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Inhibitory Mechanism of CO_2 Inhalation on Slowly Adapting Pulmonary Stretch Receptors in the Anesthetized Rabbit

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ABSTRACT

The inhibitory effects of CO_2 on slowly adapting pulmonary stretch receptors (SARs) were studied before and after administration of acetazolamide, a carbonic anhydrase inhibitor, or nifedipine, a calcium channel blocker, in anesthetized, artificially ventilated rabbits after vagus nerve section. CO_2 inhalation (maximal tracheal CO_2 concentration ranging from 7.2% to 9.5%) for approximately 60 sec decreased the receptor activity during both inflation and deflation. The magnitude of decreased receptor activity during deflation became more pronounced than that seen during inflation. Acetazolamide treatment (20 mg/kg) diminished the inhibitory responses of slowly adapting pulmonary stretch receptors to CO_2 inhalation, which were not significantly influenced by pretreatment with nifedipine (1 mg/kg). Furthermore, CO_2 inhalation before and after vagal denervation had no effect on total lung resistance and dynamic lung compliance. In another series of experiments, the staining to determine the presence of carbonic anhydrase enzymatic reaction was not found in the smooth muscle of either extrapulmonary or intrapulmonary bronchi. These results suggest that CO_2-induced inhibition of slowly adapting pulmonary stretch receptors is not related to the change in bronchomotor tone.

Inhalation of CO_2 inhibits SARs in many species (Mustafa and Purves, 1972; Schoener and Frankel, 1972; Sant'Ambrogio *et al.*, 1974; Bartlett and Sant'Ambrogio, 1976; Bradley *et al.*, 1976; Kunz *et al.*, 1976; Coleridge *et al.*, 1978), and the magnitude of the inhibitory effect depends on the location of the receptors (Mustafa and Purves, 1972; Sant'Ambrogio *et al.*, 1974; Bartlett and Sant'Ambrogio, 1976). Furthermore, it can be postulated that the variety of this inhibition reflects different concentrations of CA in the smooth muscle of different bronchi (Pack, 1981). However, no reports focus on the relationship between CO_2-induced SAR inhibition and CA activity in the airway smooth muscle.

At present, two different mechanisms on CO_2-induced SAR inhibition have been proposed: 1) The inhibitory effect is mediated through changes in bronchial smooth muscle tone (Nilsestuem *et al.*, 1979; Mitchell *et al.*, 1980). 2) The inhibitory effect of CO_2 on SARs is independent of changes in lung mechanics (Mustafa and Purves, 1972; Sant'Ambrogio *et al.*, 1974; Coleridge *et al.*, 1978). Indeed, hypercapnic inhibition of SARs appears to be nonspecific and constitutes a generalized pH effect on neural function (Mustafa and Purves, 1972; Sant'Ambrogio *et al.*, 1974; Bartlett and Sant'Ambrogio, 1976; Coleridge *et al.*, 1978). For example, administration of acetazolamide, a CA inhibitor, blocks CO_2-induced SAR inhibition (Sant'Ambrogio *et al.*, 1974). In the case of slowly adapting mechanoreceptors of the cat's vibrissae, the inhibitory action of CO_2 was attributed to the release of protein-bound calcium ions, because an increase in calcium concentration inhibited the discharge rate of those receptors (Fitzgerald, 1940). It is possible that the inhibitory response of SARs to CO_2 is mediated by ionic changes in the extracellular fluid surrounding the sensory endings (Bartlett and Sant'Ambrogio, 1976). In any event, the exact mechanism of CO_2-induced SAR inhibition is still uncertain.

To elucidate whether there is a correlation between a specific action of lung mechanics (airway smooth muscle tone) or a nonspecific action of neural function and inhibition of the SAR activity associated with CO_2 inhalation, we performed three different types of experiments in anesthetized, artificially ventilated rabbits with or without bilateral vagotomy. First, changes in R_L and Cdyn after CO_2 inhalation were examined before and after vagus nerve section. Second, responses of SARs to inhalation of CO_2 gas mixtures were examined before and after administration of acetazolamide, a CA inhibitor, or nifedipine, one of the L-subtype calcium blockers, in bilaterally vagotomized animals. In these studies, control values of tracheal CO_2 concentration were kept below 4%. The SARs have been classified into two different groups: "low-threshold" and "high-threshold" (Sant'Ambrogio, 1982; Ravi, 1986). The effects of acetazolamide and nifedipine on the responses of SARs to CO_2

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ABBREVIATIONS: SAR, slowly adapting pulmonary stretch receptor; CA, carbonic anhydrase; R_L, total lung resistance; Cdyn, dynamic lung compliance; P_T, tracheal pressure; $\dot{V}$, respiratory airflow; V_T, tidal volume; SAP, systemic arterial blood pressure.

have been examined in two different groups of receptors. Third, we evaluated the presence of CA enzymatic reaction within the smooth muscle of extrapulmonary and intrapulmonary bronchi. In additional experiments, we also determined whether the staining is due to a specific or a nonspecific activity of the CA enzyme.

Materials and Methods

Animal preparation. Thirty-seven rabbits of both sexes, weighing 3.0 to 3.5 kg, were anesthetized with urethane (1.0 g/kg) given i.p. Additional doses (0.2–0.3 g/kg/hr, i.v.) of this anesthetic agent were administered as required. The trachea was exposed through a middle incision in the neck and cannulated below the larynx, and the trachea and esophagus were rostrally retracted to obtain space for paraffin pool. In 31 of the 37 rabbits, P_T was measured by connecting a polyethylene catheter inserted into the tracheal tube to a differential pressure transducer in which one arm opened to the atmosphere. After administration of heparin (500 U/kg) into the ear vein, the femoral artery was cannulated for measurement of SAP. A polyethylene catheter was also inserted into the right external jugular vein, and its tip was advanced into the right atrium for administration of drugs or a 0.9% NaCl solution. The superior laryngeal and recurrent laryngeal nerves on both sides were identified, exposed and sectioned in advance. Then the vagus nerves were exposed and sectioned. The rectal temperature was maintained at around 37°C by a heating pad. After the administration of gallamine triethiodide (3–5 mg/kg, i.v.), animals were artificially ventilated with air. The stroke volume of the respirator was set at 10 ml/kg, and its frequency ranged from 35 to 45 cycles/min. Tracheal CO_2 concentration was measured by CO_2 gas analyzer (Respina IH 26, Sanei) and was kept at about 3.5% to 3.9% by adjusting the ventilatory rate.

