Inhibitory effect of inhaled wood smoke on the discharge of pulmonary stretch receptors in rats

C. J. LAI AND Y. R. KOU
Institute of Physiology, School of Medicine and Life Science, National Yang-Ming University, Shih-Pai, Taïpeï, Taiwan 11221, Republic of China

Lai, C. J., and Y. R. Kou. Inhibitory effect of inhaled wood smoke on the discharge of pulmonary stretch receptors in rats. J. Appl. Physiol. 84(4): 1138–1143, 1998.—We investigated the inhibition of slowly adapting pulmonary stretch receptors (PSRs) by inhaled wood smoke. Impulses were recorded from PSRs in 68 anesthetized, open-chest, and artificially ventilated rats. Eighty-one of one hundred five receptors (PSRs) by inhaled wood smoke. Impulses were recorded from PSRs in 68 anesthetized, open-chest, and artificially ventilated rats. Eighty-one of one hundred five receptors were inhibited within one or two breaths when 6 ml of wood smoke were delivered into the lungs. As a group (n = 105), PSR activity significantly decreased from a baseline of 19.0 ± 1.3 (SE) to a lowest level of 12.9 ± 1.2 impulses/breath at the fourth or fifth breath after smoke delivery. This afferent inhibition usually persisted for 5–18 breaths. In contrast, smoke delivery did not affect transpulmonary pressure. Delivery of gas-phase smoke or a hypercapnic gas mixture containing CO2 at a concentration (15%) matching that in the smoke produced a nearly identical inhibition in the same PSRs (n = 10). This afferent inhibition was largely prevented by pretreatment with acetazolamide (an inhibitor of carbonic anhydrase; n = 10) but was not affected by pretreatment with the vehicle for acetazolamide (n = 8) or isoproterenol (a bronchodilator; n = 10). These results suggest that 1) an increase in H+ concentration resulting from hydration of CO2 in the smoke may be responsible for the inhibitory effect of wood smoke on the discharge of PSRs and 2) changes in lung mechanics are not the cause of this afferent inhibition.

airway irritation; vagal sensory receptors; carbon dioxide; hydrogen ion; carbonic anhydrase

SPONTANEOUS INHALATION of wood smoke (−6 ml) via tracheostomy immediately triggers reflex changes in breathing patterns in anesthetized rats (13, 14). Both lung vagal C-fiber nerve endings and rapidly adapting receptors are believed to be involved in eliciting the immediate ventilatory responses to wood smoke (14). Indeed, electrophysiological studies in rats have shown that these two types of pulmonary receptors are stimulated by delivery of a similar amount of wood smoke into the lungs (16, 17).

Slowly adapting pulmonary stretch receptors (PSRs), the third type of lung vagal sensory receptors, also play an important role in the regulation of breathing patterns (8, 26). In contrast to the other two types of pulmonary receptors, however, PSRs are known to be more susceptible to inhibition by chemicals (8). For example, inhalation of a high concentration of CO2 inhibits the activity of PSRs in a number of mammalian species (1, 3, 9, 10, 15, 21, 23, 25, 27, 28). The inhibitory action of CO2 on PSRs has been attributed to an increase in H+ concentration at the receptor site (10, 21, 23, 25, 27) and changes in the airway smooth muscle tone (22, 25). The former is supported by the finding that the CO2-induced inhibition of PSR activity is abolished when hydration of CO2 is prevented by carbonic anhydrase inhibitors (21, 25, 27). The latter is based on the fact that CO2 alters lung mechanics (12). Because of combustion or thermal decomposition, the gas phase of wood smoke contains a high concentration of CO2 (14, 18). Therefore, it is possible that inhaled wood smoke may exert an inhibitory effect on the discharge of PSRs through the action of CO2 contained in the smoke. However, experimental evidence to support this possibility remains to be established. Furthermore, if inhaled wood smoke does produce such an effect, the underlying mechanisms warrant investigations.

