J. Physiol. (1977), **266**, *pp.* 411–421
With 2 text-figures
Printed in Great Britain

VERY SMALL, VERY SHORT-LATENCY CHANGES IN HUMAN BREATHING INDUCED BY STEP CHANGES OF ALVEOLAR GAS COMPOSITION

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(*Received 17 August 1976*)

SUMMARY

1. Three healthy young males were maintained for sessions of about 1 hr in a state of mild asphyxia ($P_{A,O_2} \sim 55$, $P_{A,CO_2} \sim 45$ torr), i.e. with moderately strong drives from both arterial and intracranial chemoreceptors. Tidal volume (V_T), breath duration (T_T) and duration of inspiration (T_I) were recorded, and ventilation (V_E) and duration of expiration (T_E) were derived breath by breath.

2. The arterial chemoreceptor component of the drive was briefly and abruptly reduced, perhaps silenced, by three separate procedures: the inspiratory pathway was connected for two breaths to a second gas supply line containing, *B*, hypoxia with P_{I,CO_2} zero (removal of hypercapnia with maintained hypoxia); *C*, pure oxygen (removal of asphyxia); and *D*, oxygen with 40 torr added P_{CO_2} (removal of hypoxia with maintained hypercapnia). In controls, *A*, the second inspiratory line contained the maintenance mixture so that the switch involved no change of inspiratory gas composition. Each type of test was repeated twenty-four times on each subject.

3. Responses attributable to silencing of arterial chemoreceptors (i.e. with $1\frac{1}{2}$–3 breath latencies, about equal to the lung-to-ear circulation time) are reported elsewhere.

4. Very small responses, occurring only half a respiratory cycle after first inhalation of the test mixture, were detected by pooling all responses

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of each kind from all subjects. When hypoxia was withdrawn, with (*C*) or without (*D*) simultaneous withdrawal of hypercapnia, V_T and $\dot{V}_E$ were reduced by 3 and 2% respectively, probably because gas mixtures containing high oxygen concentrations are appreciably more viscous than hypoxic mixtures and so require more effort to breathe in and out. When hypercapnia was withdrawn with (*C*) or without (*B*) simultaneous withdrawal of hypoxia, T_E was significantly lengthened (mean, $+65 \pm 18$ msec).

5. The change of T_E was discussed in relation to known effects of CO_2 on airway receptors in the dog.

INTRODUCTION

While great progress has been made in recent years in our understanding of the control of the duration of inspiration (T_I), the control of the duration of expiration (T_E) remains something of an enigma. In the anaesthetized cat it is regarded by Euler and his colleagues (e.g. Bradley, Euler, Marttila & Roos, 1975) as being secondary to inspiratory events and to lung volume (Knox, 1973). In man and unanaesthetized cats, however, the changes in mean total breath duration (T_T) that are associated with variations of ventilation over the normal working range are due almost entirely to change of mean T_E, T_I remaining effectively constant (Cunningham & Gardner, 1972; W. N. Gardner in preparation; Kay, Strange Petersen & Vejby-Christensen, 1975; Gautier, Remmers & Bartlett, 1973).

The value of mean T_E is related to the level of respiratory drive, itself a function of alveolar P_{CO_2} in many experimental conditions. In the anaesthetized dog on cardiopulmonary bypass the P_{CO_2} in the airways has a profound influence on T_E (Bartoli, Cross, Guz, Jain, Noble & Trenchard, 1974) which may be exerted by way of an inhibitory action of airway CO_2 on pulmonary stretch receptors (Mustafa & Purves, 1972), particularly those with a low volume threshold (Bradley, Noble & Trenchard, 1976). In the work of Bartoli *et al.* (1974) the latency of the response of T_E to change of airway P_{CO_2} was less than one respiratory cycle; the effect was most marked at very low P_{CO_2}, the upper limit being in the region of the normal alveolar P_{CO_2}. Respiratory frequency in the cat may also be influenced by CO_2-sensitive receptors in the larynx (Boushey & Richardson, 1973) but the direction of change is opposite to that of the response associated with the effect of CO_2 on the airway receptors mentioned above.

