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GABA-ergic neurotransmission in the nucleus of the solitary tract modulates cough in the cat

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ABSTRACT

GABA, muscimol, and baclofen were microinjected into the rostral (rNTS) and caudal solitary tract nucleus (cNTS) in 24 anesthetized cats. Electromyograms (EMGs) of diaphragm (DIA) and abdominal muscles (ABD), blood pressure and esophageal pressure (EP) were recorded and analysed. Bilateral microinjections of 1 mM GABA (total 66 ± 4 nl), 1 mM baclofen (64 ± 4 nl) and unilateral microinjections of 0.5 mM muscimol (33 ± 1 nl) in the rNTS significantly reduced cough number (CN), amplitudes of ABD EMGs, expiratory EP, and prolonged the duration of the cough inspiratory phase. GABA microinjections decreased the amplitudes of cough-related DIA EMGs and inspiratory EP; muscimol microinjections decreased the cough DIA EMG on the contralateral side. Only microinjections of GABA into the cNTS suppressed CN. In some cases, microinjections prolonged the inspiratory phase, lowered respiratory rate, changed the depth of breathing, and increased blood pressure and heart rate. Our results confirm that GABA-ergic inhibitory mechanisms in the rNTS can regulate coughing in the anesthetized cat.

1. Introduction

The nucleus of the solitary tract (NTS) in the dorsomedial medulla is the entry point of afferent signals from various visceral mechanosensors, chemosensors, C-fibers, including cough afferents. The NTS comprises second-order neurons receiving and transmitting afferent information for the integration of visceral reflexes via reciprocal connections with higher centres in the central nervous system (Babic et al., 2015).

The synaptic profile of NTS neurons receiving afferent excitatory drive is highly complex. It involves distinct neurotransmitters and neuromodulators, including glutamate, adenosine triphosphate and acetylcholine (Zoccal et al., 2014). Various complex respiratory/cardiovascular functions are mediated/modulated by gamma-aminobutyric acid (GABA) receptors in the NTS (Wang et al., 2010). In general, GABA release and activation of postsynaptic $GABA_A$ receptors play a crucial role in controlling neuronal excitability in adult mammals (Watanabe et al., 2002). Fast GABA-mediated synaptic inhibitory neurotransmission requires that $GABA_A$ receptors are expressed and assembled at appropriate postsynaptic sites near GABA-releasing nerve terminals (MacDonald and Olsen, 1994; Kittler et al., 2002).

Cough-related activity has been found in neurons located in the NTS (Haji et al., 2012). The multiplicity of neuronal phenotypes and responses to perturbation of NTS neurons (Lipski et al., 1991; Paton, 1998; Paton et al., 1999) reveals a complex role for the NTS in regulation of cough excitability, its sequencing, motor drive and the spatiotemporal control of cough motor pattern (Poliacek et al., 2017a,b). Mutolo et al. (2007, 2009), Mutolo (2017), Cinelli et al. (2016) have repeatedly shown in the rabbit that pharmacological perturbation of the NTS can alter the duration of the inspiratory and expiratory phases of coughing, which is strong evidence for a role of this medullary area in cough rhythmogenesis.

However, several studies support significant species differences in the anatomical arrangement of NTS elements that modulate coughing. Poliacek et al. (2017a) showed that disruption of excitatory amino acid transmission with microinjection of a broad-spectrum antagonist, kynurenic acid, in the feline rNTS, but not the cNTS, had inhibitory effects on coughing. Further, in these experiments kynurenic acid profoundly affected temporal control of this behaviour such that activation of airway mechanoreceptors that normally produce repetitive coughing induced prolonged apneusis instead.

Conversely, work in the rabbit supports an important role of the

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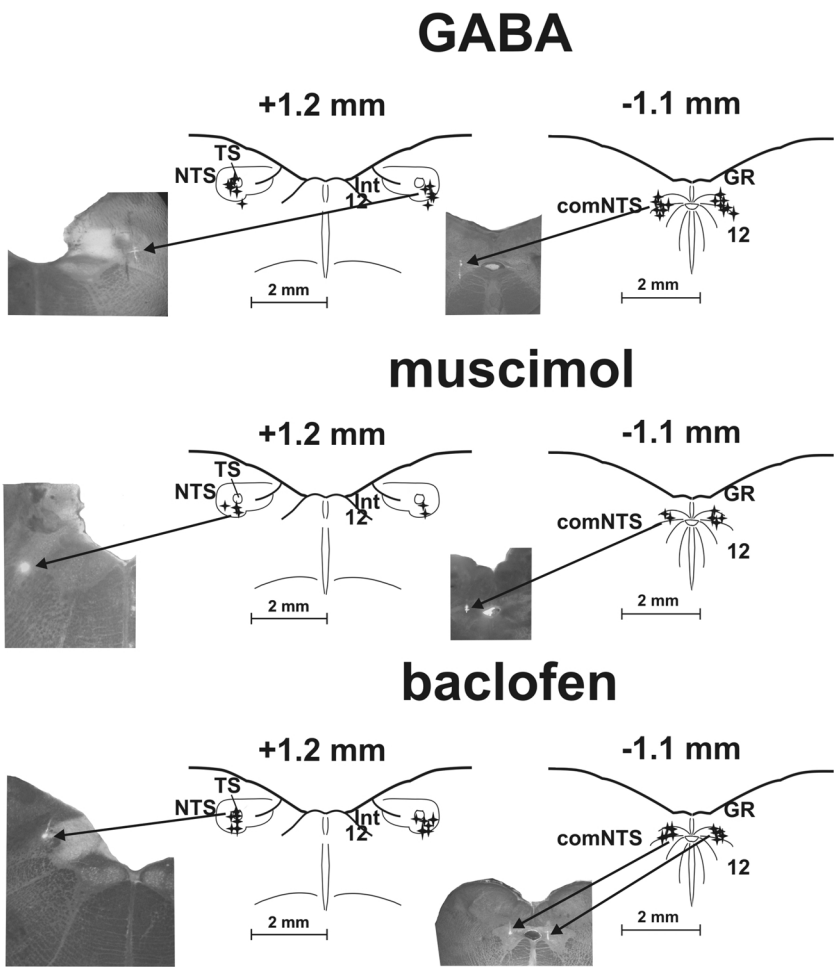

