AUGMENTED BREATH PROVOKED BY LUNG INFLATION IN CAT

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Abstract. The rate of occurrence and magnitude of provoked augmented breath (PAB) were studied as the function of lung expansion applied at different intervals (15-180 s). Together with phrenic nerve activity (Phr.) the activities of recurrent laryngeal (RL) and hypoglossal (Hyp) nerves were investigated during PAB. The experiments were carried out in 10 cats anesthetised, paralysed and artificially ventilated by means of a phrenic nerve-driven respirator. Lung expansion was performed by increasing the gain of the servorespirator for one breath. PAB could be elicited when the interval between subsequent inflations was longer than 30 s ("refractory time"). We did not find out any consistent relationship, common for all experiments, between the value of the interval (> 30 s) and the rate of occurrence of PAB as well as between the volume of lung inflation and the magnitude of PAB. During PAB registered on Phr., activities of RL and Hyp were usually inhibited. It is concluded that PAB depends upon the instantaneous balance of excitatory and inhibitory vagal influences centrally differentiated at various respiratory outputs. Its amplitude and occurrences are therefore difficult to predict. Thus, PAB can be hardly compared with spontaneous deep breath.

INTRODUCTION

Eupneic breathing is periodically interrupted by a spontaneous augmented breath (SAB), which is characterized by a burst of activity (a "gasp") appearing on the top of normal inspiration (1, 6, 28, 31). Its
incidence increases with the elevation of chemical drive and/or diminu-
tion of lung compliance (1, 37, 41). It is well documented that the aug-
mented breath is stimulated by vagal and carotid sinus nerve afferents 
(1, 17). It has also been indicated that augmentation of inspiration, similar 
to SAB, could be evoked by lung inflation (9, 16, 42) or by reopening the 
tracheal tube occluded for a few breaths (37).

In the studies concerning vagal origin of augmented breath at least 
tree different vagal reflexes should be discussed. Firstly, the inspira-
inhibiting Hering-Breuer inflation reflex which controls the duration 
and magnitude of inspiration (3, 4). Secondly, the low-threshold facili-
tation of inspiratory activity (2, 30) by slowly adapting pulmonary stretch 
receptors stimulated during lung expansion (13). Thirdly, the high-
threshold excitation of inspiration evoked by stimulation of rapidly adap-
ting pulmonary mechanoreceptors (28, 38). Two former reflexes are 
already well described in terms of stimulus-response relationships (3, 
13, 36). This is not the case with respect to augmented breath provoked 
by large inflations.

There is also an interesting problem concerning the responsiveness of 
the nerves supplying the upper airways during provoked augmented 
breath (PAB), which controls respiratory resistance (44). In contrast to 
the phrenic nerve (3) they are strongly inhibited by lung inflation through-
hout the whole inspiratory phase (7, 43). Therefore in the present paper 
we attempted to describe stimulus-response relationship for PAB with 
simultaneous recording of the activity of phrenic, recurrent laryngeal 
(R.L.) and hypoglossal (Hyp.) nerves. Preliminary report has already 
been published in an abstract form (35).

METHODS

The experiments were performed with 10 adult cats weighing between 
2.8 and 4.0 kg. Two cats were anesthetized with a mixture of urethane 
and chloralose (0.3 g + 40 mg respectively), one with pentobarbitone 
(Sagatal, M & B; 35 mg i.p.) and seven with halothane (Narkotan, Spofa; 
1.0-1.5 vol.% in a mixture of oxygen and air). One out of the latter 
seven was decerebrated at precollicular level and anesthesia was with-
held. Halothane anesthesia was introduced first in a chamber and later 
on by application of a face-mask. Preliminary preparation was performed 
in the halothane-anesthetized cats (1.0-2.5 vol.%o) with local administra-
tion of 2% Xylocaine (Astra). After tracheotomy the animals were pa-
ralyzed with gallamine (Flaxedil, D and G) and ventilated by means of 
phrenic nerve driven respirator (Medipan) (20). The animals were immo-
bilized in a supine position on a heated operating table. The efferent
activities of the C₃ phrenic root, the recurrent laryngeal (in five cats) and hypoglossal (in two cats) nerves were recorded with bipolar electrodes, amplified and integrated (2). The arterial blood pressure was recorded by means of Statham P23Db transducer connected to the femoral artery. In order to avoid blood clotting the animals were heparinized. Arterial blood was periodically analyzed for blood gases measurements (Corning electrodes); when necessary, an injection of bicarbonate solution was given i.v. Integrated activity of phrenic (Phr.), recurrent laryngeal (R.L.) and hypoglossal (Hyp.) nerves as well as volume signal (V) from the respirator were recorded on paper (Beckman R. 414).

