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Ventilatory Responses to Continuous Negative Pressure Breathing(CNPB) in Awake Dogs

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= ABSTRACT=

Ventilatory responses to inhaled CO_2 were measured during continuous negative pressure breathing (CNPB) in awake dogs. End expiratory lung volume (EELV) decreased linearly with pressure level during CNPB (correlation coefficient= 0.81, $p<0.005$) during air breathing. When CNPB was applied during 5% CO_2 inhalation, the decrease in EELV was not significantly different ($p<0.5$) from that during air breathing. As a result of a lowered EELV, tidal volume (V_T) significantly decreased by 22% and breathing frequency (f_B) increased by 68% in the steady state during air breathing ($p<0.0001$). These responses were similar during 5% CO_2 inhalation, thus the CO_2 response curve measured during CNPB shifted upward without a change in sensitivity ($p>0.05$). These results indicate additive effects of CNPB and CO_2 inhalation. The degree of hyperventilation during CNPB at eupnea was estimated to be 63% of that during control ventilation and was significantly greater than zero ($p<0.0001$), which suggests an alveolar hyperventilation due to CNPB. These results suggest that the mechanical alterations associated with a decrease in lung volume could play an important role in ventilatory control independently of chemical regulation of breathing. Thus, exercise hyperpnea, which is associated with a lowered functional residual capacity (FRC), may in part be explained by this mechanical stimulation of breathing.

Key Words: Control of breathing in awake dogs, Lung deflation, CO_2 response, Mechanical stimulation of respiration.

INTRODUCTION

Respiration is affected not only by a change in blood chemistry but also by an alteration of respiratory mechanics. A lowered lung volume is reported to stimulate the respiratory center in both experimental animals (Cherniack et al, 1973; Culver & Rahn, 1952; D'Angelo & Agostoni, 1975) and humans (McIlroy et al, 1962), presumably via the volume deflation reflex (Adrian, 1933). However, due to possible receptor adaptation, this stimulation may not persist in the steady state (Cherniack et al, 1973; Hirsch & Bishop, 1981). It is not clear if a decrease in lung volume causes any significant change in alveolar ventilation and/or breathing pattern in an awake subject, since previous results were obtained under anesthesia or without the measurement of arterial CO_2 tension. Moreover, studies on the interaction of chemical and mechanical stimuli on respiration have not been made. The present experiments were performed to examine steady state ventilatory responses to a lowered lung volume in awake dogs and to determine how the responses interact with a chemical stimulus, specifically an increased arterial CO_2 tension

($Paco_2$). Continuous negative pressure was applied at the trachea to induce a reduction in lung volume, and $Paco_2$ was increased by CO_2 inhalation.

MATERIALS AND METHODS

Animal preparation

Four mongrel dogs (23~32 kg) selected for their quiet dispositions were prepared with chronic tracheostomies performed under halothane anesthesia (Benfield et al, 1967). After a complete recovery from surgery, they were trained over a one- to two-month period to stand calmly in a Pavlov sling for 2~6 hours a day. The sling consisted of a head pad, a jacket, and a few metal frames. The animal was placed in the sling so that it could comfortably sit in the jacket by tying the jacket ropes to the frames and with its head put on the pad. Breathing pattern was monitored by an inflatable-cuff tracheal cannula (Portex, 11 mm) inserted through the tracheal opening and a pneumotachograph (Fleisch, no. 1) during daily training. Tidal volume (V_T) and breathing frequency (f_B) were measured to ensure proper training and to evaluate the resting state of each dog. During the last week of training, continuous negative pressure (−5 to −15 cmH_2O) was applied at the trachea periodically for 2 to 5 minutes. This procedure was followed to allow the animal to become accustomed to the negative pressure forcing and to our experimental environment. This training procedure resulted in no discomfort to the dogs when observed throughout the experiments.

Two (dogs no. 3 and 4) out of four dogs were also trained in a transparent whole body plethysmograph (Fig. 1). During these additional training sessions, the trachea was occluded for 2 to 3 breaths at the end of expiration. After a few trials, both dogs learned to make a spontaneous inspiratory effort against the occluded trachea, allowing the measurement of functional residual capacity (FRC) or end expiratory lung volume (EELV) which is described later.

Following the training phase, an arterial catheter made of silastic tubing (32 cm length,

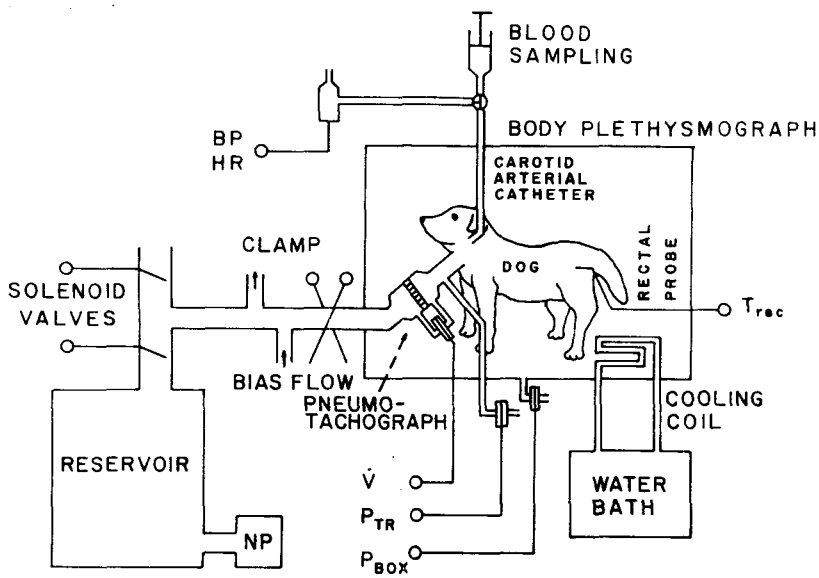

Fig. 1. Experimental set-up.