In a recent study on man (D. B. Drysdale, J. I. Jensen and D. J. C. Cunningham, in preparation) we have observed comparatively large reflex respiratory responses to step changes of alveolar P_{CO_2} and P_{O_2}, applied

separately and together; these changes occurred with a latency about equal to the lung-to-ear circulation time (4–6 sec under our conditions of driven respiration), which is consistent with their being mediated by the arterial chemoreceptors. Two of the types of test applied involved very sudden changes of upper airway P_{CO_2} from about 40 ⇋ 45 to 0 ⇋ 35 torr (the figures indicate the swings of upper airway P_{CO_2} between inspiration and expiration). The responses occurring at 4–6 sec were unequivocal despite the large breath-to-breath scatter: further analysis of the same data has revealed a very early change that is statistically significant, though small in comparison to the responses attributed to the arterial chemoreceptors. It is with this very early effect that this paper is concerned.

METHODS

The arrangement of the apparatus has been described elsewhere (Cunningham, Holson & Pearson, 1973; D. B. Drysdale, J. I. Jensen and D. J. C. Cunningham, in preparation).

The present paper is concerned with changes in times and volumes of a magnitude approaching the limit of resolution of the equipment, and the precision of measurement requires some description.

Expiratory volume was measured with a wedge spirometer (Oxford Instruments, Oxford), the calibration of which was independent of any changes in the viscosity or density of the respiratory gases. The spirometer was emptied by suction during inspiration through a solenoid-operated stopcock the opening of which was triggered by the reversal of mouth pressure at the transition from inspiration to expiration and the closing by the breaking of a contact when the emptying spirometer reached a pre-set volume. The rate of displacement of the spirometer was signalled as a linear inductance and the integral of the signal was recorded as a volume (usually 1 l. ≡ 25 mm) on moving paper (Devices hot-stylus recorder). An adjustable reciprocating pump, operating with 'tidal volumes' and cycle durations of the same order as those of the experimental subjects was used for calibration. Using a PCD trace reader (P.C.D. Ltd, Farnborough, Hants) the volumes were read to 0·5 mm (≡ 20 ml.) of record and this degree of stability was obtained between successive breaths of forty-cycle-runs with the calibrating pump.

Cycle duration, T_I and T_E were detected by the method of Howson & Gardner (W. N. Gardner, submitted). Since the subjects were in hyperpnoea throughout, the mouth-pressure swings that signalled the transitions between inspiration and expiration were sharp and so were detected with negligible delay. With the trace reader, times were read to 50 msec, which is about the magnitude of the changes to be reported.

Experimental procedure. This is described in detail by D. B. Drysdale, J. I. Jensen and D. J. C. Cunningham (in preparation). In brief, three healthy male volunteers participated in and completed the study. They were all students of physiology and had been informed of the general nature of, but not the details and aims of the experiments. Seated comfortably and reading, they were maintained mildly hypoxic and moderately hypercapnic (P_{I,O_2}, 60, leading to P_{A,O_2} ~ 55 torr, and P_{I,CO_2}, 40, leading to P_{A,CO_2} ~ 45 torr) for sessions of about an hour. After about 15 min during which no tests were performed, sudden changes of inspiratory gas were applied at the inspiratory valve (some 15–20 ml. upstream from the mouth);

