Effects of Trichloroethylene and Perchloroethylene on Muscle Contractile Responses and Epithelial Prostaglandin Release and Acetylcholinesterase Activity in Swine Trachea

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Received May 20, 2004; accepted September 6, 2004

Trichloroethylene (TCE) and perchloroethylene (PERC) have been reported to induce respiratory complications such as airway hyperactivity and asthma. The present study was designed to investigate their influence on smooth muscle contraction and epithelial release of prostanooids in swine trachea. Results showed that TCE and PERC exposure did not alter the basal tone of tracheal smooth muscle. However, TCE and PERC concentration-dependently increased both ACh-induced and high K+-induced muscle contraction. In addition to potentiation of muscle contractile responses evoked by acetylcholine or histamine, pretreatment of smooth muscle with PERC at higher concentration significantly suppressed the relaxant activity of beta-adrenergic agonists. The epithelial prostaglandin (PG)E2, but not PGD2, release from tracheal epithelium was significantly increased by TCE and PERC. In addition, the acetylcholinesterase (AChE) activity of tracheal epithelia was reduced by TCE and PERC exposure. In conclusion, our results suggest that the enhancement of spasmogen-evoked muscle contractile responses and epithelial PG E2 secretion, as well as reduction of epithelial AChE activity, may participate in airway impairment and hyperresponsiveness after TCE and PERC exposure.

Key Words: trichloroethylene; perchloroethylene; trachea; swine; prostaglandin; acetylcholinesterase.

INTRODUCTION

Trichloroethylene (TCE) and perchloroethylene (PERC), two chlorinated hydrocarbons, are widely used in industry as degreasers, dry cleaning agents, paint removers, solvents for chemical extraction, and components of adhesives and lubricants. Most significant exposure to TCE and PERC occurs in the workplace. The majority of high exposures of TCE and PERC are ascribed to metal-fabricating and degreasing operations, and then to dry cleaners (NIOSH, 1994; IARC, 1995). In addition, pollution of groundwater and drinking water by these compounds has occurred (Kilburn, 2002; Kuo et al., 1997). Although, American Conference of Governmental Industrial Hygienists (ACGIH) recommends thresholds, the limits of values for TCE are 50 ppm (time-weighted average (TWA)) and 100 ppm (short-term exposure limit (STEL)) and those for PERC are 25 ppm (TWA) and 100 ppm (STEL), and people can be accidentally exposed to high concentrations of TCE and PERC from occupational and environmental sources and from consumer products.

Since inhalation is the most important route of TCE and PERC exposure, concerns over potential adverse effects on human health, particularly the respiratory tract, have been heightened (Burg and Gist, 1999; Langworth et al., 2001). TCE affects cilia activity (Tomenius et al., 1979) and also produces a thinner tracheal epithelial layer along with intraluminal hemorrhage as well as inflammatory cell infiltration into the underlying connective tissue (Koptagel and Bulut, 1998). PERC can cause acute symptoms of cough and dyspnea in normal subject and a severe bronchospasm in asthmatic subject following an intense short-term exposure, indicating the potential to cause or aggravate asthma (Boulet, 1988). With a mild hyperresponsiveness to methacholine, the syndrome related to PERC fits the diagnostic criteria of reactive airways dysfunction syndrome and irritant-induced asthma. These two pulmonary disorders are clinically very similar; both are non-immunogenic forms of airway injury that may be associated with industrial inhalation exposure. In this situation, the direct toxic effect on the airways causes persistent airway inflammation and bronchial hyperreactivity.

The regulation of muscle tone is related to the induction of airway obstruction or hyper-responsiveness. In addition, the impaired epithelial production and/or release of inflammatory factors may contribute to airway inflammation and hyperresponsiveness (Goldie et al., 1988). The proinflammatory mediators prostaglandins have been implicated in the inflammatory cascade that occurs in asthmatic airways (Wenzel, 1997). Epithelium-derived AChE also plays an important role in cholinergic regulation in airways (Koga et al., 1992). Thus, we studied the effects of TCE and PERC on the basal and stimulant-induced contractile responses and epithelial prostaglandin release as well as acetylcholinesterase (AChE) activity.

