CORTICAL CONTROL OF HERING-BREUER REFLEXES IN ANESTHETIZED RATS

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Abstract

It had been hypothesized that the regions of prefrontal cortex which are involved in respiratory control can modulate Hering-Breuer reflexes evoked by vagal input from pulmonary stretch receptors. In the present study, experiments were performed on urethane anesthetized spontaneously breathing Wistar rats. The expiratory-promoting reflex was evaluated from changes in expiratory time immediately after airway occlusion at the end of inspiration. The inspiratory-inhibitory reflex was estimated from changes in inspiratory time provoked by airway occlusion at the end of expiration. The results indicate that electrical microstimulation of the responsive sites within the insular cortex significantly weakened both expiratory-promoting and inspiratory-inhibitory reflex. Activation of the infralimbic cortex depressed expiratory-promoting reflex, but inspiratory-inhibitory reflex was enhanced. These results suggest that stimulation of the prefrontal cortex influences vagally mediated control of the respiratory phases timing and several regions of the prefrontal cortex modulate distinct sets of neurons in the network controlling inspiratory and expiratory phases of a breath cycle.

Key words: prefrontal cortex, microstimulation, Hering-Breuer reflex

INTRODUCTION

Prefrontal cortex (PFC) has long been implicated in autonomic control playing a key role in the coordination of affective and autonomic responses [1, 2]. Dysfunction of PFC can lead to disturbances in autonomic regulation and neuroendocrine responses that are associated with mood disorders [3]. An increasing knowledge of associations between PFC lesions and stroke-induced executive deficits, such as cardiac complications and respiratory dysfunction [4-8], stimulates interest concerning the role of the central autonomic nervous system in cardiovascular and respiratory control. It has been found that there is a distinct interaction of the insular cortex (IC, lateral PFC) with heart rate and blood pressure control [9-11]. Our previous studies have confirmed that IC and infralimbic cortex (IL, ventromedial PFC) are involved in the respiratory control, because electric stimulation of these areas produced distinct patterns of respiratory responses [12, 13]. However, it remains an unresolved issue what mechanisms mediate PFC influences on the autonomic control. It is suggested that PFC influence on the cardiovascular control is mediated by the parasympathetic activity, because inactivation of the ventromedial PFC withdraws parasympathetic input to the baroreflex [14]. It is also established that stimulation of the IL cortex inhibits vagally mediated relaxation of the stomach [15]. Therefore, it seems that PFC exerts autonomic function by modulating the reflexes mediated by vagal afferents. A substantial part of vagal afferents originates from slow adapting pulmonary stretch receptors (SARs) which induce Hering-Breuer reflexes. Therefore, in the present study we investigated the hypothesis that stimulation of both IC and IL cortices will influence the Hering-Breuer reflexes.

MATERIAL AND METHODS

All animal procedures were conducted in accordance with the ethical guidelines of the European Community Council Directives 86/609/EEC and the study was approved by a local Ethics Committee. Experiments were done in 23 spontaneously breathing male Wistar rats weighing 250-300 g which were anaesthetized with urethane (1.25 g·kg^{-1}, i.p.). Anesthesia level was sufficient to eliminate pain reflexes and animal did not response to noxious stimuli (tactile pinches). Rectal temperature was monitored and maintained at $37 \pm 0.5°C$ with a thermal controller (ML295/R; ADInstruments Pty, Bella Vista NSW, Australia). The animals were tracheostomized and canulated with a respiratory flow head (MLT1L; ADInstruments Pty) which was connected to a pneumotachometer (ML141 Spirometer; ADInstruments Pty). Esophageal pressure (P_{es}) was measured in the lower third of esophagus with an air-filled latex balloon connected to a custom-made low pressure transducer. A cannula filled with heparinized saline was inserted into the femoral artery for recording of arterial blood pressure with a blood pressure transducer (MLT0670; ADInstruments Pty). Pressure signals were fed into the bridge amplifier (ML224; ADInstruments Pty). Pneumotachometer and bridge amplifier were coupled with a data acquisition system

(PowerLab 8/30; ADInstruments Pty) supplied with a conventional software. The rats were placed in a stereotaxic frame in the prone position, with the head fixed in the 'flat scull' position. Electrostimulation of the cortex was made using a lacquer insulated tungsten electrode with a tip diameter of 50 μm. Electrode was immersed into the hemisphere with steps of 250 μm by means of a stereotactic head. A trial stimulus (5-10 s train of negative 0.5 ms pulses at 60 Hz and 100 μA) was applied after each step to detect respiratory responses. Then, the electrode was left set in the most sensitive site and the experimental current strength was set at 50% above the threshold level.