0.16 cm ID, and 0.32 cm OD) was implanted under halothane anesthesia in the left carotid artery in such a manner that its tip was placed in the descending aorta approximately 2.5 cm from the aortic arch. The distal end of the catheter was extended approximately 7.5 cm from the skin and protected by a leather collar surrounding the neck area. The catheter was inspected and cleaned with sterile saline daily.

Following a complete recovery from catheter surgery, experiments were performed daily. The animal was placed in the Pavlov sling described above and the two (dogs no. 3 and 4) out of four animals in the sling were additionally placed in a whole body plethysmograph. An inflatable-cuff tracheal cannula (Portex, 11 mm) was inserted through the tracheal opening and connected to the breathing measurement and pressure control apparatus. The arterial catheter from the neck area was open and connected to the blood sampling line. During an experiment, the sling and plethysmograph were surrounded by a curtain to prevent the animal from being distracted, and the environment was kept as quiet as possible except for background music. Since no sensible stimulus was applied to the animal, which was completely adapted to, during the training phase, the animal was kept in the most comfortable condition possible during the experiment. It was sometimes observed that the animal was asleep. The detailed description of the experimental set-up and measurement procedure are provided below.

Experimental set-up and measurements

Figure 1 illustrates the experimental set-up employed in the present study. Tracheal pressure (P_{TR}) was measured by a pressure transducer (MP-45, Validyne) through a side tap made between the tracheal cannula and pneumotachograph. A pneumotachograph (Fleisch, no. 1) with another pressure transducer provided a biphasic volume flow signal. An analog integration was performed breath by breath only on inspiratory flow to obtain V_T and f_B. The pneumotachograph was connected to a T-tube with an electrically operated solenoid valves attached to each end. One end of the T-tube was open to the atmosphere and the other led to a reservoir (240 l) in which pressure was controlled at the desired level. Simultaneous operation of these two solenoid valves permitted an instantaneous application of the reservoir pressure to the animal's trachea, which in turn induced a corresponding lung volume change. Two side taps 5 cm apart were positioned between the pneumotachograph and T-tube for a bias flow. Air and Co_2 were mixed to a desired CO_2 concentration by pre-calibrated flow meters (605 and 603, Matheson), humidified, and fed in through one side tap. The other side tap was connected to an adjustable vacuum source to balance the bias flow input. The magnitude of this bias flow was 30 l/min. The arterial catheter implanted in the left carotid artery prior to the experiment was connected to another extension catheter leading to a Statham strain gauge pressure transducer (P23ID, Gould) to continuously monitor arterial blood pressure. Mean arterial blood pressure (BP) was obtained by low pass filtering of the pressure signal and heart rate (HR) was measured by counting the number of systolic peaks. The catheter was also used for sampling arterial blood via a 3-way stopcock. Each sample was 1 ml in volume drawn at a rate of 0.05 ml/sec. Duplicate samples were taken following dead space (6 ml) withdrawal and immediately analyzed for blood gases (ABL2, Radiometer America, Inc.). Rectal temperature was monitored by a thermistor probe (43TA, YSI), and remained within a 1℃ range throughout each experiment. All the signals were recorded on a multichannel strip chart recorder (200, GOULD) for analysis.

An 840 liter box made of plexiglass was custom built during the course of the study and used on two dogs (no. 3 and 4) who received the additional training, as previously described to measure steady state EELV. The animal and sling were placed in the box, but breathing was isolated by extending the tracheal cannula out of the box. Gas pressure in the box (P_{BOX}) was measured by a pressure transducer (MP-45,

Validyne). Box compliance (C_{BOX}) was obtained by injecting a known amount of air (50~100 ml) with the animal in the box during the expiratory pause at rest and by taking a ratio with the corresponding change in P_{BOX}. The temperature in the box was maintained at 20°C by introducing cold water, the temperature of which was servo-controlled by a water bath (1140, VWR), through a cooling coil placed in the box. EELV at a given experimental state was measured as follows: the animal's trachea was manually occluded for 2 to 3 breaths at end expiration followed by spontaneous inspiratory efforts, which caused changes in both P_{BOX} and P_{TR}, these changes were read and EELV was calculated as

$$EELV = -(P_B - P_W)C_{BOX}P_{BOX}/P_{TR} \quad \cdots\cdots(1)$$

where P_B and P_W are the barometric and water vapor pressures, respectively.

Protocol

An experiment involved 2 to 3 measurement sequences, each of which consisted of 7 steady state measurements. At each steady state, the data were collected as follows. When the dog was in the steady state (as judged by constancy of minute ventilation (V_E), BP, and HR) duplicate arterial blood samples were drawn. Sampling took approximately 2 minutes while respiratory variables were recorded. At the conclusion of sampling, the arterial catheter line was reconnected to the pressure transducer and BP and HR were again measured to ensure a steady state. In two dogs, steady state EELV was also measured by body plethysmography as previously described. This measurement procedure was used for all states.

