

The role of raphe and tractus solitarius neuronal structures in the modulation of respiratory pattern in rabbits

Krystyna Budzińska and Jarosław R. Romaniuk

Department of Neurophysiology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa St., 00-784 Warsaw, Poland

Abstract. We studied the effects of MNR stimulation on phrenic (Phr) electroencephalogram and external intercostal muscles (EI) electromyogram in spontaneously breathing rabbits. Additionally, experiments were performed before and after lignocaine blockade of nucleus tractus solitarius (NTS) to determine whether the information from MNR is transmitted via NTS neurones. The completeness of the blockade of NTS region was checked by studying the Hering-Breuer reflex. MNR was stimulated at the level 2-7 mm rostral to the obex. Stimulation at the rostral part of this region produced inhibition of phasic inspiratory activity, whereas stimulation in the caudal part elicited tonic activity throughout the respiratory cycle. These effects were more pronounced on EI than on Phr. Responses to MNR stimulation were attenuated after lignocaine blockade, suggesting that the neurones located in NTS take part in the transmission of the modulatory information from the MNR to respiratory motoneurones.

Key words: raphe nuclei, nucleus tractus solitarius, electrical stimulation, lignocaine blockade, phrenic nerve activity, external intercostal muscles EMG

INTRODUCTION

Respiratory pattern generated in the brainstem is under modulatory influences from many central and peripheral inputs. Respiration is integrated with other functions of the organism, such as locomotion (DiMarco et al. 1983) and swallowing (Sessle et al. 1981, Dick et al. 1993). It is controlled by specific afferent pathways (i.e. lung and chest wall mechanoreceptors and chemoreceptors) (Euler 1986). One of the relay stations for afferent inflow into the respiratory complex is a group of interneurons localized in the nucleus tractus solitarii (NTS) (Sessle et al. 1981, von Euler 1986). Specific stimuli affecting respiration are usually well recognized, but recently more interest was paid to tonic modulatory neuronal systems. One of these is composed of serotonergic neurons which in the medulla are localized mostly in the raphe nuclei and NTS (Sessle et al. 1981, Holtman et al. 1986, Lalley 1986b). The tonic modulatory system is important in transmitting any phasic motor activity. It was shown, for example, that locomotion induced by electrical stimulation of subthalamic locomotor regions depended on the tonus of the muscle prior to stimulation (Mori 1989). If the tonus of the muscle was too weak, locomotion could not be evoked. Locomotor movements were restored, however, when the tonus of the muscle was increased by electrical stimulation of medullary raphe region (Mori 1989). The effects of stimulation of raphe structures on respiration have already been documented (Brodie and Borison 1957, Anderson and Sears 1970, Bianchi 1971, Sessle et al. 1981, Polc and Monnier 1990). It was shown that stimulation of MNR may either stimulate (Millhorn 1986, Holtman et al. 1987) or inhibit (Sessle et al. 1981, Lalley 1986a) respiratory activity. The difference in the response to stimulation could be due to different localization of the stimulus site (Polc and Monnier 1990) or to the fact that the experiments were performed mostly in paralysed animals (Holtman et al. 1986, Lalley 1986, Millhorn 1986) in which there was no control of the muscle tonus. It was also suggested that some of the inspiration inhibiting influences from pontine

pneumotaxic region are transmitted to NTS *via* MNR structures (Gang et al. 1991). On the other hand, lesions of midline neuronal structures of the brainstem (Romaniuk and Bruce 1991) abolished high frequency oscillation of inspiratory activity, suggesting that these structures may be involved in the formation of respiratory rhythmicity. In this paper, we studied the effect of electrical stimulation of the raphe region on phrenic and external intercostal muscle activity. We expected different effects of raphe stimulation on these two motor outputs since the phrenic nerve transmits only respiratory activity while the intercostal muscle serves both postural and respiratory functions. We performed our experiments before and after lignocaine blockade of NTS structures, since it was suggested that NTS is a relay station for modulatory effects on respiration including inspiratory off-switch mechanism. In contrast to previous papers (Holtman et al. 1986, Lalley 1986a, Millhorn 1986, 1987), we performed our experiments with non-paralysed animals to be able to observe the effects of raphe stimulation on the tonus of skeletal muscles. Preliminary results have been presented in abstract forms (Budzińska and Romaniuk 1986a,b).

