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Stimulation of the rat medullary raphe nuclei induces differential responses in respiratory muscle activity

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ABSTRACT

Neural control circuits that coordinate the motor activity of the diaphragm (DIA) and the geniohyoid muscle (GH) are potentially involved in pathological conditions such as various forms of sleep apnea. Here we investigated a differential role of the raphe magnus (RMg), pallidus (RPa) and the obscurus (ROb) nuclei in the neural control of DIA and GH muscle activity in rats under volatile anesthesia. In order to characterize a topographical organization of the raphe nuclei we analyzed changes in DIA and GH during high-frequency stimulation (HFS, 10–130 Hz, 60 μs pulse width, 40–160 μA, 30 s). HFS of the RMg and the ROb induced apnea, in the latter case apnea was associated with massive tonic discharge in the GH. By contrast, HFS of the RPa induced tachypnea. At caudal stimulation sites the tachypnea was accompanied by tonic DIA activity and cessation of GH. These data suggest a differential distribution of inhibitory and excitatory drives of DIA and GH muscles within distinct raphe nuclei.

1. Introduction

The serotonergic raphe nuclei can be basically subdivided into two major groups: the dorsal raphe nucleus is located in the pons/mesencephalon and has mainly ascending projections to forebrain structures. In contrast the raphe nuclei found in the medulla oblongata have dense projections that target areas in the brainstem and the spinal cord (Holtman et al., 1986; Lalley, 1986a,b; Aldes et al., 1989; Li et al., 1993; Dobbins and Feldman, 1994; Haxhiu et al., 2003). The latter are known to be involved in the mediation of arousal during wakefulness but also in autonomic responses of the cardio-respiratory systems in brainstem (Jacobs and Azmitia, 1992; Jacobs and Fornal, 1999; Li et al., 2006; Comet et al., 2007). Experimental evidence also suggests that medullary raphe neurons have chemosensitive properties, although a major role in central chemosensitivity has been attributed to the retrotrapezoid nucleus (Richerson, 2004; Richerson et al., 2005; Taylor et al., 2004, 2005; Guyenet et al., 2005; Penatti et al., 2006; Mulkey et al., 2007).

Pharmacological studies performed either *in vivo* or *in vitro* showed different effects on spinal motor output related to respiratory pump activity or cranial motor output associated with upper airway patency, or both (Morin et al., 1990, 1992; King and Holtman, 1990; Sood et al., 2006). Nevertheless, the anatomical substrate and the precise mechanisms responsible for raphe-evoked modulation of cranial and spinal motor activity are not fully understood. However, a potentially differential control of spinal and cranial motor activity arising from the medullary raphe nuclei may occur during sleep-wake stage transitions. The inspiratory activity of the genioglossus muscle, which is innervated by the cranial hypoglossal nerve, is decreased in the non-REM sleep state and is often completely inhibited in the REM-sleep state. In contrast diaphragmatic activity (spinal motor output) is less affected (Haxhiu et al., 1987; Hendricks et al., 1993; Fenik et al., 1998; Lu et al., 2005; Sood et al., 2006). The same pattern of spinal and cranial respiratory motor activity is seen in humans (Sauerland and Harper, 1976; see also references cited in Feroah et al., 2001) and is closely linked with the obstructive sleep apnea syndrome (OSAS; Kurtz et al., 1978; Mezzanotte et al., 1992; Hendricks et al., 1993; Veasey et al., 1999; Fogel et al., 2003, 2005; Katz and White, 2004). OSAS is caused by upper airway obstruction and occurs particularly in REM-sleep states where the decrease in respiratory drive to upper airway muscles is most evident.

Raphe neurons directly or indirectly modulate the respiratory motoneurons via pre- and post-synaptic facilitation or inhibition (Lalley, 1986a,b; Lalley et al., 1997; Richter et al., 1997; Bouryi and Lewis, 2003). Interestingly, high-frequency electrical stimulation (HFS) of medullary raphe nuclei can induce different ventilatory responses according to the location of the stimulating electrode. Indeed, HFS of the raphe magnus (RMg) or the dorsal part of the

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raphe obscurus (ROb) depresses inspiratory phrenic motoneurons causing transient central apneas. By contrast, HFS of the raphe pallidus (RPa) or the ventral part of the ROb generates tachypnea (Lalley, 1986a,b; Millhorn, 1986; Cao et al., 2006a,b). Moreover, electrophysiological recordings of neurons located in medullary raphe showed sleep-wake state related activity (Jacobs and Fornal, 1991). Thus, it has been postulated that medullary raphe nuclei represent a key structure for the control of both cranial (upper airways) and spinal (pump muscles) respiratory motor activities during sleep-wake state transitions.