A measurement sequence started with a control (or resting) state. Then, a continuous negative pressure (NP) was instantaneously applied to the trachea at end expiration and maintained for 2 to 5 minutes until the breathing pattern was stabilized. This was followed by the steady state measurement procedure described above. The degree of NP was $-10\ cmH_2O$ in 3 dogs and $-5\ cmH_2O$ in one dog (no. 1) who showed a more sensitive response to NP (explained later). P_{TR} was raised back to atmospheric (or control) pressure, CP, and another control measurement was taken. Biphasic integration was performed on the flow for a few breaths before and after the transition of P_{TR} to obtain the transient lung volume change due to NP. 5% CO_2 in air was inhaled for 15 to 30 minutes before the CO_2 inhalation data were taken. The same degree of NP as that applied during air breathing was then superimposed for 2 to 5 minutes in order to measure the response when both CO_2 and NP forcings were applied. NP was stopped and another measurement was taken while the animal was still breathing 5% CO_2 in air. Inhaled CO_2 concentration was then lowered to zero and the last control data were taken when the animal returned to resting state. This sequence of 7 steady state measurements was repeated at least once for each experiment.

Data analysis

Given a steady state measurement period, V_T and f_B were read breath by breath and averaged over at least 10 consecutive breaths. V_E was calculated by multiplying these two variables. BP and HR were measured before and after arterial blood sampling. Duplicate blood gas analysis provided arterial CO_2 ($Paco_2$) and O_2 (Pao_2) tensions and pH data. Blood sampled outside the body was exposed to measurement errors due to metabolic chages which occur between sampling and measurement and to temperature differences between the experimental subject and the measuring electrode. The effect of metabolic change was minimized by immediately analyzing the blood samples. However, as the temperature of our electrode was maintained at 37°C, measurement error might exist with a different rectal temperature (T_{rec}). This was corrected by using Kelman and Nunn's (1966) nomograms. This was accomplished by first converting $Paco_2$ to oxygen saturation based on a normal standard dissociation curve (Fig. 7 of Kelman and Nunn, 1966). Then the empirically obtained nomograms for given oxygen saturation and rectal temperature (Fig. 5 of

Kelman and Nunn, 1966) provided the corrected $Paco_2$, Pao_2, and pH values. This correction procedure was performed for all data and enabled the most reliable blood gas analysis results.

Cardiopulmonary data obtained before and after the application of NP were averaged and considered to be the reference to the response values for the NP during either air breathing (or control) or 5% CO_2 inhalation. Thus, a measurement sequence provided data at 4 different states: air-CP (control), air-NP, 5% CO_2-CP, and 5% CO_2-NP, where CP and NP represent control (or atmospheric) and negative P_{TR} conditions, respectively. Since each experiment involved at least two measurement sequences, data at each state obtained in repeated sequences in a given experiment were averaged and were considered to represent that particular state on that day. Therefore, each variable had 4 averaged values corresponding to 4 different states, listed above, as a result of each experimental day. Statistical comparisons between two states were made assuming a null hypothesis of equal means using a paired student's t-test for all variables.

V_E and $Paco_2$ measurements of a typical experiment are shown in Fig. 2. Since we have a pair of averaged data at two different $Paco_2$ levels during either CP or NP, a CO_2 response line was constructed by connecting these two points in the V_E-$Paco_2$ domain at either pressure level. The CO_2 sensitivity during control (S_{CP}) and negative (S_{NP}) pressure was obtained by calculating the slope of both CO_2 response lines. The shift of CO_2 response line due to NP ($(\triangle V_E)_{EUP}$) was defined at the eupneic point by interpolating the CO_2 response line during NP to estimate V_E as shown in Fig. 2. Note that $(\triangle V_E)_{EUP}$ represents the degree of hyperventilation due to NP at eupnea. S_{CP}, S_{NP}, and $(\triangle V_E)_{EUP}$ were estimated in each experiment. S_{CP} was compared to S_{NP} by a paired student's t-test as the other variables and the one sample t-test was used to compare $(\triangle V_E)_{EUP}$ to zero.

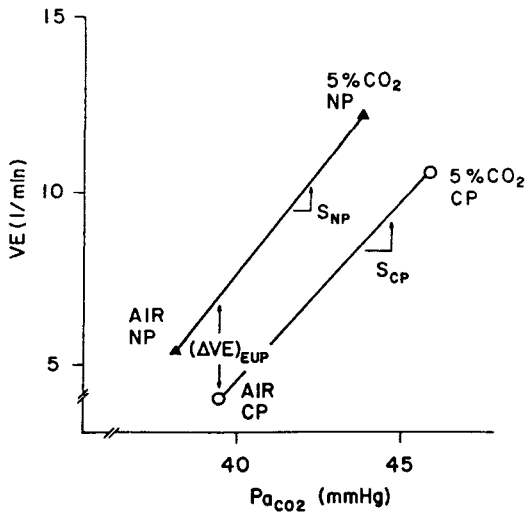

Fig. 2. Typical ventilatory response to CO_2 inhalation and CNPB. Open circles and closed triangles represent CP and NP conditions, respectively. S_{CP} and S_{NP} are slopes of the CO_2 response lines. $(\triangle V_E)_{EUP}$ is the difference in V_E between the two response lines at eupnea.

