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Isocapnic and small hypercapnic single-breath stimuli: evidence for an inhibitory inflation reflex in conscious man

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Isocapnic and small hypercapnic single-breath stimuli: evidence for an inhibitory inflation reflex in conscious man. S.S.D. Fernando, K.B. Saunders. ©ERS Journals Ltd 1994.

ABSTRACT: We wished to find out if a deep inspiration had any influence on subsequent breathing which was mediated by neural rather than chemical stimuli.

We therefore compared the effect on ventilation of a deep isocapnic breath with that of a similar breath containing 6% CO_2, and with the effect of two successive tidal volume breaths of 6% CO_2. We studied five normal subjects, each of whom repeated the three manoeuvres 20 times, and we used ensemble averaging to increase the signal-to-noise ratio.

The isocapnic deep inspiration was followed by a significant inhibition of ventilation in the group in the second post-stimulus breath, and in 4 of the 5 subjects in first and second post-stimulus breaths. This was due to an increase in both inspiratory and expiratory time, with a variable effect on tidal volume. A similar initial ventilatory inhibition was seen in the response to a deep breath of 6% CO_2. When the isocapnic response was subtracted from the hypercapnic response, the result was similar to that observed from two tidal volume breaths of 6% CO_2.

We conclude that a single deep inflation of the lungs in awake man inhibits subsequent ventilation by a neural mechanism, and that this may affect the CO_2 response measured by single-breath techniques using such manoeuvres.
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A major drawback in single-breath tests of ventilatory sensitivity to CO_2 is that, since the dose of CO_2 that can be delivered in a single tidal breath is limited by its unpleasant taste and irritant nature, either vital capacity [1, 2], inspiratory capacity, or 2–3 tidal volume breaths [3] of CO_2 have to be used in order to obtain measurable responses. We have previously observed [4] that a deep expiration to residual volume could abolish the ventilatory inhibition expected from a hypocapnic stimulus, suggesting a stimulatory effect on ventilation, making vital capacity breaths unsuitable for this purpose. An alternative is to employ inspiratory capacity (IC) breaths.

The purpose of this study was twofold. Firstly, we wished to examine the effect of a strictly isocapnic deep inspiration on the subsequent breathing pattern. Vagal reflexes that inhibit ventilation in response to lung inflation (which could very well influence the ventilatory response to CO_2) were first demonstrated in 1868 by BREUER and Hering [5]. Secondly, we wished to see whether a deep inspiration would have an influence on the subsequent ventilatory response to CO_2.

Using a fractional inspired CO_2 concentration (F_{ICO_2}) of 6%, we compared the response to two tidal breaths with a single inspiratory capacity breath. To minimize the baseline noise and increase the signal-to-noise ratio, we repeated the experimental runs many times and ensemble averaged the results.

Material and method

Subjects

Five nonsmoking, healthy volunteers (4 males, 1 female; aged 27–36 yrs) participated. Written consent was obtained after explanation prior to the study. This study had approval of the Wandsworth Health Authority Local Research Ethics Committee. The subjects took no sedative drugs and avoided caffeine on the day of an experimental session.

Methods

The subjects sat upright and breathed through a nonrebreathing valve (Hans Rudolph, No. 2700, dead space 77 ml) from an open respiratory circuit (fig. 1). Two Fleisch pneumotachographs (coupled to Validyne MP45 differential pressure transducers) continuously measured the inspiratory and expiratory air flows, and a mass spectrometer (Centronic MGA 200) continuously sampled CO_2 and O_2 at the mouth. The pneumotachographs [6] and the mass spectrometer were calibrated before each experimental session. By turning the three-way tap, which was closely adjacent to the valve, the inspirate