METHODS

Experiments were performed with 17 male rabbits weighing 2.3–3.3 kg. The animals were anaesthetized with a mixture of chloralose (33 mg/l ml) and urethane (400 mg/l ml) at a dose of 2 ml per kg b.w. intravenously. A tracheostomy was performed and the femoral artery and vein were cannulated. Both cervical vagus nerves were prepared for sectioning. After vagotomy in 5 rabbits, efferent activities of right and left vagus nerve were recorded. The C5 root of the phrenic nerve was dissected, cut and prepared for recording. Both efferent vagal and phrenic nerve activities were amplified and integrated (560 Differential Ampl.Int.-Medipan) with a time constant of 100 ms. The EMG of the external intercostal (EI) muscle from 3 to 6 intercostal spaces was recorded with concentric needle electrodes. The muscular activity was filtered (band

pass 0.1 kHz-1.0 kHz) amplified and integrated (560 Differential Ampl. Int. - Medipan). The end-expiratory CO₂ concentration and arterial blood pressure in the femoral artery were measured (Beckman Medical Gas Analyser LB-2 Statham P23Db). Body temperature in the range of normothermia (37-38°C) was maintained by an external heating. Gasometric and acid-base equilibrium measurements were carried out on an Automatic pH/Blood Gas System (Corning 175). The animals were breathing spontaneously or ventilation was assisted by a phrenic nerve driven respirator (Huszczuk 1970) without introduction of any paralysing agent. The gain of the servo-respirator, i.e. volume to integrated phrenic signal ratio, was set to obtain a tidal volume comparable to the volume recorded during spontaneous breathing (Romaniuk et al. 1976). To measure the effects of phasic vagal input on inspiratory activities (Hering-Breuer inflation reflex), we increased or decreased the gain of the servo-respirator for one breath, such that for the same signal of integrated phrenic nerve activity, the volume of inflation was higher or lower for one breath. The gain changes were performed several times with different values of volume to phrenic signal ratio. When breathing was depressed due to pharmacological blockade of NTS, respirator was switched to classical mode.

The head of the animals was immobilized in a stereotaxic apparatus. The medulla was exposed by a dorsal approach. A concentric bipolar stimulating electrode was inserted into the medulla 2-7 mm above the obex in the midline by the micromanipulator under visual control. Stimulation was performed with the intensity of 15-100 µA, frequency of 100 Hz and 0.5 ms width of pulse for the period of several seconds. Threshold stimulation was determined by noticing any changes in muscle tonus and in phrenic nerve or external intercostal EMG activities.

A micropipette 50 µm tip diameter was inserted with a micromanipulator guidance at 1-2 mm lateral to midline at a distance of 1.5 mm rostral and 1 mm caudal to the obex (NTS region) unilaterally and bilaterally. The micropipette was filled with 2% Lignocaine (Polfa). All injected solutions contained 2% Methylene Blue. Pressure injection of lignocaine was delivered at a dose of from 0.2 to 5 µl, mostly 2 µl per injection site during 10-20 s. The maximum effect was reached during 10-40 s and lasted 10-15 min. The effects of lignocaine blockade (LB) were fully reversible. Stimulation of MNR was delivered about 3 min after application of lignocaine. Hering-Breuer reflex was tested between the 3rd and 10th minute after lignocaine injection. The volumes of injections were large to be sure that the injection blocks large area of the NTS.

Both, the sites of lignocaine blockade visualized by dye and of electrical stimulation were verified histologically. A sphere of the tissue 1.5-2.0 mm in diameter was coloured with dye. No lesions were performed with pressure injections.

RESULTS

Electrical stimulation of the medullary raphe region

Continuous electrical stimulation of the neuronal structures within raphe complex 2-7 mm rostral to the obex resulted in the inhibition of integrated activity of the phrenic nerve. As it is presented in Fig. 1, the inhibitory effects were more pronounced when stimulation was performed in the rostral part of the stimulated area. Stimulation in the caudal part of this region evoked tonic activity throughout the whole respiratory cycle (Fig. 1). Activity of external intercostal muscle was more affected by MNR stimulation than phrenic nerve activity (Figs. 1 and 2). Stronger inhibition of phasic phrenic activity was often accompanied by a marked increase in tonic external intercostal activity (Fig. 2). Stimulation which evoked tonic activity throughout the respiratory cycle also produced an increase in tension (constriction) of other postural muscles (neck, chest wall) that could be easily observed since animals were not paralysed. These effects lasted only during the period of stimulation and were not accompanied by an increase in frequency of breathing.

