MOTOR CONTROL OF THE DIAPHRAGM IN ANESTHETIZED RABBITS

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Abstract

Diaphragmatic regions are recruited in a specialized manner either as part of a central motor program during non respiratory maneuvers, e.g. vomiting, or because of reflex responses, e.g. esophageal distension. Some studies in cats and dogs suggest that crural and costal diaphragm may be differentially activated also in response to respiratory stimuli from chemoreceptors or lung and chest wall mechanoreceptors. To verify whether this could occur also in other species, the EMG activity from the sternal, costoventral, costodorsal, and crural diaphragm was recorded in 42 anesthetized rabbits in response to various respiratory maneuvers, such as chemical stimulation, mechanical loading, lung volume and postural changes before and after vagotomy, or a non-respiratory maneuver such as esophageal distension. Regional activity was evaluated from timing of the raw EMG signal, and amplitude and shape of the moving average EMG. In all animals esophageal distension caused greater inhibition of the crural than sternal and costal diaphragm, whereas under all the other conditions differential diaphragmatic activation never occurred. These results indicate that in response to respiratory stimuli the rabbit diaphragm behaves as a single unit under the command of the central respiratory control system.

1. Introduction

Different regions of the diaphragm are recruited in a specialized manner during non-respiratory maneuvers such as swallowing, vomiting, eructation and regurgitation associated with rumination (Monges et al., 1978; Tan and Miller, 1986; Titchen, 1979). If the effects of swallowing might be tentatively explained by the activation of vagal afferents with esophageal distension, hiatal relaxation during vomiting, regurgitation, and eructation is thought to be part of a central motor program common to several species (Monges et al., 1978; Titchen, 1979). In cats, rabbits, and rats, the motor unit territories of phrenic nerve's primary branches appear to be highly delineated (Hammond et al., 1989; Turgut et al., 2006; Pickering and Jones, 2007), thus providing the basis for a differential motor control of the sternal, costal, and crural portions of the diaphragm. Whether the potential for regional recruitment is exploited by the central respiratory control system during breathing and in response to specific respiratory stimuli is controversial.

Earlier studies indicated synchronous activation of the diaphragm during quiet breathing in rabbits and cats (Boyd and Basmajian, 1963; Sant'Ambrogio et al., 1963) and showed uniform changes in the level of costal, and crural diaphragm activity during rebreathing and respiratory loading in dogs (Lourenço et al., 1966) and during rebreathing in rabbits, though in a very limited number of animals (D'Angelo, 1982). Subsequent studies in dogs and cats have found a much steeper CO_2 response of the crural than costal diaphragm, besides substantially different timing, both in dogs (Van Lunteren et al., 1984; Fitting et al., 1986; Darian et al., 1989) and cats (Van Lunteren et al., 1985). This led to the conclusion that costal and crural diaphragm are actually two different, separately controlled muscles even during respiratory tasks. In contrast, studies in rats have shown that the central neural inputs to the costal and crural diaphragm are similar (Pollard et al., 1985; Pickering and Jones, 2007).

The purpose of the present study was to assess whether a differential motor control of the costal and crural diaphragm occurs in rabbits, and if such differences may involve also the sternal region, as suggested in cats (Riley and Berger, 1979). To this end the electrical activity has been

simultaneously recorded from various sites of the rabbit's diaphragm, and its characteristics examined under a variety of conditions including both respiratory and non-respiratory maneuvers .

2. Methods

The experiments were performed on 42 rabbits anesthetized with intravenous urethane (0.5 g/kg body weight) and sodium pentobarbitone (15 mg/kg body weight); additional doses were given as needed using behavioral criteria (heart rate, systemic blood pressure). The level of anesthesia was such that the corneal reflex was still present. The animals were kept supine on a tilting table. Body temperature was maintained close to 38 °C with a heating pad. At the end of the experiment the animal was sacrificed with an overdose of anesthetics. All surgical and experimental procedures were carried in accordance with the principles and guidelines of the European Community for the use and care of experimental animals. The investigation was authorized by the Italian Ministry of Health.

A metal cannula, tightly fitted into the trachea, was connected to a heated pneumotachograph (Fleisch 00; HS Electronics, March-Hugstetten, Germany) and differential pressure transducer (model 270; Hewlett-Packard, Paolo Alto, CA) to measure air flow ($\dot{V}$). Tracheal pressure was recorded by means of a pressure transducer (Statham P23GB; Gould Electronics, Valley View, OH) connected to the side arm of the metal cannula. A similar transducer measured the pressure inside a catheter mounted, thick-walled balloon, placed in the thoracic esophagus. A catheter-pressure transducer system was inserted into the femoral artery to measure systemic blood pressure and to obtain blood samples for the assessment of arterial carbon dioxide (Pa_{CO_2}) and oxygen partial pressure (Pa_{O_2}), and pHa by means of a blood gas analyzer (IL 1620; Instrumentation Laboratories, Milan, Italy).

The cervical vagi were isolated and loose ligatures placed around them, for easier identification. The abdomen was opened along the mid-line and bipolar electrodes were inserted into the diaphragm. Electrodes were pairs of silver wires (0.15 mm in diameter, 3 mm in length,

2.5-3 mm inter-electrode distance) soldered to insulated copper wires. The solder was covered with enamel. The electrodes were placed into the sternal, costoventral, costodorsal and crural regions of either the left or right hemi-diaphragm through hypodermic needles; the latter were then withdrawn, and the abdominal wall was sutured. In the animals in which the effects of postural changes were studied, the electrodes were placed in the sternal, left costal, right costal, and crural regions. In 10 supine rabbits, the right phrenic nerve was cut low in the neck, its central end placed across a bipolar platinum electrode immersed in warm mineral oil, and electrodes were inserted into the left sternal, costal, and crural hemi-diaphragm. Electrodes in the crural diaphragm were inserted far from the esophageal hiatus.

The raw diaphragm EMG and phrenic ENG signals, amplified and filtered (band width: 20-1500 Hz; attenuation: 40 dB per octave), airflow, and pressures were recorded on tape. On replay, the raw EMG and ENG were full-wave rectified, fed into a Bessel low-pass filter (time constant: 52 msec.) to obtain the moving average EMG and ENG, and digitized at a sampling rate of 100 Hz together with flow and pressure signals by means of a 16-bit A/D converter (PCI 6035E, National Instrument, Austin, TX). The flow signal was numerically integrated to obtain lung volume changes.

Figure 1 helps to identify the parameters used to evaluate the EMG activity of the various diaphragmatic regions. Tidal changes of the EMG activity (AT) were quantified as difference between peak and end-expiratory level of the moving average EMG. Data obtained from a given site under a given condition were expressed relative to the corresponding mean value obtained in that animal during quiet, unobstructed breathing before vagotomy. The shape of the moving average EMG-time profile during inspiration was evaluated from the exponent of the power function $A_{(t)}=a\cdot t^{b}$, t being time from EMG onset. At each site, timing was assessed from 1) the difference between the start of the inspiratory flow and raw EMG signal (ΔT); 2) the interval between onset and peak of the moving average EMG, i.e. duration of the inspiratory ramp (TIR); and 3) the

duration of the post-inspiratory activity, i.e. the interval between the end of the inspiratory flow and the raw EMG activity (TPIIA).