the maintenance inspiratory gas mixture was restored after two inspirations of the test gas. Changes of inspiratory gas were completed during expiration while the inspiratory valve was closed. The tests were performed at intervals of about 3 min. They were of four kinds: *A*, control, in which the maintenance mixture was replaced by one of identical composition from the second supply channel; *B*, removal of hypercapnia at constant low P_{O_2} by supplying a CO_2-free mixture with unchanged P_{O_2}; *C*, removal of both stimuli by applying pure O_2; *D*, removing only hypoxia by supplying a mixture with unchanged P_{CO_2} in otherwise pure O_2. The tests were applied in random order. There was no special selection of the breaths in which the test mixtures were supplied and as a result a few tests were marred by the occurrence of large and obviously extraneous irregularities of breathing (e.g. sighs, fidgeting). Such results were discarded and sessions and testing were continued until the total number of satisfactory tests of each kind was twenty-four for each subject. No other 'editing' of results took place. As in other series, the subjects after repeated interrogation assured us that they could not detect changes in the inspiratory CO_2 in the range of concentrations we used. Thus, even if they saw, heard or felt the movement of the inspiratory gas switching device, there is really no possibility that they could detect which kind of test (*A*, *B*, *C* or *D*) was in progress in time to 'assist' us voluntarily according to their own preconceptions of what we were studying.

Processing of data. The trace reading and processing are described in detail elsewhere (D. B. Drysdale, J. I. Jensen and D. J. C. Cunningham, in preparation). End-tidal P_{CO_2} and P_{O_2}, expiratory tidal volume V_T and inspiratory and total cycle times T_I and T_T were measured for each breath for eight cycles before and eight cycles after the beginning of the test; expiratory time T_E and single-breath expiratory ventilation $\dot{V}$ ($= V_T/T_T$) were derived. The breath during which the test gas was first inspired is called breath (0) and the other breaths are numbered forward and backward from this one. The results that follow are in terms of the difference between the mean, for each run, of breaths (-8) to (-1) (the pre-switch mean) and the value at breath (0).

The differences at breath (0) were averaged, test-type by test-type, variable by variable and subject by subject, and the S.E. of mean obtained. The means and S.E. of mean were then pooled for all subjects. The significance of the differences between the grand means appearing in Table 2 was assessed using the formula for the difference between the means for large samples (Bailey, 1959); seventy-two individual values contributed to each mean.

RESULTS

A test of type *C* is illustrated in Fig. 1. P_{O_2} and P_{CO_2} at the mouth changed rapidly from a mild asphyxial combination to zero CO_2 and an off-scale high O_2; by the end of breath (0) the end-tidal composition had changed from 45 to 35 and from 55 to about 300 torr of CO_2 and O_2 respectively. In type *A* tests no such changes occurred in the pattern of gas composition; type *B* P_{CO_2} changes were as illustrated but P_{O_2} continued steady throughout, and vice versa for the type *D* tests. The time courses of the changes of airway P_{CO_2} and P_{O_2} would depend upon the position in the airways under consideration, those in the upper airway being like those shown in Fig. 1, whereas those in the terminal airways would be more like those seen in end-tidal gas only. In Fig. 1 withdrawal

of the stimuli was followed after a latent period of some 4–6 sec by an obvious reduction of V_T; the slight lengthening of T_E was not sustained in this example.

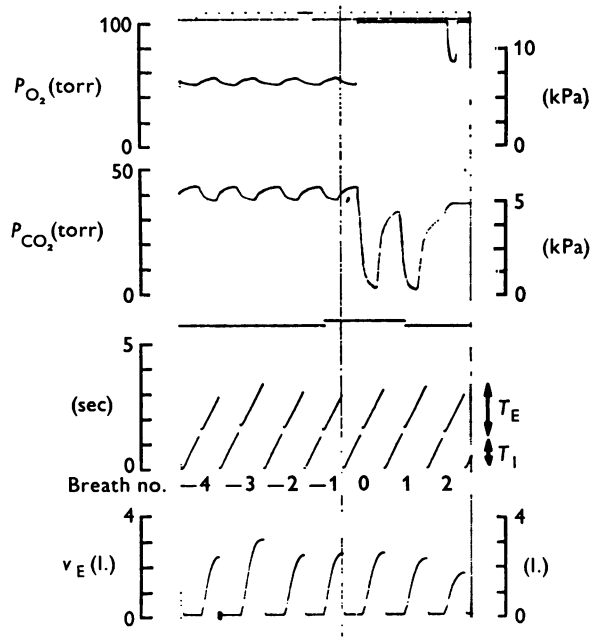

Fig. 1. Specimen experimental record. From above downwards: time marker, sec; P_{O_2} and P_{CO_2} at the mouth; event marker; breath duration (sec) split into T_I below the break and T_E above it; expiratory volume v_E, the maxima being V_T. The vertical line is the beginning of the inspiration in which the test gas was first inspired. The P_{O_2} and P_{CO_2} traces lag behind the others by a transport delay of about 1·5 sec.

