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Changes in the central respiratory rhythm following pharmacological blockade of the nucleus parabrachialis medialis in the rabbit

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Short communication

Abstract. Experiments were performed in halothane-anesthetized, paralyzed, bilaterally vagotomized and artificially ventilated rabbits. Arterial blood pressure, expiratory carbon dioxide concentration, and electrical activity of the right phrenic nerve were recorded prior to and after xylocaine microinjection to the left nucleus parabrachialis medialis (NPBM). The location of the xylocaine blockade was verified histologically. It was found that blockade of the NPBM results in a decelerated respiratory rhythm due to lengthening of both phases of the respiratory cycle. These results do not corroborate the role of NPBM in the regulation of respiration as postulated by Bertrand and Hugelin (1971).

Key words: nucleus parabrachialis medialis, respiratory rhythm, apneusis

It is assumed in the apneustic theory of respiration (Lumsden 1923) that there are two centers involved in the generation of the central respiratory rhythm: a pneumotaxic center (upper pons) and an apneustic center (lower pons), of which the first one periodically suppresses tonic activity of the other one. Lumsdens theory has later been modified by Pitts et al. (1939) who have postulated that the apneustic center is located in the medulla oblongata. Studies in rabbits (Gromysz 1984) appear to corroborate this latter proposal. Vagotomy and surgical separation of the medulla from the pons result in apneusis. Under these circumstances, inspiratory activity tonus can be interrupted by electrical stimulation of the vagal nerves. The resulting respiration is, however, of the "all or nothing" type. The medulla looses its regulatory plasticity following disconnection from the pons, and, when additionally deprived of the vagal nerves, cannot generate the respiratory rhythm.

According to a generally accepted view, duration of the inspiratory phase of the respiratory rhythm depends on the activities of both the pneumotaxic center and vagal nerves. Switching-off these structures (Lumsden 1923, Stella 1938) results in either apneusis (tonic activity of the phrenic nerve) or apneustic respiration (marked prevalence of the inspiratory phase over the expiratory one). However, there is a major disagreement concerning the location of the pneumotaxic center. In succession, this center has been located to the locus coeruleus (Johnson and Russell 1952), the dorsal quadrant of the tegmentum (Tang 1953, Wang et al. 1957), the nucleus parabrachialis medialis (NPBM) and the Kölliker-Fuse nucleus (Bertrand and Hugelin 1971, Cohen 1971), and the motor nucleus of the trigeminal nerve (NVmt) (Gromysz et al. 1988, Gromysz and Karczewski 1990, Gromysz et al. 1993). Recent reports showing deceleration of respiration following NPBM blockade in cats (Pokorski and Gromysz 1995, Pokorski and Gromysz 1997), and no inhibition of the phrenic nerve activity following stimulation of the NPBM in rabbits (Budzińska and Gromysz 1994), point at a need for further studies on the role of NBPM in the regulation of the respiratory rhythm.

For the present study, ten mongrel rabbits of either sex, 3-3.5 kg body weight, were used. Following induction of anesthesia with {4-[(diethylcarbamoil)-methoxy]-3-methoxyphenyl}-acetic acid propyl ester (Propanidid, 50 mg/kg, i.v.), the rabbits were paralyzed with tubocurarine (15 mg/kg), and artificially ventilated at eucapnic level with an air/oxygen mixture containing 0.7-1.0% (v/v) halothane. The rabbits were placed in a stereotaxic apparatus, the head being tilted ventrally (bregma 7 mm below lambda in the sagittal plane) to secure horizontal positioning of the brainstem. Occipital bone was removed. Dura, pia and both vagal nerves were cut, and arterial blood pressure, expiratory carbon dioxide concentration, and integrated activity of the right phrenic nerve (time constant: 100 ms) were recorded. After recording the above indices under control conditions, double microelectrode made of glass-embedded tungsten wire and glass capillary (tip diameter about 30 μm) filled with xylocaine solution (2%, w/v) was inserted into pontine structures, and approximately 1 μl of the xylocaine solution was injected to the following coordinates: 9.5-11.0 mm rostral to the obex, 3.0-4.0 mm left to the midline, and 1.0-2.5 mm below the dorsal surface of the brainstem. Effect of the blockade was recorded beginning 3 min after the injection. At the end of the experiment, the injection site was coagulated, and the brain was perfused with physiological saline followed by 4% formaldehyde. Frontal sections (50 μm thick) of the brainstem were evaluated using the histological atlas of the rabbit brainstem (Meessen and Olszewski 1949) and our own working atlas of the rabbit brainstem (Gromysz and Ruszczyk, unpublished) for references. Blockade of