Table 1 summarizes the experimental protocol. The response to chemical stimuli was assessed while rebreathing from eupnea or over-ventilation apnea, data being obtained at 4, roughly equally spaced levels of EMG activity, including eupnea. The effect of inspiratory elastic loading was checked by occluding the tracheal cannula at end expiration for one breath, that of posture by moving the animal from the supine to the right and left lateral decubitus, or to the head-up posture, and vice-versa, and that of lung volume by applying continuous positive (CPP) or negative pressure at airway opening (CNP), or manual rib cage compression (RCC) for 3-4 breaths. Moreover, the right phrenic nerve motor output was compared with the activity of the left sternal, costal, and crural diaphragm. During eupnea, esophageal distension was repeatedly produced in all animals by inflating the esophageal balloon at two pressure levels for 3-4 breaths. The results of an experiment were discarded if the inspiratory trajectory of the moving average EMG showed sudden, substantial changes at one or more recording sites, or after any given maneuver, the integrated EMG did not resume its initial aspect.

Data, presented as mean ±SEM, were analyzed using SPSS 11.5 (SPSS Inc., Chicago, IL). Comparisons of variables pertaining to the different sites and conditions were made using one-way ANOVA. Linear and non-linear regressions were computed using the statistical package of SigmaPlot (SS Inc., San Jose, CA), the Student's *t* test being used for the comparison of regression coefficients. Statistical significance was taken at $P \leq 0.05$.

3. Results

3.1 Timing of diaphragm activity.

Under all circumstances, timing of diaphragmatic activity never differed significantly among the various regions. No differences in EMG onset and TIR occurred among sites, independent of chemical drive and intact vagi. During eupnea and in the intact animal, the onset of costoventral,

costodorsal, and crural EMG differed from the sternal one by 1.4±3.4, -0.4±5.5 and 1.8±5.8 ms, respectively, while corresponding values for TIR were 2.4±4.4, –4.2±6.4 ms, and –1.8±4.8 ms. Similarly, TPIIA did not differ significantly among the recording sites, independent of chemical drive and intact vagi: in the intact animal, TPIIA averaged 97±4, 94±3, 95±3, and 99±4 msec in the sternal, costoventral, costodorsal, and crural region during eupnea, while at the highest level of chemical stimulation, corresponding values were 66±3, 62±2, 64±2, and 66±2 msec, i.e. ~7% of the expiratory duration at either levels of chemical drive (Table 2).

3.2 Effects of chemical drive.

On recovery from over-ventilation apnea, activity reappeared simultaneously in all diaphragmatic regions, and during rebreathing it increased in the entire diaphragm (Fig. 2). Mean values for tidal volume (VT), breath timing, Pa_{CO_2} and Pa_{O_2} occurring in the intact and vagotomized animals on recovery from over-ventilation apnea, during resting breathing, and at the end of the rebreathing tests are reported in Table 2.

With changing chemical drive, diaphragm activity changed uniformly in the various regions. Indeed, the relationships of AT of costoventral, costodorsal, and crural regions to that of the sternal computed on data from all animals was linear with an origin and slope that did not differ significantly from zero and unity, respectively, independent of intact vagi or inspiratory loading (Fig. 3). Sternal activity was taken as reference because the sternal diaphragm is easily accessible even without opening of the abdomen and hence, more commonly used when recording for general purposes. Within a given animal, however, the slope of individual AT-AT relationships could be significantly different from unity; this occurred in 2 instances for the AT_{cost_v} vs ATster (0.69±0.06; 1.31±0.11) and AT_{cost_d} vs ATster relation (0.85±0.04; 1.29±0.08), and 3 instances for the ATcrur vs ATster relation (0.69±0.05; 0.81±0.06; 1.43±0.15).

For each diaphragmatic region, the time course of the moving average EMG during inspiration was satisfactorily described by a power function of the form $A_{(t)}=a\cdot t^{b}$ (Fig. 1), with a mean value for the correlation coefficient of 0.96±0.01 (range: 0.88-0.99). The dimensionless

coefficient *b*, a shape factor describing the inspiratory time-profile of the moving average EMG, was taken to reflect the pattern of discharge frequency and motor unit recruitment and prevalent type of motor units firing during inspiration (Riley and Berger, 1979). The shape factor did not differ significantly among the various diaphragmatic regions, independent of chemical drive and intact vagi; during eupnea and in the intact animal, it averaged 0.53±0.01, 0.54±0.02, 0.54±0.02, and 0.53±0.02 for the sternal, costoventral, costodorsal, and crural EMG, respectively. The shape factor increased, however, with increasing the chemical drive and tidal diaphragm activity, a unique function describing this relationship for all diaphragmatic regions (Fig. 4).

Augmented breaths, characterized by the typical biphasic pattern of muscle activation and lung volume change, were occasionally observed at various levels of chemical drive. In 27 sighs, AT of the sternal, costoventral, costodorsal, and crural diaphragm increased relative to that of the preceding, normal inspiration by 2.02±0.08, 1.95±0.1, 1.94±0.08, and 2.01±0.09, respectively, none of these values differing significantly from the others.

3.3 Effects of elastic loading.

As expected, in intact animals airway occlusion at end-expiration prolonged inspiratory duration and increased AT without affecting the integrated EMG profile (Fig. 5), whereas in vagotomized animals, inspiratory duration was essentially unaffected and AT slightly decreased, if any. A unique linear function described the AT-AT relationship of each combination of recording sites, its coefficients being not significantly different from those of the corresponding function pertaining to unimpeded breaths (Fig. 3). The same applies to shape factor of the moving average EMG (Fig. 4), thus showing that the regional diaphragmatic response to elastic loading is homogeneous.

3.4 Effects of pressure breathing and rib cage compression.

In intact animals, application of continuous positive pressure at airway opening (CPP) caused an initial period of apnea, an enhanced chemical drive, and a subsequent increase of AT

which was homogeneous throughout the diaphragm (Fig. 6). No immediate AT changes occurred with CPP after vagotomy, as no apnea ensued.

In intact animals, application of continuous negative pressure at airway opening (CNP) or rib cage compression (RCC) increased peak EMG activity and caused the diaphragm to remain active throughout the whole expiration, these effects being prevented by vagotomy (Fig. 5). Both AT and tonic activity did not differ significantly among the various diaphragmatic regions (Fig. 6). Moreover, for the same reduction in end-expiratory lung volume, the amount of tonic activity, expressed as a fraction of control AT, was similar during CNP (0.25±0.02) and RCC (0.27±0.02).

3.5 Effects of posture.

In intact animals, moving from the supine to the right or left decubitus had no systematic or appreciable effects on regional diaphragmatic EMG activity (Fig. 6). Moreover, the AT-AT relationships obtained during rebreathing were undistinguishable from the corresponding ones obtained in the supine posture. The effects of moving to the head-up posture were not systematically studied in intact animals because of the very prolonged, life-threatening apnea that occurred often with this maneuver. In vagotomized animals, head-up tilting caused an immediate, marked decrease of VT, leading to a rapid increase in diaphragmatic EMG activity, which was, however, similar in all regions (Fig. 6).

3.6 Diaphragmatic EMG vs phrenic ENG.

The relationships between tidal or end-expiratory activity recorded from the phrenic nerve and the corresponding values recorded from the sternal, costal, and crural region of the controlateral hemi-diaphragm obtained in intact animals during rebreathing, inspiratory loading, and rib cage compression, and after vagotomy are shown in Figure 7. In each animal, the relation between phrenic and regional diaphragmatic activity was linear with an origin and slope that did not differ significantly from zero and unity, respectively. Actually, the range of the slope of the relations of the sternal (0.94-1.06), costal (0.91-1.15), and crural EMG activity (0.90-1.19) to the phrenic ENG activity was substantially smaller than that of the AT-AT relationships in Figure 3. Furthermore, the

shape factor of the moving average EMG or ENG did not differ significantly among the various diaphragmatic regions and the phrenic nerve, independent of chemical drive, inspiratory loading, rib cage compression, and intact vagi; during eupnea and in the intact animal, it averaged 0.55±0.02, 0.57±0.04, and 0.53±0.04 for the sternal, costal, and crural EMG, respectively, and 0.56±0.03 for the phrenic ENG.