Measurement of lung mechanics. In 7 of the 31 rabbits, $\dot{V}$ was measured by connecting the tracheal tube to a pneumotachograph and a differential pressure transducer, and the flow signal was integrated to obtain V_T. P_T was measured by using the technique described previously. R_L and Cdyn were calculated by using the manual graphic method reported by Norlander *et al.* (1968).

Measurements of SAR activity. The peripheral end of the cut left vagus nerve was desheathed. To record single unit activity of SARs, thin strands containing afferent nerve fibers were separated, placed on a unipolar silver electrode and submerged in a pool of warm liquid paraffin (37°C–38°C). The SARs were identified, on the basis of their firing behavior during lung inflation, as follows: 1) The SARs increased their discharge during inflation and decreased their discharge during deflation. 2) The increase in SAR activity was proportional to the increase in inflation volume of the respirator. 3) The discharge of SARs continued as long as the tracheal tube was occluded in a hyperinflated condition. The SAR activity was amplified and selected by a window discriminator for counting the number of impulses. It was also monitored on an oscilloscope and recorded on a polygraph. The location of the receptors obtained was determined by means of a balloon catheter. When the tip of the catheter reached the carina, the balloon was inflated and pulled rostrally. If the receptors were located below the carina, they were not stimulated by pulling the inflated balloon catheter. The 24 SARs located below the carina were confirmed in 24 rabbits. In these receptors, 14 were low-threshold receptors that fired during the whole respiratory cycle, and the remainders were classified as high-threshold receptors that fired only during inflation.

Examination of an enzymatic reaction of CA in the airway smooth muscle. In 6 of the 37 rabbits, under artificial ventilation the chest was widely opened in the midline. A polyethylene catheter was inserted into the left ventricle, and its tip was advanced into the ascending aorta. After the incision in the right appendage, a 0.9% NaCl solution (about 500 ml) was perfused, and it was followed by a fixative consisting of 4% paraformaldehyde, 3% glutaraldehyde and cacodylate buffer (0.05 M, pH = 7.4). The trachea and lung tissues were excised immediately, and air from the lungs was sucked by a vacuum pump. The trachea and lung tissues were stored for 2 hr in the same fixative and then were immersed in a 20% sucrose solution, embedded in ornithine carbamoyl transferase compound and frozen. Frozen sections with a thickness of 20 μm were made on a cryostat and reacted in Hansson's solution for 8 to 13 min (Hansson, 1967; Sugai *et al.*, 1981). Finally, we examined an enzymatic reaction of CA in the smooth muscle located in both extrapulmonary and intrapulmonary bronchi in four rabbits. We also determined whether there was a specific reaction of the CA enzyme in two rabbits. Frozen sections were initially incubated according to the cobalt capture method of Hansson (1967). Afterwards, histological sections were incubated in cobalt phosphate containing 10^{-5} M acetazolamide.

Experimental design. The experiments were designed to examine the effect of CO_2 on lung mechanics, to test the role of a CA inhibitor in the responses of SARs to CO_2 and to estimate any dependence of calcium influx on CO_2-induced SAR inhibition. The following experiments were performed: 1) In seven rabbits, before and after vagal denervation, the effects of CO_2 inhalation for about 60 sec on P_T, R_L and Cdyn were examined. 2) In 14 SAR fibers (low-threshold receptors = 9, high-threshold receptors = 5) in 14 rabbits, the effects of CO_2 inhalation on SAR activity were determined. Five minutes after the administration of acetazolamide (20 mg/kg, i.v.), the same tests were repeated under the same conditions. 3) In 10 SAR fibers (low-threshold receptors = 5, high-threshold receptors = 5) in 10 rabbits, the changes in SAR activity in response to inhalation of CO_2 gas mixtures were examined. Ten minutes after administration of nifedipine (1 mg/kg, i.v.), the same sets of experiments were repeated again.

Drugs. Acetazolamide (Takeda Pharmaceutical Cooperation, Japan, 500 mg) was diluted with 0.9% NaCl (20 mg/ml). Nifedipine (Sigma, USA, 10 mg) was dissolved in a small amount (1 ml) of ethanol and diluted with a 0.9% NaCl solution (1 mg/ml). The stock solution of nifedipine was kept in a bright protective glass container.

Statistical analysis. During control conditions, V_T, $\dot{V}$ and P_T were measured over several respiratory cycles, and R_L and Cdyn were calculated. The average values of P_T were expressed as cm H_2O, and the average values of R_L and Cdyn were expressed as cm H_2O/l/sec and ml/cm H_2O, respectively. The responses of P_T, R_L and Cdyn to CO_2 inhalation for approximately 60 sec (maximal tracheal CO_2 concentration ranging from 7.2% to 9.5%) were also calculated by measuring all three respiratory parameters at 10-sec intervals and by performing the measurements over 120 sec. Similarly, control firing rates of the SARs during both inflation and deflation were averaged over several respiratory cycles and expressed as imp/sec. The SAR responses to CO_2 inhalation were obtained by counting the firing rates of receptors at 10-sec intervals and by performing the measurements over 120 sec, and the average activities of SARs during both inflation and deflation were expressed as imp/sec. Concerning the four measured parameters (P_T, R_L, Cdyn and SAR activity), the time-dependent difference was compared between control and CO_2-inhaled rabbits as well as before and after vagotomy by using a one-way analysis of variance for repeated measurements. The effects of acetazolamide and nifedipine on the responses of SAR activities during inflation and deflation to CO_2 inhalation were also analyzed by means of the modified t statistics. Then we used the Bonferroni test for one comparison (k = 1) with the control. All values were expressed as means ± S.E. A P value of less than .05 was considered statistically significant.