Lignocaine blockade of NTS region

Neuroanatomical localization of the sites of lignocaine injections is presented in Fig. 3. All injections were performed between 1 and 2 mm lateral to midline and 1.5 mm rostral and 1 mm caudal to the obex. It is known that within the NTS structures, there are interneurons integrating the Hering-Breuer inflation reflex (von Euler 1986). To study the completeness of lignocaine blockade of NTS, we measured the intensity of the Hering-Breuer reflex before and after blockade. Changes

in lung volume for the period of one breath were performed during assisted ventilation by means of a phrenic nerve driven servo-respirator (Huszczuk 1970). Changes in the amplitude of integrated phrenic nerve activity during tracheal occlusion or changes in the gain of servo-respirator (see Methods) were related to changes in transpulmonary pressure (PTP). As an effect of decreasing the gain of the respirator ($PTP < 100\%$ of control gain) or tracheal occlusion ($PTP = 0$), the amplitude of integrated phrenic nerve activity increased. Increase in gain ($PTP > 100\%$) inhibited inspiratory activity.

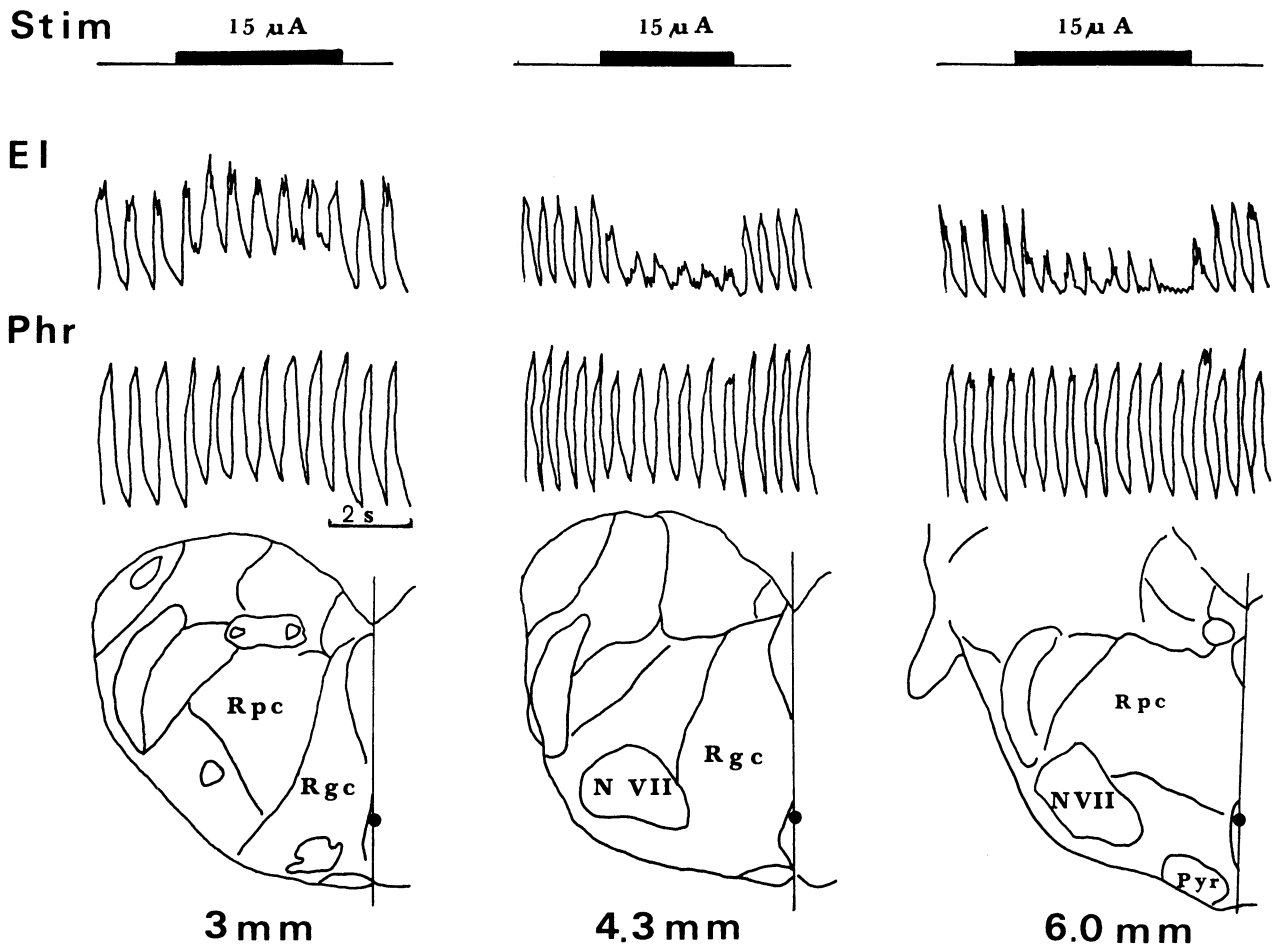


Fig. 1. Effects of medullary raphe nuclei (MNR) stimulation on integrated electromiogram of external intercostal muscles (EI) and phrenic nerve (Phr) activities. Stimulations were performed 3 mm (left panel), 4.3 mm, (medium) and 6 mm (right panel) rostral to the obex. Below the recordings there are transverse sections of the brain stem representing sites of stimulation (filled circles). Stimulation applied in caudal part of MNR produced