Effects of Potassium Channel Blockers on CO₂-Induced Slowly Adapting Pulmonary Stretch Receptor Inhibition

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ABSTRACT

In anesthetized, artificially ventilated rabbits with vagus nerve section, inhalation of CO₂ gas mixtures (tracheal CO₂ concentration ranging from 8.0 to 10.2%) for 60 s decreased slowly adapting pulmonary stretch receptor (SAR) activity during both inflation and deflation. The magnitude of decreased receptor activity during deflation had a more pronounced effect than that seen during inflation. CO₂ inhalation did not cause any significant change in tracheal pressure (P₁) as an index of bronchomotor tone. Intravenous administration of 4-aminopyridine (0.7 and 2.0 mg/kg i.v.), a K⁺ channel blocker, which dose-dependently increased SAR activity during deflation and had no effect on P₁, abolished or attenuated the decrease in SAR activities induced by CO₂ inhalation in a dose-dependent manner. The K⁺ channel blocker tetraethylammonium (2.0 and 6.0 mg/kg i.v.) that did not significantly alter either basal SAR discharge or P₁ had no effect on the inhibitory responses of receptor activity to CO₂ inhalation. These results suggest that the inhibitory mechanism of CO₂ inhalation on SARs may be involved in the activation of 4-aminopyridine-sensitive K⁺ channels in the nerve terminals of SARs.

Inhalation of CO₂ gas mixtures inhibits slowly adapting pulmonary stretch receptor (SAR) activity, and this inhibition is not related to the change in lung mechanics (Sant’Ambrogio et al., 1974; Coleridge et al., 1978; Matsumoto et al., 1994). After delivery of wood smoke containing a high concentration of CO₂, the SARs decrease their activity, and the wood smoke-induced SAR decrease is not significantly influenced by pretreatment with a bronchodilator isoproterenol (Lai and Kou, 1998). The administration of acetazolamide, a carbonic anhydrase (CA) inhibitor, blocks or attenuates the inhibitory responses of SARs to CO₂ inhalation (Sant’Ambrogio et al., 1974; Matsumoto et al., 1996) and wood smoke delivery (Lai and Kou, 1998). From these observations, it is most likely that the inhibitory effect of CO₂ inhalation on SAR activity may be related to an increase in the H⁺ concentration at the receptor site but does not depend on the change in lung mechanics.

Blockade of CA-dependent CO₂ hydration is thought to decrease both the rate of change of [H⁺] and the responsiveness of SARs to rapid changes in CO₂, but the exact mechanism by which an increase in the H⁺ concentration inhibits the receptor discharge remains to be determined. Nevertheless, in neurons of the marine mollusk Aphysia californica, hyperpolarizing responses of the neural structures to CO₂ may be caused by an increase in Cl⁻ or K⁺ conductance (Brown, 1972). Based on evidence showing that no CA enzymatic reaction is found in the smooth muscle of the bronchi, a similar mechanism to explain the inhibitory action of CO₂ has been suggested by Matsumoto et al. (1996). Because myelinated afferent fibers in peripheral nerves contain CA activity (Cammaer and Transey, 1987; Riley et al., 1988; Szaboks et al., 1989), Matsumoto et al. (1996) also postulated that the existence of CA enzymes would be expected in airway afferent fibers. It is therefore possible that the blockade of CA hydration by acetazolamide acts to prevent hyperpolarization of the membrane potential of the SAR terminal. Concerning the generation of action potentials, K⁺ conductance is thought to play a significant role in repolarization of the nerve cell membranes, which regulates the number of spikes with a cycle Na⁺ inflow and K⁺ outflow. We hypothesized that CO₂ inhalation may exert an inhibitory effect on the activity of SARs through the modification of K⁺ channel activity; however, no studies have examined this hypothesis.