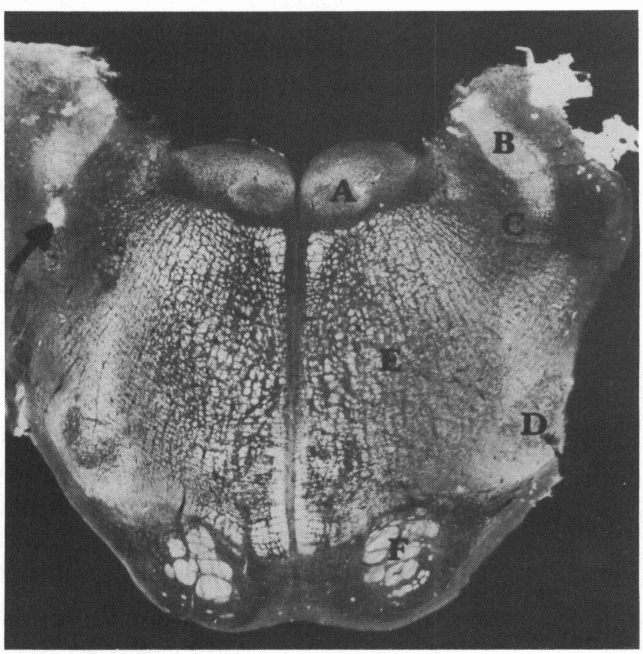

Fig. 1. Frontal section of the rabbit brainstem. A, central gray matter; B, brachium conjunctivum; C, nucleus parabrachialis medialis; D, nucleus lemnisci lateralis; E, nucleus reticularis pontis oralis; F, tractus pyramidalis. The arrow points to the location of the xylocaine blockade (coagulation trace).

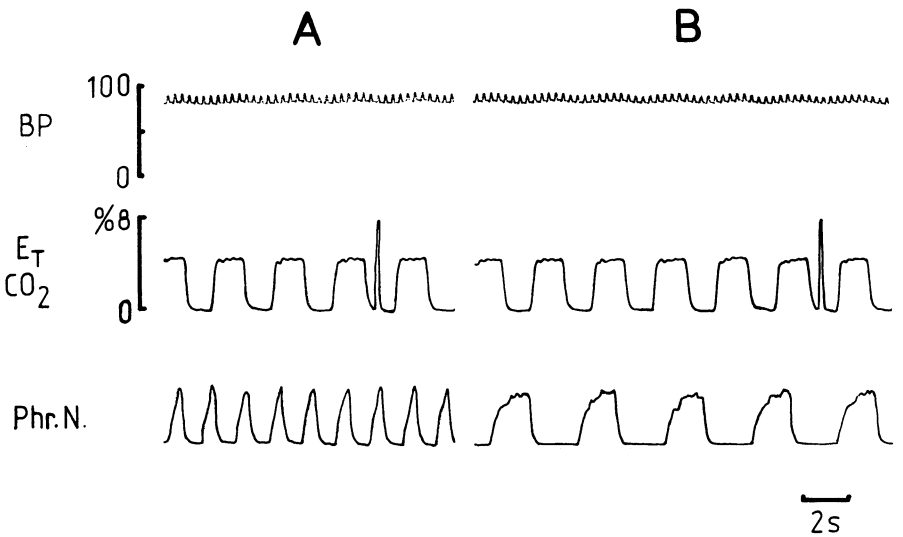

Fig. 2. Effects of xylocaine blockade of the nucleus parabrachialis medialis on selected respiration-related indices. Panel A, control records; panel B, records obtained following xylocaine microinjection. Upper traces, arterial blood pressure; middle traces, expiratory CO_2 concentration; lower traces, integrated activity of the phrenic nerve.

the NPBM has been confirmed histologically in 8 out of 10 rabbits used for the present study (Fig. 1). In the remaining two rabbits, injection site has been located to the caudal part of the reticular formation. Similarly to the control injections of physiological saline, xylocaine blockade of the reticular formation did not affect the contralateral phrenic nerve activity.

Xylocaine blockade of the NPBM caused deceleration of the central respiratory rhythm (Fig. 2) in all 8 rabbits. This was due to lengthening of both phases of the respiratory cycle. The inspiratory and expiratory phases were prolonged by 137% and 152%, respectively, compared to the controls, while amplitude of the phrenic nerve discharges oscillated slightly at the respective control value. Relative changes in the phrenic nerve discharges following the NPBM blockade are shown in Fig. 3.

The putative pneumotaxic center should satisfy two conditions at least: (1) it should suppress inspiration when electrically stimulated, and (2) switching it off by either coagulation or pharmacological means should result in apneustic respiration and significant changes in the Hering-Breuer inflation reflex. While supposedly being a principal candidate for this role (Bertrand and Hugelin 1971, Cohen 1971), nucleus parabrachialis medialis fulfills neither of these conditions. Electrical stimulation of the NPBM at more dorsal points has inspiratory-facilitatory effetcs, while stimulation at more ventral points has expiratory facilitatory effects (Cohen 1970). Considering the localization of anatomical pontine structures at the NPBM level in the cat (see plate 17 of the Berman's atlas), and comparing it with the localization of stimulation points used by Cohen (Cohen 1970), the expiratory-facilitatory effect of this stimulation should be ascribed to the motor nucleus of the trigeminal nerve. According to the Bermans atlas, this nucleus is located just below the NPBM and not above the locus coeruleus as shown in the respective figure in Cohens report (Cohen 1970, Fig. 12). Moreover, no inhibitory response in the phrenic nerve activity was observed following electrical stimulation of the NPBM in rabbits (Budzińska and Gromysz 1993).