3.7 Effects of esophageal distension.

In intact animals, esophageal distension invariably decreased diaphragm activity (Fig. 8). Inhibition was similar in the sternal, costoventral, and costodorsal diaphragm, but systematically larger in the crural diaphragm: relative to control, AT decreased in the sternal, costoventral, costodorsal, and crural diaphragm to 0.87±0.02, 0.93±0.02, 0.89±0.02, and 0.59±0.05 when the esophageal balloon was inflated to 10 cmH_2O, and to 0.73±0.05, 0.79±0.06, 0.71±0.07, and 0.44±0.04 when the esophageal balloon was inflated to 30 cmH_2O. Vagotomy abolished the effect of esophageal distension on diaphragm activity.

4. Discussion

The results of the present study show that, although the regional activity of the rabbit diaphragm can be differentially modulated, as in response to esophageal distension (Fig. 8), this ability is not exploited during respiratory tasks. When under the command of the brain stem respiratory controller, as in response to chemical stimuli, the various regions of the diaphragm not only undergo the same level of activation (Fig. 3), but also the timing (Fig. 1), pattern of discharge frequency, motor unit recruitment, and type of recruited motor units (Fig. 4) are similar in each of the diaphragmatic regions supplied by the four primary branches of the rabbit phrenic nerve. Furthermore, reflexes from lung mechanoreceptors that are known to be mediated through the brain stem respiratory network also cause changes that uniformly involve the entire diaphragm (Fig. 5 and 6), while posture does not interfere with the regional distribution of the central motor command (Fig. 6).

Though AT changes during respiratory maneuvers were on average similar in all diaphragmatic regions (Fig. 3, 5, and 6), regional AT changes in a given animal and condition were rarely the same, especially during rebreathing from hyperventilation apnea when these changes were the largest. Nevertheless, the AT-AT relationships were always linear with a slope that did not differ from unity, except in 3 rabbits. No explanation was found for the latter observation, also because timing, inspiratory profile of the moving average EMG, post-inspiratory activity of the various regions, and their dependency on chemical drive were indistinguishable from those of the animals in which the slope of the AT-AT relationships was not significantly different from unity. Probably, this might have been the occasional manifestation of the various artifacts that can affect intramuscular EMG recordings (Kim et al., 1985; Brancatisano et al., 1989; Hodges and Gandevia, 2000). Indeed, in each of the nine rabbits in which phrenic and diaphragm activity were simultaneously recorded, regional diaphragmatic activity closely resembled, both in amplitude and shape, that of the phrenic nerve (Fig. 7), indicating that in anesthetized rabbits EMG recording from any portion of the diaphragm generally provides a good index of the central motor command.

The shape factor describing the inspiratory time-course of the integrated EMG was seldom the same for all diaphragmatic regions of a given animal; moreover it varied substantially both within and among the diaphragmatic regions (Fig. 4). However, under all conditions the average value of the shape factor did not differ significantly among the diaphragmatic regions, and in fact, the dependence of the shape factor on the amplitude of EMG activity was the same for all regions (Fig. 4). This indicates a uniform pattern of discharge frequency and motor unit recruitment among diaphragmatic regions, and, to the extent that the shape factor also reflects the prevalent type of recruited motor units, it suggests a uniform distribution of fiber types. Riley and Berger (1979) have directly related the regional inspiratory profile of the moving average EMG during quiet breathing to the fiber type composition of the corresponding region of the cat diaphragm. In spite of the marked variability of both fiber types and shape of the integrated EMGs among and within regions, their results can be taken to indicate an essentially uniform fiber type composition of the sternal,

costal, and crural diaphragm, the striking differences being confined to the hiatal region. Indeed, the shape factor computed from their records of integrated EMG activity was as low as 0.2 for the portion close to the esophageal hiatus, whereas it ranged from 0.42 in the crossing band, to 0.52 in the sternal portion, 0.57 in the left costal region, and 0.65 in the left crus. In the present rabbits under the same condition, the range of the shape factor in the sternal, costoventral, costodorsal, and crural diaphragm was 0.46-0.63, 0.4-0.68, 0.44-0.67, and 0.46-0.69, respectively.

Earlier studies performed in anesthetized rabbits, cats, and dogs (Boyd and Basmajian, 1963; Sant'Ambrogio et al., 1963, Lourenço et al., 1966) during chemically stimulated or loaded breathing, suggested that regional diaphragm activation is essentially uniform. Later studies reported that the response to chemical stimulation differs in terms of amplitude, timing, or post-inspiratory activity between the crural and costal diaphragm both in anesthetized dogs (VanLunteren et al., 1984; Fitting et al., 1986; Darian et al., 1989) and cats (VanLunteren et al., 1985), thus supporting the hypothesis of a differential central motor control of the costal and crural diaphragm. In contrast, studies in awake or anesthetized dogs (Smith et al., 1989; Abe et al., 1993; Easton et al., 1995) and cats (Oyer et al., 1989) have demonstrated an essentially uniform activation of the diaphragm in response to hypercapnia and/or hypoxia. No acceptable explanations have been found for these discrepancies (crf. Oyer et al., 1989). However, the notion of the diaphragm acting as a single functional unit when under the respiratory control system is also supported by the results obtained in awake or anesthetized sheep (Henderson et al., 1982; Cooke et al., 1993; Torres et al.,1993) and rats (Pollard et al., 1985; Pickering and Jones, 2007), as well as by the present observations in rabbits. Moreover, our results show that the pattern of regional diaphragmatic activity is independent of posture (Fig. 6), thus supporting the suggestion from a study on anesthetized dogs (Brancatisano et al., 1989). It appears, therefore, that in animals the prevalent evidence is in favor of an essentially uniform activation of the diaphragm during respiratory tasks. Interestingly, it has been shown in man that during inspiratory maneuvers, the supraspinal control of the costal and crural diaphragm is identical (Sharshar et al., 2005).

The possibility of a differential control of the costal and crural diaphragm has been suggested by the segmental nature of diaphragm innervation (Sant'Ambrogio et al., 1963; De Troyer et al., 1982) and the sharp delimitation of the motor unit territories of phrenic nerve's primary branches (Hammond et al., 1989). While the latter observation has found general consensus (Turgut et al., 2006; Pickering and Jones, 2007), the former has not been confirmed (Fournier and Sieck, 1988). Furthermore, the finding that motoneurons projecting to the sternal, costal and crural diaphragm are intermingled throughout the entire phrenic nucleus (Gordon and Richmond, 1990) indicates that there are no evident differences between the motor innervation of the various diaphragmatic portions also at the spinal cord level. Nevertheless, motoneurones innervating the hiatal fibers should be selectively controlled by supra-spinal networks during non-respiratory maneuvers such as swallowing, vomiting, eructation and regurgitation associated with rumination (Monges et al., 1978; Tan and Miller, 1986; Titchen, 1979). An alternative is represented by the recent demonstration that motor vagal fibers innervate the hiatal portion of the crural diaphragm in addition to phrenic motoneurons (Young et al., 2009). This seems of particular interest because under the conditions above the motor control of the diaphragm hiatal fibers is usually coupled with that of the lower esophageal sphincter. On the other hand, a selective activation of the crural diaphragm does not occur in the vagally mediated response to esophageal distension: in the rabbit, as in other species (Cherniack et al., 1984; Oliven et al, 1989), this reflex affects in fact all diaphragmatic regions, though more markedly the crural one (Fig. 8).