Results

Effect of CO_2 on lung mechanics. The values of P_T in the control were 9.1 ± 0.2 cm H_2O. Base-line R_L was 21.6 ± 2.9 cm H_2O/l/sec, and Cdyn was 2.7 ± 0.3 ml/cm H_2O. CO_2 inhalation for about 60 sec did not produce any significant

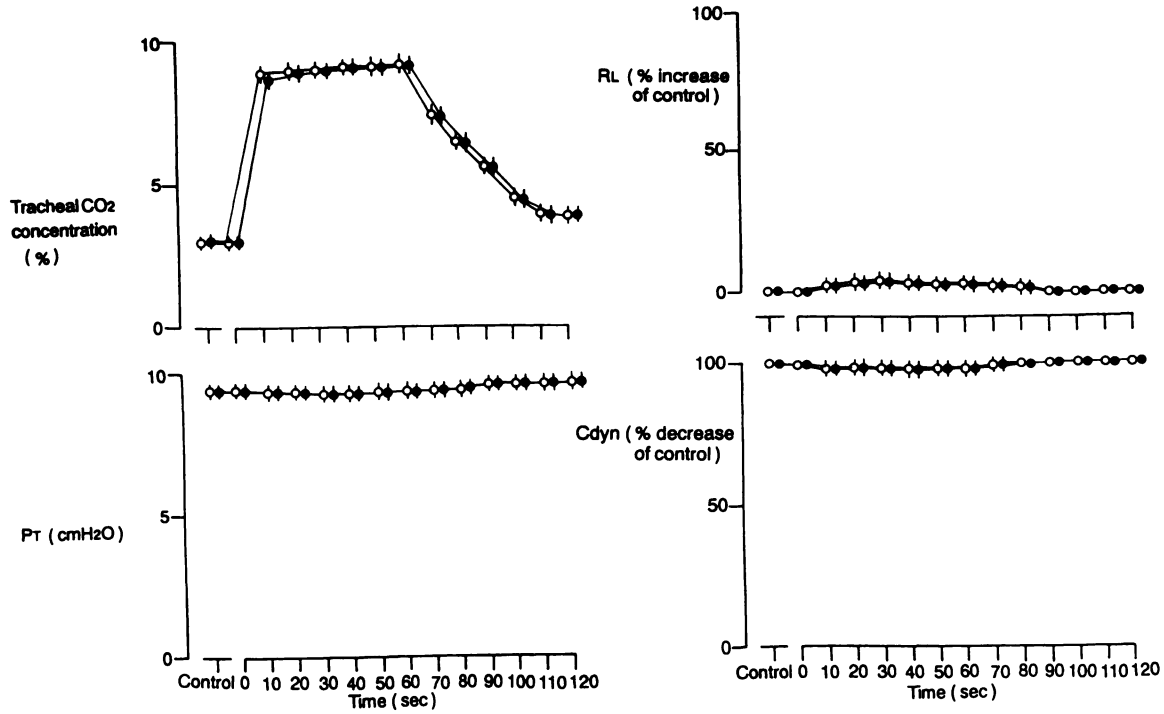

Fig. 1. Changes in P_T, R_L and Cdyn in response to CO_2 inhalation before (open circles) and after (closed circles) vagal denervation. 0, the onset of increased tracheal CO_2 concentration. Values are means ± S.E.; n = 7.

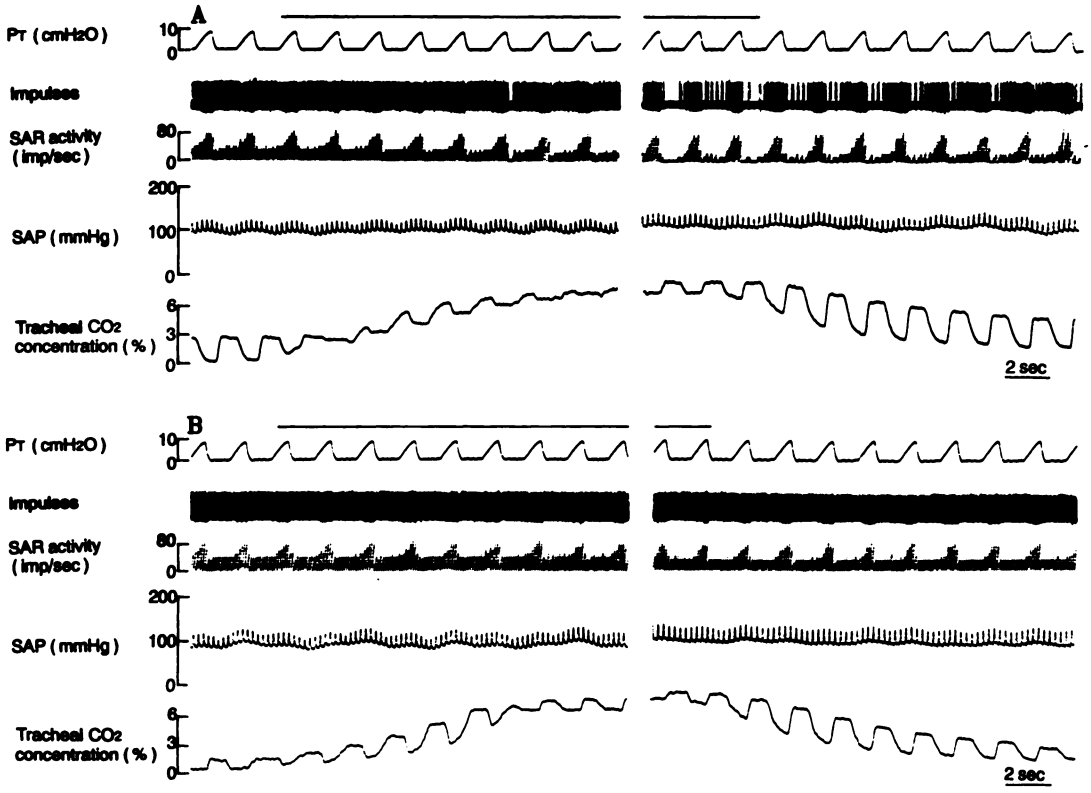

Fig. 2. Effect of acetazolamide on the responses of P_T, SARs (low-threshold receptors) and SAP to CO_2 inhalation. A) Control. B) After administration of acetazolamide (20 mg/kg). —, period of increased tracheal CO_2 concentration.

change in P_T, R_L or Cdyn in seven rabbits before and after vagal denervation, which indicated that inhalation of CO_2 gas mixtures does not result in either bronchoconstriction or bronchodilation (fig. 1).