The aim of this study was to investigate the concomitant changes in breathing patterns of the geniohyoid and diaphragm muscles, which are innervated respectively by cranial and spinal motoneurons, in response to HFS of various medullary raphe nuclei. Contraction of the geniohyoid muscle contributes to pharyngeal dilatation. By analyzing the frequency- and intensity-related effects of HFS we tested the hypothesis that the various medullary raphe nuclei operate differently to coordinate cranial and spinal motor activity.

2. Methods

2.1. General procedures

Experiments were carried out in accordance with European Communities Council Directive 86/6609/EEC as well as French law. Male Sprague–Dawley rats (250–360 g, Janvier, France) were group housed (5–6) under conditions of constant temperature (21 °C ± 1 °C) and humidity under a 24-h light-dark cycle (lights on 08:00–20:00 h) with food and water freely available. The rats were anesthetized with isoflurane inhalation. Volatile anesthesia was initiated in a sealed chamber at 3.5% in oxygen (flow rate 2 l/min) and maintained at 1% in oxygen (flow rate 0.8 l/min) and nitrogen protoxyde (flow rate 0.6 l/min). The nitrogen protoxyde was administered in order to decrease nociception due to surgery. The state of anesthesia was adjusted to inhibit the withdrawal reflex. Rats were breathing spontaneously under anesthesia, and their body temperature was kept at 37 °C using a rectal probe connected to a servo controlled heating pad (CFP, USA).

2.2. Recording of cardio-respiratory parameters

The geniohyoid (GH) recording electrodes (insulated sliver wire, tip diameter 0.25 mm) were placed via midline incision in the ventral aspect of the mandible. In addition, after a hemilaparotomy electrodes were attached to the costal diaphragm (DIA), and the abdominal wall was sutured. Muscle activities recorded from the GH and DIA were amplified and filtered using appropriate settings (AM system Inc., USA). In addition, the oxygen saturation ($SatO_2$) and heart rate (HR) were monitored via an impedance plethysmographic sensor placed at the left paw (NELLCOR, Pulse oxymeter, USA). Signals from GH, DIA, $SatO_2$ and HR were digitized (sampling frequency 5 KHz), recorded and displayed in real-time on a computer using the SciExperimenter software (DataWave Technology, USA).

2.3. Electrical stimulation of raphe nuclei

In order to place the bipolar micro-electrodes (tip diameter 200 μm, impedance 0.5 MΩ, FHC, USA) for HFS, three small midline craniotomies were performed. The electrodes were guided into the medullary raphe (Stereotaxic coordinates according to Paxinos and Watson, 1998; 9.5–12.5 mm caudal to bregma, 9–11 mm ventral from surface) using a micromanipulator (Unimecanique, France). The stimulation tracks were separated by 1 mm on order to map respiratory responses from RMg, ROb, and RPa. Stimulation tracks for respiratory responses were aiming for dorsal to ventral parts of the raphe nuclei. For each individual track, electrical stimulation was delivered with step width of 500 μm.

2.4. Experimental protocols

Muscle activities from the GH and DIA, the respiratory rate (RR), HR and $SatO_2$ were analyzed before (1 min), during (30 s) and after (1 min) HFS (LeadPoint, Medtronic, USA) of the raphe nuclei. Stimulus intensity- and frequency-related effects were tested for each rat according to the following protocol.

Stimulation sites were tested for frequency- and intensity-related effects on the cardio-respiratory parameters. First simulation trial varied the stimulus intensity of 30 s stimulus trains (130 Hz, 30 s, pulse width 60 μs). The intensity was then successively increased in 40 μA steps from 40 to 160 μA. In a second stimulation trial, the frequency of the stimulus train (30 s, pulse width 60 μs, 120 μA) was progressively increased from 10, 30, 70, 100 to 130 Hz. Each individual stimulus trial was separated by 60 s. The stimulus intensities and train frequencies were chosen according to previous publications (Holtman et al., 1986; Lalley, 1986a,b; Dostrovsky and Lozano, 2002; Cao et al., 2006b).