An additional anatomical feature that could suggest the presence of a peculiar motor control of the crural diaphragm, is represented by the nearly exclusive location of muscle spindles in this region (Duron et al., 1978). This, together with the observation that in cats the density of diaphragm muscle spindles is the same as in other skeletal muscles (Balkowiec et al., 1995), suggests that muscle spindles could play an important role in diaphragm motor control, especially in the presence of non uniform changes in muscle length, as during CNPB, CPPB, rib cage compression, postural changes, and inspiratory efforts. However, under none of these circumstances did the behavior of

EMG differ among diaphragmatic regions (Fig. 3, 5, and 6), indicating lack of muscle spindle intervention, in line with the results obtained during high-frequency vibration of the central tendon in spontaneously breathing, anesthetized rabbits (Jammes et al., 2000). The effects of CNPB and rib cage compression on diaphragm EMG were, in fact, due to pulmonary vagal afferents, being prevented by vagotomy (Fig. 5).

The suggestion that the costal and crural diaphragm might need a separate motor control in performing respiratory tasks followed the demonstration that these two parts have different mechanical effects on the lower rib cage (De Troyer et al., 1981; 1982). In principle, differences in regional cross-sectional area, *in situ* length, posture dependence of operating length, force-length and force-frequency characteristics, and fiber type composition both within and among species, could represent potential reasons for a differential motor control of the sternal, crural and costal diaphragm. Nevertheless, the same studies (De Troyer et al., 1981; 1982) have shown that the increase in lung volume produced in the apneic dog by the separate stimulation of the crural and costal diaphragm is comparable. To the extent that the respiratory action of the diaphragm consists of increasing the lung volume, and the above observation in dogs also applies to the other species, it does not seem necessary to differentially control the two parts of the diaphragm during respiratory tasks. This conclusion would be strengthened further if the lung inflationary effects of crural and costal diaphragm contraction were additive, an aspect that has never been investigated.

In conclusion, in the present study the rabbit diaphragm was stimulated in several ways, and its activation was studied measuring a number of parameters. None of these parameters allowed the identification of regional differences in diaphragmatic activation in any of the conditions studied, except stimulation of vagal afferents by means of esophageal distension. The results of this study, in line with those of the majority of the studies performed in dogs, cats, sheep, and rats, support the notion of a uniform activation of the diaphragm when functioning as an inspiratory muscle under the command of the central respiratory control system.

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Table 1. Experimental protocol[a]

maneuver	position	N of animals	N of tests per animal	N of data per test
rebreathing from over-ventilation apnea	supine	16	6	34-38
single breath inspiratory elastic load	supine	16	10-12	1
postural change	supine; decubitus; head-up	16	12	10-15
rebreathing from eupnea	supine; decubitus	12	12	28-32
positive and negative pressure breathing, rib cage compression	supine	14	24	3-4
rebreathing from eupnea, inspiratory elastic load, rib cage compression	supine	10	6 10-12 4	28-36 1 3-4
esophageal distension	supine	42	6	3-4

[a]EMG was recorded from sternal, costoventral, costodorsal, and crural diaphragm, except in the last but one case when recordings were from the phrenic nerve and the sternal, costal, and crural region of the controlateral hemi-diaphragm. Tests were performed both before and after vagotomy, except in the head-up posture when they were made only after vagotomy.

Table 2. Breathing pattern and arterial blood gasses and pH on recovery from over-ventilation apnea, during eupnea, and at the end of the rebreathing test.

V_T ml	T_I s	T_E s	PaO_2 mm Hg	$PaCO_2$ mm Hg	pHa
intact					
10.2±0.6	0.74±0.04	0.94±0.05	119±3	19.1±1.9	7.56±0.07
16.8±0.4	0.54±0.02	0.80±0.04	99±5	32.7±1.4	7.38±0.09
27.4±1.3	0.38±0.02	0.48±0.03	55±4	58.5±2.0	7.27±0.07
vagotomized					
19.5±1.7	2.01±0.33	1.08±0.11	122±5	18.6±1.7	7.63±0.08
28.3±1.6	1.49±0.19	0.99±0.09	102±6	29.5±1.4	7.39±0.08
43.4±1.9	0.94±0.11	0.91±0.09	53±5	60.2±2.4	7.23±0.07

Values are mean±SE of data obtained during 3 rebreathing tests performed both before and after vagotomy in each of the 16 rabbits.

Legends

Fig. 1. Tracheal flow, lung volume change, raw and moving average EMG of sternal (ster), costoventral ($cost_v$), costodorsal ($cost_d$), and crural diaphragm (crur) in an anesthetized, intact, supine rabbit during eupnea, from which onset (ΔT), duration of post-inspiratory activity (T_{PIIA}), amplitude (A_T), duration of the inspiratory ramp of the moving average EMG (T_{IR}), and shape of the inspiratory trajectory of the moving average EMG were assessed. Numbers indicate the exponent (±SE; $P<0.001$ in all cases) of the power function (dashed line) used to fit the inspiratory trajectory of the moving average EMG.

Fig. 2. Lung volume change and moving average EMG of sternal (ster), costoventral ($cost_v$), costodorsal ($cost_d$), and crural diaphragm (crur) in an anesthetized, intact, supine rabbit during rebreathing from eupnea, or recovery from over-ventilation apnea. Arrows indicate onset of rebreathing through the dead space (left) and end of mechanical hyperventilation (right).

Fig. 3. The relationships between the tidal changes of moving average EMG (A_T) of sternal and costoventral, or costodorsal, or crural diaphragm in 16 anesthetized, supine rabbits during rebreathing from overventilation apnea before and after vagotomy. Before vagotomy, data of both unimpeded (open) and loaded (occluded) breaths are presented. In each animal and recording site, A_T pertaining to a given condition was expressed relative to eupneic A_T of the intact animal. Numbers indicate the slope (±SE) of the linear function used to fit each combination of regional activities.

Fig. 4. The relationship between tidal changes of the moving average EMG (A_T) and the shape factor (s.f.) describing its inspiratory trajectory of the sternal and costoventral, or costodorsal, or crural diaphragm in 16 anesthetized, supine rabbits during rebreathing from overventilation apnea before and after vagotomy. In each animal and recording site, A_T pertaining to a given condition was expressed relative to eupneic A_T of the intact animal. Symbols as in Fig. 3. Data from all recording sites could be fitted with a unique function: $s.f = 0.38\pm0.02 + 0.16\pm0.01 \cdot A_T^{0.66\pm0.07}$ ($R=0.862$; $P<0.001$).

Fig. 5. *Left panels*: the effect of complete airway occlusion at end-expiration on the moving average EMG of the sternal, costoventral, costodorsal, and crural diaphragm in an anesthetized, intact, supine rabbit during eupnea. The trajectory of the moving average EMG of the unimpeded breath preceding the occlusion (dotted line) was superimposed to that of the inspiratory effort. *Middle and right panels*: the effect of rib cage compression on lung volume change and moving average EMG of the sternal, costoventral, costodorsal, and crural diaphragm during eupnea in an anesthetized, supine rabbit before and after vagotomy.