To elucidate whether there is a correlation between a generalized action of K⁺ channels and inhibition of the SAR activity associated with CO₂ inhalation, we performed two different types of experiments in anesthetized, artificially ventilated rabbits after vagus nerve section. First, the responses of SARs to CO₂ inhalation were examined before and after the administration of 4-aminopyridine (4-AP), a well known K⁺ channel blocker. Second, the responses of SARs to CO₂ inhalation before and after the administration of tetra-

ABBREVIATIONS: SAR, slowly adapting pulmonary stretch receptor; P₁, tracheal pressure; BP, blood pressure; MBP, mean blood pressure; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; CA, carbonic anhydrase; I₉, fast transient outward K⁺ current.
ethylammonium (TEA), a K⁺ channel blocker, were compared. In the present experiments, control tracheal CO₂ concentrations were kept below 4%. Furthermore, we selected “low-threshold” receptors (Sant’Ambrogio, 1982; Ravi, 1986; Matsumoto et al., 1996) that were more sensitive to CO₂ than “high-threshold” receptors (Sant’Ambrogio et al., 1974; Coleridge et al., 1978; Matsumoto et al., 1996).

**Materials and Methods**

**Animal Preparation.** Sixteen rabbits, weighing 2.5 to 3.0 kg, were anesthetized with urethane (1.0 g/kg i.p.). The trachea was exposed through a middle incision in the neck and cannulated below the larynx. The trachea and esophagus were dissected free and retracted rostrally to obtain a wide space for liquid paraffin. Tracheal pressure (P₂) was measured by connecting a polyethylene catheter inserted into the tracheal tube to a pressure transducer. After the administration of heparin (500 U/kg) into the ear vein, the femoral artery and vein were cannulated for measurement of blood pressure (BP) and for administration of anesthetic agents, respectively. Additional doses (0.2–0.3 g/kg i.v.) of urethane were administered as required. A polyethylene catheter was also positioned in the right atrium through the jugular vein for the administration of drugs or a 0.9% NaCl solution. Then the vagus nerves were exposed and sectioned. The rectal temperature was maintained at approximately 37°C by means of a heating pad. The animals were paralyzed with an initial i.m. administration of suxamethonium (20 mg/kg) followed by a continuous infusion at 10 μg/kg/min. The stroke volume of the respiratory was set at 10 ml/kg, and its frequency ranged from 35 to 40 cycles/min. Tracheal CO₂ was monitored and maintained at approximately 3.5 to 3.9% by adjusting the ventilatory rate. The tracheal CO₂ concentration began to increase. The magnitude of the decrease in SAR activity during deflation was greater than that seen during inflation. The response was not associated with any significant change in P₂, as an index of global bronchomotor tone. After CO₂ inhalation stopped, SARs returned to the control activity within 30 s (Fig. 1A). The inhibitory effect of CO₂ inhalation on SAR activities was abolished by pretreatment with 4-AP (2.0 mg/kg), which produced a significant increase in the SAR activity during deflation and had no significant effect on the receptor activity during inflation and P₂ (Fig. 1B). The responses of eight different SARs to CO₂ inhalation before and after pretreatment with 4-AP at 0.7 and 2.0 mg/kg were compared (Fig. 2). The average inspiratory discharges of SARs in the control and 4-AP (0.7 and 2.0 mg/kg)-treated animals were 59.5 ± 2.3, 60.3 ± 2.9, and 61.2 ± 2.8 imp/s, respectively, and the average expiratory discharges of receptors in those animals were 19.7 ± 1.6, 25.8 ± 1.5, and 28.9 ± 1.6 imp/s, respectively. 4-AP treatment at the dose of 2.0 mg/kg caused a significant increase in the SAR activity during deflation. At 10 s after CO₂ inhalation, the inspiratory discharge of SARs was decreased from 59.5 ± 2.3 to 51.2 ± 1.7 imp/s, and the expiratory discharge of receptors was decreased from 19.7 ± 1.6 to 10.6 ± 1.4 imp/s. The decreases in SAR activities during inflation and deflation had more pronounced effects at 40 s after CO₂ inhalation. The K⁺ channel blocker 4-AP (0.7 and 2.0 mg/kg) significantly reversed the inhibitory effect of CO₂ inhalation on SAR activities during inflation (percent inhibition: absence, 32.4 ± 1.5, n = 8; in the presence of 4-AP, 0.7 mg/kg, 18.9 ± 0.9, n = 8, P < 0.05; 2.0 mg/kg, 3.8 ± 1.3, n = 8, P < 0.05) and deflation (percent inhibition: absence, 83.5 ± 3.2, n = 8; in the presence of 4-AP, 0.7 mg/kg, 24.8 ± 1.1, n = 8, P < 0.05; 2.0 mg/kg, 1.5 ± 1.4, n = 8, P < 0.05). CO₂ inhalation before and after pretreatment with 4-AP (0.7 and 2.0 mg/kg) had no significant effect on P₂. The increase in BP occurred after the administration of 4-AP, but this pressor effect was transient. The mean BP (MBP) values during