RESULTS

Pressure-Volume (P-V) relationship

In dogs no. 3 and 4, the transient change in EELV due to NP was compared to the steady state change in FRC measured by body plethysmography during air breathing. The resultant points fell close to the identity line as shown in Fig. 3, and the paired student's t-test resulted in no statistical difference in the mean ($p > 0.2$). Therefore, both transient and steady state changes in EELV were pooled in all dogs.

Fig. 4 shows the decrease in EELV due to NP during air breathing in all 4 dogs. Note that $-5\ cmH_2O$ (NP) was used in dog no. 1, whereas $-10\ cmH_2O$ was used in the others. Dogs no. 1~3 appeared to share a common linear P-V relationship as shown by the high correlation coefficinet of 0.81 ($p<0.0005$). Dog no. 4 showed a much larger decrease in EELV presumably because of its larger body size (32 kg) compared to the others (23~25 kg). Therefore,

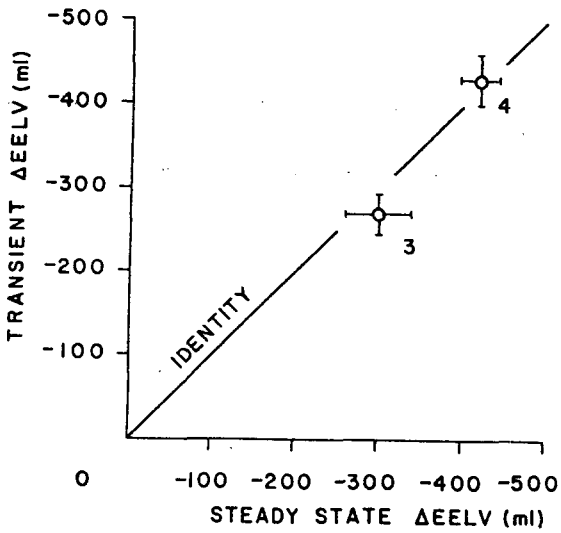

Fig. 3. Changes in EELV during transient and steady state CNPB in dogs no. 3 and 4. Open circles and bars are means and standard errors (S.E.), respectively.

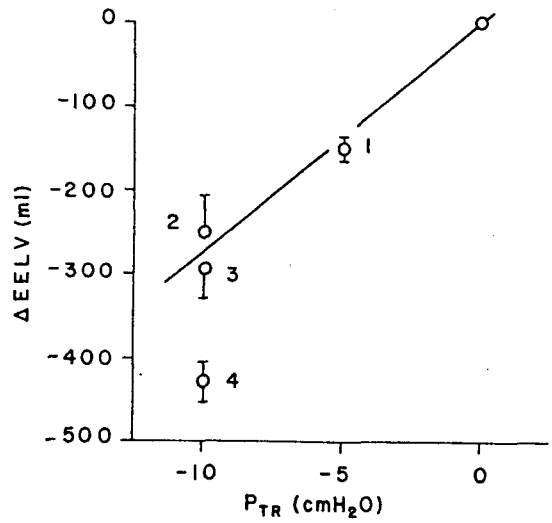

Fig. 4. Changes in EELV from control due to NP during air breathing shown with dog numbers. Solid line was fitted to the data from dogs 1~3 using linear regression.

the P-V data from dogs no. 1~3 were pooled. The mean changes in EELV from the three dogs were −150 and −270 ml at −5 and −10 cmH_2O, respectively.

During 5% CO_2 inhalation, the decrease in EELV due to NP in dogs no. 1~3 was similar to that during air breathing, and was not statistically significant ($p>0.5$). However, dog no. 4 showed a much smaller decrease in EELV in response to NP during CO_2 inhalation compared to that during air breathing (only 25% of the change during air breathing).

Data pooling

Mean S_{CP} showed similar values in dogs no. 1~3 (1.13~1.34 l/min · mmHg), but an almost doubled value (2.36 l/min · mmHg) in dog no. 4, who also showed a different P-V relation, especially during CO_2 inhalation. The three dogs, no. 1~3, also responded with a qualitatively and quantitatively similar ventilatory pattern to both CO_2 and NP, despite the fact that a lower NP level was used in dog no. 1 compared to the other two dogs. An NP level of −5 cmH_2O was used in dog no. 1 to obtain comparable ventilatory responses to dogs no. 2 and 3, since it showed a higher response sensitivity to NP. This is shown in Fig. 5 where the increase in V_E due to NP obtained from these three dogs during air breathing showed comparable values with a relatively narrow 95% confidence interval (0.87 l/min, 20% of control V_E). However, dog no. 4 showed a much larger increase in V_E associated with a larger decrease in EELV due to NP. The mean increase in V_E was higher in dog no. 4 by 1.57 l/min as compared to the upper limit of the 95% confidence intervals calculated from the data in the other three dogs (shown in Fig. 5). A similar pattern was also observed in V_T and f_B. Moreover, the decrease in EELV in response to NP in dog no. 4 was not maintained during CO_2 inhalation. Therefore, the data obtained from dogs no. 1~3 were pooled for CO_2 response analysis. Although the data from dog no. 4 were excluded from statistical analysis, they will be presented when necessary and were qualitatively consistent with those obtained in the other