Effect of acetazolamide on the responses of SARs to CO_2 inhalation. After CO_2 inhalation the SARs decreased their activity during both inflation and deflation. The decrease in the SAR activity occurred approximately 5 sec after

the onset of increased tracheal CO_2 concentration. The magnitude of decreased SAR activity during deflation became more prominent than that during inflation. The response was not associated with any significant change in P_T. After CO_2 inhalation ceased, the SARs returned to their control activity within 30 sec (fig. 2A). The inhibitory effect of CO_2 inhalation on SAR activities was diminished by pretreatment with acetazolamide (20 mg/kg), which elicited severe hypocapnia (tracheal CO_2 concentration < 2%) and had no significant effect on SARs and P_T (fig. 2B). We compared the responses of nine different SARs (low-threshold receptors) to CO_2 inhalation before and after pretreatment with acetazolamide (20 mg/kg), a CA inhibitor (fig. 3). At 10 sec after CO_2 inhalation, the inspiratory discharge of SARs was decreased from 62.2 ± 2.2 to 55.0 ± 2.3 imp/sec, and the expiratory discharge of receptors was decreased from 30.6 ± 1.0 to 22.2 ± 1.5 imp/sec. The decrease in SAR activities during inflation and deflation became more prominent at 40 sec after CO_2 inhalation. The inhibitory effect of CO_2 inhalation on SAR activities during inflation and deflation was significantly diminished by the treatment with acetazolamide. The average discharges of SARs (high-threshold receptors, n = 5) in control animals and acetazolamide (20 mg/kg)-treated animals were 64.7 ± 2.4 and 62.1 ± 2.3 imp/sec, respectively. The CO_2-induced SAR inhibition reached a plateau at 10 sec after CO_2 inhalation, and the effect of CO_2 on SARs was abolished by pretreatment with acetazolamide (fig. 4).

Effect of nifedipine on the responses of SARs to CO_2 inhalation. Administration of nifedipine (1 mg/kg), a potent calcium channel blocker, had no significant effect on the inhibitory effect of CO_2 inhalation on low-threshold SARs (n = 5) (fig. 5). The CO_2-induced SAR (high-threshold receptors, n = 5) inhibition was not significantly altered by pretreatment with nifedipine at 1 mg/kg (fig. 6).

Histochemical examination of CA activity in the airway smooth muscle. The smooth muscle in the extrapulmonary bronchi located below the carina did not exhibit any CA activity (fig. 7A). CA activity was also not found in the smooth muscle of intrapulmonary bronchi, although the alveolar epithelial cells exhibited CA activity (fig. 7B). To determine whether the positive staining was due to a specific reaction of the CA enzyme, we stained adjacent sections in the absence and presence of acetazolamide (10^{-5} M). As shown in figure 8A–D, the staining of erythrocytes in the blood vessels and of lung epithelial cells disappeared after inhibition of the CA activity with acetazolamide. Under these

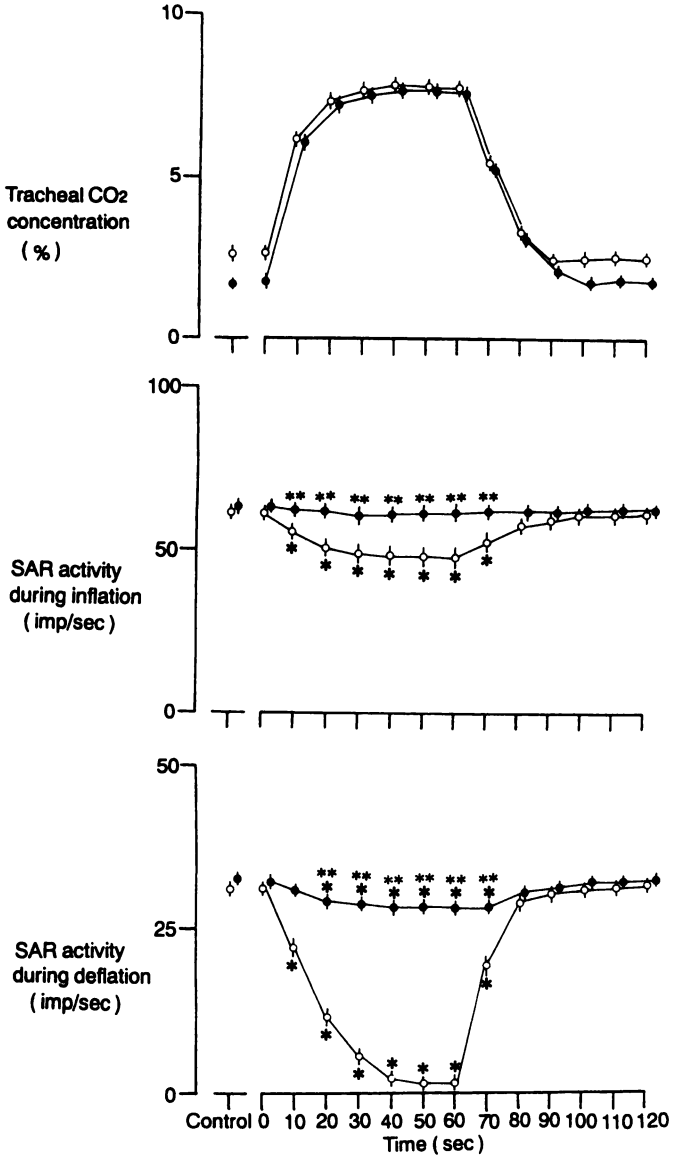

Fig. 3. Changes in SAR activity (low-threshold receptors) in response to CO_2 inhalation before (open circles) and after (closed circles) administration of acetazolamide (20 mg/kg). 0, the onset of tracheal increased CO_2 concentration. Values are means ± S.E.; n = 9. * P < .05 for significant difference from control values. ** P < .05 for significant difference from acetazolamide effects.