**Statistical Analysis.** During control conditions, firing rates of the SARs during inflation and deflation were measured over several respiratory cycles and expressed as imp/s. The SAR responses to CO₂ inhalation for 60 s (tracheal CO₂ concentration ranging from 8.2 to 10.2%) were obtained by counting the firing rates of receptors at 10-s intervals and by performing the measurements over 120 s, and the average activities of SARs during inflation and deflation were expressed as imp/s. Similarly, control values for P₂ were averaged over several respiratory cycles and expressed as cm H₂O. The responses of P₂ to CO₂ inhalation were obtained by measuring the respiratory parameter at 10-s intervals and by performing the measurements over 120 s. The statistical significance of the time-dependent effects of 4-AP and TEA on the responses of SAR activities and P₂ to CO₂ inhalation was first calculated by a one-way ANOVA for repeated measurements. In addition, the maximum decreases in baseline SAR activities during inflation and deflation produced by CO₂ inhalation in the absence and presence of 4-AP (0.7 and 2.0 mg/kg) and TEA (2.0 and 6.0 mg/kg) were also analyzed by a paired t test. All values were expressed as mean ± S.E. A value of P < .05 was considered statistically significant.

**Results**

**Effect of 4-AP on Responses of SARs to CO₂ Inhalation.** Inhalation of CO₂ gas mixtures caused decreases in SAR activity during both inflation and deflation. The decrease in SAR activities occurred immediately after the tracheal CO₂ concentration began to increase. The magnitude of the decrease in SAR activity during deflation was greater than that seen during inflation. The response was not associated with any significant change in P₂, as an index of global bronchomotor tone. After CO₂ inhalation stopped, SARs returned to the control activity within 30 s (Fig. 1A). The inhibitory effect of CO₂ inhalation on SAR activities was abolished by pretreatment with 4-AP (2.0 mg/kg), which produced a significant increase in the SAR activity during deflation and had no significant effect on the receptor activity during inflation and P₂ (Fig. 1B). The responses of eight different SARs to CO₂ inhalation before and after pretreatment with 4-AP at 0.7 and 2.0 mg/kg were compared (Fig. 2). The average inspiratory discharges of SARs in the control and 4-AP (0.7 and 2.0 mg/kg)-treated animals were 59.5 ± 2.3, 60.3 ± 2.9, and 61.2 ± 2.8 imp/s, respectively, and the average expiratory discharges of receptors in those animals were 19.7 ± 1.6, 25.8 ± 1.5, and 28.9 ± 1.6 imp/s, respectively. 4-AP treatment at the dose of 2.0 mg/kg caused a significant increase in the SAR activity during deflation. At 10 s after CO₂ inhalation, the inspiratory discharge of SARs was decreased from 59.5 ± 2.3 to 51.2 ± 1.7 imp/s, and the expiratory discharge of receptors was decreased from 19.7 ± 1.6 to 10.6 ± 1.4 imp/s. The decreases in SAR activities during inflation and deflation had more pronounced effects at 40 s after CO₂ inhalation. The K⁺ channel blocker 4-AP (0.7 and 2.0 mg/kg) significantly reversed the inhibitory effect of CO₂ inhalation on SAR activities during inflation (percent inhibition: absence, 32.4 ± 1.5, n = 8; in the presence of 4-AP, 0.7 mg/kg, 18.9 ± 0.9, n = 8, P < 0.05; 2.0 mg/kg, 3.8 ± 1.3, n = 8, P < 0.05) and deflation (percent inhibition: absence, 83.5 ± 3.2, n = 8; in the presence of 4-AP, 0.7 mg/kg, 24.8 ± 1.1, n = 8, P < 0.05; 2.0 mg/kg, 1.5 ± 1.4, n = 8, P < 0.05). CO₂ inhalation before and after pretreatment with 4-AP (0.7 and 2.0 mg/kg) had no significant effect on P₂. The increase in BP occurred after the administration of 4-AP, but this pressor effect was transient. The mean BP (MBP) values during