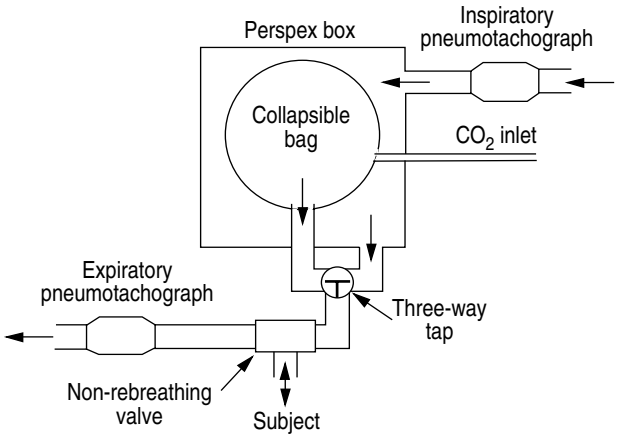

Fig. 1. – The respiratory circuit. The inspirate could be switched from room air to CO_2 mixture in the bag, whilst the inspiratory flow is being continuously registered in the pneumotachograph.

could be switched from room air to the test gas mixture (and *vice versa*) without being noticed by the subject. The pneumotachographs were unheated and the inspired gas was not humidified.

Signals from the pressure transducers and the mass spectrometer were recorded on a pen-chart recorder (Gould 2600s) and on a magnetic tape (Racal FM tape recorder), which was later digitized and analysed on a PDP 11/23 computer. The computer algorithm measured breath-by-breath values for the inspiratory (T_I) and expiratory (T_E) times, inspiratory tidal volume (V_T), end-tidal carbon dioxide tension (P_{ETCO_2}) and end-tidal oxygen tension (P_{ETO_2}), and derived the minute ventilation ($\dot{V}_E$) and the mean inspiratory flow ($\dot{V}_I$). Each breath was placed on its mid-breath time ([T_I+T_E]/2). The flow and its derived variables were calculated from integrated signals from the inspiratory pneumotachograph. The expiratory pneumotachograph was used only for timing purposes.

Study design

Each subject was tested with three types of CO_2 stimuli:
1. A voluntary deep inhalation (from functional residual capacity (FRC) to total lung capacity (TLC)) of 6% CO_2 in air ($IC_{HYPERCAP}$).
2. Two successive spontaneous tidal inhalations of 6% CO_2 in air ($TV_{HYPERCAP}$).
3. A voluntary deep inhalation of 3–5% CO_2 in air, adjusted to maintain isocapnia during the deep breath (IC_{ISOCAP}). The exact value of the F_{ICO_2} was determined by previous trials for individual subjects.

The experiment was conducted on two days, each subject performing six experimental runs, four hypercapnic and two isocapnic, on each day. A hypercapnic run consisted of five test breaths, either $IC_{HYPERCAP}$ stimuli or $TV_{HYPERCAP}$ stimuli, in random order. Isocapnic runs consisted of only IC_{ISOCAP} test stimuli. The test breaths were placed at 3 min intervals. The order of the runs were randomized. Thus, at the end of the experiment, each subject had performed 20 each of IC_{ISOCAP}, $IC_{HYPERCAP}$ and $TV_{HYPERCAP}$ manoeuvres.

During the experiment, the subjects listened to taped music *via* headphones and were separated from the investigator and the apparatus by a screen. No coffee, tea or alcohol was allowed for at least 10 h before the experiment.

Analysis

Variables measured for each breath were placed on the mid-point ([T_I+T_E]/2) of the relevant breath on a time scale. The test breath(s), 10 preceding breaths and the breaths within 60 s after the test breath (post-test breaths) were considered as a single experimental run. The runs were ensemble averaged as shown in fig. 2.

Breaths in each run were numbered sequentially, starting from the test breath (IC or first breath of TV) which was allocated zero. The runs from each subject were then aligned and ensemble averaged on a breath-number basis, as shown in Step A of figure 2. The breaths were placed on their mean mid-breath times and presented on a time base (onset of inspiration of the test breath is time zero). The mean values from Step A were taken as the signal for that particular individual. The 10 control "breaths" (Step B) were averaged to obtain the final control. The test breath and each of the post-test breaths were tested against the control by Student's t-test (degrees of freedom (df) = 9) and also by calculating the 95% confidence interval. For group results, we used paired t-tests for each post-test breath against control (n=5; df=4).