During the experiments the animals were ventilated in three different modes: (i) controlled ventilation, in which the volume of air inflated to the lungs was proportional to the integrated activity of the phrenic nerve (2), (ii) triggered ventilation, in which inflation with the given constant airflow was triggered with some delay (about 100 ms) from the onset of phrenic signal (34) and (iii) classical ventilation, when the volume and frequency were set arbitrarily. During controlled ventilation changes in the gain of the servomechanism were performed for one breath, so that for the same Phr. signal different volume (V) of air inflated to the lungs could be obtained in accordance with the equation: Gain = V/Phr. (2). The manoeuvres were performed in series with a different time interval: 15, 30, 60, 120 and 180 s.

We compared the changes of integrated nerves’ activity during changes in gain to the breaths preceding the manoeuvre (control breaths). Since changes of the amplitude of integrated phrenic nerve activity showed quite a large scatter in relation to changes in gain, we accepted a linear correlation, in the first approximation, between studied parameters (Phr. vs. Gain). We assumed the control value of Phr. and Gain as 100%o. During the series in which the gain of the servorespirator was increased for one breath we made pauses to avoid hyperventilation. The first breath in the series was omitted in the process of analysis.

RESULTS

Stimulus-response relationship

When the gain of servorespirator was larger than 150%o of the control value an excitation of phrenic nerve activity was observed which to some extent resembled the spontaneous augmented breath (SAB) with the characteristic phase I (when the slope of Phr. is similar to control inspiration) and phase II (“gasp” at the top of normal inspiration) (6, 31) as it is shown in Fig. 1. (gain up.) Usually this distinction between these two phases was less obvious, since the duration of phase I and amplit-
tude of phase II varied with the gain of the servorespirator. For this type of high-threshold response to lung expansion we will use the term "provoked augmented breath" (PAB) to distinguish it from SAB and deep breath elicited by chemical stimuli via the carotid sinus nerve (17). In a practical sense, all responses to an increase in the servorespirator gain in which Phr. amplitude exceeded 100% of the control value were considered as a PAB.

**Fig. 1.** Integrated activities of recurrent laryngeal (R.L.) (upper record) and phrenic (Phr.) (lower record) nerves during: control breath, switching off the pump for one breath and during increase in the gain of the servorespirator, inducing provoked augmented breath (PAB). At the bottom — time scale.

During a run with 15 s interval between the manoeuvres only the pure inhibition of Phr. was observed, which was proportional to lung expansion. The correlation between Phr. and pump gain is presented in Fig. 2. (line I). High threshold excitation of phrenic nerve activity was observed for intervals longer than 20 s. In the range of intervals (t) used in our experiments (30 s < t < 180 s) there was no significant correlation between the incidence of PAB and the width of interval. Only very weak positive correlation between gain and amplitude of Phr. was found for PAB (compare line II and III in Fig. 2 as well as Fig. 5A). Detailed analysis of Phr. responses to changes in gain which exceeded 150% showed (see, eg. Fig. 2) that for the same value of gain a variety of effects could be observed: (i) inhibition of Phr. in accordance with Phr. vs. Gain negative relationship (line I), (ii) no response in Phr. amplitude as a result of mutual abolition of inhibitory and excitatory influences and (iii) strong excitation of Phr. which resembles SAB as well as all intermingled responses.

The duration of PAB in comparison with the control inspiratory time
(T₁) varied in different experiments; it was shorter or longer than T₁
(see Fig. 1. and Fig. 3.). These discrepancies were probably due to in-
dividual differences in sensitivity to vagal excitation.