In this study, we recorded afferent activity arising from PSRs in anesthetized rats to determine 1) whether these pulmonary receptors are inhibited by delivery of wood smoke into the lungs and, if so, 2) whether CO2 in the gas phase of wood smoke is responsible for this afferent inhibition, 3) whether this afferent inhibition is associated with hydration of CO2, and 4) whether this afferent inhibition is linked to changes in lung mechanics after smoke delivery.

METHODS

Sprague-Dawley rats (weight 326 ± 6 g) of either sex were anesthetized with intraperitoneal injection of chloralose (100 mg/kg; Sigma Chemical) and urethan (500 mg/kg; Sigma Chemical). A polyethylene catheter was inserted into the jugular vein and advanced until the tip was close to the right atrium for intravenous administration of pharmacological agents. The right femoral artery was cannulated for measuring arterial blood pressure. During the course of the experiments, supplemental doses of chloralose (20 mg·kg−1·h−1·iv) and urethan (100 mg·kg−1·h−1·iv) were administered to maintain abolition of the corneal reflex and pain reflexes induced by tail pinch. During the recording of vagal action potentials, the rats were paralyzed with pancuronium bromide (0.05 mg/kg iv; Orgon Teknika). Periodically, the effect of pancuronium was allowed to wear off so that the depth of anesthesia could be checked.

The rats were tethered in a supine position, and the trachea was cannulated below the larynx with a short tracheal tube via a tracheotomy. A midline thoracotomy was performed, and the edges of the rib cage were retracted. The lungs were ventilated by a respirator (model 683, Harvard) at a constant volume of 2 ml. The frequency of the respirator was set at 65–75 breaths/min and was kept constant in each experiment. The expiratory outlet of the respirator was placed under 3–4 cm of water to maintain a near-normal functional residual capacity. Tracheal pressure (Ptr; i.e., transpulmonary pressure in open-chest preparation) was monitored by a pressure transducer (MP45-28, Validyne) via a side tap of the tracheal cannula. Body temperature was maintained at ~36°C by a servo-controlled heating blanket throughout the experiment.
Recording of afferent activity of PSRs. Afferent activity arising from PSRs was recorded by using techniques described elsewhere (19). Briefly, a fine afferent filament was split from the desheathed nerve trunk of the right vagus and was placed on a platinum-iridium recording electrode. Action potentials were amplified (PS11K, Grass), monitored by an audio monitor (AM8, Grass), and displayed on an oscilloscope (model 420, Gould). The fine nerve filament was subdivided until activity from only one or two units was obtained. Afferent activity was counted by a rate meter (RIC-880, CWE), the window discriminators (model 121, WP1) of which were set to accept action potentials of a selected amplitude. All physiological signals were simultaneously recorded by a thermal-array recorder (TA11, Gould) and recorded on tape (DR-890, Neurorecorder) for later analysis.

PSRs were easily recognized by their distinct baseline discharge in phase with ventilatory cycles. To confirm the receptor type, the lungs were hyperinflated in a stepwise manner to four times the tidal volume (4 × VT) or by constant pressure inflation (~20 cmH2O), which was maintained for 10–15 s. The receptor responses to lung deflation were also studied by exposing the expiratory outlet of the respirator to atmospheric pressure for a period of 10–15 s. Conduction velocity of the afferent fiber arising from the individual receptor was measured in the majority (71 of 105) of the receptors studied by the method described by Bergren and Peterson (2). Two criteria were used to consider the receptors as PSRs in this study: 1) an adaptation index to maintained lung inflation was <50% (30) and 2) conduction velocity, whenever measured, was within the range of myelinated fibers (~2 m/s). Before the end of each experiment, the general location of the receptors studied was identified within the lung structure by gently probing the tissues with a polyethylene rod (2-mm diameter).