The test was of type *C* (removal of both hypoxia and hypercapnia). Note the variation from breath to breath and the substantial change, here seen first at breath (2). The small change of T_E seen at breath (0) in this example is about four times as large as the significant change in mean T_E reported in this paper.

The changes in volumes and times following such tests show much scatter but the major patterns which emerge when many individual tests are averaged are well established (e.g. Bouverot, Flandrois, Puccinelli & Dejours, 1965; Miller, Cunningham, Lloyd & Young, 1974; D. B. Drysdale, J. I. Jensen and D. J. C. Cunningham, in preparation). The changes in T_E averaged for all subjects for test types *B* and *C* combined are shown in Fig. 2. The earliest substantial change was in breath (1) and the change was larger in breath (2). The small change that concerns us now occurred

at breath (0), i.e. during the expiratory half of the cycle in which the test gas was first inspired.

The mean changes ± S.E. of mean at breath (0) for each variable and pooled for all subjects are shown for each kind of test in Table 1. None of the changes in T_I approached significance and the changes in T_T mirrored those seen in T_E.

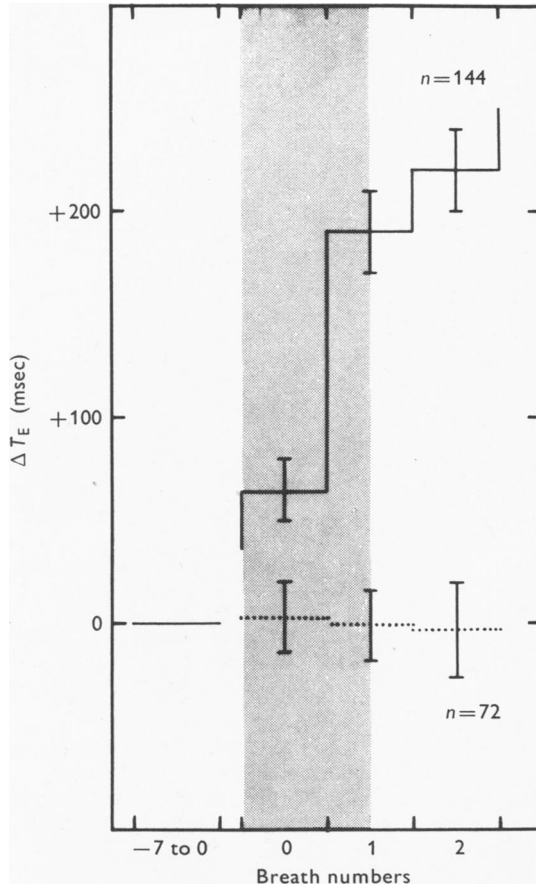

Fig. 2. Mean changes in the duration of expiration (T_E), ± 1 S.E. of mean, associated with removal of hypercapnia (continuous lines) compared with control runs (dotted lines). Results of test-types *B* and *C* were combined to give the continuous line; controls were of type *A* only. The shaded area shows the period over which CO_2 was absent from the inspiratory gas. Mean pre-test T_E in tests and controls were 1422 ± 8 and 1410 ± 13 msec respectively ($n = 8 \times 144$ and 8×72).

The change at breath (0) was about one-third of those at breaths (1) and (2), which are attributed to arterial chemoreceptor activity.