The existence of two antagonistically acting influences on inspiratory
activity can theoretically be a source of oscillations in the controlledphe-
nomenon. In fact, in a few experiments such oscillations were seen, espe-
cially for high gains (> 200%/s); see Fig. 3.

Fig. 2. Relationship between changes of the amplitude of integrated phrenic nerve
activity (Phr.) and increase in gain (in Δ%/s) imposed for one breath. Three lines
represent the series with 15 (■), 60 (x) and 120 s (o) intervals. n, numbers of
manoeuvres in a series; r, correlation coefficient for linear regression. Asterisk,
threshold for high-gain excitation of Phr.

Fig. 3. Comparison between the responses of integrated recurrent laryngeal (R.L.)
and phrenic (Phr.) nerve activities during control (solid line) and provoked aug-
mented breath (dotted line). A-D, examples taken from different experiments.
Refractory period

It was already stated, that when the interval between the manoeuvres was shorter than 20 s the PAB could not be elicited. However, if we assume that all changes in the shape of the integrated phrenic nerve activity similar to those presented in Fig. 3. reflect the effect of high threshold excitation, it could be demonstrated that such response can be elicited in two succeeding breaths for high gains (over 200%) as shown in Fig. 4.

![Fig. 4. Changes in the amplitude and slope of integrated activities of phrenic (Phr.) and recurrent laryngeal (R.L.) nerves during two successive manoeuvres of gain increase (the same gain, over 160% shown by arrows).](image)

Using the triggered mode of ventilation (36) we could apply lung inflation with a constant airflow during the inspiratory phase independently of Phr. amplitude. It was shown that such a manoeuvre can elicit PAB in every breath when the airflow is sufficiently high.

The effect of anesthesia

In all experiments, independently of the type of anesthesia, we could as a rule, elicit, PAB with the same characteristics. However, the halothane anesthesia was best, since the pattern of breathing was most stable during the series of manoeuvres. The shape of PAB in other types of anesthesia was often less regular (eg. short lasting gasps during the whole respiratory cycle) suggesting that cough or aspiration reflex could be involved in the overall response (26). In this respect, we cannot agree with the conclusion of Younes and Youssef (46) that halothane anesthesia should not be used for the study of vagal reflexes (see also 21). In the case of halothane anesthesia, the basal respiratory rhythm was fast, and this could have been of some significance for the study concerning the refractory period.
Activities of recurrent laryngeal and hypoglossal nerves during provoked augmented breath

As it was already shown in the presented figures the excitation of recurrent laryngeal nerve activity (R.L.) is weak or even absent during a provoked augmentation of phrenic nerve activity. The amplitude of R.L. during PAB is usually unchanged or lower than the control value (see Fig. 5) in spite of evident changes in the shape of the envelope of integrated activity which reflect the effects of excitation (see eg. Fig. 3.).

Fig. 5. Amplitudes of integrated (A) — phrenic (Phr.) and (B) — recurrent laryngeal (R.L.) nerve activities in relation to changes in gain of the servorespirator. Numbers show the ratio of breaths with augmented activities to all studied breaths in a series.

Fig. 6. Superimposed integrated activities of recurrent laryngeal (R.L.) and phrenic (Phr.) nerves during control breath (dotted line), switching off the pump for one breath (pump off, continuous line) and provoked augmented breath (gain up, dashed line). A and B, different experiments, in B also hypoglossal (Hyp.) activity is presented.
Figure 6A presents integrated phrenic and recurrent laryngeal nerves activities during the control breath (dotted line), switching off the pump for one breath (solid line) and during PAB (dashed line). In the control breath, the slope of Phr. is steeper and maximal amplitude lower than after the elimination of phasic vagal feedback. The R.L. activity shows, in agreement with the data of Cohen (7), only inhibition during the whole respiratory cycle. During PAB the inspiratory time ($T_I$) is shorter but the amplitude of PHR is larger than in the control breath. These changes of $T_I$ and Phr. in response to an increase in the pump gain (which do not follow the normal positive correlation Phr. vs. $T_I$) demonstrate a transition from low- to high-threshold facilitation of inspiratory activity, as shown by DiMarco et al. (13). The activity of recurrent laryngeal nerve shows, however, further inhibition in response to an increase in the gain of the pump.