Fig. 6. Effects of continuous positive (CPPB) or negative pressure breathing (CNPB) or rib cage compression on the tidal changes (AT) and end-expiratory (tonic) level of the moving average EMG of the sternal, costoventral, costodorsal, and crural diaphragm in 14 anesthetized, intact, supine rabbits, and the effects of changing posture on AT of the sternal, crural, left (L) and right costal (R), diaphragm in 16 anesthetized, intact (decubitus) or vagotomized rabbits (head-up). For each animal and recording site, AT pertaining to a given condition was expressed relative to eupneic AT of the intact, supine animal.

Fig. 7. The relationships between the tidal changes (open symbols) or end-expiratory level of the moving average ENG of the right phrenic nerve (closed symbols) and those of the moving average EMG of the left sternal, costal, and crural diaphragm in 10 anesthetized, intact, supine rabbits during rebreathing before (squares) and after vagotomy (diamonds), and airway occlusion at end-expiration (triangles). For each animal and recording site, AT pertaining to a given condition was expressed relative to eupneic AT of the intact animal (circles). Numbers indicate the slope (±SE) of the linear function used to fit each combination of regional activities.

Fig.8 The effect of esophageal distension on the moving average EMG of the sternal, costoventral, costodorsal, and crural diaphragm in an anesthetized, intact supine rabbit during eupnea. The lowermost tracing is pressure inside the esophageal balloon (Pes).

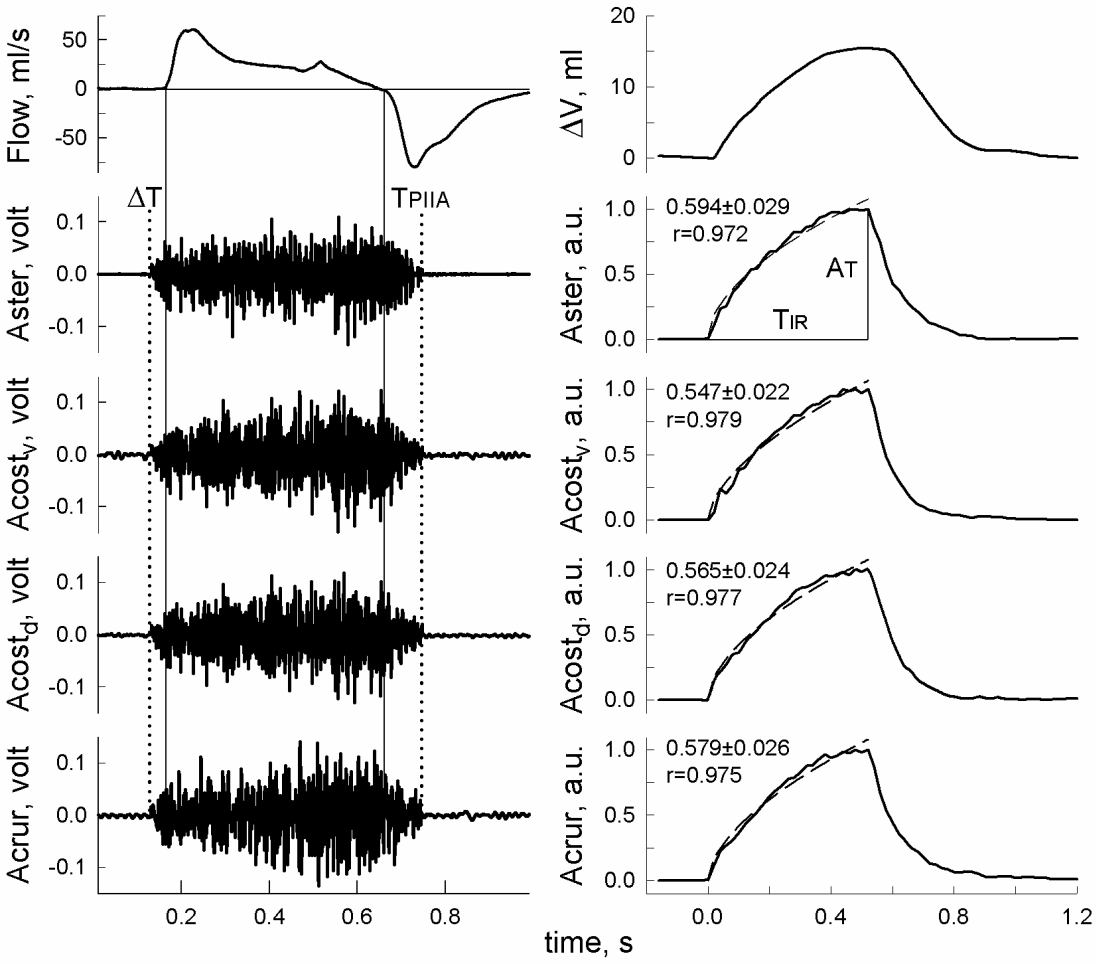
Flow, ml/s
50
0
-50
ΔT
TPIIA
Aster, volt
0.1
0.0
-0.1
Acost$_v$, volt
Acost$_d$, volt
Acrur, volt
0.2
0.4
0.6
0.8
time, s
ΔV, ml
20
10
0
Aster, a.u.
1.0
0.5
0.0
0.594±0.029
r=0.972
AT
TIR
Acost$_v$, a.u.
0.547±0.022
r=0.979
Acost$_d$, a.u.
0.565±0.024
r=0.977
Acrur, a.u.
0.579±0.026
r=0.975
0.0
0.4
0.8
1.2