After surgery, animals were allowed 30 min for stabilization. The pneumotachogram, P_{es} and arterial blood pressure were continuously recorded. The experiment started with a recording of the respiratory response to cortical stimulation and the effects of expiratory and inspiratory occlusions were tested afterwards. Then, the stimulation current was switched on again and, after 2-3 breaths, airway occlusion was superimposed on cortical stimulation. The above protocol was repeated several times before the electrode was moved to another cortical site. At the end of the experiment, an electrolytic lesion was made at the bottom of the last electrode track. The animal was killed with an overdose of urethane and perfused with 100 ml of 0.9% saline followed by 250 ml of 10% formalin solution. Cortical tissue specimens were prepared and histological verification of the stimulated sites was performed as previously described [13].

Pneumotachography was used for measuring maximal inspiratory flow (V_{Imax}), tidal volume (V_T), inspiratory time (T_I), and expiratory time (T_E). Inspiratory swings of esophageal pressure were recorded to estimate total inspiratory effort (P_{es}) and to approximate T_I and T_E during airway occlusion. Airway occlusions were performed either at end-inspiration or at end-expiration. Expiratory occlusions were used for the purpose of estimating the strength of the Hering-Breuer inspiratory-inhibitory (T_I-inhibitory) reflex, whereas inspiratory occlusions enabled to assess the Hering-Breuer expiratory-promoting (T_E-promoting) reflex. In order to obtain quantitative characteristics of these reflexes, the absolute values of T_I or T_E were estimated in the first post-occlusive breath and expressed as a percent of the corresponding last pre-occlusive breath. The magnitude of these indexes was taken as reflecting the strength of the reflexes. Statistical analyses were performed using the non-parametric Wilcoxon signed-rank test. $P<0.05$ were considered significant.

RESULTS

The results demonstrate that the same stimulus train applied to the infralimbic or insular cortex altered the pattern of breathing in a different way. The responsive sites in the IL cortex were located between 1.5-2.5 mm rostral to bregma at a depth of 4.5-5.0 mm from the brain surface. The IL responses consisted of a progressive decrease of T_T and V_T (Fig. 1A). In the first breath after the start of stimulation, T_T and V_T fell to 58.0 ±4.7%, and 70.0 ±7.7 % of the control level, re-

spectively. The changes stabilized during the successive 3-4 breath and remained so until the end of the stimulus period ($P<0.05$). Stimulation of the responsive sites in the IC cortex 0.5-1.5 mm rostral to bregma produced inhibitory responses manifested in a decrease of V_{Imax}, V_T, and P_{es} (Fig. 1B). In the first breath, V_{Imax}, V_T, and P_{es} decreased to 53.0 ±5.6%, 43.0 ±3.6%, and 57.0 ±5.4 % of the control level, respectively ($P<0.05$). The magnitude of the decreases tapered off in the successive respiratory cycles during the stimulation period, until the variables returned to the control levels. Therefore the IC responses were transient character.

During control conditions, airway occlusion at end-inspiration prolonged T_E and increased P_{es} in the first and following breaths, but the T_E of the first expiration was prolonged to a greatest extent (Fig. 2A-a). The T_E increased by 500-700% of the control value. Airway occlusion at end-expiration evoked a prolongation of T_I and an increase of P_{es} in the first and following occluded breaths (Fig. 2B-a). Within the first occluded inspiration, the increment of the inspiratory time was 170-250% of the control level. Stimulation in the prefrontal cortex modified the effects of occlusions. In particular, IL and IC stimulations shortened T_E which was already prolonged by inspiratory occlusion (Fig. 2A-b, c). On the other side, stimulation of the IL enhanced the effect of expiratory occlusion which manifested itself in the increase in T_I (Fig. 2B-b). Stimulation of the IC had a similar effect on T_I (Fig. 2B-c). Further analysis permitted to make more definite conclusions about the effects of the stimulation of either prefrontal cortex exerted on the Hering-Breuer reflexes. It was confirmed that stimulation of IL, just as that of IC, significantly weakened the T_E-promoting reflex (Fig. 3A). As to the T_I-inhibitory reflex, IL promoted and IC depressed it, but the latter effect turned out to be insignificant (Fig. 3B).
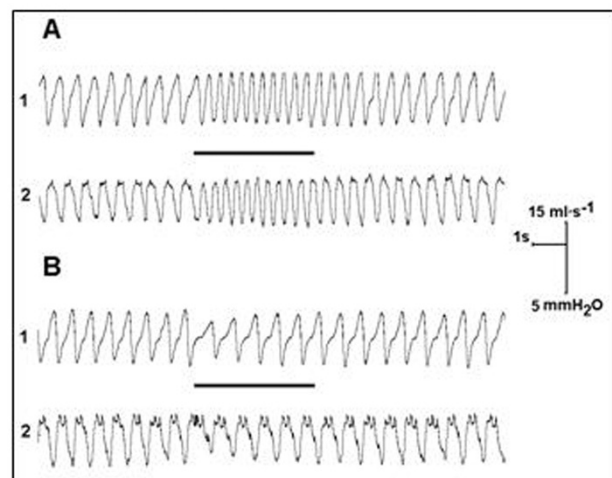