2.5. Data analysis

Muscle activities from the GH and DIA were integrated in order to analyze respiratory burst areas. The burst areas were measured during baseline activity prior to stimulation (control period), during the 30 s stimulation period, and during the post-stimulation period. The post-stimulation period was further subdivided into an immediate (0–30 s) and a late phase (30–60 s) in order to analyze respiratory parameters during compensation of HFS effects (rebound), and during progressive return to baseline activity (recovery), respectively. Burst areas measured from the integrated DIA/GH were normalized, and expressed as % changes from baseline (±S.E.M.). Statistical comparison was performed with a Wilcoxon sign rank test. The respiratory rate (RR) was calculated from the DIA, and HR from the plethysmographic sensor signal. In cases where raphe stimulation caused transient apnea, the duration of the evoked apnea was measured. Statistical analyses of changes in RR, HR, $SatO_2$ and apnea duration were performed with ANOVA followed by a Fisher LSD post-hoc test (StatView).

Furthermore, to analyze intensity- and frequency-related changes of cardio-respiratory parameters, Pearson correlations and linear regression (StatEL Base) were performed to examine changes in GH, DIA, RR, HR (see Supplementary Figs. 1 and 2). In these analyses, effects were considered to be intensity- or frequency-related when a statistical level of significance of $p < 0.01$ was reached.

In addition changes in RR evoked by raphe stimulation were analyzed for a quantal relation to the baseline respiratory rhythm. To do so, the instantaneous frequency was normalized to the mean respiratory cycle length.

2.6. Tissue processing

After the end of the physiological experiments each rat was deeply anesthetized with pentobarbital (100 mg/kg) and intracardially perfused with 300 mL of phosphate-buffered saline (PBS; 0.1 M, pH 7.4) followed by 300 mL of 4% paraformaldehyde in phosphate buffer. Brains were removed and fixed for 2 h in 4% paraformaldehyde, and then in 30%sucrose solution for 24 h. Neural tissues were cut into 40 μm coronal sections on a microtome at −20 °C and collected in PBS. The sections were then mounted on gelatin-coated slice and stained with cresyl-violet. Sections were examined on an Olympus microscope. Each track and final depth of the electrodes were visualized and documented in semi-schematic

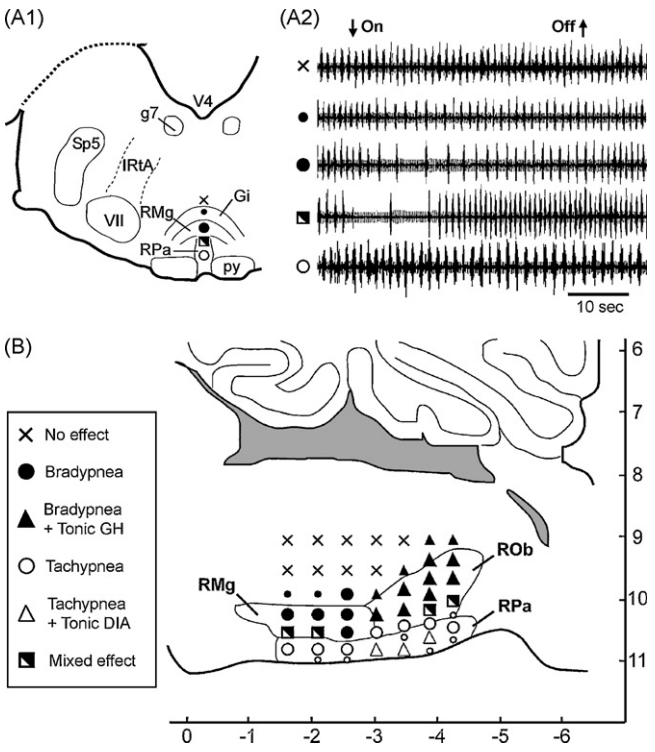

Fig. 1. Topographical organization of respiratory effects triggered by electrical stimulation (130 Hz/160 μA) of medullary raphe nuclei. (A) 1–2: Illustration of DIA responses evoked by an individual stimulation track trough the raphe magnus (RMg) and pallidus (RPa). Please note that the stimulation site at the border between RMg and RPa triggered a mixed response comprising an initial bradypnea followed by tachypnea. Stimulation sites within the RMg caused a pure bradypnea and stimulation of RPa caused a pure tachypnea. (B) Semi-schematic drawing summarizing the respiratory effects triggered by stimulation of the medullary raphe nuclei. *Abbreviations:* ROb, raphe Obscurus; Gi, gigantocellular reticular nucleus; Sp5, spinal trigeminal nucleus; VII, facial motor nucleus; IRtA, intermediate reticular nucleus pars alpha; V4, fourth ventricle; g7, genu of the facial nerve; py, pyramidal tract.

drawings of the raphe nuclei according to the Stereotaxic Brain Atlas of Paxinos and Watson (6th edition, 2007, see Fig. 1).