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Toxicological Sciences vol. 83 no. 1 © Society of Toxicology 2005; all rights reserved.
in swine trachea in vitro. These endpoints may provide more inclusive toxicological profiles of these two chlorinated organic solvents on trachea to predict their potential to produce hyper-responsiveness and reactive airways dysfunction syndrome.

MATERIALS AND METHODS

Preparation of tracheal epithelial and smooth muscle strips. A number of evidence shows that there are many similarities in airways between porcine and humans (Roperto et al., 1993; Fabst, 1996). Therefore, swine airway is often used and proved to be a useful animal model for human disease. In this study, male Lanyu swine (15–20 kg) were purchased from Taiwan Livestock Research Institute (Taitung, Taiwan). The experimental protocol was approved by Committee of the Tzu Chi University for the Use of Animal Subjects. Swine were fed standard laboratory chow and tap water and were housed indoors under automatically controlled temperature and light cycle conditions in the animal facilities at Tzu Chi University. The preparation of tracheal tissue was performed as described previously (Lin et al., 2002). After anesthesia with 5% isoflurane and exsanguination, the trachea was removed immediately and transported in HEPES buffer solution containing the antibiotic penicillin (100 U/ml) and streptomycin (100 µg/ml). The trachea was opened longitudinally along the cartilage rings opposite the muscle and mounted lumen side up in a stretched position on a dissection tray. The epithelium was isolated from whole trachea (~20 rings) and cut into 4 segments. Each segment (about 0.1–0.2 g) was removed to culture dish with 3 ml HEPES buffer solution and incubated at 37°C. Then tracheal smooth muscle was left attached to small sections of the cartilage ring and almost cleaned of all connective tissue, epithelium, and mucus glands from both the mucosal and serosal surfaces. The small cartilage pieces were present to permit attachment to the force transducer and mounting block without damaging the muscle. The trachea muscle was then cut into separate individual muscle strips.

Measurement of isometric tension. Muscle tension was measured using an isometric force transducer interfaced to the computer via A/D converter. The smooth muscle strips were mounted vertically in a 10-ml water-jacketed organ bath filled with Krebs-bicarbonate buffer and gassed with a mixture of 95% O₂ and 5% CO₂ at 37°C. The bottom end of the strip was fixed to an L-shaped hook, and the top end was tied to a stainless-steel wire attached to a force displacement transducer (Grass FT.03) for monitoring changes in isometric force. Each strip was subjected to a load of 3 g and was allowed to equilibrate for at least 40–60 min before the drugs were applied to the organ bath. This was determined to be optimal for force generation. Muscle contraction is expressed as percentage of the contractile response initially contracted by KCl (40 mM) or acetylcholine (ACh, 1 µM), which is taken as 100%.

Enzyme immunoassays (EIA) of prostaglandins. After treatment of tracheal epithelial segments with TCE (100 ppm), PERC (100 ppm) or other agents for 2h, HEPES supernatants from the epithelial segments incubated at 37°C were collected and initially stored at −20°C until assayed for prostaglandins (PGs) and acetylcholinesterase (AChE) activity. For PGD₂ and PGE₂ measurements, an enzyme immunoassay (EIA) technique was used. In briefly, samples from previous supernatants were thawed slowly at 4°C and then centrifuged at 1200 g for 10 min. After centrifugation, 50 µl supernatant and 50 µl prostaglandin tracer were added to each well of a 96-well plate coated with anti-mouse IgG antibody, followed by prostaglandin monoclonal antibody (50 µl), and then the plate was incubated at 4°C for 18 hr. Next, each well of the plate was washed with the wash buffer 5 times and added with 200 µl Ellman’s reagent. Then the plate was shaken in the dark for 60 min and absorbance at 405 nm was determined using a microplate reader (Molecular Devices Corp., Sunnyvale, CA). The amount of PG was calculated from a standard curve of PGs.