Fig. 1. Changes in breathing pattern during electrical stimulation of different prefrontal areas.
A – infralimbic cortex (IL); B – anterior part of insular cortex (IC); 1– respiratory flow and 2- inspiratory swings of esophageal pressure. Horizontal lines indicate duration of stimulation consisting of 5 s train of 0.5 ms pulses at 60 Hz and 150 mA.
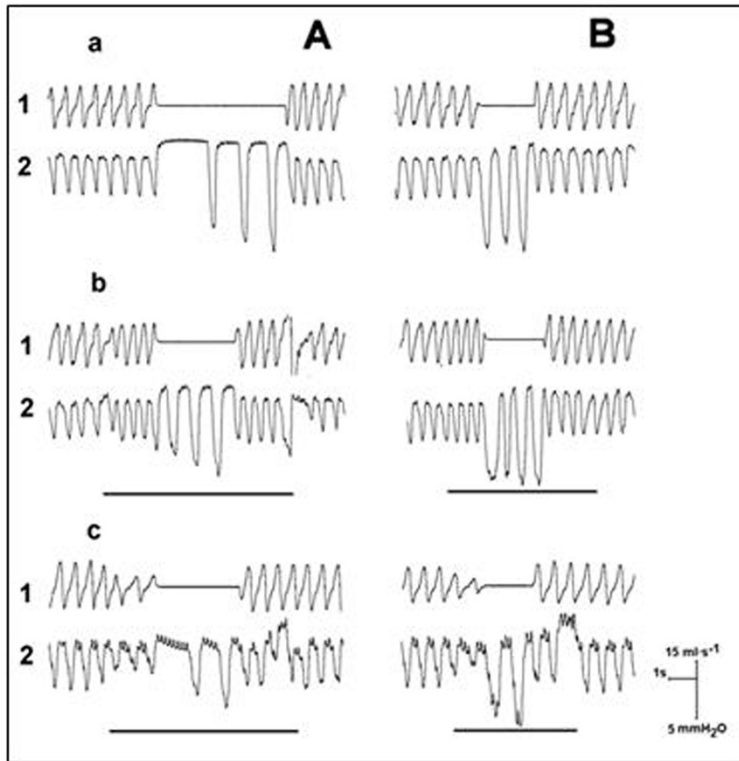
Fig. 2. Influence of cortical stimulation on the effects of airway occlusions. A - occlusions at the end-inspiration and B - occlusions at end-expiration; a – control, b – stimulation of infralimbic cortex (IL), and c – stimulation of insular cortex (IC); 1 – respiratory flow and 2 - inspiratory swings of esophageal pressure. Stimulus characteristics are the same as in Fig.1. Horizontal lines indicate duration of stimulation.
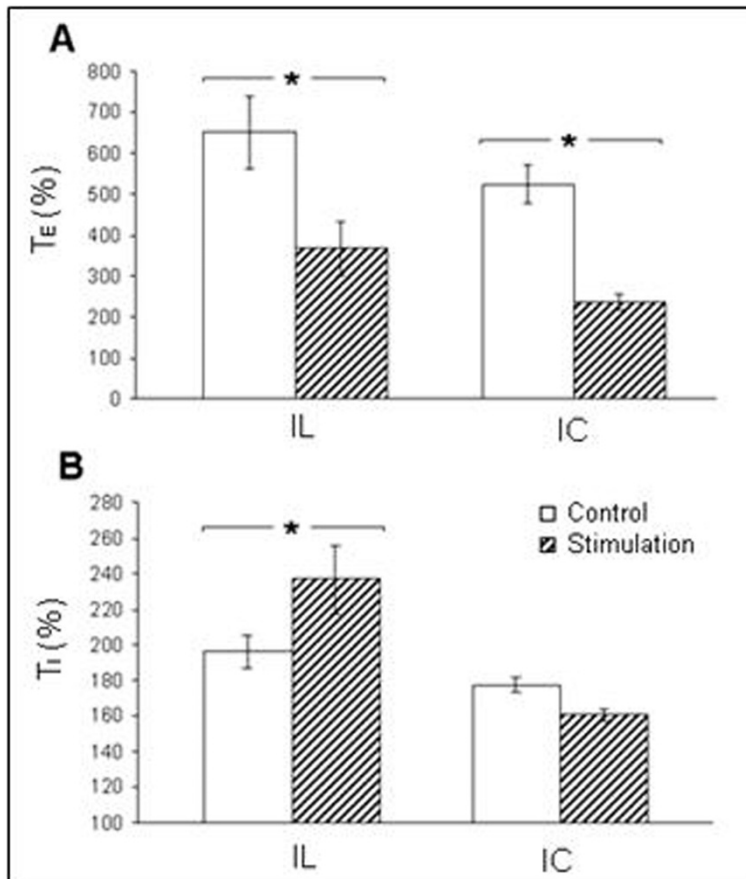