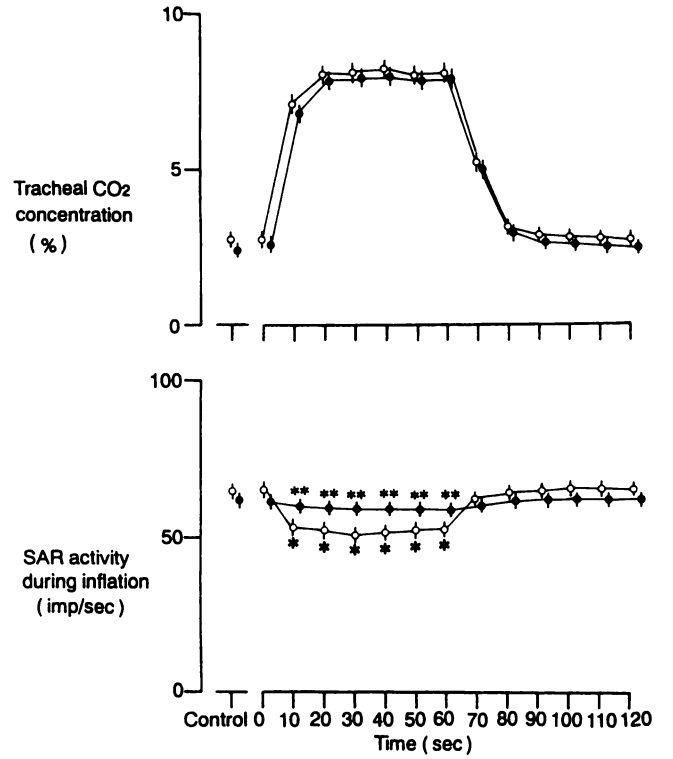

Fig. 4. Changes in SAR activity (high-threshold receptors) in response to CO_2 inhalation before (open circles) and after (closed circles) administration of acetazolamide (20 mg/kg). 0, the onset of increased tracheal CO_2 concentration. Values are means ± S.E.; n = 5. * P < .05 for significant difference from control values. ** P < .05 for significant difference from acetazolamide effects.

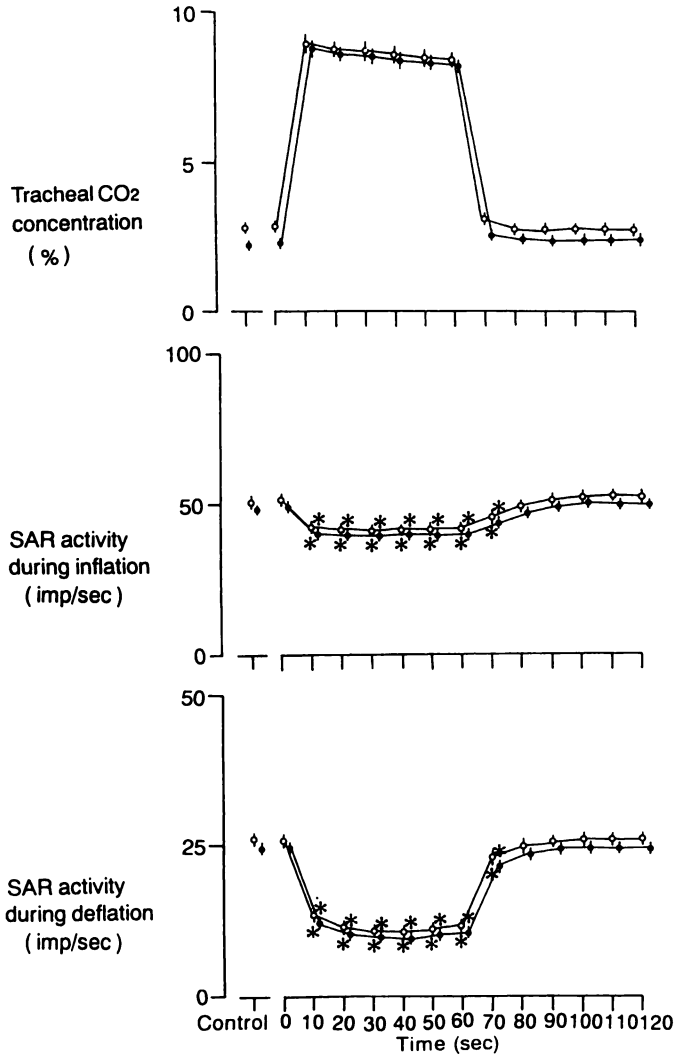

Fig. 5. Changes in SAR activity (low-threshold receptors) in response to CO_2 inhalation before (open circles) and after (closed circles) administration of nifedipine (1.0 mg/kg). 0, the onset of increased tracheal CO_2 concentration. Values are means ± S.E.; $n = 5$. * $P < .05$ for significant difference from control values.

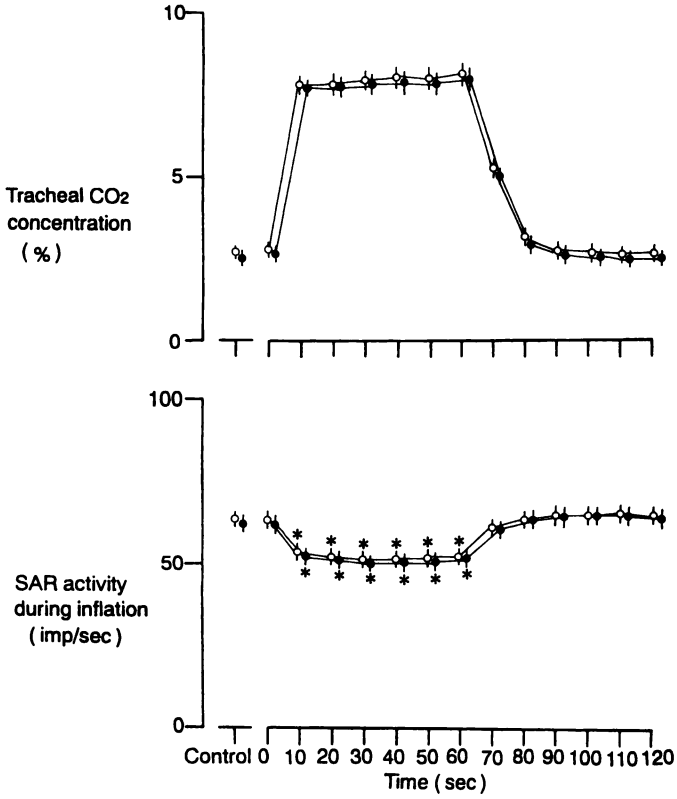

Fig. 6. Changes in SAR activity (high-threshold receptors) in response to CO_2 inhalation before (open circles) and after (closed circles) administration of nifedipine (1.0 mg/kg). 0, the onset of increased tracheal CO_2 concentration. Values are means ± S.E.; $n = 5$. * $P < .05$ for significant difference from control values.

conditions, the smooth muscle in both intrapulmonary and extrapulmonary bronchi did not show any specific reaction of the CA enzyme.