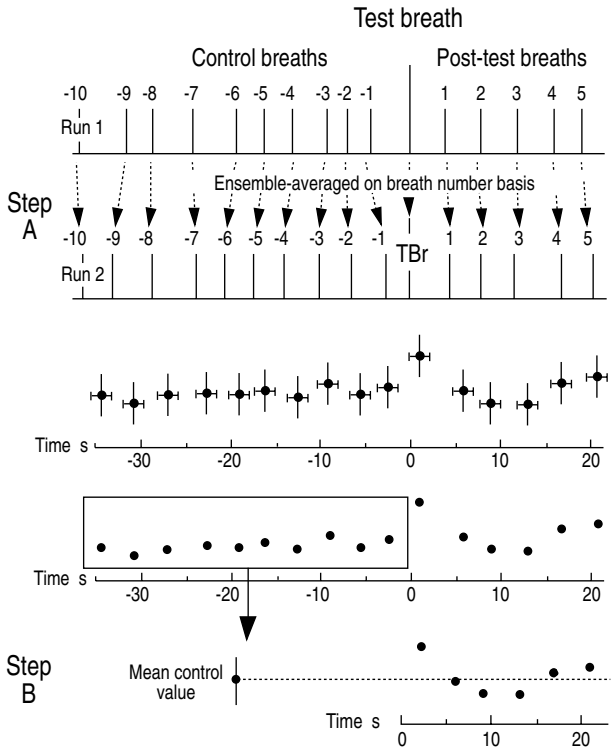

Fig. 2. – A schematic diagram to illustrate ensemble averaging in a single subject (Step A) and derivation of the control for subsequent testing against each post-test breath (Step B). TBr: test breath. Time zero is the beginning of the test breath. Bars represent SD. This example must represent a test breath of a single deep inhalation, with points representing successive value of $\dot{V}_E$.

Table 1. – The control values for $\dot{V}_E$ and P_{ETCO_2} for individual subjects and the peak change in P_{ETCO_2} in the test breath

Subject No.	Control $\dot{V}_E$ $l \cdot min^{-1}$	Control P_{ETCO_2} mmHg	Control P_{ETCO_2} kPa	P_{ETCO_2} in test breath* mmHg	P_{ETCO_2} in test breath* kPa	ΔP_{ETCO_2}* mmHg	ΔP_{ETCO_2}* kPa	ΔV_T† l
IC$_{HYPERCAP}$								
1	10.9 (0.13)	41.2 (0.15)	5.5 (0.02)	43.8 (2.27)	5.8 (0.30)	2.6	0.35	1.6
2	10.6 (0.34)	41.3 (0.16)	5.5 (0.02)	44.7 (1.20)	6.0 (0.16)	3.4	0.45	1.7
3	9.3 (0.22)	38.3 (0.08)	5.1 (0.01)	42.4 (3.41)	5.7 (0.45)	4.1	0.55	2.4
4	10.4 (0.46)	38.9 (0.58)	5.2 (0.08)	43.0 (1.22)	5.7 (0.16)	4.1	0.55	1.6
5	12.9 (0.21)	36.8 (0.16)	4.9 (0.02)	41.7 (0.79)	5.6 (0.11)	5.0	0.67	1.7
TV$_{HYPERCAP}$								
1	10.6 (0.21)	41.4 (0.12)	5.5 (0.02)	45.6 (2.39)	6.1 (0.32)	4.2	0.56	
2	10.8 (0.30)	41.6 (0.05)	5.5 (0.01)	47.1 (1.41)	6.3 (0.19)	5.5	0.73	
3	9.4 (0.19)	37.7 (0.12)	5.0 (0.02)	42.8 (3.42)	5.7 (0.46)	5.1	0.68	
4	9.9 (0.19)	39.3 (0.10)	5.2 (0.01)	44.7 (1.56)	6.0 (0.21)	5.4	0.72	
5	12.4 (0.24)	37.8 (0.13)	5.0 (0.02)	42.6 (1.46)	5.7 (0.19)	4.7	0.63	
IC$_{ISOCAP}$								
1	10.3 (0.25)	41.4 (0.16)	5.5 (0.02)	41.1 (1.13)	5.5 (0.15)	-0.24	-0.03	1.6
2	10.5 (0.28)	41.2 (0.14)	5.5 (0.02)	41.1 (1.36)	5.5 (0.18)	-0.14	-0.02	1.6
3	9.4 (0.23)	38.0 (0.10)	5.1 (0.01)	38.6 (2.61)	5.1 (0.35)	0.62	0.08	2.7
4	10.9 (0.47)	39.4 (0.14)	5.3 (0.02)	39.4 (1.57)	5.3 (0.21)	-0.02	-0.003	1.7
5	11.5 (0.28)	37.4 (0.14)	5.0 (0.02)	37.6 (0.72)	5.0 (0.10)	0.14	0.02	1.7

