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Brief exposure of air-filled guinea-pig isolated trachea to low levels of toluene diisocyanate (TDI) vapor in vitro increases reactivity to methacholine

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Abstract

Toluene diisocyanate (TDI) causes occupational asthma characterized by inflammation and hyperreactivity of airways to irritants and bronchoconstrictor drugs. We examined the non-immune, direct effect of TDI on airway reactivity in vitro in the absence of an inflammatory response using the guinea-pig isolated, perfused trachea preparation to measure reactivity to methacholine (MCh), and fixed point ion mobility spectrometry to measure moment to moment levels of TDI vapor in air that was delivered to the tracheal mucosa. MCh was added to the mucosal modified Krebs–Henseleit (MKH) perfusing solution to generate control concentration–response curves for contractile responses. The lumen was then emptied and perfused with air or air containing 5, 20 or 70 ppb TDI vapor, after which the trachea was perfused with MKH solution and reactivity to MCh was re-examined. After only 30 min of treatment, TDI vapor concentration-dependently increased reactivity of the trachea to MCh (2.4- and 2.9-fold, respectively, for 20 and 70 ppb TDI; 5 ppb TDI and air alone had no effect). In tracheas treated in vitro with 2 μM capsaicin to deplete tachykinins, TDI caused the same (4-fold) increase in reactivity to MCh that was observed in control tracheas. However, TDI vapor (70 ppb) no longer enhanced reactivity to MCh in tracheas from which the epithelium had been removed. Our results indicate that a direct, non-immune, non-inflammatory action of TDI on respiratory epithelium leads to hyperreactivity of airways in vitro. Published by Elsevier Science Ireland Ltd.

Keywords: Toluene diisocyanate; Airway; Hyperreactivity; Methacholine; Epithelium; Smooth muscle; Perfused trachea; Guinea-pig; Occupational asthma; Irritant; Capsaicin

1. Introduction

Toluene diisocyanate (TDI) is used widely in plastic and paint industries and is a leading cause

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of occupational asthma. Repeated airway exposures in the work place to TDI may result in the development of nonspecific airway hyperresponsiveness to methacholine (MCh) or histamine in humans (Cartier et al., 1989; Baur, 1990; Burge, 1991) as well as in animal models (Cibulas et al., 1988; Gagnaire et al., 1997).

An immunologically-mediated mechanism has been thought to be involved in the etiology of TDI-induced airway hyperresponsiveness, based on the finding of specific antibodies (IgE or IgG) against isocyanates in affected subjects and mucosal inflammation characteristic of an influx of polymorphonuclear leukocytes. However, the underlying pathophysiological mechanisms of TDI-induced asthma remain controversial because only 10–30% of the subjects with the disease demonstrated the specific antibodies (Karol and Jin, 1991; Baur et al., 1994; Fabbri et al., 1994). Recent evidence indicates that TDI probably stimulates the release of tachykinins in the airways, initiating a cascade of events that result in the development of airway hyperresponsiveness (Thompson et al., 1987; Mapp et al., 1991; Marek et al., 1996; Gagnaire et al., 1997). The involvement of a chemical, irritant effect of TDI, a reactive chemical, in the development of airway hyperreactivity is suspected but the relative importance of this effect in the presence of an inflammatory response and in relation to tachykinin release is unclear.

In addition to the difficulty of understanding the irritant effect of TDI in vivo in the face of inflammation, it has not been readily possible previously to examine the effect of TDI vapor in airway preparations which are bathed in physiological salt solutions, because the compound is unstable in water. To circumvent these limitations, we examined the direct effects of TDI vapor on reactivity of the airways using a novel in vitro preparation of air-filled whole trachea, using controlled delivery of airborne TDI in low concentrations that are relevant to work place exposures. We investigated in vitro for the first time the interaction of TDI vapor with airway epithelium and smooth muscle, and the effects of a brief exposure of low concentrations of TDI on reactivity of the intact trachea to MCh, using the isolated, perfused trachea preparation (Munakata et al., 1988; Fedan and Frazer, 1992). It was also of great interest to compare the effects of TDI on reactivity in untreated tracheas with that of tracheas treated in vitro with capsaicin to deplete the organ of tachykinins.

The respiratory epithelium is intimately involved in the regulation of airway smooth muscle reactivity. The epithelium is a source of degradative enzymes, such as neutral endopeptidase, and it produces substances such as epithelium-derived relaxing factor (EpDRF) and prostanoids that downregulate smooth muscle reactivity (Flavahan et al., 1985; Hay et al., 1986; Fedan et al., 1988; Goldie and Hay, 1997). Airway hyperreactivity caused by TDI might be induced by interfering with these mechanisms, as well as by disrupting the diffusion barrier afforded by the epithelium (Frossard et al., 1989; Tschirhart et al., 1990; Lamport and Fedan, 1990). The isolated, perfused trachea preparation has provided much information on the various reactivity-modulating roles of respiratory epithelium in health (Fedan and Frazer, 1992) and disease (Smith et al., 1993; Nijkamp et al., 1993; Fedan et al., 1995). This technique allows agents to be applied separately to the mucosal or serosal surfaces of the airway; the difference in reactivity to agents applied to each surface is a characteristic of epithelial integrity and the degree to which the epithelium is modulating reactivity of the smooth muscle in normal tracheas and in tracheas from animal models of human disease (Fedan and Frazer, 1992; Smith et al., 1993). For example, reactivity to agents such as MCh when applied to the serosal surface of the trachea, where there is no barrier to the diffusion of drugs to the smooth muscle, is greater than that seen after application of the agent to the mucosal perfusing solution, after which the agents must diffuse across the epithelium to gain access to the smooth muscle. However, in tracheas from which the epithelium has been mechanically removed, reactivity to mucosally-applied MCh is raised to the level seen after application to the serosal surface (Munakata et al., 1989; Pavlovic et al., 1989; Fedan and Frazer, 1992; Nijkamp et al., 1993). Thus, the perfused trachea preparation was employed be-