Figure 1

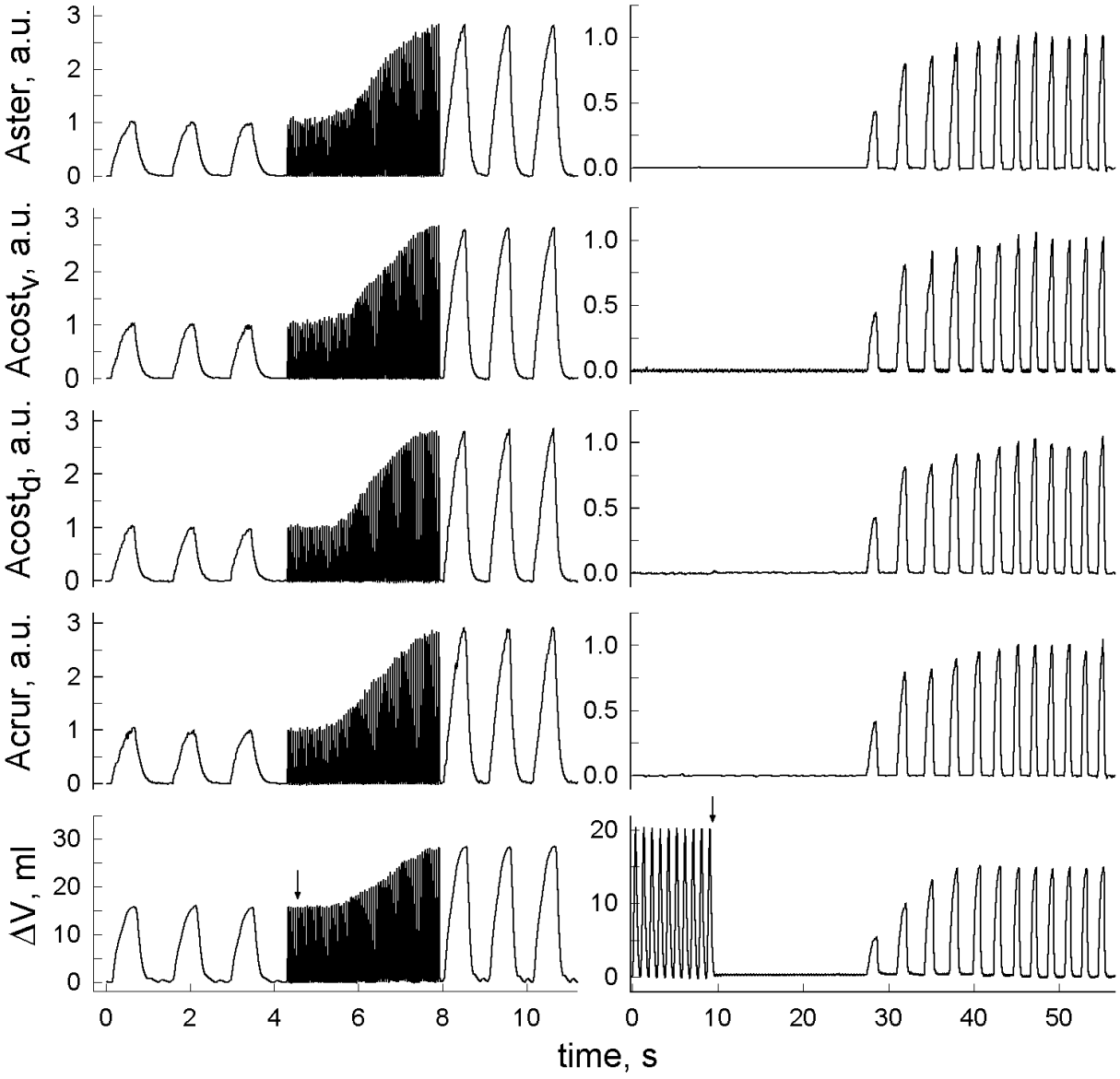

Figure 2

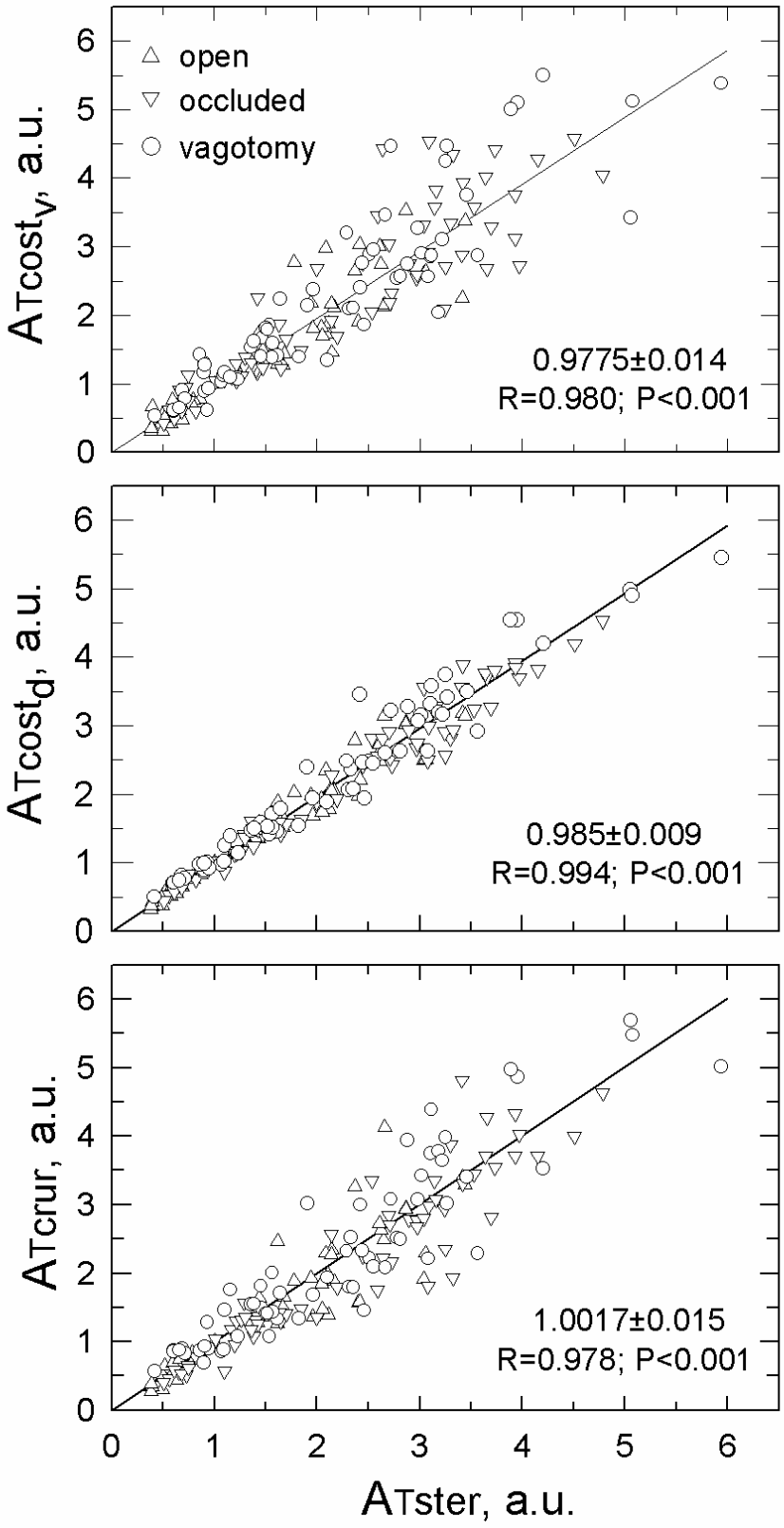
open
occluded
vagotomy
ATcost$_V$, a.u.
0.9775±0.014
R=0.980; P<0.001
ATcost$_d$, a.u.
0.985±0.009
R=0.994; P<0.001
ATcrur, a.u.
1.0017±0.015
R=0.978; P<0.001
ATster, a.u.
0
1
2
3
4
5
6

Figure 3

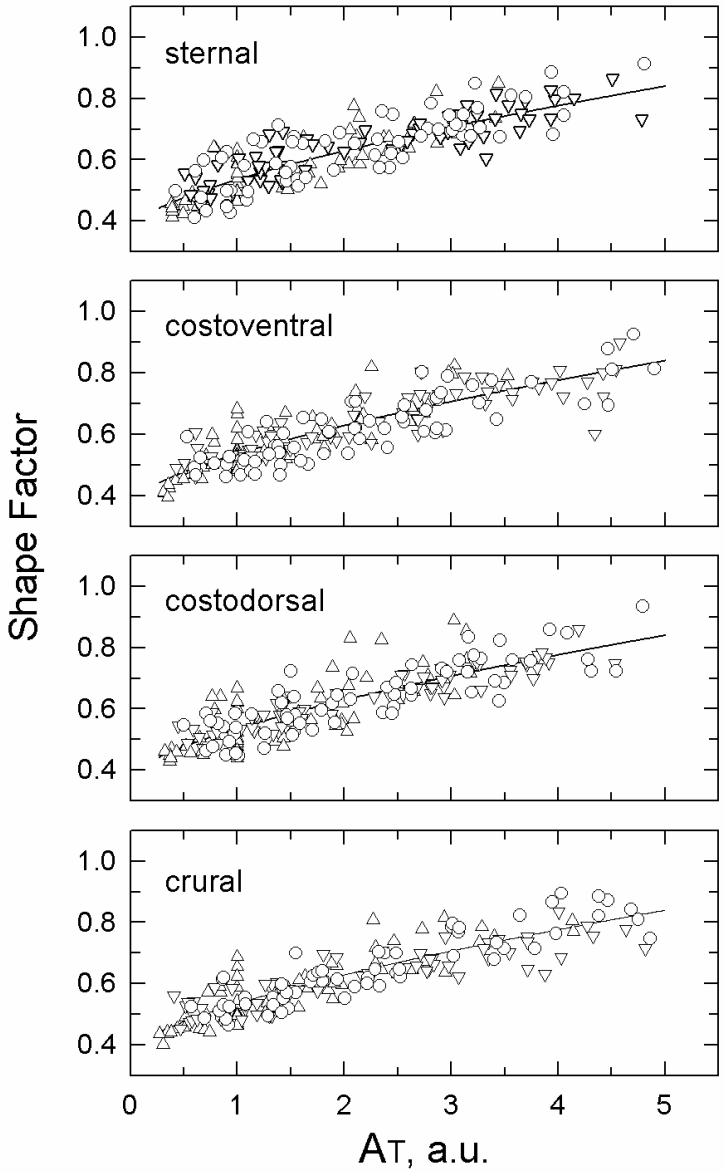
sternal
costoventral
costodorsal
crural
Shape Factor
1.0
0.8
0.6
0.4
0
1
2
3
4
5
AT, a.u.