an excitation of tonic activity with an enhancement of amplitude of integrated phrenic nerve and external intercostal muscles activities. More rostrally stimulation produced inhibition of phasic EI activity, decrease in frequency of breathing and (medium) inhibition of phasic Phr activity. Intensity of stimulation $15 \mu A$. Recordings are retouched. Abb.: Rpc, nucleus reticularis parvocellularis; Rgc, nucleus reticularis gigantocellularis; NVII, nucleus facialis; Pyr, pyramides.

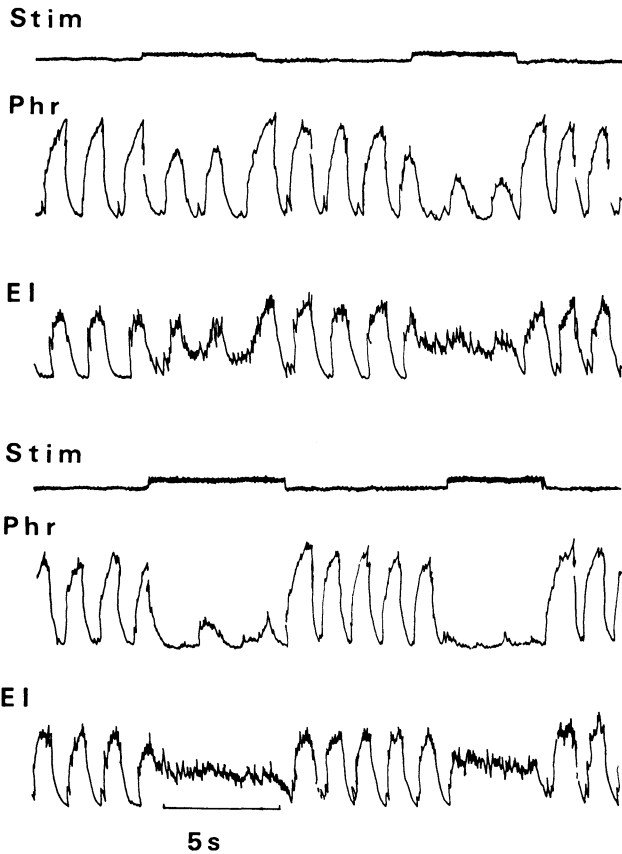


Fig. 2. Effect of an increase in the intensity of MNR stimulation on Phr and EI activities. With increasing intensity of stimulation, phasic Phr and EI activities are more inhibited, whereas, tonic activity of EI increases. Intensity of stimulation: A, 10 and 20 μ A; B, 30 and 40 μ A. Abbreviations as in Fig. 1.

Figure 4 presents the linear relationship (filled circles) between changes in the amplitude of integrated phrenic nerve activity and peak inspiratory transpulmonary pressure during maneuvers of tracheal occlusion and gain changes expressed in percent of PTP during control gain. After lignocaine blockade, this relationship was abolished, i.e. there was no effects of PTP changes on the amplitude of integrated phrenic nerve activity (open circles in Fig. 4). Elimination of the Hering-Breuer inflation reflex is evidence that lignocaine injection was sufficient to block the transmission of vagal afferent information.

The effects of injection of lignocaine into NTS on inspiratory motor activity strongly resembled the effect of vagotomy - there was prolongation of