Data are presented as mean and SD in parenthesis, of 20 tests in each subject. *: for TV$_{HYPERCAP}$, the values given are for the second CO_2 breath. †: in test breath; $\dot{V}_E$: minute ventilation; P_{ETCO_2}: end-tidal carbon dioxide tension; V_T: tidal volume; IC$_{HYPERCAP}$: single voluntary deep inhalation from functional residual capacity to total lung capacity of 6% CO_2 in air; TV$_{HYPERCAP}$: two successive spontaneous tidal inhalations of 6% CO_2 in air; IC$_{ISOCAP}$: single voluntary deep inhalation of 3–5% CO_2 in air, adjusted to maintain isocapnia during the deep breath.

This procedure was designed to test the null hypothesis that "following the stimulus, no subsequent breath is different from the control steady-state". In group results, we also tested the null hypothesis "the overall response to the stimulus is not discernible above the noise", by performing an analysis of variance (ANOVA) between 10 control and 10 post-test breaths (2nd to 11th breaths for IC stimuli and 3rd to 12th breaths for TV stimuli). Since we were interested in the breaths immediately after the IC$_{ISOCAP}$ stimulus, ANOVA was performed between control and five post-test breaths.

For hypercapnic manoeuvres, P_{CO_2} stimulus was defined both as peaks and as time-integrals. The integral was defined as the envelope enclosed by significantly different post-stimulus P_{ETCO_2} points.

Results

The control values for $\dot{V}_E$ and P_{ETCO_2} for individual subjects and the change in P_{ETCO_2} in the deep breath are shown in table 1. Although we attempted to achieve identical P_{CO_2} stimuli, the peak P_{ETCO_2} produced by the TV$_{HYPERCAP}$ stimulus (defined as ΔP_{ETCO_2} in the second CO_2 breath) was significantly higher than that obtained by the IC$_{HYPERCAP}$ stimulus (mean±SD 5.0±0.5 *vs* 3.8±0.9 mmHg; (0.7±0.07 *vs* 0.5±0.12 kPa) p=0.047, paired t-test). However, there was no difference between the P_{CO_2} integrals (57.1±11.9 *vs* 57.4±24.3 mmHg·s^{-1} (7.6±1.6 *vs* 7.7±3.2 kPa·s^{-1}); p=0.98, paired t-test). During IC$_{ISOCAP}$