3. Results

Systematic investigation of high frequency stimulation (HFS) of different medullary raphe nuclei revealed a clear topography of evoked cardio-respiratory effects documented by recordings of GH/DIA muscle activity, RR, HR and $SatO_2$. The different respiratory responses evoked from rostro-caudal and dorso-ventral variation of the stimulation sites are summarized in Fig. 1. All stimulation sites were tested with varying stimulation frequency and intensity (See Supplementary Figs. 1 and 2). Nevertheless, it turned out that a stimulation intensity of 160 μA and a frequency of 130 Hz yield the best effects. Therefore the following results are based on cardio-respiratory effects triggered by 160 μA/130 Hz HFS.

3.1. Cardio-respiratory effects evoked by stimulation of the raphe magnus

Significant frequency- and intensity-related cardio-respiratory effects were observed during stimulation of the raphe magnus (RMg; Supplementary Fig. 1A and B). The highest stimulation intensity and frequency (160 μA/130 Hz) initially caused apnea followed by a decreased RR leading to a drop in oxygen saturation (Fig. 2A–C; Table 1). The decrease in RR was never accompanied by significant changes of amplitude, burst duration or area as measured from the integrated DIA. The GH remained synchronized with DIA during stimulation but showed an increased burst amplitude leading to a significant increase in the integrated GH (Fig. 2A–C; Table 1). During the post-stimulation rebound, the integrated GH and HR were still significantly elevated. An increase in integrated DIA and RR was also observed during this rebound period, although $SatO_2$ remained significantly depressed. All parameters returned to baseline about 30 s after the end of the stimulation (Table 1).

Analysis of a relation to the normalized instantaneous respiratory cycle length showed no evidence for a quantal slowing of the respiratory rhythm during stimulation of the RMg.

3.2. Cardio-respiratory effects evoked by stimulation of the raphe obscurus

HFS of the raphe obscurus (ROb) revealed effects similar to those observed with HFS of the RMg (Fig. 2D–F). In particular, the integrated DIA did not change significantly during stimulation when respiratory activity resumed after apnea. However, apnea duration was slightly more pronounced compared to RMg stimulation (Fig. 2D–F; Table 1).

During ventilatory depression induced by HFS of ROb, different effects were observed for the GH activity. Contrary to stimulation of the RMg, activation of the ROb caused a marked tonic activation of GH (Fig. 2D–F; Table 1). The evoked tonic activation was intensity- and frequency-related (Supplementary Fig. 1C and D). Analyses of the parameters during the rebound period showed that integrated DIA/GH activities were significantly elevated, although RR and $SatO_2$ remained depressed immediately after stimulation (Table 1). These parameters returned to baseline about 30 s after the end of the stimulation (Table 1). The cardiac response to HFS of ROb also clearly differed from that observed during RMg stimulation, since no significant change in HR was measured during or after ROb stimulation (Table 1). Again, no quantal relation between baseline and stimulus-associated respiratory rhythm was detected.

3.3. Cardio-respiratory effects evoked by stimulation of caudal and rostral raphe pallidus

Stimulation of the rostral parts of raphe pallidus (rRPa) induced significant intensity- and frequency-related increases in RR (Supplementary Fig. 2A and B), as indicated by an increase in GH/DIA amplitude and burst rate (Fig. 3A–C; Table 1). The integrated DIA and GH, HR and $SatO_2$ remained unchanged during and after rRPa stimulation (Table 1). The RR remained elevated and then progressively returned to baseline during the post-stimulation periods (Table 1).

Stimulation of more caudal aspects (see Fig. 1B) of raphe pallidus (cRPa) also triggered significant tachypnea in relation to stimulation intensity and frequency (Supplementary Fig. 2C and D). However, the simultaneous recording of GH and DIA revealed a differential response pattern. While DIA showed a massive increase in bursting frequency on top of tonic discharge, phasic GH activity was clearly depressed or even switched off (Fig. 3D–F). The HR and $SatO_2$ were unchanged during HFS (Table 1). During the initial post-stimulation period, the RR and DIA activity returned to baseline, whereas GH activity remained decreased. All parameters were similar to the control period 30 s to 1 min after the end of the stimulation (Table 1).