Generation and delivery of smoke. The electric furnace and the methods for generating wood smoke have been described in detail in our previous study (13). In brief, 100 g of dry wood dust (lauan wood) were thermally decomposed by the furnace at a core temperature maintained at 500 ± 8°C for 5 min, and the effluent smoke was collected in a 25-liter plastic balloon attached to the furnace outlet. Gas-phase smoke was generated by passing the wood smoke through a glass-fiber Cambridge filter, which removed >99% of the smoke particulates (14). The smoke was sampled and analyzed for its O2 (OM-11, Beckman), CO2 (L-2, Beckman), CO (model 961, Neotronics), and particulate (P-SH2, Sibata) concentrations. Unfiltered smoke generated by this method contains ~1.5% O2, 15% CO2, 24% CO and 25 mg/l particulates (13, 14). The gas-phase smoke contains similar concentrations of these gases but is free of particulates (14). Immediately after its generation, 6 ml of unfiltered smoke or gas-phase smoke at a temperature of ~25°C were delivered by the respirator in three ventilatory cycles by using a circuit similar to that described previously (19). To avoid contamination, the tubing of the circuit was replaced after each smoke delivery.

Pharmacological agents. Acetazolamide (an inhibitor of carbonic anhydrase; Sigma Chemical) was first dissolved in 1 N NaOH (200 mg/ml) and then diluted in isotonic saline to a concentration of 20 mg/ml. Isoproterenol (a bronchodilator; Sigma Chemical) was dissolved in isotonic saline to a concentration of 0.1 mg/ml. The stock solution of acetazolamide (20 mg/kg) or isoproterenol (0.1 mg/kg) required for each animal was further diluted in saline to a final volume of 0.7 ml and was slowly injected into the vein over a period of 20 s. The dose of acetazolamide has been reported to inhibit 99.99% of carbonic anhydrase (20). The dose of isoproterenol has been shown to block the bronchoconstriction evoked by vagal stimulation in rats (11).

Experimental procedures. A total of 105 PSRs were recorded from 68 rats to study their control afferent responses to unfiltered wood smoke. In 10 PSRs recorded from 10 rats, afferent responses to deliveries of gas-phase smoke, a hypercapnic gas mixture (15% CO2–20% O2–balance N2), and air were compared with those to delivery of unfiltered smoke. The concentration of CO2 in the gas mixture was chosen to match that existing in the unfiltered or gas-phase smoke. The challenges of gas-phase smoke, the gas mixture, and air were conducted in an alternate sequence to achieve a balanced design. In another 28 PSRs recorded from 28 rats, challenges of unfiltered smoke were repeated after the animals had been pretreated with acetazolamide (n = 10), the vehicle for acetazolamide (n = 8), or isoproterenol (n = 10). Before each test of delivery of smoke, the gas mixture, or air, the animal’s lungs were hyperinflated (4 × VT) to maintain a constant volume history. An interval of at least 30 min elapsed between two deliveries of smoke or deliveries of smoke and the hypercapnic gas mixture to avoid tachyphylaxis; our preliminary data indicated that the receptor responses to smoke or to the gas mixture were reproducible when this period of recovery time was allowed. A 10-min period elapsed before the study was resumed after administration of vehicle, acetazolamide, or isoproterenol.

Data analysis and statistics. PSR discharges that occurred during inflation, deflation, and both phases and Ptr were measured on a breath-by-breath basis. Baseline data of these physiological parameters were calculated as the average values over the 10-breath period immediately preceding the challenge of smoke, the hypercapnic gas mixture, or air. A PSR was judged to be inhibited by the smoke or the gas mixture when its activity was reduced by at least 20% of its baseline for three consecutive breaths or more. These physiological parameters were analyzed by using a computer equipped with an analog-to-digital converter (DASA 4600, Gould) and software (BioCybernatics 1.0). Results obtained from the computer analysis were routinely checked for accuracy with those calculated manually. Results were analyzed by a paired t-test or a one-way repeated-measures analysis of variance followed by Duncan’s test when appropriate. P < 0.05 was considered significant. All data are presented as means ± SE.