**Measurement of SARs.** The peripheral end of the cut left vagus nerve was desheathed. To record the single-unit activity of SARRs, thin strands containing afferent nerve fibers were separated, placed on a unipolar silver electrode, and submerged in a pool with warm liquid paraffin (37–38°C). The SARs were identified, on the basis of their firing behavior during lung inflation, as follows: 1) the SARs (low-threshold) increased their discharge during inflation and decreased their discharge during deflation, 2) the increase in SAR activity was proportional to the increase in the inflation volume of the respirator, and 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 means of a window discriminator for counting the number of impulses. It was also monitored on a unipolar silver electrode, and submerged in a pool with warm liquid paraffin. The SAR responses to CO₂ inhalation for approximately 60 s on SAR activity were determined. Ten minutes after i.v. administration of 4-AP (0.7 and 2.0 mg/kg), the same tests were repeated under the same conditions. The effectiveness of 4-AP was confirmed by repeating the experiments under the same conditions. The effectiveness of 4-AP was confirmed by repeating the experiments under the same conditions.
control and after 4-AP treatment were 98.4 ± 3.6, 98.7 ± 3.5, and 99.2 ± 3.5 mm Hg with 0.7 and 2.0 mg/kg, respectively, and 118.5 ± 4.3, 117.8 ± 4.5, and 117.3 ± 3.9 mm Hg after CO₂ inhalation, respectively. The maximal changes in MBP in response to CO₂ inhalation were not significantly altered by 4-AP treatment.

Fig. 1. Effect of 4-AP on the responses of P₄, SAR activity, and BP to CO₂ inhalation. A, control. B, after i.v. administration of 4-AP (2.0 mg/kg). Straight line, period of increased tracheal CO₂ concentration.

Fig. 2. Changes in P₄ and SAR during both inflation and deflation in response to CO₂ inhalation before (●) and after i.v. administration of 4-AP at 0.7 (▲) and 2.0 (■) mg/kg. 0, the onset of increased tracheal CO₂ concentration. Values are mean ± S.E.; n = 8. *P < .05, significant difference from control values.
Effect of TEA on Responses of SARs to CO₂ Inhalation. Typical examples of the effect of TEA (6.0 mg/kg), a K⁺ channel blocker, on the responses of SAR activity, P₄, and BP to CO₂ inhalation are shown in Fig. 3, A and B. Pretreatment with TEA did not significantly alter either the control activity of SARs or the CO₂-induced SAR inhibition. The effects of TEA at different doses (2.0 and 6.0 mg/kg) on the responses of SAR activities and P₄ to CO₂ inhalation in eight different SAR fibers in eight rabbits are summarized in Fig. 4. TEA (2.0 and 6.0 mg/kg) did not significantly attenuate the inhibitory effect of CO₂ inhalation on SAR activities during inflation (percent inhibition: absence, 29.3 ± 1.3, n = 8; in the presence of TEA, 2.0 mg/kg, 28.6 ± 1.4, n = 8, P > .05; 6.0 mg/kg, 27.8 ± 1.3, n = 8, P > .05) and deflation (percent inhibition: absence, 90.8 ± 2.8, n = 8; in the presence of TEA, 2.0 mg/kg, 90.9 ± 2.6, n = 8, P > .05; 6.0 mg/kg, 88.4 ± 3.1, n = 8, P > .05). Inhalation of CO₂ gas mixtures in the absence and presence of TEA (2.0 and 6.0 mg/kg) had no effect on P₄. The administration of TEA caused a hypotensive effect, but the hypotension evoked by TEA was restored to the control level within 5 min. The MBP values were 95.4 ± 3.7, 95.6 ± 4.2, and 96.2 ± 4.5 mm Hg during control and after TEA treatment at 2.0 and 6.0 mg/kg, respectively, and 114.3 ± 3.9, 114.2 ± 4.1, and 116.3 ± 4.4 mm Hg during CO₂ inhalation, respectively. TEA treatment had no significant effect on the maximal changes in MBP induced by CO₂ inhalation.