4. Discussion

In this study, electrical stimulation of the raphe nuclei was used to analyze concomitant changes in respiratory activity of the geniohyoid (GH) and diaphragm (DIA) muscles in the anesthetized rat. Basically in accordance with previous studies (Holtman et al., 1986; Cao et al., 2006b), electrical stimulation of various raphe nuclei

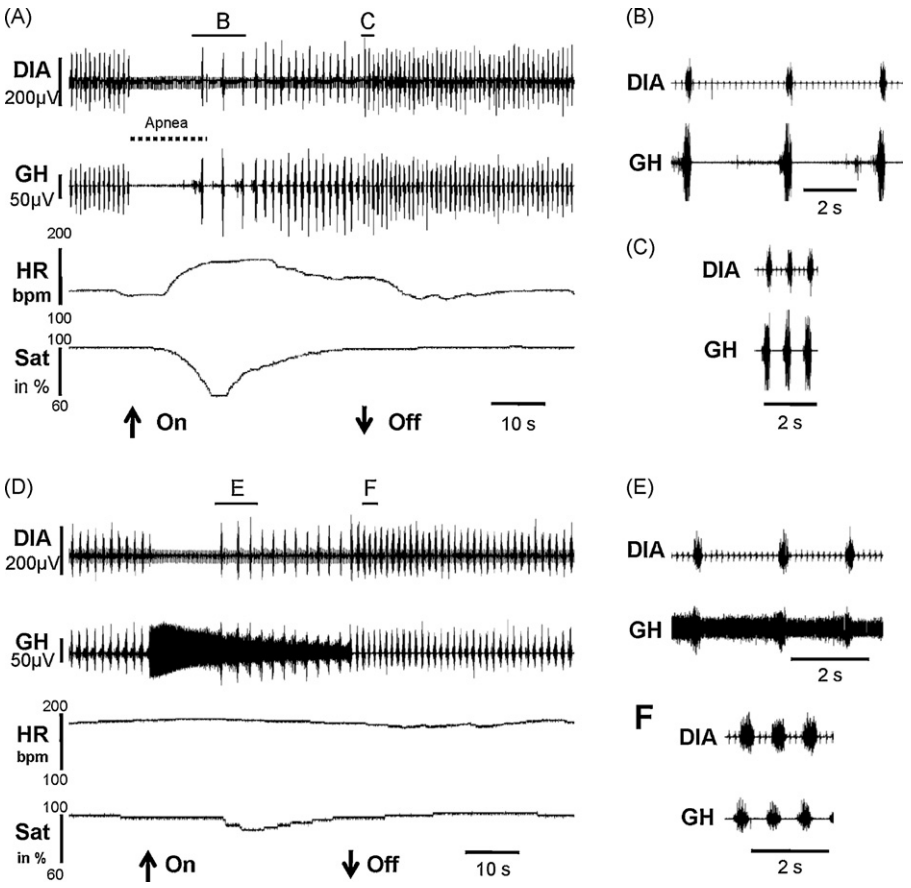

Fig. 2. Original recording of the cardio-respiratory response pattern to electrical stimulation (130 Hz/160 μA) of the raphe magnus (RMg) and obscurus (ROb). (A–C) Overview of the cardio-respiratory response to stimulation of the RMg. (A) From top to bottom: diaphragm EMG (DIA), geniohyoid EMG (GH), heart rate (HR) and oxygen saturation (Sat). Enlarged traces of DIA and GH are shown during the stimulation (B), and during the post-stimulation rebound (C). (D–F) Original recording of the response to ROb stimulation, with the same organization and abbreviations as in (A–C). Please note the differential response of DIA and GH induced during ROb stimulation.

Table 1
Summary of cardio-respiratory effects triggered by electrical stimulation of the raphe magnus, obscurus, rostral and caudal pallidus during stimulation, post-stimulation rebound, and recovery. All presented data relate to stimulation with 130 Hz/160 μA and are presented as % change to baseline, except for heart rate (HR) expressed as beats per minute (bpm). *Abbreviations:* DIA, diaphragm EMG; GH, geniohyoid EMG; RR, respiratory rate; $SatO_2$, oxygen saturation.