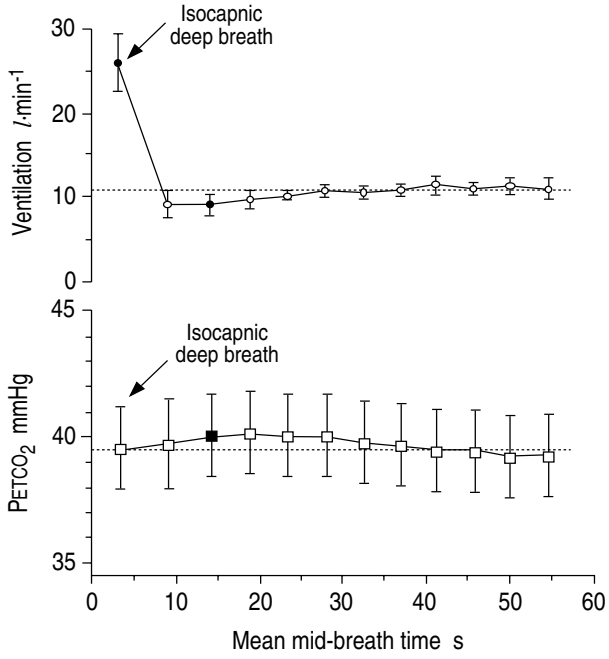

Fig. 3. – The ventilatory response to an isocapnic deep inspiration. Ensemble-averaged results for five subjects. Each point represents one "breath" and is placed on its mean mid-breath time. Error bars 1 SD. Broken horizontal line indicates the control. Data points significantly different (paired t-test; df=4) are filled. The overall $\dot{V}_E$ in the first five post-deep breaths is significantly below the baseline noise ($p<0.001$, ANOVA). P_{ETCO_2}: end-tidal carbon dioxide tension; df: degrees of freedom; ANOVA: analysis of variance; $\dot{V}_E$: minute ventilation. 7.5006 mmHg = 1 kPa.

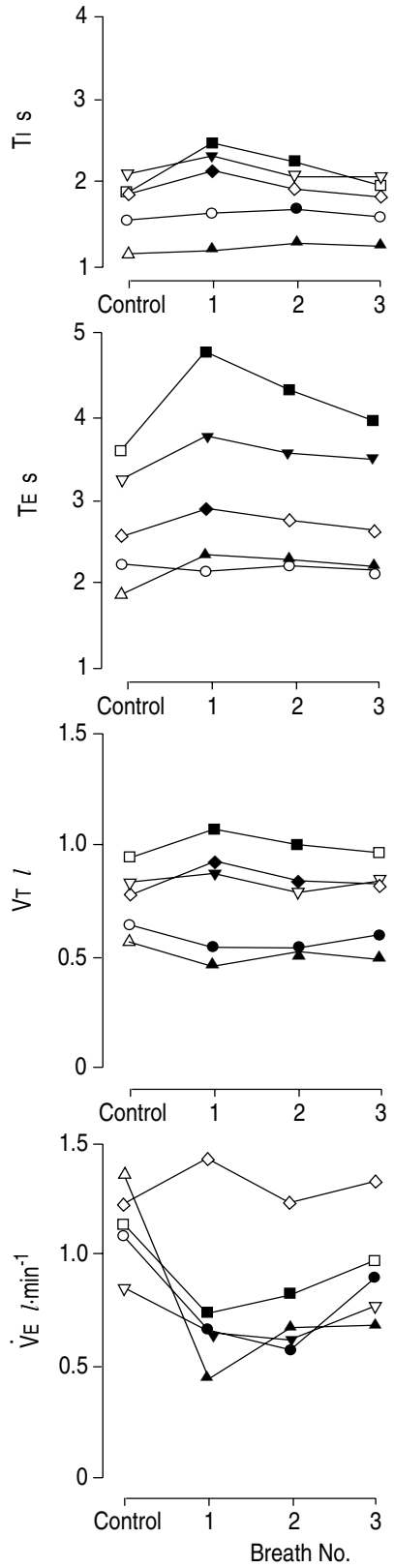

Fig. 4. – TI, TE, VT and V̇E in the first three breaths following an isocapnic deep inspiration (not shown in the figure) compared with the control. Ensemble-averaged results of 20 tests for individual subjects. Data points significantly different (Student's t-test) from the control are filled. TI: inspiratory time; TE: expiratory time; VT: tidal volume; V̇E: minute ventilation; —○—: No. 1; —□—: No. 2; —▽—: No. 3; —◇—: No. 4; —△—: No. 5.

breath, P_{ETCO_2} was kept close to the control (ΔP_{ETCO_2} mean±SD 0.07±0.34 mmHg (0.009±0.05 kPa); p=0.6, paired t-test).