Discussion

The inhibitory effects of CO_2 inhalation on SARs were diminished by acetazolamide, whereas nifedipine had no significant effect on CO_2-induced SAR inhibition. Because CA activity was not found in the smooth muscle of the extrapulmonary and intrapulmonary bronchi, it is most likely that inhibition of SAR activity by CO_2 does not involve changes in the airway smooth muscle tone that depend upon the CA activity. This is further confirmed by the fact that CO_2 inhalation does not significantly alter either R_L or Cdyn.

The inhibitory effect of CO_2 on SARs is known to become more prominent under hypocapnic conditions (Mustafa and Purves, 1972; Sant'Ambrogio *et al.*, 1974; Bartlett and Sant'Ambrogio, 1976; Bradley *et al.*, 1976). Thus tracheal CO_2 concentration was held below 4%, in the present experiments, by adjusting the ventilatory rate and/or the inflation volume of the respirator. The SARs recorded in this study were similar to those of the receptors obtained in previous studies (Matsumoto *et al.*, 1990; 1993). Thus the SARs in this study may belong to the same category of type II receptors as obtained in the SAR responses to static pressure inflations (Miserocchi and Sant'Ambrogio, 1974). Sant'Ambrogio *et al.* (1974) demonstrated that the inhibitory action of inhaled CO_2 on bronchial SARs appeared faster than that on extrapulmonary SARs. Furthermore, in an earlier study, Bradley *et al.* (1976) reported that CO_2 inhalation did not significantly influence the activity of tracheal SARs. We therefore decided to investigate the inhibitory effect of CO_2 in our SAR preparations. Moreover, CO_2-induced SAR inhibition is observed even when the change in the airway CO_2 is isolated from that in the blood CO_2 (Sant'Ambrogio *et al.*, 1974; Bradley *et al.*, 1976; Coleridge *et al.*, 1978). The rapid reaction of SARs after CO_2 inhalation in this study may be explained by the change in the bronchial lumen CO_2 concentration rather than by variation in the blood CO_2 concentration.

During CO_2 inhalation, the SARs that were classified as low-threshold receptors decreased their activity during both inflation and deflation. The magnitude of decreased SAR activity during deflation became more pronounced than that during inflation. These results are in agreement with observations indicating a greater inhibition of SAR activity during deflation (Mustafa and Purves, 1972; Sant'Ambrogio *et al.*, 1974; Bradley *et al.*, 1976; Coleridge *et al.*, 1978). The difference in the inhibitory action of CO_2 on SARs is probably due

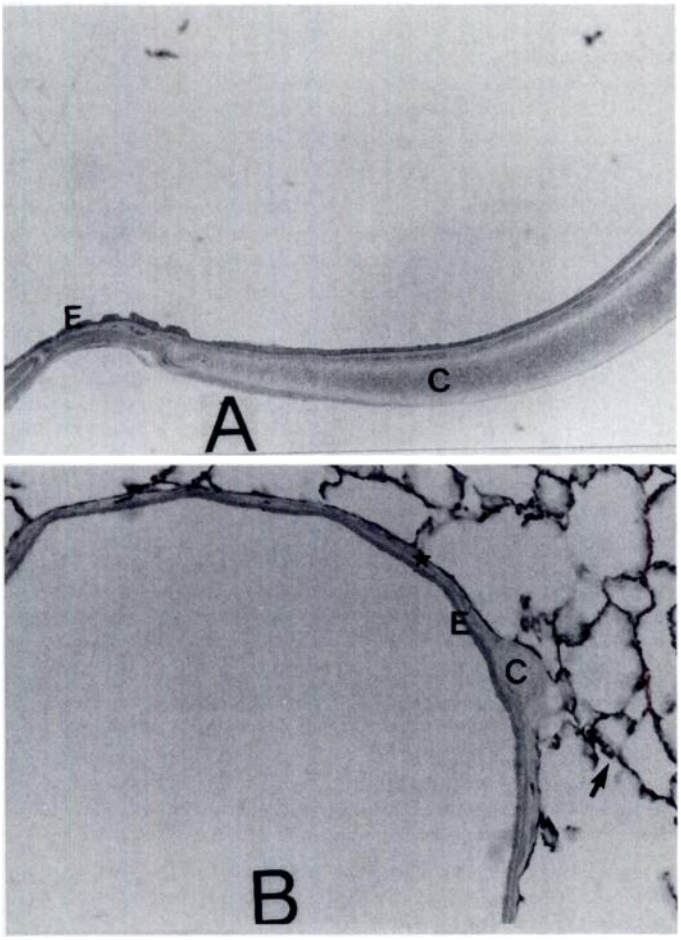

Fig. 7. A) Light micrograph of the extrapulmonary airways for CA activity. B) Light micrograph of the intrapulmonary airways for CA activity. Lung epithelial cells (arrow) show intense staining, but smooth muscles (star) do not show staining (×100). C) C-shaped tracheal cartilage. E) Epithelium of trachea.

to the difference in transmural pressure between inflation and deflation. This interpretation is further supported by evidence that the inhibitory effect of CO_2 on SARs is weaker at the higher transmural pressures (Mustafa and Purves, 1972; Bradley *et al.*, 1976). In addition, during CO_2 inhalation the peak inspiratory discharge of SARs in both high-threshold and low-threshold receptors decreased. The decrease in high- and low-threshold SAR responses to CO_2 inhalation could be explained by reduction of the transmural pressure that passes through the nerve endings of receptors during inflation.