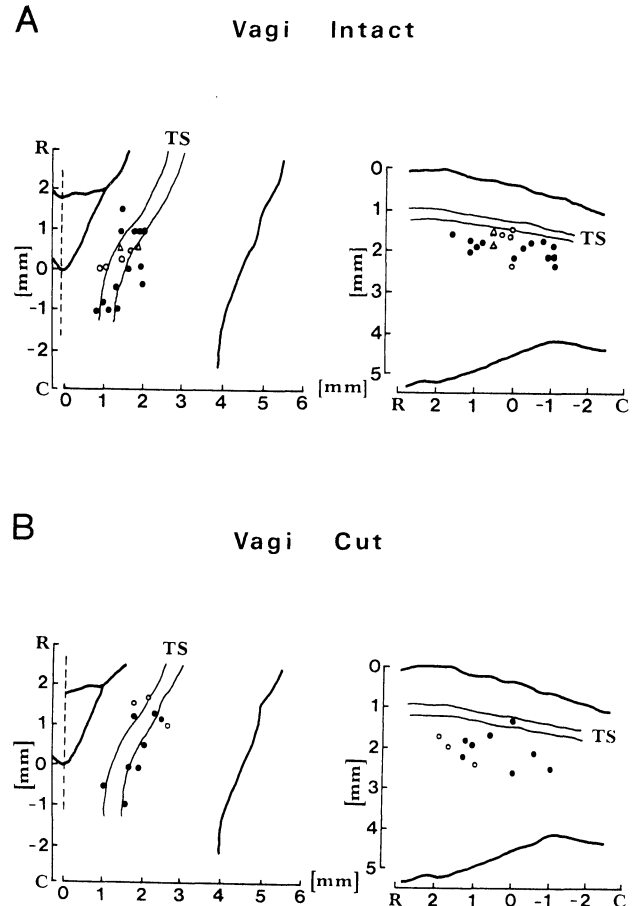


Fig. 3. Localization of lignocaine injection within NTS area shown at schematic horizontal and sagittal section of the medulla. All marks show sites of injections. A, vagi intact: filled circles, show points where after lignocaine injection Hering-Breuer reflex persisted; open circles, show sites where Hering-Breuer reflex was abolished by lignocaine blockade; triangle, indicate apneustic pattern of breathing. B, vagi cut: open and filled circles, show all sites where unilateral blockade by lignocaine was performed; open circles, indicate points where ipsilateral efferent vagus nerve activity was blocked; obex was referred as zero level; R, rostral; C, caudal.

inspiratory time (T_I) and an increase (or no changes) in the amplitude of integrated phrenic and external intercostal activities (Figs. 5 and 6). The T_I prolongation was, however, still present when blockade was repeated after bilateral vagotomy (Fig. 6). Apneustic pattern of breathing was seen occasionally (see Fig. 3).

The effect of NTS blockade on descending motor activity could be observed by recording efferent vagal activity since vagal motoneurons are localized in close vicinity of the site of injection

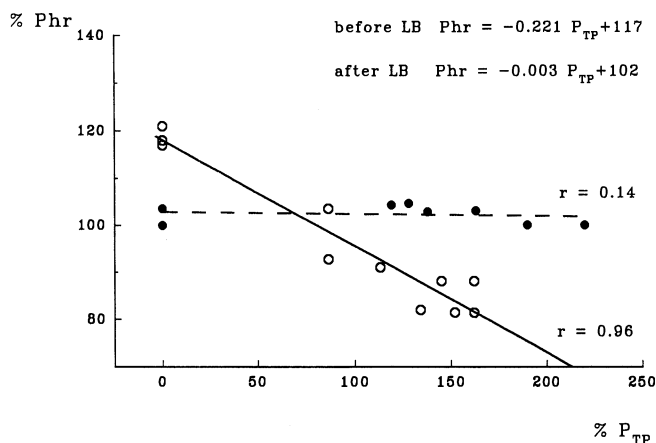


Fig. 4. Relationship between transpulmonary pressure (P_{TP} in % of control) and integrated phrenic nerve amplitude (Phr in % of control) as a measure of Hering-Breuer reflex intensity. Values of P_{TP} below 100% were obtained for decreased gain of servo-respirator and $P_{TP} = 0$ for tracheal occlusion. Open circles, before lignocaine blockade (LB) of NTS; filled circles, after lignocaine blockade (LB) of NTS. After blockade, relationship Phr *versus* P_{TP} was abolished.

(Bianchi 1971). Figure 7 presents the effect of lignocaine blockade on efferent vagal activity recorded ipsilaterally to the site of injection. Administration of lignocaine into NTS region abolished phasic efferent vagal activity ipsilaterally to the side of blockade.

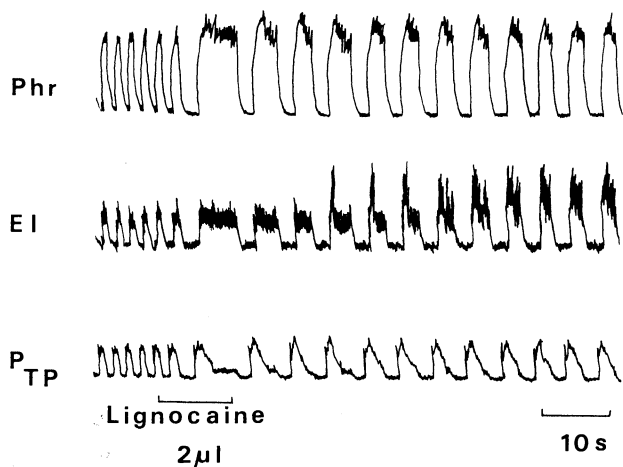


Fig. 5. Effect of lignocaine blockade of NTS on integrated phrenic nerve (Phr) and external intercostal muscles (EI) activities during assisted ventilation. After lignocaine injection into NTS, there was a decrease in frequency of breathing, and an increase in amplitude of recorded integrated activities. Note apneustic inspiration initially after blockade.