The isocapnic deep inspiration was followed by a drop in ventilation in the next breath, which was statistically significant ($\Delta\dot{V}_E$ mean±SD 1.4±1.0; p=0.04, paired t-test) in the second post-test breath in overall grouped data (fig. 3). This inhibition of ventilation was associated with a small, but statistically significant, overshoot in P_{ETCO_2}. Results for individual subjects are shown in figure 4. Except in one subject, a significant fall in ventilation can be seen in the first and second post-test breaths. Both TI and TE were prolonged, lengthening of TE being more consistent between the subjects. The effect on VT was variable.

Figure 5a and b show breath-by-breath plots of mean ventilation (n=5) against the breath number following ICHYPERCAP and TVHYPERCAP stimuli, respectively, expressed

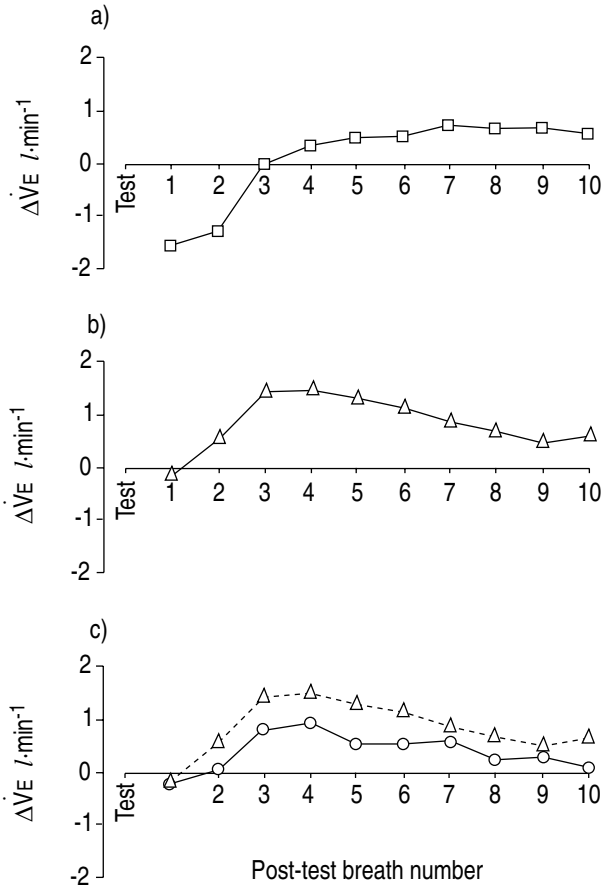

Fig. 5. – The ventilatory response to: a) ICHYPERCAP (❑); and b) TVHYPERCAP (Δ) stimuli. c) The "isocapnic deep breath" element has been subtracted from ICHYPERCAP. Averaged results for five subjects expressed as the difference from control and plotted against the breath number. When "isocapnic deep breath" effect is removed from ICHYPERCAP response, the curve (○) can be seen to run parallel to the TVHYPERCAP (superimposed as a broken line in (c)). V̇E: minute ventilation; ICHYPERCAP: voluntary deep inhalations from functional residual capacity to total lung capacity of 6% CO_2 in air; TVHYPERCAP: two successive spontaneous tidal inhalations of 6% CO_2 in air; ICISOCAP: voluntary deep inhalations of 3–5% CO_2 in air, adjusted to maintain isocapnia during the deep breath.

as the difference (n=5) from control. Immediately after the ICHYPERCAP stimulus, ventilation falls, and then reaches a peak about 7th and 8th post-test breaths. Following the TVHYPERCAP stimulus, there is no initial fall in ventilation, the peak is higher and occurs sooner, in the third post-test breath. When the "deep breath effect" was removed from the ventilatory response curve to ICHYPERCAP stimulus by subtracting the response to an isocapnic deep breath (ICISOCAP, not shown here), the residual can be seen to shift towards the ventilatory plot for TVHYPERCAP stimulus (fig. 5c).