As evidenced by the present report and former studies in cats (Pokorski and Gromysz 1995, Pokorski and Gromysz 1997), pharmacological blockade of the NPBM causes deceleration of the respiratory rhythm due

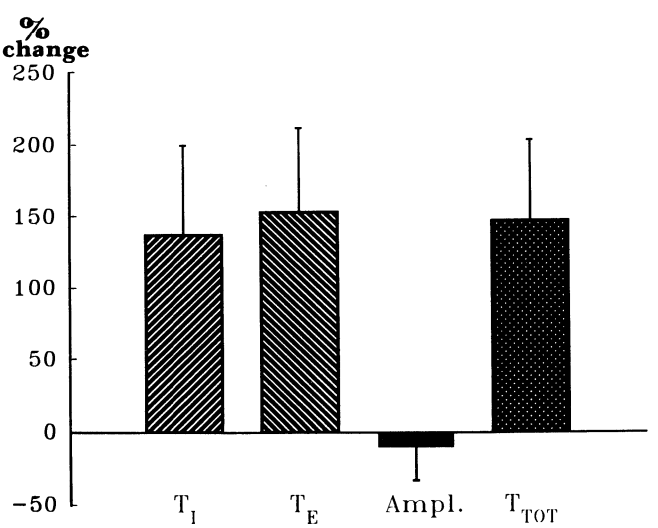

Fig. 3. Relative changes (mean ± SD) in the duration of inspiratory phase (T_I), expiratory phase (T_E) and the total duration of respiratory cycle (T_{TOT}), and in the amplitude of phrenic nerve activity (Ampl.), following xylocaine blockade of the nucleus parabrachialis medialis.

to the lengthening of both phases of the respiratory cycle. Switching the NPBM off by either coagulation (St. John 1972), or cold block (Berger et al. 1978), or kainic acid microinjections (Denavit-Saubié et al. 1980) results, according to these authors, in apneustic respiration in the cat. However, close examination of the respective original recordings shows that lengthening of the inspiratory phase (T_I) is associated with lengthening of the expiratory phase (T_E) which may even be more pronounced (see Berger et al. 1978, Fig. 1). Simultaneous lengthening of both T_I and T_E represents a slow respiration which is a quantitatively different state from that of apneustic respiration. According to the apneustic theory of respiration (Lumsden 1923, Stella 1938), switching the pneumotaxic center off and transsection of the vagal nerves result in either apneusis (tonic activity of the phrenic nerve) or apneustic respiration (sustained inspiratory discharges interrupted by short expiratory pauses). The definition of apneustic respiration which was accepted by St. John et al. (1972) (a prolongation of inspiration for ten seconds or longer) and did not take into account the duration of the expiratory phase is in conflict with the apneustic theory of respiration, and makes understanding of pneumotaxic mechanism of respiration regulation difficult.

The above data show that respiratory effects induced by pharmacological (xylocaine) blockade of the NPBM in the rabbit (present study) and cat (Pokorski and Gromysz 1997) are not different from those obtained following either coagulation (St. John et al. 1972), or cold block (Berger et al. 1978), or kainic acid injections (Denavit-Saubié 1980). In the cat, there is also no significant change in the Hering-Breuer inflation reflex following switch-off of the NPBM by coagulation (Feldman and Gautier 1976).

Based on the results of most recent studies in cats and rabbits, the inhibitory control of inspiration can be ascribed to the NVmt (with a contribution from the vagal nerves). Those studies have demonstrated that: (1) NVmt produces lung mechanoreceptor-induced expiratory activities (Gromysz et al. 1988, Gromysz and Karczewski 1990); (2) electrical stimulation of the NVmt using a single stimulus results in inhibition of phrenic nerve activity, and in a switch-over from inspiratory to expiratory phase (Gromysz et al. 1988, Budzińska and Gromysz 1993); (3) pharmacological blockade of the NVmt results in apneustic respiration and abolishes the Hering-Breuer inflation reflex (Gromysz and Karczewski 1990, Pokorski and Gromysz 1995, Pokorski and Gromysz 1997).

In summary, the results of the present study corroborate the hypothesis set forth by Gromysz and Karczewski (1990), according to which NVmt is the anatomical substrate of the pneumotaxic center, and a relay station for the Hering-Breuer inflation reflex. This hypothesis is also in agreement with the results of long forgotten studies by Marckwald (1887) showing that damage to the nucleus of the trigeminal nerve in the rabbit results in death.

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