The reverse response of Phr. and R.L. to lung inflation during the control as well as during provoked augmented breath is also presented in Fig. 6B and Fig. 7C, where additionally the integrated activity of the hypoglossal nerve (Hyp.) is shown. The inhibition of Hyp. during PAB is even more pronounced than in R.L.

Fig. 7. Records of the stroke volume (V), integrated activities of hypoglossal (Hyp.), recurrent laryngeal (R.L.) and phrenic (Phr.) nerves during “classical” (constant volume) ventilation. A, increase in the stroke volume performed for one inflation evoked augmentation of all recorded activities; B, effect of switching off the pump for one cycle; C, as in A, but in this case only Phr. was excited by hyperinflation (Records retouched).
In some circumstances, during strong excitation of Phr. we observed an excitation of the activity of all recorded nerves (Fig. 7A). In this case PAB strongly resembled the spontaneous augmented breath.

DISCUSSION

The phenomenon of “paradoxical” inspiratory augmentation elicited by lung inflation has been well known since the classical work of Head (19). His paper started a long discussion about the type of vagal fibres transmitting this reflex (see 8, 22, 28). Different types of experiments were performed with an application of lung inflation (12, 23) or electrical vagal stimulation (22) with a combination of blockades of the transmission from slowly adapting pulmonary receptors (10, 18).

On the basis of these experiments it could be concluded, that inspiratory-inhibiting and expiratory-prolonging vagal reflexes are transmitted via the afferents from slowly adapting pulmonary mechanoreceptors (22). It was shown also that the low-threshold facilitation of inspiratory activity is proportional to the level of stimulation of slowly adapting receptors (13). When the stimulation of lung receptors or myelinated vagal fibres is followed by an inspiratory time prolongation (12) or a shortening of expiratory phase (22), it might be concluded that these reflexes are transmitted through afferents of rapidly adapting pulmonary mechanoreceptors (38). In the last years it is rather commonly accepted that the augmented breath provoked by lung expansion is evoked by an excitation of irritant receptors (1, 6, 8, 33) i.e., rapidly adapting pulmonary receptors (28).

The application of respirators steered by the activity of phrenic nerves (20, 25, 30) allowed to study vagal reflexes under conditions of closed vagal feedback loop (2). An increase in the gain of servorespirator above 150% of the control value can elicit an augmented breath (2, 6, 30). With further increase in the gain above the threshold for PAB, the slope of Phr. rises and the peak activity of the augmented breath could be achieved earlier in the inspiratory phase. Figure 8. shows the results of such an experiment conducted by Bruce, Romaniuk and von Euler (unpublished), using servorespirator introduced by Knox (25). During this experiment a cold block of the vagal transmission was performed. It was interesting to compare the effects of lung deflation during control condition with the responses to lung inflation after cooling the vagus nerve to 14°C (Fig. 8.). Considering the similarities of the obtained effects, it could be concluded that the shape of Phr. during the manoeuvre of “gain up” is the sum of excitatory effects from the rapidly adapting receptors and inhibitory influences from slowly adapting receptors, since the latter are attenuated during both vagal cooling and deflation.
Stimulus-response relationship

The difficulties which we have encountered during this work with a clear documentation of stimulus-response relationship for provoked augmented breath appear to arise from the fact that the formation of the inspiratory activity during PAB is under two counteracting vagal influences. Both of them are increasing with stimulus intensity and become stronger with developing inspiratory activity (inspiratory modulation — 3). A larger gain of the respirator evokes an excitation of Phr. earlier during inspiration, but with a smaller amplitude (see Fig. 8.). The augmentation of Phr. elicits further increase in volume which, in turn, produces a stronger reciprocal inhibition. For a lower gain (but still higher than 150%/) the excitation is evoked later during inspiration, but since the volume is relatively smaller, the augmentation is less manifested. Hence the overall response is hard to predict due to the exi-

![Fig. 8. Changes in the shape of integrated phrenic nerve activity (Int. Phr.) during an increase of respirator gain above 150% of the control value (C.). O, switching off the pump for one breath (gain zero). Defl., deflation and Infl., inflation of the lungs. Cont. temp., without and 14°C, with cooling the vagus nerve to 14°C.](image)
stence of inhibitory and excitatory vagal influences acting simultaneously. Also the phenomenon of inspiratory modulation of vagal reflexes can play its part here. For these reasons it is difficult to show a real distinction between phase I (control inspiration) and phase II ("gasp") for PAB. As a consequence, the amplitude of PAB is not constant, in contrast to the stability of phase II in SAB (44). It was pointed out (6) that phase II in SAB has no stimulus-response relationship.