RESULTS

All the PSRs studied exhibited a baseline activity in phase with ventilatory cycles. Fifty-five, three, and forty-seven receptors fired during inflation, deflation, and both phases, respectively. The evoked discharge of each receptor in response to maintained lung inflation was adapted slowly, and the mean adaptation index reached only 11.8 ± 0.9% (range: 1.0–36.6%; n = 105). Nineteen PSRs were stimulated by lung deflation, whereas the other 86 were inhibited. The average conduction velocity of the afferent fibers conducting impulses from 71 of these PSRs was 32.1 ± 1.0 m/s (range: 15.5–48.0 m/s); the conduction velocity of the remaining 34 was not measured. All receptors were localized within the lung structure, and their physiological properties were consistent with those previously reported in rats (2) and in other species (8, 26).

Of the 105 PSRs studied, 81 were inhibited within one or two breaths when 6 ml of unfiltered wood smoke were delivered into the lungs. When inhibited, the
Discharge of PSRs either totally vanished or was largely reduced (Fig. 1A). This afferent inhibition reached its maximum at the fourth or fifth breath after smoke delivery and usually persisted for 5–18 breaths before the activity returned to its normal baseline (Fig. 2A). In 37 PSRs having baseline activity that occurred during both ventilatory phases, the receptor discharge was more depressed by wood smoke during deflation phase than during inflation phase (Fig. 1A); their activity was reduced maximally by 88.3 ± 3.5% (n = 37) of the baseline value for deflation phase and 38.1 ± 4.4% for inflation phase. In the remaining 24 PSRs, their activity was not significantly affected by smoke challenge (Fig. 2B). For the whole group of 105 PSRs, the receptor discharge significantly decreased from a baseline of 19.0 ± 1.3 impulses/breath to a lowest level of 12.9 ± 1.2 impulses/breath during this initial period after smoke delivery.

In 10 PSRs initially inhibited by unfiltered smoke, their afferent responses were compared with those evoked by deliveries of gas-phase smoke, a hypercapnic (15% CO₂-20% O₂-balance N₂) gas mixture, and air. These two types of smoke and the hypercapnic gas mixture essentially produced a similar pattern of afferent inhibition in the same PSRs (Figs. 1 and 3A). In contrast, delivery of air did not significantly affect the discharge of PSRs (Fig. 3A). On average, the maximum reduction in PSR activity produced by unfiltered smoke (45.1 ± 9.5% of the baseline activity; n = 10) was not significantly different from that produced by gas-phase smoke (46.4 ± 9.6%) or by hypercapnic gas mixture (42.9 ± 9.7%).

In 28 PSRs initially inhibited by unfiltered smoke, smoke challenges were repeated after the animals had been pretreated with acetazolamide (n = 10), vehicle for acetazolamide (n = 8), or isoproterenol (n = 10). Ten minutes after pretreatment with vehicle or these chemicals, the average baseline activity of these PSRs did not change significantly (P > 0.05; Fig. 4). In the acetazolamide-treated group, a repeated smoke challenge no longer reduced the receptor activity in seven PSRs (Fig. 4A) while it produced a very mild inhibition in the other three. As a result, the overall responses of PSRs to wood smoke were largely diminished (Fig. 5A). In contrast, in the vehicle- (Fig. 5B) or isoproterenol-treated group (Figs. 3B and 4B), a repeated smoke challenge produced an afferent inhibition of similar amplitude and time course in the same PSRs compared with their control responses. On average, the maximum reduction in PSR activity produced by unfiltered smoke after acetazolamide reached only 12.9 ± 2.5% of the baseline activity (n = 10), which was significantly smaller than that before acetazolamide (53.4 ± 9.4%). Furthermore, the maximum reduction in PSR activity produced by unfiltered smoke after vehicle (37.9 ± 4.3% of the baseline activity; n = 8) or after isoproterenol (68.5 ± 7.4%; n = 10) was not significantly different from the control response (before vehicle, 34.4 ± 4.1%; before isoproterenol, 66.7 ± 8.5%).