TABLE 1. Mean changes ± S.E. of mean at breath (0); twenty-four tests of each kind on each of three subjects

	ΔV_{T_0} (ml.)	ΔT_{I_0} (msec)	ΔT_{E_0} (msec)	$\Delta \dot{V}_{E_0}$ (ml.min^{-1})
Removal of				
A, neither stimulus	−8	−1	+2	−220
	±21	±13	±17	±450
B, hypercapnia	+37	−1	+76	−470
	±28	±14	±20	±540
C, hypercapnia, hypoxia	−27	−7	+55	−1380
	±21	±10	±15	±480
D, hypoxia	−49	+12	+1	−1470
	±22	±12	±18	±490

where ΔV_{T_0} is the change in tidal volume; ΔT_{I_0}, change in duration of inspiration; ΔT_{E_0} change in duration of expiration; and $\Delta \dot{V}_{E_0}$, change in single-breath expiratory ventilation.

TABLE 2. Differences between mean changes at breath (0). *n* is the number of observations

	n	ΔV_{T_0} (ml.)	ΔT_{E_0} (msec)	$\Delta \dot{V}_{E_0}$ (ml. min^{-1})
Comparison				
B − *A*, − hypercapnia	72	−45	+75	−250
		±35	±26	±700
		n.s.	$P < 0.01$	n.s.
C − *A*, − hypercapnia and hypoxia	72	−19	+54	−1160
		±29	±23	±660
		n.s.	$P < 0.02$	($P < 0.10$)
D − *A*, − hypoxia	72	−41	−1	−1260
		±30	±24	±660
		n.s.	n.s.	($P < 0.10$)
C − *B*, − hypoxia in hypercapnia	72	−64	−21	−910
		±34	±25	±720
		($P < 0.10$)	n.s.	n.s.
(*B* + *C*) − (*A* + *D*), − hypercapnia	144	+33	+65	−80
		±24	±18	±490
		n.s.	$P < 0.001$	n.s.
(*C* + *D*) − (*A* + *B*), − hypoxia	144	−52	−11	−1080
		±24	±18	±490
		$P < 0.05$	n.s.	$P < 0.05$

Abbreviations as in Table 1.

Table 2 shows comparisons of test means with the appropriate controls for V_T, T_E and $\dot{V}_E$. The first four lines of the Table are concerned with comparisons of one test type with another. In the fifth line all the tests in which hypercapnia was removed are compared with all those in which it was left unchanged (*B* and *C* versus *A* and *D*); in the sixth line the corresponding trial for the effectiveness of removal of hypoxia is shown (*C* and *D* versus *A* and *B*).

In general, all the groups in which CO_2 was removed showed significant lengthening of T_E in breath (0); the first and second lines show that the effect on T_E was present whether hypoxia was removed simultaneously or not. The mean change of T_E was in the range 50–80 msec in a total of 1500–2500 msec, i.e. it was close to the limit of resolution of the method of measurement and much smaller than the changes occurring in subsequent breaths. Removal of CO_2 was not itself associated with any significant change of volume or ventilation at breath (0).

Table 2 also shows one significant change in ventilation and one in volume at breath (0), both associated with removal of hypoxia with or without simultaneous removal of hypercapnia. A few more differences between means approached significance ($P < 0{\cdot}1$), all of them in the V_T and $\dot{V}_E$ columns.

Two other subjects were studied completely with respect to test-types *A*, *B* and *C* but satisfactory tests of type *D* were not sufficient. Combining these extra data, where appropriate, with those shown in the tables makes little difference to the statistical results.

DISCUSSION

In general the data are consistent with the view that removal of hypercapnia is associated with immediate slight lengthening of T_E with no change of volume or ventilation, while our method of removal of hypoxia, though without effect on T_E, affects expiratory V_T and $\dot{V}_E$.

