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BRAIN RESEARCH

Research Report

Brainstem regions involved in the expiration reflex A *c-fos* study in anesthetized cats

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ABSTRACT

Expression of the immediate-early gene c-*fos*, a marker of neuronal activation, was employed to localize brainstem neuronal populations functionally related to the expiration reflex (ER). Twelve spontaneously breathing, non-decerebrate, pentobarbital anesthetized cats were used. The level of Fos-like immunoreactivity (FLI) in 6 animals with repetitive ERs mechanically induced from the glottis (296±9 ERs) was compared to FLI in 6 control non-stimulated cats. Respiratory rate, arterial blood pressure, and end tidal CO_2 concentration remained stable during the experiment. In the medulla, increased FLI was found in the region of nucleus tractus solitarii ($p<0.001$), in the ventrolateral medulla along with the lateral tegmental field ($p<0.01$), and in the vestibular nuclei ($p<0.01$). In the pons, increased FLI was detected in the caudal extensions of the lateral parabrachial and Kölliker–Fuse nuclei ($p<0.05$). Within the rostral mesencephalon, FLI was enhanced in the midline area ($p<0.05$). A lower level of ER-related FLI compared to control animals was detected in the pontine raphe region ($p<0.05$) and the lateral division of mesencephalic periaqueductal gray ($p<0.05$). The results suggest that the ER is coordinated by a complex long loop of medullary–pontine–mesencephalic neuronal circuits, some of which may differ from those of other respiratory reflexes. The FLI related to the expulsive behavior ER differs from that induced by laryngeal stimulation and laryngeal adductor responses, particularly in ventrolateral medulla and mesencephalon.

1. Introduction

The expiration reflex (ER) is a defensive reflex of the airways characterized by a solitary brief and intense expiratory effort without a preceding inspiration. It is a significant aspiration prevention behavior restricting penetration of foreign bodies into the lower respiratory tract and expelling them by a fast expiratory airflow (Korpas and Tomori, 1979). The reflex is

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Abbreviations: AGN/H, average group number of immunoreactive neurons at particular area / hemisection; AR, aspiration reflex; BP, blood pressure; ER, expiration reflex; $ETCO_2$, end tidal CO_2 concentration; ER FLI, expiration reflex-related FLI; FLI, Fos-like immunoreactivity; FTL, lateral tegmental field; KF, Kölliker–Fuse nucleus; LAR, laryngeal adductor response; LRN, lateral reticular nucleus; NPBL, lateral parabrachial nucleus; NTS, solitary tract nucleus; PAG, periaqueductal gray; PBS, phosphate buffer; ResR, respiratory rate; SLN, superior laryngeal nerve; VLM, ventrolateral medulla; VRG, ventral respiratory group

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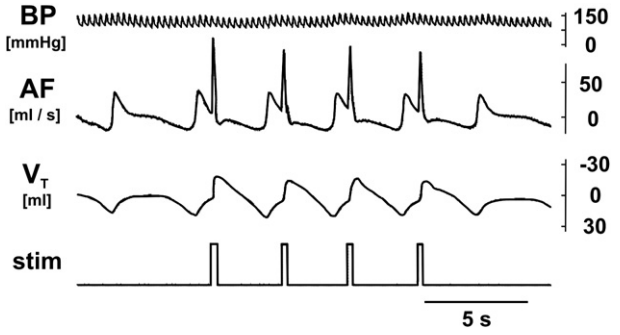

Fig. 1 – An original record of blood pressure (BP), airflow (AF; expiration up) and tidal volume (V_T; expiratory volume up) during expiration reflexes induced by mechanical stimulation (stim) of the glottis.

frequent in many animal species as well as in humans from the very early postnatal period (Korpas and Tomori, 1979; Nishino et al., 1990). ER is easily produced by mechanical stimulation of the medial margin of the vocal folds (Korpas and Tomori, 1979; Korpas and Jakus, 2000). Sensory afferents from laryngeal mucosa project to the solitary tract nucleus containing second-order neurons of defensive airway reflexes such as cough or ER (Kalia and Mesulam, 1980; Dyachenko, 1988). The primary efferent pathway begins in the region of the caudal ventral respiratory group (VRG). The expiratory pre-motor neurons in the caudal VRG drive spinal thoracic and abdominal pools of expiratory motoneurons during active expiration resulting in strong expiratory motor output during ER, cough, or sneeze (see review of Iscoe, 1998). The neuronal network that integrates the powerful expulsions during these reflexes is poorly understood. Unlike cough and sneeze, ER is a model of a simple expulsive expiratory activation with no preparatory inspiration. Previous electrophysiological studies reported alterations in the neuronal activities of both the respiratory and non-respiratory modulated neurons in ER, predominantly in the medullary lateral and gigantocellular tegmental fields (Dyachenko, 1990). Kainic acid microinjections into the neurons of the medullary raphe (Jakus et al., 1998), the lateral tegmental field (FTL) of medulla (Jakus et al., 2000) and the rostral dorsolateral pons (Poliacek et al., 2004) were shown to preclude the motor signs of the reflex. Similarly, focal cold block in the rostral VRG led to the reversible suppression of ER (Jakus et al., 1996). Recent studies confirmed that the activity of the rostral and caudal respiratory neurons of VRG are markedly modified during the fictive ER (Baekey et al., 2004); however, these studies are not sufficient to identify all brainstem components of the neurogenic mechanisms responsible for the ER.