The CO_2-induced SAR inhibition is mediated by augmentation of bronchial smooth muscle tone (Nilsestuem *et al.*, 1979; Mitchell *et al.*, 1980). Conversely, inhibition of SARs by CO_2 is independent of changes in lung mechanics (Mustafa and Purves, 1972; Sant'Ambrogio *et al.*, 1974; Coleridge *et al.*, 1978). Studies in the presence of intact vagal efferent innervation to the airways have demonstrated that hypercapnia causes a cholinergic-mediated increase in R_L (Widdicombe, 1966; Martin *et al.*, 1995). In this study, we found that CO_2 inhalation had no significant effect on lung mechanics (R_L and Cdyn) in rabbits before and after vagus nerve section. This finding implies that the increase of bronchomotor tone induced by CO_2 is attenuated by reflex airway smooth muscle relaxation secondary to elevation in blood pressure (Nadel and Widdicombe, 1962) and/or by stimulation of sympathetic outflow to the airways. Therefore, it is reasonable to assume that the mechanism by which CO_2 inhibits SARs is not related to changes in bronchial smooth muscle tone. On the other hand, there is evidence that the sensitivity of SARs to CO_2 depends on their location in the tracheobronchial tree (Sant'Ambrogio *et al.*, 1974; Bradley *et al.*, 1976). According to Pack (1981), who suggested that the differences in sensitivity may be related the different concentrations of CA activity in the walls of different bronchi. However, there are no studies to confirm this suggestion. In this study, the positive staining of the erythrocytes in blood vessels and the alveolar epithelial cells was based on a specific reaction of the CA enzyme. Because there was no significant staining for CA enzymatic reaction in the smooth muscle of extrapulmonary and intrapulmonary bronchi, we concluded that CO_2-induced SAR inhibition is not related to the CA activity in the airway smooth muscle.

Sant'Ambrogio *et al.* (1974) reported that administration of acetazolamide (35 mg/kg) blocked the inhibitory effects of CO_2 on the discharge of SARs in dogs. However, they did not examine whether the effect of acetazolamide at a higher dose is due to a specific action of the drug. We showed that the inhibitory effect of CO_2 inhalation for approximately 60 sec on SARs (low-threshold and high-threshold receptors) was reduced by the administration of acetazolamide, a CA inhibitor, at 20 mg/kg. Maren (1977) demonstrated that the dosages of acetazolamide of 20 mg/kg or less inhibit 99.99% of the enzyme and do not have any nonspecific effect. Thus CO_2-induced SAR inhibition is closely related to the presence of the CA enzyme in the lung, except for the smooth muscle. Because CA activity has been identified in myelinated afferent fibers in peripheral nerves (Cammaer and Transey, 1987; Riley *et al.*, 1988; Szaboks *et al.*, 1989), similar localization of the CA enzyme would be expected in airway afferent nerves. However, studies to investigate the CA localization of afferent nerves in the airways have not been reported.

We believe the proposed model shown in figure 9 explains Cl^- transport involving both $Cl^- \leftrightarrow Cl^-$ and $Cl \leftrightarrow HCO_3$ exchange systems. The cell membranes are known to be freely permeable to CO_2 but relatively impermeable to HCO_3^-. The intracellular CO_2 is hydrated to $HCO_3^- + H^+$ and is greatly accelerated by the intracellular CA. Thus there will be a tendency for HCO_3^- to exchange with external Cl^- *via* $HCO_3^- \leftrightarrow Cl^-$ exchange carrier, and this process depends on the magnitude of increased CO_2 levels. Hence the increased intracellular Cl^- levels cause hyperpolarization of the membrane potential of the SAR terminal and elevate the threshold of action potential generation, resulting in a greater inhibition of the expiratory discharge of SARs during CO_2 inhibition. Therefore, it is more plausible that blockade of CO_2 hydration after the administration of a CA inhibitor acts to depolarize hyperpolarization of the membrane potential of the SAR terminal. Although the cellular mechanism mediating the mechanosensitivity of SAR endings has not yet been determined, those receptors are believed to have stretch-activated (SA) channels on the endings. In whole-cell patch-clamp experiments using the labeled aortic baroreceptor neurons in the nodose ganglion, Cunningham *et al.* (1995) have demonstrated that the changes in conductance induced by hypoosmotic stretch appear to be mediated by an increase in inward current ranging from −100 to −10 mV. Evidence that

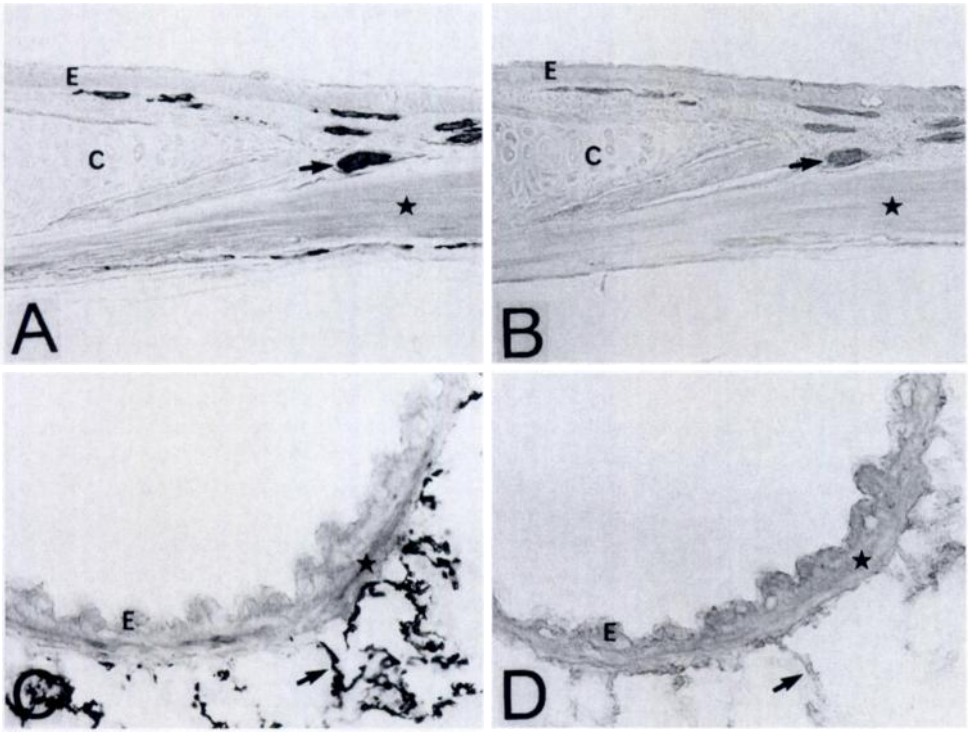

Fig. 8. A) Light micrograph of the extrapulmonary airways for CA activity (×100). The erythrocytes of blood vessels show intense staining (arrow), but smooth muscles (star) do not show staining. B) Serial section of panel A, incubated with 10^{-5} M acetazolamide, an inhibitor of CA. The erythrocytes of blood vessels are CA-negative (arrow). C) Light micrograph of the intrapulmonary airways for CA activity (×100). Lung epithelial cells (arrow) show intense staining, but smooth muscles (star) do show any significant staining. D) Serial section of panel C, incubated with 10^{-5} M acetazolamide. Lung epithelial cells are CA-negative (arrow). C, Bronchial cartilage. E, Epithelium of bronchiole.