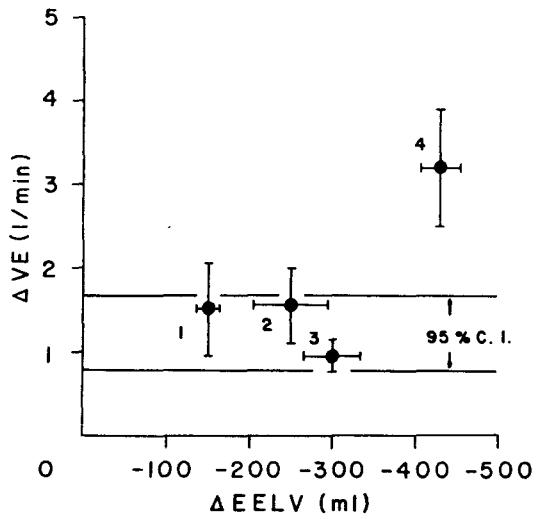

Fig. 5. V_E responses to CNPB during air breathing plotted against the corresponding changes in EELV in each dog. The two horizontal lines represent the 95% confidence interval of V_E calculated from the data in dogs no. 1~3. Note a large deviation of the data from dog no. 4 from the others.

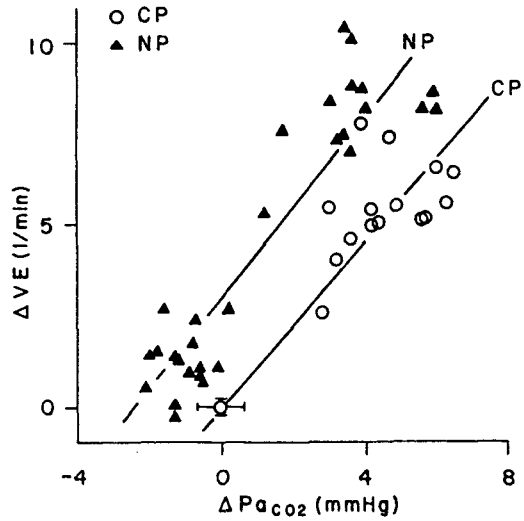

Fig. 6. Ventilatory response to inhaled CO_2 during CP and NP in 15 successful experiments obtained from dogs no. 1~3. Open circles represent data during CP and closed triangles, data during NP. The control point is shown with ±S.E. of the individual measurements. The lines were fitted by linear regression.

three dogs except for one state in which both NP and CO_2 were applied. The reason for this will be discussed later.

CO_2 responses

The deviations of V_E and $Paco_2$ from control were measured to minimize day to day and interindividual variances. The data in 15 successful experiments in dogs no. 1~3 are plotted in Fig. 6. Mean V_E and $Paco_2$ at rest (or control) were 4.45 l/min and 40.0 mmHg, respectively. During air breathing, V_E increased little, but significantly by 1.20 l/min (27% control) and $Paco_2$ decreased by 1 mmHg ($p < 0.0001$) due to NP, indicating that NP caused a small degree of hyperventilation. $Paco_2$ and pH also increased slightly. Similar alveolar hyperventilation was observed due to NP while breathing 5% CO_2, suggesting an additive effect of NP and CO_2 inhalation. This is further supported by comparing the CO_2 sensitivities in S_{NP} was not significantly different from S_{CP} ($p > 0.05$). Therefore, the CO_2 response line during NP was a shifted version of that during CP. The degree of the shift is defined by $(\triangle V_E)_{EUP}$, which also represents the degree of hyperventilation due to NP at eupea. Mean $(\triangle V_E)_{EUP}$ was about +2.78 l/min (63% control increase) and was significantly greater than zero ($p < 0.0001$). Data of dog no. 4 which was excluded from this analysis were qualitatively different as the response due to NP resembled the response described above during air breathing. However, both V_E and $Paco_2$ did not change when NP was applied during CO_2 inhalation, resulting in a lowered S_{NP} compared to S_{CP}.

Breathing pattern

Fig. 7 shows V_T and f_B responses to CO_2 inhalation and CNPB. The increase in V_E during CO_2 inhalation was mainly due to an $V_T : f_B$ increased only by 8% with no statistical signifi-