Measurement of acetylcholinesterase activity. Acetylcholinesterase activity was measured by a modified method of Ellman and coworkers (Ellman and Callaway, 1961). Before assay, samples were thawed slowly at 4°C and then centrifuged at 1200 g for 15 min. The supernatant (200 µl) was then diluted with 600 µl phosphate buffer (pH 8), added with 100 µl dithiobisnitrobenzoic acid (DTNB, 0.01 M) and 100 µl acetylthiocholine (0.075 M) for assaying AChE activity, which was read at the wavelength of 412 nm using a spectrophotometer (Beckman Instruments, Irvine, CA). One unit (U) of enzyme activity is defined as 1 micromoles of acetylthiocholine hydrolyzed per minute at pH 8 and 25°C.

Materials. All dilutions of drugs were prepared on the day of the experiments. Stock solutions were made by dissolving TCE and PERC (Ridel-de Haen) in dimethylsulfoxide (DMSO, J. T. Baker) at 1% (v/v) or 10% (v/v). Acetylcholine (Sigma) was dissolved in distilled water. The PGD₂ and PGE₂ EIA kit was purchased from Cayman Chemical (Ann Arbor, MI). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

The HEPES buffer consisted of the following (mM): NaCl 140, KCl 5, CaCl₂ 2, glucose 5.5, HEPES 10, and pH was adjusted to 7.4. The Krebs-bicarbonate buffer was composed of (mM): NaCl 113, KCl 4.8, CaCl₂ 2.5, NaHCO₃ 18, KH₂PO₄ 1.2, MgSO₄ 1.2, glucose 5.5, mannitol 30, and pH was adjusted to 7.4.

Statistical analysis. Results were expressed as means ± S.E.M. Statistical significance of difference between groups was determined by ANOVA followed by Tukey’s multiple comparison test. Values of p < 0.05 indicate significant difference.

RESULTS

Effects of TCE and PERC on Muscle Contractile Responses

TCE and PERC at the concentrations of 3–1000 ppm did not alter the basal tension of tracheal muscle strips (data not shown). Neither did dimethylsulfoxide (DMSO) blank (0–200 µl), the vehicle of TCE and PERC, alter the basal tension of smooth muscle strips. Figure 1A shows the tracings of ACh- and KCl-evoked smooth muscle contractile responses altered by TCE in swine trachea. TCE at the concentrations of 10, 30, 100, and 300 ppm significantly increased the KCl-induced and ACh-induced contractions in tracheal smooth muscle (Fig. 1B). The KCl- and ACh-induced muscular contractile responses were also enhanced by PERC (10–300 ppm) (Fig. 1C). However, TCE and PERC at the higher concentration (1000 ppm) examined provoked a relaxant response in ACh- and KCl-induced muscle contractions (Fig. 1A). This relaxation response for TCE and PERC was maintained less than 10 min followed by gradually increasing tension.

As the muscle strips were pretreated with either TCE or PERC at the concentration of 100 or 1000 ppm for 20 min, the ACh-evoked and histamine-evoked muscle contractions were significantly potentiated by PERC at 1000 ppm but not at 100 ppm, whereas the ACh- and histamine-elicited responses were not significantly affected by TCE pretreatment at 100 and 1000 ppm (Fig. 2).

Effects of TCE and PERC on β-Adrenergic Agonist-Produced Smooth Muscle Relaxation

As previously reported, significant relaxation occurred with cumulative administration of 10⁻⁸ to 10⁻⁶ M isoproterenol (beta-adrenergic agonist) in ACh-induced muscle contraction. Pre-exposure of smooth muscle to TCE at 1000 ppm for 20 min did not significantly alter the isoproterenol-induced relaxation, whereas pretreatment of 1000 ppm PERC significantly reduced
the relaxant effects of isoproterenol at the concentrations tested (Fig. 3).