There is also another important point which should be taken into consideration. The rapidly adapting receptors respond to applied stimuli with much bigger scatter than the slowly adapting ones, (8, 29, 38). The same stimulus applied at different moments of the experiment can evoke different patterns of their firing. This could be one of the reasons why the amplitude of Phr. can achieve any value ranging from pure inhibition (line I, Fig. 2.) to a maximal excitation for the same gain repeated several times. Another mechanism of this unpredictability may depend upon the central organization of PAB (see below).

Refractory period

It is well known that PAB can be elicited when the stimulus is repeated after a certain delay — "refractory period" (31) — which in our experiments was about 20-30 s. This refractory period can be linked with the short-lasting (maximal changes appear during first 3 min) increase in lung compliance, which is observed after a large inflation (45) and — consequently — weaker excitation of irritant receptors (47). However, there is also evidence that the refractory period is a central phenomenon which follows augmented breath (6, 32). It was already demonstrated that central effects of stimulation of rapidly adapting pulmonary receptor afferents last several seconds (21, 22). Similar mechanisms of central short-term plasticity (24, 32) can take place after SAP.

There are still some discrepancies in the literature about the existence of the refractory period observed in animals paralyzed with gallamine (6, 31). On the basis of our results obtained with an application of a respirator working in controlled (2) or triggered (36) mode (closed or open vagal feedback loop), we conclude that PAB could be evoked in every breath only when the airflow does not depend upon the phrenic signal (open vagal feedback loop). This takes place after muscle paralysis during the classical (31) or triggered (this paper) ventilation.

Recurrent laryngeal and hypoglossal nerves activities during PAB

Activities of the nerves supplying the upper airways are under continuous inhibitory vagal influences (40, 43). This inhibition is not modulated during the inspiratory phase (27). In that respect this reflex is
similar to a low-threshold facilitation of phrenic and external intercostal nerves activities (13). It acts, however, in the opposite direction on the rate of rise of the respective inspiratory activity. During PAB the inhibitory effects of lung expansion on R.L. and especially on Hyp. are larger than during quiet breathing and the dissociation of responses of Phr. on one hand and R.L. and Hyp. on the other are more clear. A similar differentiation of responsiveness of various respiratory outputs to applied peripheral and central stimuli has also been described by Bruce et al. (5), DiMarco et al. (14, 15) and Sica et al. (40). It might be suggested that central differentiation of responses takes place possibly at the level of premotoneurons (see also 39, 40). However, there are rare situations, when during PAB all recorded activities respond in synchrony, all with an augmentation of their activity. This can be seen sometimes in the case of large inflations (see Fig. 5 and 7A). Only this type of response is similar to spontaneous augmented breaths, in which the activities of all inspiratory nerves are enhanced (44). It could be postulated that inspiratory excitation is transmitted through the neuronal network which generates inspiratory action. Possibly such augmentation which really resembles SAB, has a longer central refractory period, which in the case of experiments presented in this paper would exceed 3 min. Such long a duration of refractory period for SAB could be explained by a low chemical drive, which developed in our experiments as a result of controlled ventilation with the mixture of air oxygen and halothane anesthesia (11). Also, the type of manoeuvres performed during the experiments (gain up) prevented lung atelectasis which, otherwise, is the stimulating condition for SAB (1, 37).

On the basis of the presented results it could be concluded that the augmented breath provoked by an increase in the gain of the servorespirator has no clear stimulus–response relationship due to unpredictable behaviour of high-threshold excitation combined with IIering–Breuer inflation reflex. Since the nerves supplying the upper airways show a different pattern of activation compared to the phrenic nerve activity during spontaneous and provoked augmented breath, it is worthwhile to differentiate them as two distinct phenomena.

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