Under control conditions, delivery of unfiltered wood smoke did not cause any detectable change in P trị (Figs. 1A and 4). The response of P trị, averaged over the 10-breath period immediately after the smoke delivery, was 8.4 ± 0.1 cmH₂O (n = 105), which was not significantly different from its baseline value (8.5 ± 0.1 cmH₂O).

DISCUSSION

Results of this study demonstrate that a majority of the PSRs studied (77%) were promptly inhibited when three tidal breaths of wood smoke were delivered into the lower airways and lungs. This afferent inhibition seems to be linked to the components in the gas phase of wood smoke because the PSR response was not affected by removal of smoke particulates. Additionally,

Fig. 1. Afferent responses of a pulmonary stretch receptor to unfiltered wood smoke (A) and to a hypercapnic gas mixture (B) in an anesthetized rat. Six milliliters of smoke or gas mixture were delivered in 3 ventilatory cycles into the lungs, as indicated by horizontal bars. Gas mixture (15% CO₂-20% O₂-balance N₂) contained CO₂ at concentration matching that in wood smoke. Thirty minutes elapsed between delivery of smoke and gas mixture. AP, action potential; P trị, tracheal pressure; FA, fiber activity expressed as impulses (imps)/0.1 s.
delivery of a hypercapnic gas mixture containing CO₂ at a concentration matching that in the smoke produced an almost identical afferent inhibition in the same receptors. Hence, our results suggest that CO₂ may be the causative smoke component responsible for the inhibitory action of wood smoke on PSRs. Indeed, the inhibition of PSR activity became apparent within the first two ventilatory cycles after smoke delivery and was more prominent in the deflation phase than in the inflation phase. These notable features are very similar to those of the CO₂-induced inhibition of PSR activity reported by other investigators (9, 15, 21, 23, 27). The effect of wood smoke was rapid probably because PSRs are readily accessible to the increased CO₂ in the airway lumen (1, 26). The differential effect of wood smoke on the discharge occurring during inflation and deflation phases is presumably due to the fact that the inhibitory action of CO₂ is weaker at a higher transmural pressure (1, 23).

Several mechanisms have been suggested to explain how CO₂ in the airways diminishes the discharge of PSRs, including a direct effect of CO₂ mediated by an increase in H⁺ concentration (21, 23, 25, 27) and an indirect effect resulting from changes in airway smooth muscle tone produced by CO₂ (22, 25). In this study, pretreatment with acetazolamide largely prevented the smoke-induced inhibition of PSR activity, whereas pretreatment with its vehicle failed to do so. Acetazolamide is an inhibitor of carbonic anhydrase, a key enzyme that catalyzes the hydration of CO₂ to yield H⁺ and HCO₃⁻ (20). Therefore, our findings suggest that the inhibitory action of wood smoke on PSRs may arise from the ability of CO₂ in the smoke to produce H⁺. The exact mechanics by which an increase in H⁺ concentration elicits a decline in PSR activity still remain obscure. However, in other neural structures, a decrease in extracellular pH produced by CO₂ may lead to increases in Cl⁻ and K⁺ conductances, which in turn would induce hyperpolarization of the neuronal membrane (4). In fact, a similar mechanism has been recently proposed by Matsumoto et al. (21) to explain the inhibitory action of CO₂ on PSRs. These investigators (21) found no evidence of carbonic anhydrase activity in the smooth muscle of bronchi and postulated that this enzyme may localize in the nerve terminals of PSRs to catalyze the reaction. This postulate was based on the finding that carbonic anhydrase activity has been identified in myelinated afferent fibers of peripheral nerves in rats (5, 29). On the other hand, delivery of wood smoke did not produce any detectable change in Ptr when PSR activity was inhibited, suggesting that changes in lung mechanics may not be the cause of this afferent inhibition. This notion is further supported by the present finding that the smoke-induced inhibition of PSR activity was not affected by prior administration of isoproterenol. The observation is compatible with the findings that the use of bronchodilators does not prevent the inhibition of PSR activity by CO₂ (9, 27). Kunz et al. (15) have suggested the possibility that the inhibitory action of CO₂ on PSRs may in part be due to local changes in the airway smooth muscle tone at the receptor site, even though the overall Ptr is not altered. However, unless wood smoke could alter the airway smooth muscle tone even after pretreatment with isoproterenol, this possibility is questionable.
We found that acetazolamide did not significantly alter the average baseline activity of PSRs in this study; this is in fact a net result from a slight increase in baseline activity in six receptors and a decrease in the other four. Our result is consistent with the finding in rabbits that acetazolamide did not significantly modify the baseline PSR activity occurring during both inflation and deflation (21). The effect of acetazolamide on the baseline PSR activity in dogs (27) and in cats (25), however, is not known because it was not reported. In other CO₂-sensitive receptors, an inhibition of carbonic anhydrase has been shown to change the baseline receptor discharge (see references of Ref. 7). For example, Coates et al. (7) reported that systemic administration of acetazolamide produced an increase in baseline discharge in laryngeal CO₂ receptors, which was attributed to a rise in receptor or tissue pH because of the suppressive effect of H⁺ on the receptor activity. On the other hand, systemic administration of acetazolamide may result in tissue hypercapnia, which would decrease baseline receptor discharge. Perhaps our finding that the effect of acetazolamide on the baseline PSR activity varied among the animals studied may reflect the difference in receptor or tissue pH caused by the carbonic anhydrase inhibitor.