Discussion

The present study provided evidence that the inhibitory responses of SAR activity to CO₂ inhalation were diminished by a well known K⁺ channel blocker, 4-AP, whereas TEA, a K⁺ channel blocker, had no effect on CO₂-induced SAR inhibition. Because blockade of CA-dependent hydration due to acetazolamide is known to attenuate the inhibitory responses of SAR activity to CO₂ inhalation (Sant’Ambrogio et al., 1974; Matsumoto et al., 1996) and wood smoke delivery (Lai and Kou, 1998), it is most likely that an increase in the H⁺ concentration at the receptor site inhibits the activation of 4-AP-sensitive K⁺ channels in the SAR terminals.

CO₂ inhalation usually induced a pressor effect. This effect was not significantly altered by pretreatment with either 4-AP or TEA. Hargreaves et al. (1991) reported that during
graded increases in mean left atrial pressure in the rabbit, there was a small but statistically significant increase in SAR activity during inflation. Pulmonary venous congestion is known to increase the pressure in both the left atrium and the pulmonary veins (Braunwald, 1988). If CO₂ inhalation is permitted to cause the development of pulmonary venous congestion, one can expect that an increase in SAR activity during inflation actually occurs during CO₂ inhalation, but such no effect was observed in this study.

Regarding several K⁺ channels with different kinetic and pharmacological properties, the three most prevalent types of K⁺ channels have been classified as “delayed rectifier” (Hodgkin and Huxley, 1952), “Ca²⁺-activated K⁺” (Meech and Standen, 1975), and “fast transient outward” (Connor and Stevens, 1971) currents. The fast transient outward K⁺ currents (Iₒs) are identified in invertebrates (Hagiwara et al., 1961; Connor and Stevens, 1971; Neher, 1971) as well as in vertebrate neurons (Adams et al., 1982; Gustafsson et al., 1982). Furthermore, frog myelinated axons have at least three different and distinct types of K⁺ channels, which are characterized by the two fast and slow K⁺ conductances; 4-AP blocks the fast conductances and TEA blocks the fast and slow conductances (Dubois, 1981; Grissmer, 1986). In mammalian myelinated axons, the two pharmacologically different types of K⁺ channels are also identified in the peripheral (Baker et al., 1987; Kocsis et al., 1987) and central (Kocsis et al., 1986; Gordon et al., 1988; Thorn et al., 1991) nervous systems: one is sensitive to 4-AP, but the other is sensitive to TEA. There are functional differences between 4-AP- and TEA-sensitive K⁺ channels in the myelinated axons of the rat sciatic nerve fibers because the 4-AP-sensitive K⁺ channels are related to action potential repolarization, but the TEA-sensitive K⁺ currents cause the afterhyperpolarization after repetitive activity (Kocsis et al., 1987). Although the application of 4-AP results in the broad spike of action potentials (Kocsis et al., 1987; Thorn et al., 1991; Poulter and Padjen, 1995), such an effect could not be confirmed in this study because we were measuring extracellular action potentials. In addition, we found that the administration of 4-AP increased the discharge of SARs during deflation in a dose-dependent manner and caused a pressor effect reported by other investigators (Yanagisawa and Taira, 1979; Chung et al., 1996), but the latter effect was very short lasting. The former effect is probably explained by evidence demonstrating that 4-AP can elicit both membrane depolarization and repetitive firing in squid axons (Yeh et al., 1976a,b). Presumably, specific actions of 4-AP on the expiratory discharge of SARs readily appear in the condition in which there is no mechanical deformation due to lung inflation. In the whole-cell patch-clamp study with nerve terminals of the rat posterior pituitary, which are acutely dissociated and identified by both morphological and immunohistochemical techniques, 4-AP and cesium block Iₒs in a dose-dependent manner, but TEA (100 mM) and charybotoxin at a concentration (4 μg/ml) that blocks Ca²⁺-activated K⁺ currents (Miller et al., 1985) have no effect on Iₒ (Thorn et al., 1991). They also found no evidence of a delayed rectifier K⁺ current (Thorn et al., 1991). In this study, 4-AP at a relatively smaller dose of 0.7 mg/kg significantly suppressed the inhibitory responses of SAR activity to CO₂ inhalation, and the administration of 4-AP up to 2.0 mg/kg abolished CO₂-induced SAR inhibition. Because pretreatment with a CA inhibitor prevents CO₂-induced SAR inhibition (Sant'Ambrogio et al., 1974; Matsumoto et al., 1996), the results of this study led us to suggest that an increase in [H⁺] at the receptor site may involve inhibition of Iₒ of the SAR terminals, which are the major currents responsible for terminal repolarization after a spike. In other words, the blocking effect of K⁺ efflux attenuates the repolarization of the membrane potential of SAR terminals and, as a result, decreases the number of action potentials. In other neuronal structures, a decrease in extracellular pH induced by CO₂ would cause hyperpolarization in accordance with increases in Cl⁻ or K⁺ conductances (Brown, 1972). However, further studies are needed to define the relationship between the Cl⁻ transport coupled to Cl⁻/HCO₃⁻ and/or Cl⁻/HCO₃⁻ exchanger systems and the inhibition of SAR activity associated with CO₂ inhalation.