Effects of TCE and PERC on PGD₂ and PGE₂ Release from Tracheal Epithelium

To study the effects of TCE and PERC on epithelial release, the tracheal epithelium segments were pretreated with TCE and
PERC at 100 ppm for 2 h. TCE and PERC significantly enhanced the PGE2 release from tracheal epithelia. The increases in PGE2 release induced by TCE and PERC were inhibited by indomethacin (10 μM), which also suppressed the basal release of PGE2 from epithelium (Fig. 4B). Alternatively, epithelial PGD2 release was not significantly increased by TCE and PERC, whereas indomethacin decreased the PGD2 release in the absence and presence of TCE and PERC treatment (Fig. 4A).

**Effects of TCE and PERC on Acetylcholinesterase Activity**

The activity of acetylcholinesterase (AChE) was determined in the supernatant obtained from tracheal epithelium pretreated with TCE (100 ppm), PERC (100 ppm), or neostigmine (1 μM, an anticholinesterase agent) for 2 h. Significant decreases in AChE activity were observed in the neostigmine, TCE, and PERC treated groups compared with control group, which were 60.9 ± 3.5%, 82.8 ± 3.6%, and 83.9 ± 7.8%, respectively (Fig. 5). The AChE activity for control was 116.8 ± 9.8 mU per mg of epithelium segments (n = 5).

**DISCUSSION**

The relationship between exposures of volatile compounds and asthma has been reported (Burg and Gist, 1999). Acute exposure to PERC induced hyperreactive responsiveness in human airways (Boulet, 1988). Our data showed that acute exposure of tracheal smooth muscle denuded of epithelia to TCE and PERC did not directly alter the basal muscle tension, but concentration-dependently provoked an augmentation of contractile responses in muscle strips precontracted with KCl and ACh. High concentrations of PERC pretreatment enhanced the muscular contractile responses induced by spasmogens as well as suppressed the beta-adrenergic function in swine trachea, but TCE did not. It appears that PERC is more effective than TCE to potentiate the muscle contractile responses. Furthermore, both TCE and PERC increased tracheal epithelial PGE2 release but decreased the epithelial AChE activity. The present findings indicated that TCE and PERC interfere with both smooth muscle and epithelia. Therefore, airway hyper-responsiveness or reactive airways dysfunction syndrome might occur after exposure to these two volatile chlorinated hydrocarbons.

As the applied concentrations of TCE and PERC were raised to 1000 ppm, TCE and PERC transiently induced the relaxant...
response on smooth muscle precontracted by KCl and ACh. The relaxing action of TCE and PERC on muscle tension is consistent to that of other aliphatic chlorinated hydrocarbons in our previous study (Chan et al., 2002). The mechanism of their actions may not involve stimulation of beta-adrenergic receptors, since TCE and other volatile anesthetics were demonstrated to cause a marked positive chronotropic effect, which was unaltered in the presence of beta-adrenergic blocker propranolol in rat atrial preparations (Kriska and Paradise, 1977). Alternatively, TCE and PERC have been reported to decrease the amplitude of electrically induced intracellular free Ca\(^{2+}\) transients in rat cardiac myocytes (Hoffmann et al., 1994). The question is raised whether the relaxation response in airways exposed to chlorinated hydrocarbons is due to the decrease in intracellular Ca\(^{2+}\) mobilization. Assessing the degree of Ca\(^{2+}\) mobilization following treatment with TCE and PERC would help to address the question of mechanisms here.