Figure 4

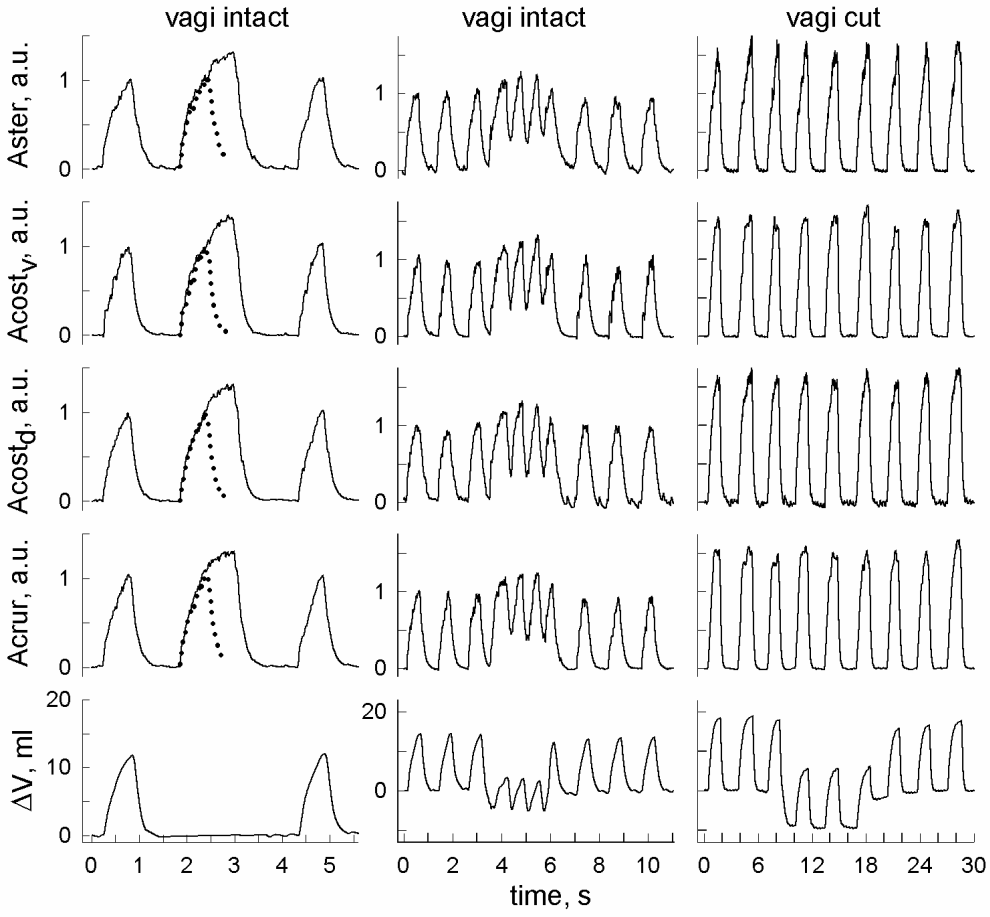
vagi intact
vagi intact
vagi cut
Aster, a.u.
Acost$_V$, a.u.
Acost$_d$, a.u.
Acrur, a.u.
ΔV, ml
time, s

Figure 5

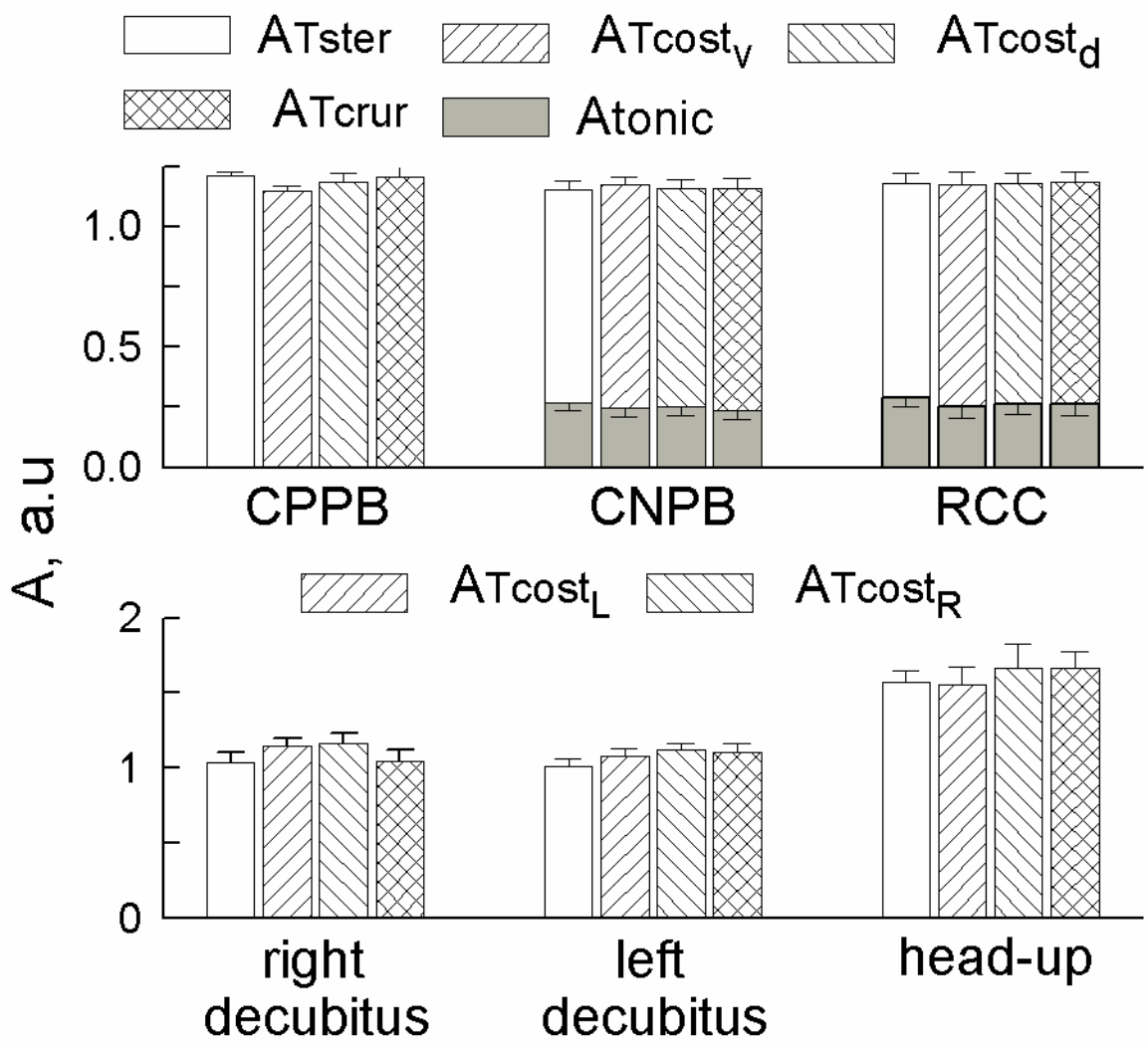
ATster
ATcost$_v$
ATcost$_d$
ATcrur
Atonic
A, a.u
1.0
0.5
0.0
CPPB
CNPB
RCC
ATcost$_L$
ATcost$_R$
2
1
0
right decubitus
left decubitus
head-up