The Fos method is based on induced expression of an immediate early gene c-*fos*, by neuronal depolarization during extra-cellular synaptic activation (Dragunow and Robertson, 1988; Morgan and Curran, 1991). The products of c-*fos* can be detected by an immunohistochemical method (Dragunow and Faull, 1989). The method was successfully used in the study of functional neural pathways in e.g. laryngeal stimulation (Tanaka et al., 1995), cardiac sympathoexcitatory reflexes (Guo et al., 2002a,b), sneezing (Wallois et al., 1995), coughing (Gestreau et al., 1997), and aspiration reflex (AR; Jakus et al., 2004a).

The present study was designed to determine the neuronal populations and the brainstem areas involved in ER using the Fos method. We hypothesized that the frequent ERs would increase the Fos-related expression in several areas of the medulla and pons mostly involved in production of forceful expulsions. Activation of motor pathways involved in ER would presumably expose differences in Fos-like immunoreactivity (FLI) distribution compared to laryngeal stimuli that did not induce ER (Tanaka et al., 1995; Ambalavanar et al., 1999, 2004). Furthermore, we expected to detect differences in ER-related distribution of FLI relative to that found in coughing (Gestreau et al., 1997), sneezing (Wallois et al., 1995), and in the AR (Jakus et al., 2004a,b).

2. Results

The stimulation (and ERs) had little effect on breathing. A slight shortening of the expiratory phase was usually observed (Fig. 1). The respiratory rate (ResR) returned to the baseline within 5–10 s after a series of ERs. As shown in Table 1, there was no significant difference in the mean ResR, blood pressure (BP), and end tidal CO_2 concentration ($ETCO_2$) during and post-stimulation referred to the baseline and compared to the control, sham-operated animals.

Fos-like expression was detected as a dark brown staining of variable intensity in inspected brainstem slices. The quantitative analysis of the number of immunolabeled neurons at the brainstem regions with significant differences between the groups of stimulated and control cats is shown in Table 2. The control and ER-related FLI (ER FLI) distribution within the medulla oblongata and pons Varoli (at 1, 5, and 10 mm rostral to the obex) as well as at the mesencephalon (at 13 and 17 mm rostral to the obex) are compared on diagrammatic reconstructions of transverse brainstem sections (Fig. 2). Examples of significantly enhanced or diminished FLI in some medullary and mesencephalic structures

Table 1 – Cardiorespiratory parameters in ER-induced and sham-operated animals

Period	ER-induced			Sham-operated	
	Control	Stim	Survival	Control	Survival
ResR [cycles/min] (range)	17.5±1.8 (13–25)	18.0±1.9 (14–27)	20.2±2.5 (14–29)	18.0±1.8 (13–23)	19.0±0.7 (17–21)
BP systolic [mm Hg] (range)	147±15 (120–190)	162±13 (125–190)	151±11 (120–180)	165±15 (120–190)	161±15 (120–190)
BP diastolic [mm Hg] (range)	97±8 (65–120)	109±8 (80–120)	104±8 (80–130)	112±5 (95–120)	119±7 (95–130)
$ETCO_2$ [%] (range)	4.7±0.2 (4.0–5.3)	4.3±0.2 (3.5–4.8)	4.1±0.3 (3.6–4.8)	4.8±0.3 (4.1–5.3)	4.3±0.4 (3.5–5.3)

Respiratory rate (ResR), systolic and diastolic blood pressure (BP), and end tidal CO_2 ($ETCO_2$) before stimulation (Control), at the intervals of repetitive glottal stimulations (Stim), and during survival period (Survival) in the group of stimulated animals (ER-induced). The same parameters analyzed in the early (Control) and late (Survival) period in a group of sham-operated, non-stimulated cats.