Stimulation at 130 Hz/160 μA	Stimulation	Rebound	Recovery
Raphe magnus			
DIAemg (%)	+1.7 ± 17.7	+11 ± 8*	+3.7 ± 5.3
GHemg (%)	+33.2 ± 28.6*	+13.3 ± 14.7*	−2.1 ± 2.9
RR (%)	−58.9 ± 18.9*	+11.2 ± 15*	−0.6 ± 1.1
Apnea (s)	13 ± 5	–	–
Sat02 (%)	75.7 ± 21.8*	88.8 ± 11.9*	98.5 ± 1.7
HR (bpm) (basal = 160.8 ± 25.8)	201.4 ± 17.1*	186.7 ± 11.1*	162 ± 16.0
Raphe obscurus			
DIAemg (%)	−7.1 ± 27.7	+31 ± 21.3*	+3.1 ± 5.4
GHemg (%)	+146.1 ± 84.3*	+47.3 ± 36.2*	+2.1 ± 3.4
RR (%)	−50.4 ± 17.8*	−39.7 ± 20*	0.0 ± 0.9
Apnea (s)	19 ± 5	–	–
Sat02 (%)	75.6 ± 14.6*	85.3 ± 20.5*	99 ± 0.8
HR (bpm) (basal = 173.2 ± 26.7)	179.9 ± 28.1	176.8 ± 28.6	184.9 ± 16.9
Rostral raphe pallidus			
DIAemg (%)	+6.7 ± 3.3	+13.1 ± 4.4	+1.4 ± 2.1
GHemg (%)	−4.9 ± 13.8	−4.6 ± 15.1	−1.8 ± 3.3
RR (%)	+20.4 ± 10.3*	+24.2 ± 13.8*	+0.3 ± 0.2
Sat02 (%)	98 ± 0	98 ± 0	98 ± 0
HR (bpm) (basal = 173.4 ± 12.6)	168.1 ± 21.6	160.8 ± 9.1	167.2 ± 11
Caudal raphe pallidus			
DIAemg (%)	+82.1 ± 49.1*	+15.8 ± 13.8	+0.3 ± 0.4
GHemg (%)	−55.3 ± 15.6*	−27.3 ± 18*	−1.4 ± 2.1
RR (%)	+66.3 ± 32.1*	−8.2 ± 6.6	−1.0 ± 0.6
Sat02 (%)	98 ± 0	99 ± 0.5	98 ± 0
HR (bpm) (basal = 166.0 ± 30.9)	195.5 ± 42	196.3 ± 39.6	177.9 ± 17.4

* $p < 0.05$.

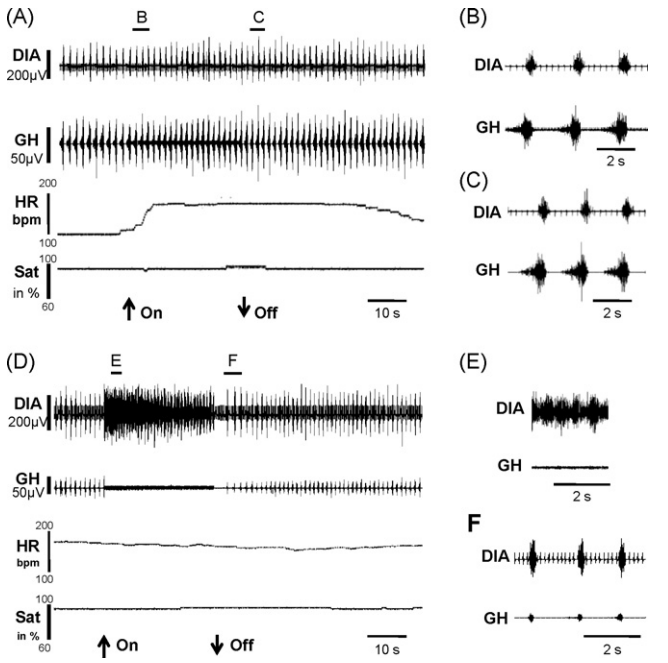

Fig. 3. Original recordings of the response pattern to electrical stimulation of the rostral (A–C) and caudal (D–F) raphe pallidus (RPa). Same organization and abbreviations as in Fig. 2. Please note the clear differential response of DIA and GH during tachypnea evoked by stimulation of the caudal RPa.

revealed opposite influences on respiratory frequency. Stimulation of the raphe magnus (RMg) and raphe obscurus (ROb) generated apneas and respiratory depression, while stimulation of the raphe pallidus (RPa) always triggered tachypnea. However, the topographical analysis of the cardio-respiratory effects evoked by raphe stimulation, showed for the first time that stimulation sites within the ROb and caudal RPa were associated with differential modulation of spinal (DIA) and cranial (GH) respiratory motor outputs. These findings are of potential relevance for the understanding of both fundamental and pathological aspects of breathing.