Our results furthermore demonstrate that pretreatment of high concentrations of PERC enhanced ACh- and histamine-evoked muscle contraction and decreased the relaxant effects of beta-adrenergic activation on muscle precontracted by ACh. Since activation of muscarinic M2 receptors has inhibitory effect on beta-adrenoceptor agonists effects in tracheal smooth muscle (Zhang et al., 1996), it is possible that the reduction of the beta-adrenergic effect by PERC may be through, at least in part, activation of muscarinic M2 receptors. Alternatively, it cannot be excluded the possibility that beta-2 receptor activation is less effective under the larger muscle contraction potentiated by PERC. In contrast, TCE pretreatment did not alter the spasmodogen-stimulated muscle contraction and beta adrenoceptor activation. It seems that PERC pretreatment is more effective than TCE particularly in smooth muscle contractile responses. Our previous study also demonstrated that the aliphatic chlorinated hydrocarbons such as dichloromethane, dichloroethane, and trichloromethane exerted differential effects on the enhancement of tracheal muscle contractions induced by stimulants (Chan et al., 2002). Furthermore, these aliphatic chlorinated hydrocarbons directly provoke muscle contractile responses, but TCE and PERC did not. Thus, it is important to note that exposure of distinct chlorinated hydrocarbons to airways may produce differential effects on muscle contractile responses in trachea.

Airway epithelial injury and inflammation are involved in airway diseases such as asthma (Goldie et al., 1988). Prostaglandins, the endogenous proinflammatory mediators, have been implicated in the inflammatory cascade that occurs in airway allergic asthma (Raskovic et al., 1998; Wenzel, 1997). Our present results showed that TCE and PERC increased the prostaglandin release from tracheal epithelium, particular PGE\(_2\). This augmentation suggested to be mediated by the influence of enzyme cyclooxygenase or prostaglandin biosynthesis, since indomethacin inhibited TCE- and PERC-induced prostaglandin release from epithelium. Besides the increase in epithelial prostaglandin secretion, TCE was reported to induce inflammatory cell infiltration into the underlying connective tissue in rat tracheal mucosa (Koptagel and Bulut, 1998). Thus TCE and PERC appear to enhance the release of metabolites of arachidonic acid, resulting in promoting inflammation. Further study is required to elucidate whether the prostaglandin production from airway epithelia exposed to TCE and PERC results in airway inflammation.

Chlorinated aliphatic hydrocarbons including TCE and PERC have been shown to diminish human erythrocyte membrane AChE activity (Korpela and Tahti, 1986). TCE and PERC significantly decreased the AChE activity in swine tracheal epithelium. Since neostigmine, an AChE inhibitor, enhanced the muscle contractile response to Ach (1 \(\mu\)M) in the presence of epithelia (preliminary data), it is reasonable to speculate that the reduction in AChE activity from airway epithelium exposed to TCE and PERC may lead to the decrease in hydrolysis of ACh, resulting in augmenting cholinergic stimulation in airways. It is noteworthy that the effect of organic solvents on muscle contractility in the present study is not due to epithelial release of PGs or AChE, since the epithelia were denuded. Thus, the influence of epithelia exposed to chlorinated hydrocarbons on muscle contraction required further investigation.

In conclusion, the current findings demonstrate that exposure of smooth muscle to TCE and PERC enhanced the precontracted muscle tension in swine trachea. Pretreatment with TCE and PERC also potentiated the muscle contractile responses stimulated by spasmodogens and suppressed beta-adrenoceptor induced relaxation. Furthermore, TCE and PERC increased PGE\(_2\) release from tracheal epithelium but decreased epithelial AChE activity. Thus, inhalation of these two chlorinated hydrocarbons during occupational exposure may induce airway hyper-responsiveness triggered by spasmodgens, mediate beta-adrenoceptor hypofunction, and lead to airway inflammation. Therefore, high concentrations of exposure to TCE and PERC should be prevented, especially in the subjects exhibiting airway hyperresponsiveness, such as asthmatics.

ACKNOWLEDGMENTS

We are grateful to Dr. H. I. Chen for his kind equipment support and to S.-C. Jong for her technical assistance. This research was supported by grants NSC 89-EPA-Z-320-001 from the National Science Council, Taiwan.

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