Figure 6

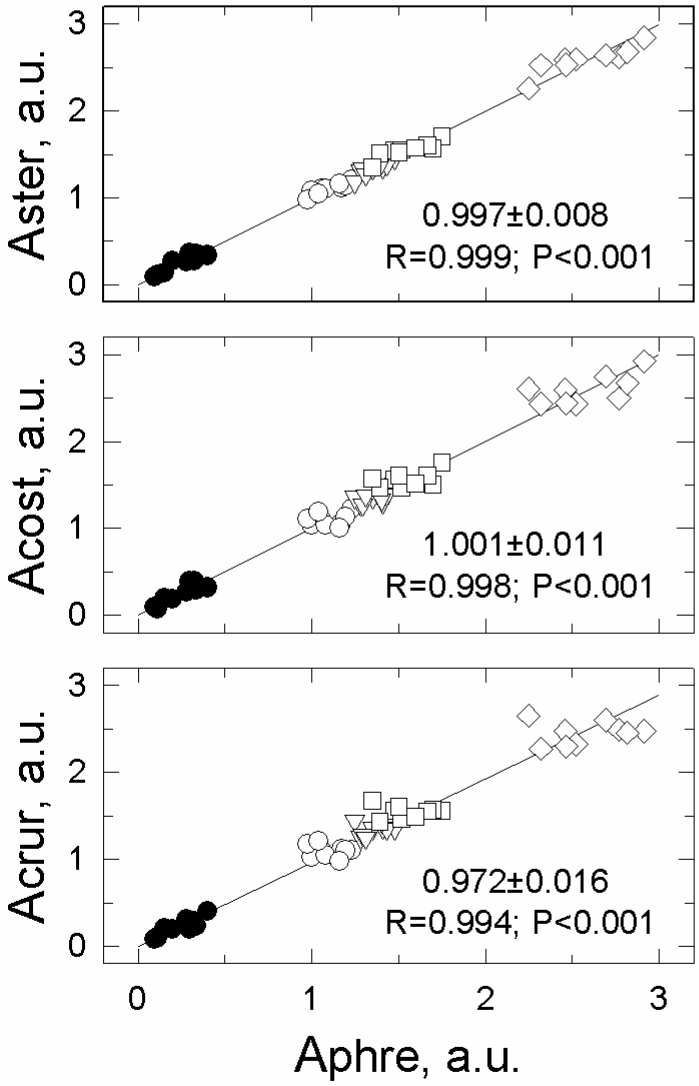
Aster, a.u.
0.997±0.008
R=0.999; P<0.001
Acost, a.u.
1.001±0.011
R=0.998; P<0.001
Acrur, a.u.
0.972±0.016
R=0.994; P<0.001
0
1
2
3
Aphre, a.u.

Figure 7

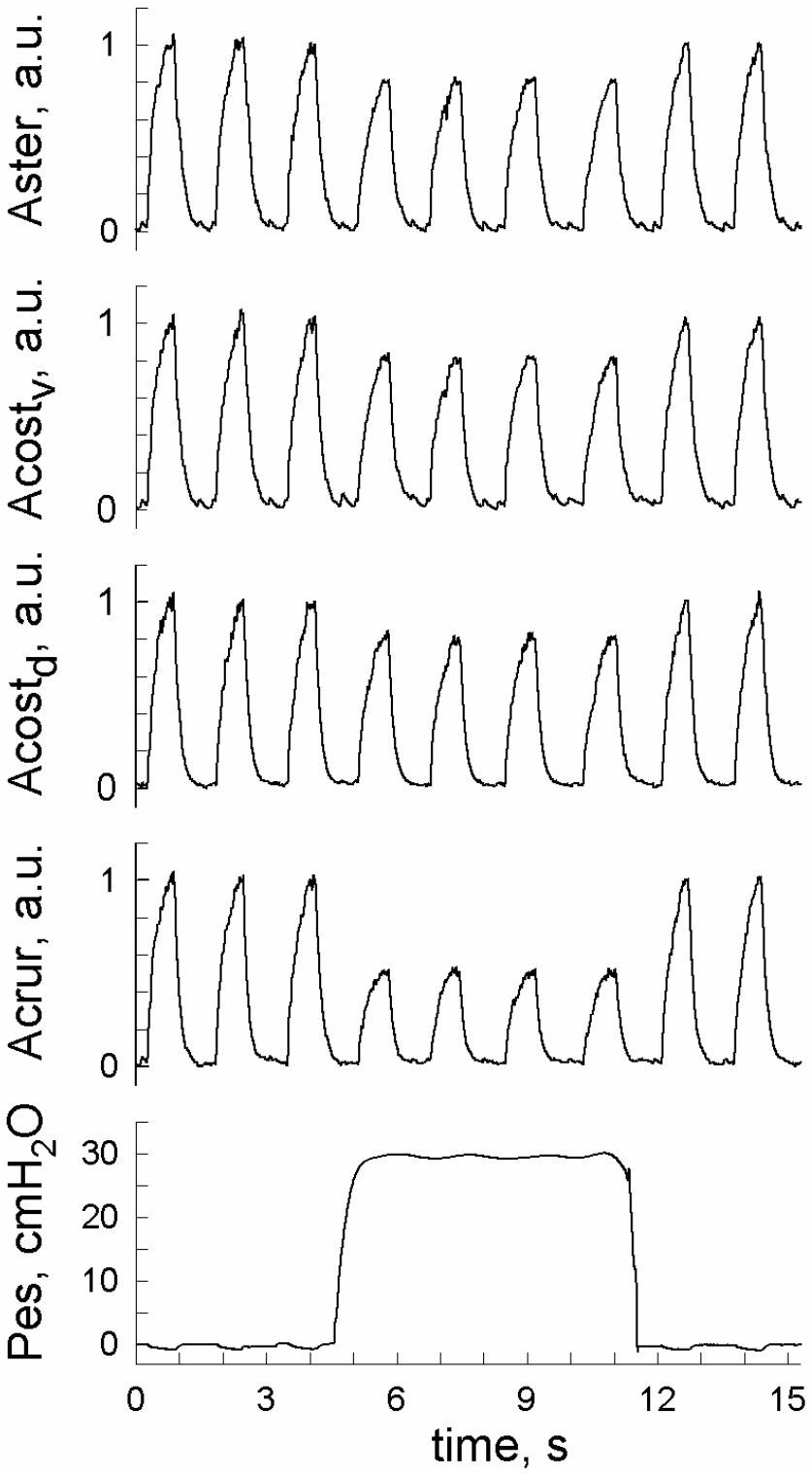
Aster, a.u.
Acost$_V$, a.u.
Acost$_d$, a.u.
Acrur, a.u.
Pes, cmH$_2$O
1
0
30
20
10
0
0
3
6
9
12
15
time, s

Figure 8