4.1. Technical considerations

It cannot be excluded that electrical stimulation could have excited raphe neurons as well as other neuronal populations located in the close vicinity of the stimulating electrode. Nevertheless, the clear topography of the effects and mixed response from stimulation at nuclear borders suggest that current diffusion is rather restricted. Also, coherent intensity- and/or frequency-relationships found after HFS of individual raphe subnuclei (Supplementary Figs. 1 and 2) further suggest that the reported effects can be attributed to stimulation of raphe neurons located around the tip of the electrode. Glutamate microinjection into the medullary raphe nuclei can cause similar ventilatory and cardiovascular changes (Man and Liu, 1992; Dong and Liu, 1994; Mohammed et al., 1995; Haxhiu et al., 2003; Alvarenga et al., 2005) as we found with HFS. Therefore, HFS used in the present study probably excited similar populations of raphe neurons without stimulating adjacent fiber tracts. Moreover, the analyses of the topography of raphe-evoked responses do not support a functional separation of dorsal and ventral ROb as suggested in rats by Cao et al. (2006b). In all cases, stimulation sites that were clearly identified within the ROb triggered apneic responses. By contrast, only those ventral stimulation sites located near the border between ROb and RPa evoked tachypnea (Fig. 1). Since all responses triggered by HFS of RPa evoked tachypnea, similar effects obtained with stimulation sites in the ventral-most aspects of the ROb were likely due to activation of RPa neurons.

Another limitation of HFS is due to the simultaneous activation of neurons with different phenotypes that are known to be present in raphe nuclei. Thus, the effects reported in this study may not fully correspond to the cardio-respiratory effects triggered by more physiological inputs to the various medullary raphe nuclei.

4.2. Differential effects on GH and DIA during raphe-evoked bradypnea or apnea

Apneas followed by strong decreases in respiratory frequency were evoked during HFS of the RMg and ROb. There are numerous anatomical and electrophysiological data suggesting that this effect on the respiratory rhythm arising from raphe nuclei is mediated via direct influences on neurons located within the ventral respiratory group (Lalley, 1986a,b; Holtman et al., 1986; King and Holtman, 1990; Richter et al., 1997; Haji et al., 2000; Wang et al., 2005; Feldman and Del Negro, 2006). For example, it has been shown that stimulation of the ROb nucleus can abolish phrenic nerve activity and hyperpolarize other respiratory-related neurons via activation of 5-HT1A receptors (Lalley et al., 1997). Oxygen desaturation was associated with apnea or bradypnea suggesting secondary hypoxic stimulation of peripheral carotid bodies chemoreceptors during the stimulation and post-stimulation rebound. These changes in blood gas levels are therefore expected to increase muscle contraction of the DIA (Vizek and Bonora, 1998), however, this may account in particular for the post stimulation period. Since GH inspiratory bursts were significantly increased at RMg and ROb stimulation sites, we propose that during the stimulation the response to an increase in chemical drive to the DIA was blunted, while that of the GH was not. This may be reminiscent from the observation that the hypercapnic threshold of DIA is higher than those of the UAM (Dreshaj et al., 1998). Chemoreceptor inputs are mediated and integrated within the nucleus tractus solitarius (NTS). At the level of the NTS the mediation of chemoreceptor input could be modulated via the release of 5-HT in response to raphe stimulation (Kubin et al., 2006) leading to blunted hypoxic augmentation of DIA activity. However, tonic activation in GH was triggered with onset of stimulation of ROb. This suggests differential alteration of the firing of motoneurons controlling the GH and DIA via direct projections onto the corresponding motor nuclei (Aldes et al., 1989; Holtman et al., 1990; Li et al., 1993; Dobbins and Feldman, 1994).

Our results also showed a clear tonic activity in GH after HFS of ROb, whereas such an effect was never triggered after HFS of RMg. Considering the differential responses of DIA and GH evoked by HFS of RMg/ROb, we propose that both nuclei exert an inhibitory drive on phrenic motoneurons, while ROb neurons exert an excitatory drive on hypoglossal motoneurons.

Our data indicate that the cardiac response to HFS of ROb differed from that observed during RMg stimulation. Inputs from baroreceptors are integrated within medullary raphe nuclei, and both RMg and ROb neurons have been shown to contribute to baroreceptor-induced changes in respiratory activity (Arata et al., 2000; Curran and Leiter, 2007). Therefore, it is possible that the differential effects observed on GH/DIA activity during HFS of RMg and ROb reflect alterations in cardio-respiratory coupling. This is in line with the fact that raphe and ventral medullary neurons form a dynamic distributed network, engaged in a concurrent processing of information from chemoreceptors and baroreceptors (Li et al., 1999; Arata et al., 2000).