The changes described in this paper are at or even beyond the limits of resolution of the recording equipment. If the 'quanta' of measurement are taken to be about 50 msec and 20 ml. respectively, an increase of a so-called mean time or volume implies that with repeated measurement of quantities which vary greatly from one measurement to the next, the test procedure, when effective, was associated more often than not with the inclusion of an extra 'quantum' of time or volume in an individual measurement. The quantitative significance of the word 'mean' is not altogether clear in the present context but that changes actually occurred seems to be beyond reasonable doubt. We may conclude first, that the effects are real and, secondly, that they are so small as to be outside the

limits usually regarded as worth measuring in human respiratory physiology. Nevertheless, improved precision of measurement might not yield better data; breath-to-breath scatter remains the main difficulty to be overcome in this branch of physiology and for most purposes the precision of the present apparatus is sufficient.

Removal of hypoxia

The expiration with which we are concerned started about 1 sec after the first change of P_{O_2} in the lungs: the gas drawn in contained nearly 100 % O_2 and the gas driven out through the airways contained 40–50 % O_2; the inspirate and expirate would have viscosities respectively some 9–11 % and 4–5 % greater than those of the preceding cycle. The simplest way of accounting for the small decreases in V_T and $\dot{V}_E$ is that there was no active physiological response whatever, with no change in time, inspiratory and expiratory effort and airway calibre. In such circumstances the small increase of viscosity would result in proportional changes in airway resistance and hence in tidal volume. With all the reservations expressed above about the meaning of 'mean' in mind, it is nevertheless reasonable to regard the values shown in Table 2 as being of a magnitude consistent with the explanation put forward above. In other words, given the change of viscosity, constancy of V_T and $\dot{V}_E$ would have implied an active physiological response.

Removal of hypercapnia

During breath (0), though P_{A,CO_2} fell by some 10 torr, mixed venous P_{CO_2} presumably remained constant; the diffusion gradient for CO_2 from blood to gas, and with it the flux of CO_2, presumably increased about threefold. No short-latency *increase* of breathing was associated with the change and it may therefore be argued that any short-latency receptors excitatory to ventilation and responsive to the 'rate of CO_2 flow to the lungs' (Wasserman, Whipp & Castagna, 1974; Wasserman, Whipp, Casaburi, Huntsman, Castagna & Lugliani, 1975) are not stimulated by increase of CO_2 flux across the alveolar capillary walls.

Receptors in the airways. The timing and direction of the changes in T_E are consistent with their being initiated reflexly by CO_2-sensitive, hypoxia-insensitive receptors in the airways such as are referred to at the beginning of this paper. According to the descriptions of Bartoli *et al.* (1974) and of Bradley *et al.* (1976), the sharp changes of airway P_{CO_2} we have imposed are of a magnitude and cover a range such that they would, if applied to a dog on cardiopulmonary bypass, induce a step change from an environment causing a marked inhibition of the receptors by CO_2 (with upper airway P_{CO_2} swinging between 40 and 45 torr) to a nearly

normal environment (upper airway P_{CO_2} 0 $\rightleftharpoons$ 35 torr), at least for the two breaths of the test. We are unable to say what happens to this effect after breath (0) in our subjects because changes attributable to arterial chemoreceptors dominate the response, usually from the second half of breath (1) onwards.

The importance of the present finding is twofold. In the first place, it probably provides the first evidence that the effects of CO_2 described by Bartoli *et al.* (1974) in the dog could be applicable to man. Secondly, in that conditions were probably ideal for its demonstration, it indicates that under our conditions the proposed mechanism is too weak to account for more than a small fraction of the normal control of mean T_E in man. In this respect, mechanisms found in the experimental anaesthetized animal may have a quite different quantitative importance in man. It seems, therefore, that the large effects of increasing respiratory drive in shortening T_E and increasing respiratory frequency are mediated mainly by mechanisms that have not yet been demonstrated.

We are grateful to the Medical Research Council for the award of a project grant which enabled us to carry out this work. We thank the subjects for their cheerful co-operation. Technical services were provided by Messrs E. Aldsworth, D. V. Goff, M. G. Howson, T. J. Meadows and Miss L. M. Castell.

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