In the nodal membrane of rat myelinated nerve fibers, the slow TEA-sensitive K⁺ conductance is prevalent (Roeppe and Schwarz, 1989). A similar localization of TEA-sensitive channels was reported by Baker et al. (1987). In the sucrose gap recordings obtained from sciatic nerves of immature and
mature rats, TEA (10 mM) application alone has little effect on the wave form of the compound action potential at any age but blocks 4-AP-induced postspike positivity (hyperpolarization; Eng et al., 1988). The results indicate that the slow K⁺ channels sensitive to TEA are not responsible for repolarization after single action potentials. Indeed, in this study, pretreatment with TEA (2.0 and 6.0 mg/kg) that did not significantly alter the basal activity of SARs had no effect on CO₂-induced SAR inhibition. Furthermore, there is evidence that TEA only slightly reduces Iₚs. TEA-sensitive K⁺ channels might, therefore, not play a significant role in the inhibitory response of SARs to CO₂ inhalation, but the possibility that TEA did not reach the SAR endings at concentrations sufficient to block K⁺ channels cannot be completely excluded.

TEA blocks voltage-dependent K⁺ conductances as well as some Ca²⁺-activated K⁺ conductances (Rudy, 1988). In particular, the large conductance Ca²⁺-activated K⁺ channels (Miller et al., 1985) are known to be blocked by TEA (Blatz and Magleby, 1987), and this blocking effect acts more effectively at the external membrane surface than at the internal membrane surface (Adams et al., 1982; Latorre et al., 1982). In this study, however, the administration of TEA at a higher dose (6 mg/kg) did not significantly modify the inhibitory responses of SAR activity to CO₂ inhalation, so the large conductance Ca²⁺-activated K⁺ channels sensitive to TEA might not contribute greatly to the mechanism of CO₂-induced SAR inhibition. Further studies are necessary to determine whether the inhibitory effect of CO₂ on SAR activity is related to the functioning of the stretch-activated channels on the receptor endings.

In conclusion, the inhibitory responses of SAR activity to CO₂ inhalation were blocked by pretreatment with 4-AP, but TEA treatment did not significantly alter the CO₂-induced inhibition of SAR activity. The results suggest that inhibition of SARs by CO₂ inhalation may be mediated by the stimulating action of 4-AP-sensitive K⁺ currents in the nerve terminals of SARs.

References