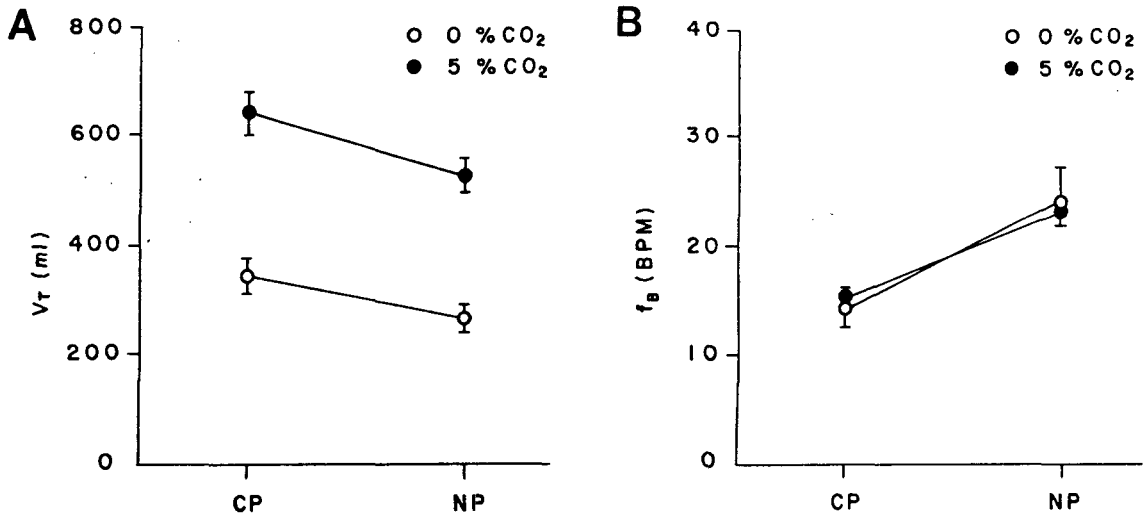

Fig. 7. Responses of V_T and f_B to CNPB and CO_2 inhalation. Open and closed circles represent 0 and 5% CO_2 breathing, respectively.
(A) V_T, (B) f_B

cance ($p>0.3$), whereas V_T significantly increased by 85% ($p<0.0001$). The hyperventilation caused by NP during air breathing was achieved by a net result of a decreased V_T by 22 % and an increased f_B by 68%, and both changes were statistically significant ($p<0.0001$). When NP was applied while breathing 5% CO_2, the responses were similar to those during air breathing both qualitatively and quantitatively except in dog no. 4 above, suggesting that the breathing pattern responses are also additive when these two forcings exist together.

Blood pressure and heart rate

Both BP and HR increased slightly in response to NP during both air and 5% CO_2 breathing. These responses were small, but consistently observed in all experiments. Mean BP and HR at control were 120 mmHg and 55 pulses/min, respectively. During CNPB, they increased by 6 mmHg (5%, $p<0.0001$) and 5 pulses/min (9%, $p<0.05$), respectively.

DISCUSSION

Instrumentation

A pneumotachograph-integrator combination to obtain breath by breath tidal volume was calibrated by a manual input of known volumes using a syringe with steps of 100 ml. The response was linear (correlation coefficinet = 1.0000, $p<0.0001$) and the mean standard deviation at a given stroke volume was only 13 ml, which was negligible compared to the tidal volumes of the dogs. The transient change in EELV due to NP was estimated by integrating both inspiratory and expiratory air flows over a few breaths before and after the transition. The drift involved in this biphasic integration was found to be linear and was subtracted before the change in EELV was read. NP was instantaneously applied at end expiration before the next inspiratory effort began. Thus, the change in EELV could be read on the corrected volume signal at the time of the application of

NP. To test the maintenance of EELV during CNPB, EELV was directly measured in the steady state in two dogs (no. 3 and 4) by body plethysmography. These two dogs were trained in the body box to become accustomed to tracheal occlusion at end expiration. Neither discomfort nor excitement was observed while occluding the trachea for 2 to 3 breaths. The same technique in chronic awake dogs has been employed previously (Bouverot & Fitzgerald, 1969). The steady state change in EELV due to NP measured by a body plethysmographic method showed no statistical difference when it was compared with the change estimated from the integral of flow during the transient period ($p>0.2$), confirming that EELV remained unchanged in the steady state (Fig. 3). Arterial blood was withdrawn at a slow rate (0.05 ml/sec) while collecting ventilatory data to ensure a complete mixing, and also, duplicate samples were taken to enhance reliability. Blood gas tensions and pH are affected by temperature, the time between the sampling and the analysis due to metabolic changes in blood (Kelman & Nunn, 1966; Nunn et al, 1965). These artifacts were minimized by analyzing the blood immediately after sampling and by correcting the values according to rectal temperature. The air volume of the pressure reservoir could act as an elastic load, which might have affected the subject. This possibility was tested in one dog by collecting ventilatory data before and after opening the reservoir to the trachea while maintaining atmospheric pressure in the reservoir. Neither V_T nor f_B changed significantly when the dog breathed while connected to the reservoir ($p>0.1$). Since the reservoir volume was quite large (240 l) and elastic loading decreases f_B (Kosch et al, 1986), in contrast with the present observation that f_B significantly increased during CNPB maintained by the reservoir, the elastic loading effect does not appear to have been a major part of the response. Based on the above considerations, the results obtained in the present study could not have been due to the errors in instrumentation or measurement technique. Our results are also free from the artifacts due to anesthesia or surgical trauma, which could modulate the responses to a significant degree, since experiments were performed on chronic well-trained awake dogs after complete recovery from surgery.

Effects of increased BP and HR

A small but significant increase in both BP and HR was observed during CNPB. Baroreceptor reflexes following an increase in aortic pressure decreased V_E and f_B (Grunstein et al, 1975), whereas both variables actually increased during CNPB in this study. An increase in cardiac output (CO), which was expected from the increase in HR (Asmussen, 1965) could induce a secondary hyperpnea. The CO contribution to V_E was estimated to be approximately 6% based on Greco et al.'s (1978) results, this contribution is substantially smaller than the $(\triangle V_E)_{EUP}$ of 63% calculated from the present CO_2 response lines. Therefore, BP and HR did not seem to be important factors in the ventilatory responses observed in the present study.