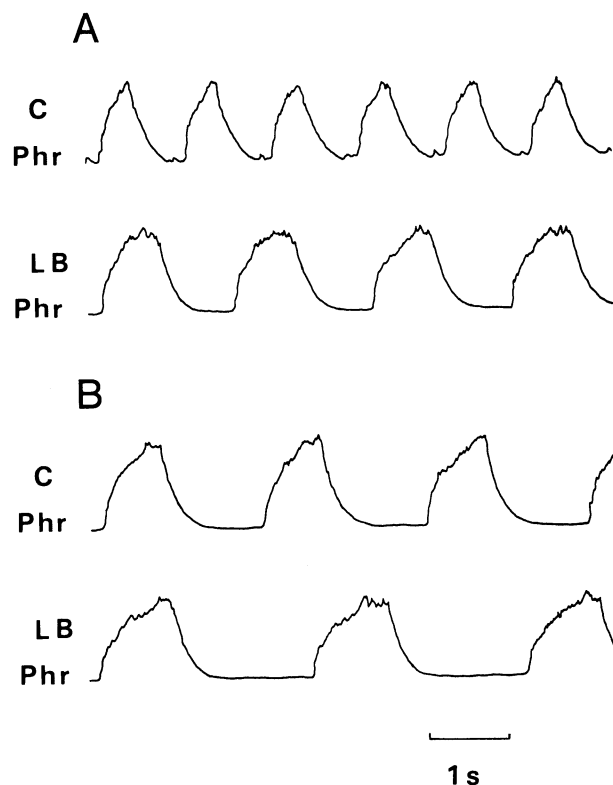


Fig. 6. Effect of lignocaine blockade (LB) before and after vagotomy on the respiratory pattern represented by integrated phrenic nerve activity. Lignocaine blockade produced prolongation of both inspiratory (T_I) and expiratory (T_E) time before (A) as well as after vagotomy (B).

Responses to MNR stimulation after lignocaine blockade

Both tonic and phasic responses to electrical stimulation of MNR were affected by lignocaine blockade of NTS at all studied regions of MNR. However, the respiratory response to MNR stimulation still persisted and it was similar from rostral to caudal part of MNR. When stimulation was applied ventrally in the midline structures after NTS blockade, the respiratory response became less pronounced (Fig. 8). Stimulation in caudal regions of MNR produced less tonic activity of external intercostal muscles being about $57\% \pm 17$ SD for $n=8$ of pre-blockade values. Stimulation in rostral regions of MNR, where evoked tonic activity was low, cause no or very low tonic activity ($5.2\% \pm 3.5$ SD for $n=9$) after NTS blockade.