In this investigation, 23% of the PSRs studied did not respond to delivery of wood smoke. There are at least four possible explanations for the absence of afferent inhibition in these PSRs. First, the transduction properties of these PSRs might be different from those of the PSRs inhibited by wood smoke. Second, these PSRs might be localized in a lung region that was poorly ventilated and thus were not accessible to the smoke. Third, it has been found in dogs that PSRs localized in extrapulmonary airways are relatively insensitive to CO₂ compared with those localized in intrapulmonary airways (1, 27). It is very plausible that the PSRs with activity that was not affected by wood smoke were localized in extrapulmonary airways. Unfortunately, this possibility could not be confirmed in each of these PSRs because of the small body size of the animals and the limitation of our preparation. Fourth, although the distribution of carbonic anhydrase in the lungs is still not clear, there may be a regional difference in the activity of this enzyme in pulmonary tissues (24). Accordingly, the absence of afferent inhibition in these PSRs could possibly be attributed to a lack of carbonic anhydrase at receptor sites.

In our previous studies in rats (13, 14), we reported that the acute ventilatory responses to inhalation of wood smoke consisted of two phases: either a slowing of respiration, or an augmented breath triggered within one or two breaths after the smoke was inhaled and then a delayed tachypnea. The slowing of respiration and the augmented breath may originate from the stimulation of lung vagal C-fiber nerve endings and
Effect of CO\(_2\) on arterial peripheral chemoreceptors (13, 14). It is generally believed that the afferent information arising from PSRs exerts an inhibitory influence on the respiratory center (8). A decrease in PSR discharge in acute ventilatory responses, it seems reasonable to postulate that, although the diminished PSR activity observed in this study may dispute the occurrence of the slowing of respiration, it may contribute to the elicitation of the augmented breath and the delayed tachypnea after smoke inhalation.

In summary, inhaled wood smoke inhibits the discharge of PSRs, and this afferent inhibition may be mediated through an increase in H\(^+\) concentration resulting from hydration of CO\(_2\) in the smoke. Inhalation of wood smoke immediately evokes airway irritations (18), but the underlying mechanisms are not fully understood. The observations made in this and our previous studies (16, 17) provide the electrophysiologic evidence that all three major types of lung vagal sensory receptors play an important role in detecting the airway assault by wood smoke.

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