Discussion

The question we asked at the beginning of this experiment was whether a deep voluntary inspiration would have an effect on the subsequent breathing pattern, and if so, whether this would affect the single-breath ventilatory sensitivity to CO_2 measured using inspiratory capacity breaths. The deep voluntary inspiration was consistently followed by a significant fall in ventilation in the next breath, even though hypocapnia was successfully avoided by adjusting the F_{ICO_2} of the inspirate. This cannot be a chemoreflex, since the time-delay is too short.

Lung inflation leading to vagally-mediated apnoea is well-described in several animal species [7]. In conscious awake man, however, the results have been conflicting. CHRISTIANSEN and HALDANE [8] demonstrated apnoea in a few subjects but this finding was not confirmed in subsequent studies [7, 9]. A weak inflation reflex has been shown to be present in anaesthetized [7, 9, 10] man. One of the problems of testing the inflation reflex in conscious man is reflex glottic closure. HAMILTON *et al*. [11] avoided this by inflating lungs of laryngectomized subjects, but could not demonstrate apnoea in the awake state.

Our subjects clearly showed a non-chemically induced inhibition of ventilation, immediately following the voluntary deep inspiration, which was 3–4 times above the tidal range. This suggests that the inflation reflex operates even in awake subjects, although the possibility of effect on the behavioural control of breathing cannot be excluded. It is interesting to note, however, that in animals and in anaesthetized man, the main effect of lung inflation was a lengthening of the respiratory cycle, and in our subjects, the inhibition of ventilation was principally due to consistent and significant prolongation of T_I and T_E.

Change in lung volume could alter the measured output variables, such as $\dot{V}_E$, for a given level of respiratory drive for purely mechanical reasons. For example, deep inspirations are known to increase lung compliance in dogs [12], and lower the airway resistance in asthmatics [13], and in normal subjects with induced bronchoconstriction [14]. Any of these factors would increase the measured ventilation, a change in the opposite direction to that observed in our study. We cannot say whether any "after-discharge" phenomenon [15] is initiated by a single deep inspiration in man, but again this would cause stimulation of ventilation in the following breaths, not inhibition. Our evidence is, therefore, against "after-discharge" in these experiments, or if present it must be outweighed by opposing neural mechanisms. Finally, during the deep inspiration, P_{ETO_2} rose from 107 to 112 mmHg (14.3 to 14.9 kPa), but an increase of this magnitude would not account for a fall in ventilation.

The results from the isocapnic deep breaths, which indicate transient voluntary inhibition, are further supported by the difference between ventilatory responses to a deep breath of 6% CO_2 compared to two tidal volume inhalations, which presented an approximately equivalent hypercapnic stimulus. The former shows an immediate inhibition of ventilation, but this is removed and the whole response to ICHYPERCAP shifts closer to that of the TVHYPERCAP response when the deep breath effect was removed from the former by subtracting the ICISOCAP response. At F_{IO_2} of 8%, we previously [4] observed no difference in the CO_2 sensitivity measured as the ratio of peaks or integrals between IC and TV methods. It seems that the "deep breath induced ventilatory inhibition" is comparatively more important at lower levels of F_{ICO_2}, probably because this is masked by chemical ventilatory stimulation at higher levels of F_{ICO_2}. Whatever the reason for the fall in ventilation, it has a bearing on the single-breath ventilatory response CO_2 measured using inspiratory capacity breaths.

The mechanism we describe will improve the stability of respiratory control of P_{CO_2} in conscious man. The disturbance caused by a sudden deep inspiration or sigh is eventually corrected by hypocapnic inhibition of chemoreceptor drive, but additionally, and more rapidly, by the inhibitory inflation reflex, which we take to be the same as that demonstrated with different techniques by HERING and Breuer [5].

Finally, it is often thought that the vagus plays no part in control of respiratory pattern at low levels of respiratory drive. Our findings are not entirely discordant with that view. We find that the vagus may be stimulated by a single large breath of high voluntary drive, but may then continue to affect ventilation in subsequent breaths at low drive levels.

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