4.3. Differential effects on GH and DIA during raphe-evoked tachypnea

Consistent increases in respiratory frequency were induced during HFS of the RPa. As mentioned previously, similar effects were also observed at ventral-most aspects of the ROb. In both

cases, however, no obvious sign of long-term phrenic potentiation (or facilitation) was noticed following cessation of the stimulus (Millhorn, 1986). This lack of potentiation could be due to differences in protocols of stimulation and/or experimental models between the two studies. Under normal conditions, tachypnea is expected to induce hypocapnia which in turn should lead to a suppression of GH/DIA inspiratory bursts. However, phasic GH activity was immediately inhibited or even suppressed right at the onset of stimulation of the caudal RPa while the DIA activity was increased. This finding strongly suggests that termination of the GH activity and concomitant increases in DIA activity induced by caudal RPa stimulation were not secondary to changes in respiratory chemical drive. Instead, these ventilatory effects are likely mediated via specific projections from RPa nuclei to various elements of the respiratory network. The increase in respiratory frequency evoked after RPa stimulation may arise from direct excitation of ventral respiratory group neurons (Holtman and King, 1994). The release of serotonin and subsequent activation of various pre- and post-synaptic 5-HT receptors of medullary respiratory neurons can profoundly alter membrane potentials and neuronal discharge activity (Richter et al., 1997). Alternatively, tachypnea and the concomitant increase in DIA activity may be due to indirect excitation of the ventral respiratory group. Such an effect could be mediated by retrotrapezoid neurons which are known to receive dense serotonergic inputs, and to exert an excitatory influence on the ventral respiratory group (Rosin et al., 2006). We can also speculate that the termination of inspiratory phasic GH activity upon stimulation of caudal RPa is also due to the activation of an indirect pathway. Indeed, glutamate injection within the Kölliker–Füse nucleus (KF) has been shown to selectively inhibit the inspiratory discharge of the XIIth nerve while almost no effect could be detected on the phrenic nerve (Gestreau et al., 2005). The KF belongs to the respiratory CPG and hypoglossal premotoneurons have been described in this nucleus (Roda et al., 2004). Thus, the stimulation-evoked suppression of inspiratory GH activity after HFS of the caudal RPa may be mediated by serotonergic inputs on KF neurons.

4.4. Implications for non-respiratory behaviors

Raphe neurons contribute to regulate the gain of respiratory motor output and have reciprocal interactions with the ventrolateral medullary respiratory network (Morris et al., 1996; Lindsey et al., 1994, 1998). Lesions of the medullary raphe have been shown to eliminate cough patterns in phrenic and lumbar nerve neurograms (Jakus et al., 1998), and activities of RMg and RPa neurons are altered during fictive cough (Baekey et al., 2003). Therefore, it is possible that the respiratory effects described in the present study are relevant for non-respiratory behaviors that require contraction of the respiratory muscles, such as coughing or swallowing. Swallowing is always associated with suppression of inspiratory DIA activity although upper airway muscles including the tongue must contract to propel the food into the esophagus. However, the precise contribution of the various medullary nuclei in these motor activities remains to be investigated.

4.5. Clinical relevance of the findings

Our data based on HFS of the medullary raphe nuclei provided evidence for opposite ventilatory effects and differential respiratory modulation on GH/DIA. In particular, HFS of the ROb was shown to specifically increase tonic GH activity, while HFS of the caudal RPa evoked a clear increase in tonic DIA activity. In agreement with our findings, previous studies using pharmacological manipulation of medullary raphe nuclei, or application of various serotonergic agonists have also demonstrated a differential modulation of cranial and spinal motor outputs (Morin et al., 1992; Morin, 1993; Hilaire et al., 1993; Haxhiu et al., 2003; Sood et al., 2006). Interestingly, a similar effect is observed in both animals and humans during NREM and REM sleep states, and neuronal activity of raphe neurons is known to be dependant on sleep-wake states (Jacobs and Fornal, 1991, 1999). A decrease in activity of ROb neurons could result in a decreased GH muscle tone, leading to an increase in upper airway resistance. Therefore, we propose that RPa and ROb could represent key structures involved in the differential control of respiratory-related muscles associated with the physiological sleep-wake cycle. In patient suffering from sleep apnea syndrome, sleep fragmentation may alter the differential on/off-switching of upper airway muscle respiratory activity from raphe nuclei, thereby increasing the risk to develop obstructive sleep apneas.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.resp.2008.12.004.

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