Breathing pattern responses

A decrease in V_T and an increase in f_B observed in the present study generally agree with previous investigations in anesthetized animals (Culver & Rahn, 1952; D'Angelo & Agostoni, 1975) and in humans (Hirsch & Bishop, 1981). These responses are mediated by the vagi, since they are abolished by a bilateral cervical vagotomy. Slowly adapting pulmonary stretch receptors (Paintal, 1973) or a separate group of receptor cells called "the deflation receptors" (Koller & Ferrer, 1970) are known to be responsible. Although the present study does not permit an indentification of the exact mechanisms involved, we demonstrated that lung deflation could have a significant influence on breathing in awake dogs comparable to that reported in anesthetized animals. We believe that the present responses to CNPB originated from the same lung volume related vagal reflex for the following reasons. First, the pattern of

the ventilatory responses to NP in our awake dogs resembled those obtained from anesthetized dogs in previous studies, which disappeared following bilateral cervical vagotomy or cold blockade of the vagi. Secondly, a particular animal (dog no. 4) changed neither ventilation nor arterial CO_2 tension due to NP during CO_2 inhalation, only EELV slightly decreased, whereas similar responses to the other dogs were observed during air breathing when EELV decreased as much as expected from the P-V relationship obtained from the other dogs. In other words, no response to CNPB was observed only when EELV did not decrease due to NP, demonstrating an association of the response with lung volume change. Nevertheless, the data from dog no. 4 were consistent with the others during air breathing but were of larger magnitude probably due to its greater body weight (approximately 1.5 times as great as the other dogs).

Although it seems likely that the present changes in breathing pattern were reflexly mediated, breathing under NP is also subject to other mechanical changes. A decrease in EELV elevates the diaphragm, lengthening its muscles and an increased V_T is expected due to an enhanced mechanical efficiency since a motor unit operating at a longer length generates more tension for a given neural drive (Marshall, 1962). On the other hand, breathing against an external NP requires more elastic work to achieve the same V_T, since the P-V relation of the lungs and thorax has a curvilinear shape near the normal FRC level (Kraemer et al, 1983). In other words, additional energy is needed to overcome the external force, which has changed the end expiratory position, thus compensating the advantage expected from the length-tension relationship of the diaphragm mentioned above. These mechanical factors complicate the interpretation of the response. Nevertheless, the increase in f_B under lung deflation was probably due to a reflex mechanism.

Cherniack et al. (1973) reported that the integrated phrenic nerve activity immediately increased in response to negative end expiratory pressure and recovered to the original level in 3 to 8 minutes. They concluded that the phasic rather than the tonic component of lung volume change was much more important. In contrast, we found sustained responses in both V_T and f_B after a 2 minute transient period without an indication of further adaptation in agreement with D'Angelo & Agostoni (1975). It might be argued that a possible discomfort arising from an externally applied NP could have accelerated breathing in our awake dogs. We excluded this possibility, since they were trained under NP prior to the experiments, and no indication of any irritation was noticed. The maintained ventilatory responses in the steady state suggest that the tonic component in lung volume change may also be important in determining the breathing pattern. Since the experiments were performed in an awake condition, the present results should be more physiological than those obtained in artificially ventilated animals under anesthesia and paralysis.

Alveolar hyperventilation

V_T increased by 27% during CNPB as a net result of a decreased V_T and an increaed f_B. Although $Paco_2$ decreased only to a small degree of 1 mmHg, it was statisticaslly significant ($p<0.001$) which suggests alveolar hyperventilation. This hypothesis was also supported by a slight increase in Pao_2 and pH (respiratory alkalosis). While similar alveolar hyperventilation has been previously observed when EELV was lowered by chest compression (Culver et al, 1952; McIlroy et al, 1962), Hirsch & Bishop (1981) found no change in V_E and end tidal CO_2 during CNPB in humans, in disagreement with the present results. There is the obvious difference in species, but major difference in preparation and technique also exist, which might explain this disparity, They applied a continuous positive pressure (PP) around the body surface, whereas we directly applied NP to the trachea. PP has to be transmitted through the impedance of the chest wall to reach the pulmonary receptor sites. If the recep-

tors respond mainly to alveolar rather than transpulmonary pressure, a smaller response is expected, since the coupling through respiratory system compliance would limit the changes in alveolar pressure due to a maintained PP around the body surface (a mean pressure component cannot be transmitted through a compliant element). On the other hand, NP applied at the trachea directly acts on these receptors through airway resistance. Moreover, the dogs in the present study were prepared with a tracheostomy eliminating the large airway resistance, and reducing the conductive dead space. A reduced dead space would make our minute ventilation value closer to alveolar ventilation as compared to intact subjects. The dead space is known to decrease during chest compression (McIlroy et al, 1962). If this is also true during CNPB, minute ventilation may not be an appropriate measure of alveolar hyperventilation. Since the intact humans studied by Hirsch & Bishop (1981) should have a relatively larger dead space than our tracheostomized dogs, alveolar hyperventilation might have occured without being detected in minute ventilation measurements. The lack of $Paco_2$ measurement in their study makes it difficult to interpret the data. We found a small but significant drop in $Paco_2$ associated with a corresponding increase in V_E during CNPB, indicating that an alveolar hyperventilation actually occurred in our awake dogs. This relatively small degree of hyperventilation might have been hidden in the larger inter-individual variances observed in their study. They also employed a bag-in-box system to monitor the ventilatory variables, which probably involved a larger resistive and/or elastic loads on the subjects compared to our experimental set-up. When we tested the loading effect of our breathing apparatus including the reservoir, no statistically significant differences was detected in any of the variables ($p>0.1$).