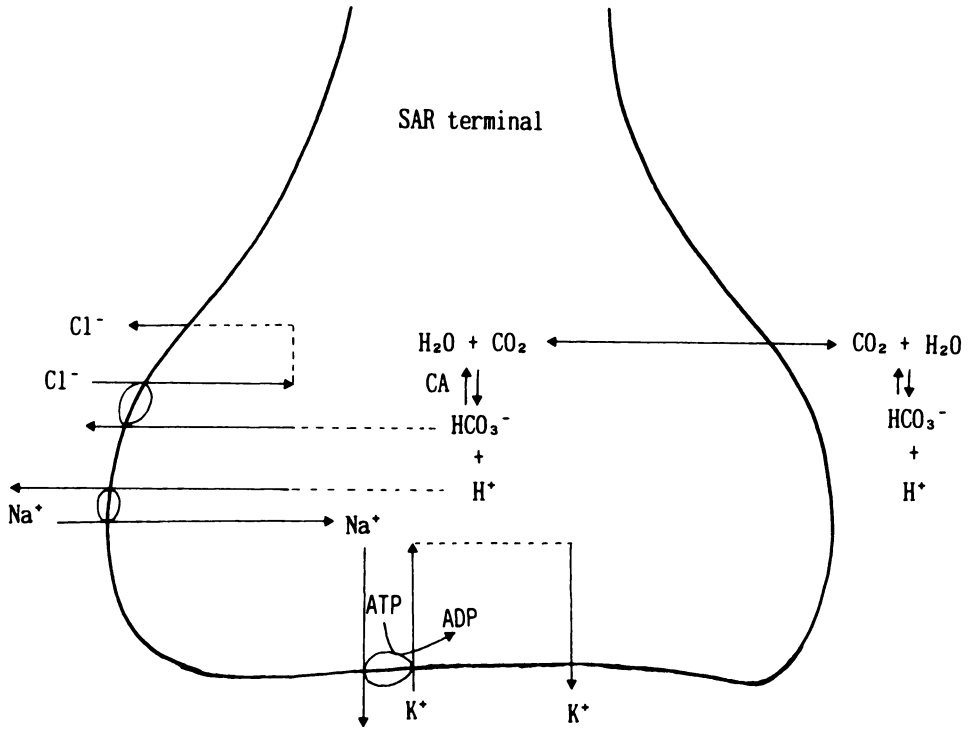

Fig. 9. Proposed model of the ion movements for CO_2 hydration in an SAR terminal.

there is a close interaction between the Cl^- efflux and the macroscopic mechanosensitive current has been reported in chick heart (Hu and Sachs, 1994). Considering these observations, an intracellular Cl^- transport coupled to the $Cl^- \leftrightarrow Cl^-$ and $Cl^- \leftrightarrow HCO_3^-$ exchange systems may be related to the functioning of the SA channels on the SAR endings. Presumably, inhibition of these SA channels occurs at hyperpolarized voltages resulting from increased intracellular Cl^- levels. This inhibition would disappear when CA-dependent CO_2 hydration in the SAR terminals was blocked by administration of a CA inhibitor. As a generalized phenomenon, inhibition of CA activity is thought to elicit a disequilibrium in the CO_2 transport system, giving rise to increased CO_2 tensions and decreased pH levels in the tissue. In addition, acetazolamide has the effect of decreasing the availability of protons for Na^+-H^+ exchange. Blockade of CA-dependent CO_2 hydration is also considered to decrease pH in the SAR endings as well as in the vicinity of the endings, and this decrease in pH would inhibit the activity of SA channels on the SAR endings. Further studies are needed to determine

whether intracellular or extracellular acidosis inhibits the stretch sensitivity of mechanosensitive channels of SARs.

Fitzgerald (1940) observed that the discharge rate from slowly adapting mechanoreceptors of the cat's vibrissae was diminished by an increase in extracellular calcium concentration. If such an effect elicits the CO_2-induced SAR inhibition, then one would expect that administration of calcium channel blockers, which are known to prevent the influx of calcium ions, at least in part inhibits the inhibitory responses of receptors to inhalation of CO_2 gas mixtures. However, in this study the administration of nifedipine, a potent L-subtype calcium channel blocker (Miller, 1987; Matsumoto *et al.*, 1993), had no effect on CO_2-induced SAR inhibition. Because there is evidence that CO_2 inhalation for a short period does not cause either bronchoconstriction or bronchodilation, the results of this study suggest that inhibition of SARs by CO_2 is independent of the influx of calcium ions into the receptive terminals. However, further studies are needed to elucidate the effect of other calcium channel blocks, such as ω-conotoxin, on the mechanism of CO_2-induced SAR inhibition.

In anesthetized animals, the SARs are responsible for the Hering-Breuer inflation reflex to terminate inspiration and to prolong expiration, and the receptors have prominent mechanisms for regulating the depth and rate of breathing (Coleridge and Coleridge, 1986). The strength of the Hering-Breuer inflation reflex would therefore be expected to be reduced by CO_2-induced SAR inhibition. Indeed, the duration of inspiratory inhibition during lung inflation is increased by inhalation of hypercapnic gas mixtures (Bouverot *et al.*, 1970; Younes *et al.*, 1974). Furthermore, the increase in SAR activity is known to reduce the efferent vagal excitatory activity to airway smooth muscle (Widdicombe and Nadel, 1963), which indicates that SARs can optimize the conflicting influences of dead space and airway resistance on alveolar ventilation. From the standpoint of bronchomotor activity, inhibition of SARs by CO_2 inhalation would impair a mechanism capable of limiting bronchoconstriction.

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