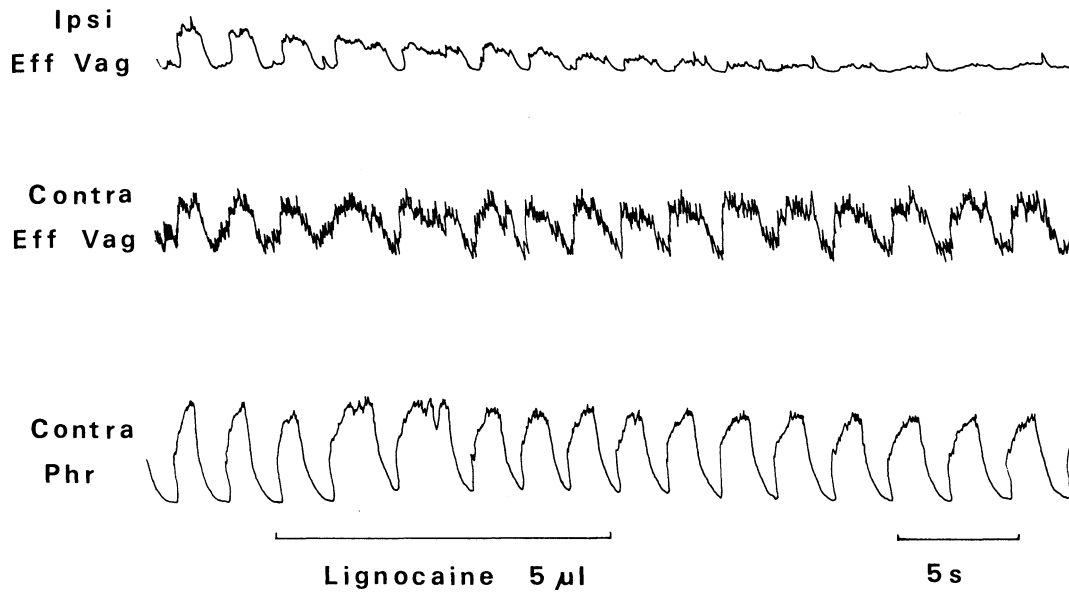


Fig. 7. Elimination of ipsilateral efferent vagal activity by lignocaine injection into NTS region.

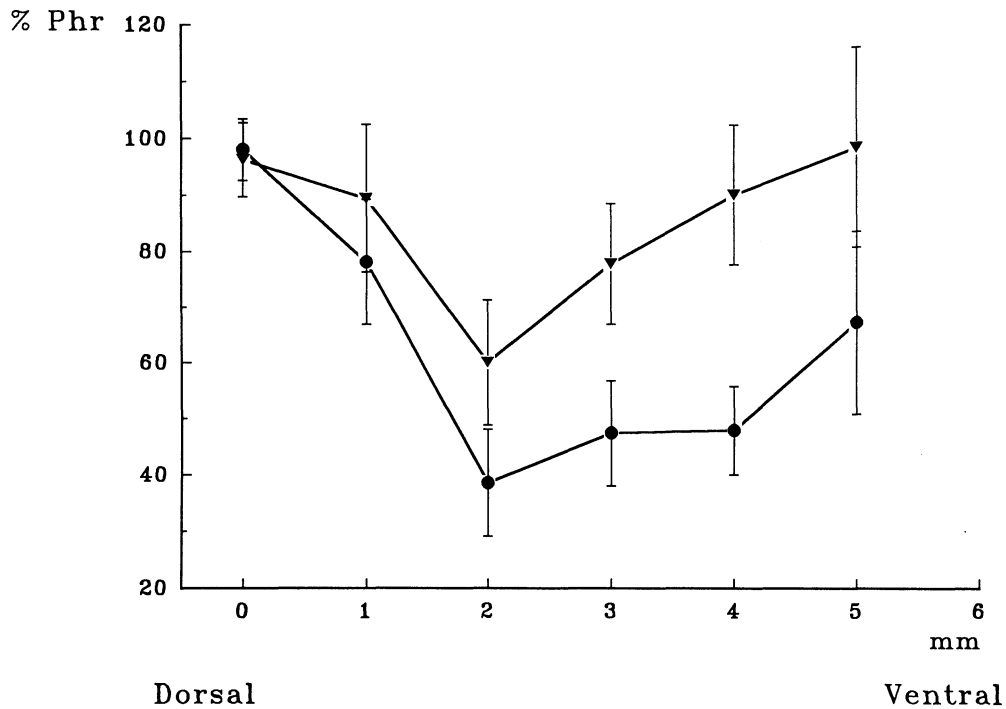


Fig. 8. Effect of midline stimulation before (circles) and during (triangles) NTS blockade in five experiments. Stimulation was performed every 1 mm at six different levels in the dorso-ventral plane (abscissa). 100% Phr, expresses the amplitude of integrated phrenic nerve activity before stimulation and blockade (ordinate). The strongest inhibition of Phr was seen when stimulation was performed 2 mm below the dorsal surface of the medulla and the effect of stimulation slightly declined towards the ventral surface. During NTS blockade the response to stimulation became less and less pronounced when more ventral sites were stimulated. Intensity of stimulation 40 μ A. Bars represent SD.