Fig. 3. Alterations in Hering-Breuer reflexes in response to cortical stimulation. A - expiratory-promoting reflex; B – inspiratory-inhibitory reflex. T_E (%) - duration of the first expiration after inspiratory occlusion expressed as a percent of the T_E of the last pre-occluded breath; T_I (%) – duration of the first inspiration after expiratory occlusion expressed as a percent of the T_I of the last pre-occluded breath. *$P<0.05$.

DISCUSSION

In agreement with our previous studies [12, 13], the present study demonstrate that distinct patterns of respiratory responses localize to different parts of the prefrontal cortex in the rat. The present findings show that stimulation of different PFC fields produced specific alterations in the respiratory pattern.

The SARs are activated by lung inflation and afferent input descending from SARs is important for the timing of respiratory phases. Afferent traffic from SARs delivered during inspiration terminates inspiratory phase [16], and that during expiration prolongs the expiratory period [17]. These reactions are referred to as Hering-Breuer inspiratory-inhibitory (TI-inhibitory) and Hering-Breuer expiratory-promoting (TE-promoting) reflex, respectively. If the airway occlusion is performed at end-expiration, lung volume remains at the level of functional residual capacity. Afferent traffic from SARs is minimal under such conditions, therefore, the TI-inhibitory reflex weakens and TI of the immediately following inspiration increases. On the other hand, airway occlusion at end-inspiration keeps afferent traffic high and increases the strength of the TE-promoting reflex. Therefore, it is obvious that both TI-inhibitory and TE-promoting reflex were evoked in the present study.

This study describes a novel phenomenon which consists of alterations in the Hering-Breuer reflexes in response to electrical microstimulation applied to sites in the prefrontal cortex. This fact suggests that the prefrontal cortex is able to modulate respiratory reflexes evoked by SARs. The present study indicates that stimulation of the IC weakens both TE-promoting and TI- inhibitory reflexes, and activation of the IL depresses the TE-promoting reflex. Thus, prefrontal cortex effects on the Hering-Breuer reflexes are inhibitory for the most part. This fact is in accordance with the results of the previous studies which have demonstrated an inhibitory action of the prefrontal cortex on autonomic reflexes [14, 15]. In the present study, however, the IL increased the strength of the TI-inhibitory reflex, which suggests that PFC effects on the autonomic reflexes may be excitatory.

It seems clear that the anatomic substrate for this cortical action on respiratory reflexes is formed by extensive direct projections from the PFC to the nucleus tractus solitarius (NTS) [18-22]. The NTS is the main brainstem recipient of vagal afferents which conduct viscerosensory information from various viscera, including lungs, and a number of autonomic reflexes, including Hering-Breuer reflexes, integrate within the NTS [23-25]. Therefore, PFC may influence transmission through the various reflex pathways which starts with the vagal afferents and switches in the NTS onto the neurons of the dorsal respiratory group. Besides, respiratory neurons mediating the Hering-Breuer TE-promoting reflex have been identified in the ventral respiratory group (VRG) situated in the ventrolateral medulla [26]. The ventrolateral medulla, in turn, receives afferents from the PFC and other autonomic structures related with it [22, 27]. Therefore, PFC effects on the Hering-Breuer reflexes may be mediated by neurons of the DRG and VRG.

In summary, the present study demonstrates that impulses originating from the prefrontal cortex can influence the vagally-mediated control of the respiratory phase timing mechanism. It is suggested that several regions in the prefrontal cortex modulate distinct sets of neurons in the network controlling inspiratory and expiratory phases. This mechanism could play an important role in the cortical control of breathing patterns associated with emotional and stressful behavioral disturbances, such as dyspnea or breathlessness.

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