Although a statistically significant difference was found, an argument on alveolar hyperventilation might arise from the small decrease in $Paco_2$ due to NP. Since CNPB changed both V_E and $Paco_2$, alveolar ventilation must be evaluated not by either, but by both variables. This was attempted by running CO_2 responses, which would provide an overall and more complete ventilatory response to CNPB. The application of NP during both 0% (air) and 5% CO_2 inhalation enabled us to estimate CO_2 response lines for both CP (normal EELV) and NP (lowered EELV) conditions by linear regression (Fig. 6). Since we had only two different inhaled CO_2 levels, the possibility of non-linearity in the response to CO_2 was excluded by trying an intermediate CO_2 level (3%) in three experiments. In these experiments the (V_E, $Paco_2$) points fell very close to the response line estimated from the 0 and 5% CO_2 inhalation data. A high correlation coefficient of 0.92 was also found. To ensure a linear CO_2 response, the degree of any changes in alveolar ventilation due to NP was evaluated by calculating $(\triangle V_E)_{EUP}$. $(\triangle V_E)_{EUP}$ representing the estimated shift of CO_2 response line due to NP at the eupneic point. This should make the most accurate and reliable measure of any changes in control characteristic of alveolar ventilation at eupnea. The mean $(\triangle V_E)_{EUP}$ was significantly greater than zero by 63% control V_E ($p<0.0001$), which reflected the degree of hyperventilation that should occur when $Paco_2$ is truely controlled such as in exercise hyperpnea (discussed later). Therefore, we maintain that a true alveolar hyperventilation has occured during CNPB in awake dogs.

Interaction between CNPB and CO_2 inhalation

It is clear, from this study, that not only chemical stimuli, but also mechanical alterations could have a significant influence on determining ventilatory responses. We intended to examine possbile interactions between these two different types of forcing. One type of forcing was an increased $Paco_2$ by inhaling CO_2, and the other, a lowered EELV by CNPB, these corresponded to chemical and mechanical stimuli, respectively. At both (0 and 5%) levels of CO_2 inhalation, the application of NP resulted in a similar decreased V_T, approximately 20%,

and an increased f_B, of approximately 70% (Fig. 7). These results suggest that breathing pattern variables respond to CNPB to the same degree, regardless of $Paco_2$ level changed by a chemical stimulus, which implies an additive effect of CNPB and CO_2 inhalation. This was further explored by examining CO_2 responses. CO_2 response line during NP did not change its sensitivity (S_{NP}) compared to CP (S_{CP}) ($p>0.05$) shifted upwards, reflecting an alveolar hyperventilation as previously discussed. Thus, the CO_2 response to steady state CNPB was simply a shifted version of that with atmospheric pressure, resulting in the same increase in ventilation at any $Paco_2$ level. This iterates an additive effect of CNPB and CO_2 inhalation.

Integrated phrenic nerve activity was more sensitive to $Paco_2$ change during transient lung deflation than at normal FRC in artificially ventilated dogs (Cherniack et al, 1973). In other words, alveolar ventilation would increase more than that expected from the sum of the individual responses when the lung was deflated under hypercapnea. This would increase CO_2 sensitivity during NP compared to CP as opposed to our results. However, it is unreasonable to directly compare their experiments under artificial ventilation with the lungs paralyzed to our data, obtained from conscious spontaneously breathing dogs. Furthermore, their results are confined only to a transient state, whereas our study is focused to steady state responses. Since our experiments were free from any possible non-physiological factors, such as anesthesia, we believe that our results are more reliable.

Physiological importance

The present study demonstrated that mechanical alterations such as a lowered EELV (or FRC) could play an important role in ventilatory control independently of the chemical regulation of breathing. This maybe of physiological significance under conditions when both chemical and mechanical stimuli exist, eg. in exercise hyperpnea, as discussed below. FRC is known to decrease during exercise (Grimby et al, 1968; Lind & Hesser, 1984; Linnarson, 1974). Expiratory muscles become more active during muscular exercise, lowering the end expiratory position of the lungs (Dimarco et al, 1983). A lowered FRC may cause a secondary alveolar hyperventilation, since the same reflex stimulation of respiration as described in the present study should also be operating in an intact exercising subject. When the decrease in FRC during moderate exercise was approximately the order of tidal volume (Lind & Hesser, 1984), which is a similar value for the NP level we used, approximately 63% increase in ventilation (the degree of hyperventilation at eupnea during CNPB) could be attributed to the reduced FRC, since isocapnea is maintained during exercise. In addition, since a reduction in FRC occurs due to active expiration during exercise, recovered elastic work can be added to inspiration, making it more efficient (Yamashiro & Grodins, 1973), whereas the subject has to overcome the external negative force during CNPB. Therefore, the contribution of this mechanical change could be even larger during an exercising condition. Although the application of CNPB to induce a lung deflation is different from the exercising condition, where a lowered lung volume is achieved by an active increase in neuromuscular activity, the respiratory stimulation via a lowered FRC during exercise warrants further investigation.

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