Fig. 1. Reconstruction of microinjection sites.
Stars represent highlighted positions of the micropipette tip during microinjections of GABA, muscimol and baclofen in the rostral (rNTS; +1.2 mm to the obex) and caudal nucleus of the solitary tract (cNTS; −1.1 mm to the obex) as determined by fluorescent marker. Reference points: comNTS, 12, central canal, medullary surface for the cNTS; TS, NTS, 12, Int, the bottom of the 4th ventricle, medullary surface for the rNTS.
Microinjections of **GABA**. All 14 microinjection locations were found within or near the commissural subnucleus of the NTS in the cNTS. All 12 microinjections were positioned in or near ventrolateral subnucleus of the NTS in the rNTS.
Microinjections of **muscimol**. All 6 locations where muscimol was delivered in or near comNTS were identified. In the rNTS 5 out of 6 microinjection locations were positively identified in the ventrolateral region of the NTS.
Microinjections of **baclofen**. All 10 Baclofen microinjections were positioned in or near comNTS in the cNTS. All 12 rNTS microinjection were identified in the area of TS and ventrolateral region of the NTS. 12: hypoglossal ncl., comNTS: commissural subnucleus of the NTS, GR: gracile ncl., Int: ncl. intercalatus, NTS: ncl. tractus solitarius, TS: tractus solitaries
Inset photographs demonstrate the process. The arrows 'injection' point out light spots of a spread of fluorescent marker.

cNTS but not the rNTS in regulation of cough. Mutolo et al. (2007, 2009, 2017) and Cinelli et al. (2016) in rabbit studies demonstrate effects of neuroactive and cough modulating drugs on neurons in the cNTS for modulation of cough expression and execution.

More recently, Cinelli et al. (2016) investigated the role of GABA receptors in the cNTS of the rabbit in the production of coughing. GABA receptors, especially the $GABA_A$ subtype, are important in the production of rhythmic respiratory behaviors (Bongianni et al., 2010; Anderson et al., 2016; Marchenko et al., 2016); but may not be essential for breathing to occur (Janczewski et al., 2013). Microinjection of the $GABA_A$ receptor antagonist, bicuculline, into the cNTS in the rabbit enhanced coughing and shortened cough inspiratory but not expiratory phase duration (Cinelli et al., 2016). Based on these previous data and the fact that systemically administered GABA-ergic agents suppress cough (Bolser et al., 1994; Nosalova, 1998; Mutolo et al., 2008) we attempted to modulate cough responses by local delivery of GABA, the $GABA_A$ receptor agonist muscimol and the $GABA_B$ receptor agonist baclofen in the NTS. It was hypothesized that $GABA_A$ as well as $GABA_B$ agonists in the rNTS would suppress coughing induced by mechanical stimulation of the tracheobronchial airways in cats. Further, we speculated that these GABA receptor agonists would perturb cough phase timing, consistent with a role of synaptic inhibition in the NTS in the regulation of cough rhythmogenesis. We also expected a limited effect of GABA-ergic agents on cough when administered in cNTS in the cat model.

2. Material and methods

2.1. Ethical approval

Animal care as well as all procedures were performed in accordance with the Animal Welfare Guidelines of the Comenius University and the legislation for animal use and welfare of Slovak Republic and European Union (Directive 2010/63/UE).

2.2. Animal preparation and recording procedures

The experiments were carried out on 24 male cats (4.06 ± 0.16 kg). The animals were anesthetized with sodium pentobarbital (Pfannenschmidt GmbH; initial dose 38 mg/kg, i.p., 1–3 mg/kg i.v. supplementary as needed). At the beginning of the experiment atropine (Biotika; 0.15 mg/kg, i.v.) was administered to reduce the mucosal secretion in the airways and hydrocortisone (VUAB Pharma a.s.; 2 mg/kg, i.v.) to reduce brain swelling. The level of anaesthesia was assessed regularly by the absence of reflex withdrawal of the hind limb in response to noxious pinching of the paw. The other criteria were the presence of palpebral reflex and jaw tone. The cats spontaneously breathed oxygen-enriched air (30–40%). The trachea, femoral vein and artery were cannulated. During the experiments respiratory rate (RR), end-tidal CO_2 concentration ($ETCO_2$), arterial blood pressure, and rectal temperature were continuously monitored. The animal's temperature was maintained within the range 37.5–38.5 °C, using a heating pad and a lamp. Arterial blood samples were removed periodically to perform gas and pH analysis. For the measurement of intrathoracic pressure (esophageal pressure, EP) a soft balloon was inserted into the