments, Union City, CA, USA) and appropriate software (Axoscope, Axon Instruments).

2.3. Microinjection procedures

Microinjection procedures have been fully described in previous reports [8,32,33,35]. Bilateral microinjections were performed into the caudal mNTS and the lateral comNTS. The following drugs (Tocris Cookson, Bristol, UK) were used: kynurenic acid (KYN, 50 mM), a broad-spectrum EAA receptor antagonist, D(−)-2-amino-5-phosphonopentanoic acid (D-AP5, 10 mM), a NMDA receptor antagonist, and 6-cyano-7-nitroquinoxaline-2,3-