DISCUSSION

Electrical stimulation of neuronal structures in the midline of the brainstem 4-7 mm rostral to the obex inhibited phasic inspiratory activity of phrenic nerve and external intercostal muscles. Stimulation at more caudal levels, 2-3 mm rostral to obex, evoked tonic activity of external intercostal EMG. With an increase in the intensity of stimulation, phasic activity was more inhibited or even abolished while tonic activity was enhanced. External intercostal muscle units, which also subserve postural functions, showed stronger excitation of tonic activity compared to phrenic motoneurons. It was observed during earlier experiments (DiMarco et al. 1983, Kasicki et al. 1991) that an increase in the intensity of electrical stimulation of subthalamic locomotor regions may terminate stepping movements by an increase in muscle tone. One could speculate that strong excitation of tonic activity of skeletal muscles attenuates transmission of phasic motor activities. On the other hand tonic activity of different intensities can be released both in phrenic nerve as well as in intercostal and abdominal muscles activities when the respiratory drive decreases by focal cold block of the ventrolateral rostral regions of the medulla. The cold block causes simultaneously a strong depression of rhythmic inspiratory activity (Budzińska et al. 1985a,b). In this case, however, there was no systematic difference in the response of tonic and rhythmic activity of both phrenic nerve and external intercostal muscles. Also if the chemical drive is sufficiently lowered and rhythmicity fades to apnoea low level tonic activity persists (Cherniack et al. 1979). Release of tonic activity in the expiratory muscles did not correspond closely to the variations in the inspiratory activity and might represent some other, non-respiratory functions of the respiratory muscles (Budzińska et al. 1985c). However, the above observations and our present results suggest that the proper balance between phasic and tonic motor activities is generally required to maintain rhythmic movements (Mori 1989). Different regions of neuronal raphe complex exert different effects on

muscle tones. This may be the reason why raphe stimulation facilitates or disfacilitates the transmission of phasic activity, depending on the site of stimulation (Holtman et al. 1986, Lalley 1986). The modulatory effects of raphe stimulation on respiration may be transmitted by interneurons of NTS. Pharmacological blockade of NTS attenuated both tonic and phasic components of the response produced by raphe stimulation. However, some effects still persisted after NTS blockade. Persistence of the response after blockade of NTS suggests that although NTS interneurons take part in the transmission of modulatory effects from MNR, other pathways from MNR to respiratory motoneurons bypassing the NTS structures are involved. Transmission through ventrolateral rostral medulla cannot be excluded. Another possibility is that the information is sent *via* direct connections from the raphe nuclei to respiratory motoneurons at the spinal level (Holtman et al. 1984). Pharmacological blockade of NTS in the rabbit causes different changes in the respiratory pattern according to the site of the injection, i.e. the prolongation of inspiratory and expiratory phase or apneustic pattern. Apneustic respiration probably is an effect of blocking the afferent terminals of the vagus nerve that give the input to inspiratory off-switch mechanism. In the rabbit, the area where apneustic breathing could be evoked by lignocaine blockade seems to be smaller, than that in the cat described by focal cooling, (see Fig. 2 in Budzińska et al. 1985a). The difference between the cat and the rabbit might be due to blocking by lignocaine both of somas and axons while focal cooling at 20° blocks only somas. However, this may reflect also the observation that NTS in the rabbit contains a great amount of expiratory neurones intermingled with inspiratory neurones (Fallert and Wassermeyer 1977) and blockade in this area eliminates neurones of both pools. It is of interest that pharmacological blockade of the NTS region in the rabbits prolonged both inspiratory and expiratory time without significant changes in the amplitude of integrated phrenic nerve activity. These results support the conclusion that general inspiratory off-switch mechanism cannot be located

in NTS region and may be in any other specific region of the brainstem (Euler 1986) or perhaps it is property of the whole system of respiratory network. There are, however, specific pathways known for transmission of the inspiration inhibitory influences through NTS interneurons. Inspiratory inhibition is transmitted to NTS interneurons by vagal afferents and descending pathways from pontine pneumotaxic region and nucleus reticularis gigantocellularis, partially *via* the raphe structures (Euler 1986, Richard and Stremel 1990, Gang et al. 1991). Elimination of these inhibitory inputs to NTS by lignocaine blockade led to the prolongation of inspiratory time (before and after vagotomy) observed in this study.

Disappearance of vagal motor activity without significant attenuation of amplitude of integrated phrenic and external intercostal activities (Fig. 7) supports the hypothesis that NTS is not the major source of phrenic and EI premotoneurone activity. Instead of being a crucial part of the respiratory pattern generator (including off-switch mechanism) or motor output, the NTS region is, rather, the relay station for modulating the respiratory pattern (Lucier and Sessle 1981, Euler 1986, Monteau et al. 1990). Lack of significant changes in the amplitude of integrated phrenic nerve activity could be the result of two opposite effects of the blockade: the elimination of some inspiration-inhibiting inflows allowing to develop inspiratory amplitude and the blockade of inspiratory premotoneurons located in NTS that cause a decrease of the amplitude (Euler 1986). In summary results presented in this paper suggest that neuronal structures of raphe system exert inhibitory effects on phasic inspiratory activity and stimulate tonic activity mostly in the muscles subserving postural functions (see also Monteau et al. 1990, Morin et al. 1990). Interneurons located in the NTS region take part in the transmission of raphe inflow on respiratory activity.

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