cause it is well-suited to examine whether the modulatory influence of the epithelium on reactivity of the smooth muscle is affected by TDI, and it also is a preparation that enables delivery of TDI vapor to the lumen of the same airway.

Therefore, our experiments tested the hypotheses that TDI has non-immune, direct actions in the airways which do not involve inflammatory cells, but which can increase reactivity to MCh. This hyperreactivity could involve effects of TDI on the epithelium or on neuropeptide-containing nerves in the airway wall, and would not be attributable to an inflammatory response in the absence of recruitable inflammatory cells.

2. Materials and methods

2.1. Perfused trachea preparation

Specific pathogen-free male English short-hair guinea pigs (450–680 g) were obtained from Harlan Sprague–Dawley (Indianapolis, IN). The animals were anesthetized (50 mg/kg sodium pentobarbital, i.p.) and exsanguinated. The preparation of tracheas for perfusion has been described in detail (Fedan and Frazer, 1992). Briefly, a 4-cm-long tracheal segment was removed and cleaned in modified Krebs–Henseleit solution (MKH; bubbled with 95% O_2–5% CO_2). The segment was mounted to a perfusion holder which contained side-hole catheters that were inserted into the lumen from each end. The holder was placed in a 25-ml bath of gassed MKH solution (37°C) (the extraluminal (EL) bath). The inlet and outlet ends of the indwelling catheters were connected to the positive and negative sides, respectively, of a differential pressure transducer. The trachea was perfused at a rate of 34 ml/min with gassed, recirculating MKH solution (37°C) from a separate, 30-ml bath (the intraluminal (IL) bath) with transmural pressure adjusted to zero. Responses were measured as changes in the inlet minus outlet pressure difference (ΔP in cm H_2O). The preparations were equilibrated for 1 h before experimental manipulations were begun, during which time they were washed at 15-min intervals by changing the EL and IL MKH solutions.

2.2. Epithelium removal

The epithelium was removed mechanically in some experiments to evaluate whether changes brought about by exposure to TDI vapor involved an interaction with the epithelium. Before mounting the trachea, a 5–6 cm piece of pipe cleaner (Fisher Scientific, Springfield, NJ), which had been trimmed with scissors to a size slightly larger than the trachea's diameter, was inserted slowly until the trailing end nearly entered the lumen. It was then withdrawn slowly while being rotated. This method has been demonstrated previously with histological analysis to remove > 95% of the epithelium (Fedan and Frazer, 1992) and to alter the response to intraluminally-added KCl (see Section 2.3).

2.3. Functional examination of epithelial integrity or effectiveness of epithelium removal using intraluminal addition of KCl

In preparations at the baseline ΔP level or after contraction with MCh, an elevation in the KCl concentration of the MKH perfusate does not cause a contractile response of epithelium-containing tracheal preparations; instead, intraluminal application of KCl to MCh-contracted trachea gives rise to a relaxation response (Munakata et al., 1988; Fedan et al., 1990; Fedan and Frazer, 1992). This relaxation is dependent upon the release of EpDRF by the epithelium. In contrast, intraluminally-applied KCl contracts epithelium-denuded tracheas in the same manner as serosally-applied KCl (Munakata et al., 1988; Fedan and Frazer, 1992). This behavior formed the basis of a routine, functional test to confirm epithelial integrity in intact tracheas, and to validate the effectiveness of epithelium removal in denuded tracheas. Thus, in every experiment, KCl (120 mM) was administered intraluminally at the end of the MCh concentration–response curves.

2.4. MCh concentration–responses curves; effect of intraluminal TDI exposure

Stepwise-increasing, cumulative additions of MCh were made to the IL bath to generate a

control concentration–response relationship. After the maximum response was reached, the preparation was washed at 15 min intervals for 1.5 h until ΔP returned to resting values. The MKH solution was then removed from the IL compartment, allowing the lumen of the trachea and the holder to contain only air. With the trachea still mounted to the holder and bathed in MKH solution, the lumen of the trachea was perfused with air (controls) or TDI vapor (see below) for 30 min. After the exposure, the trachea was perfused again with MKH and following a 1-h equilibration a second IL MCh concentration–response curve was obtained.

Attention was paid to whether or not air or TDI exposure affected basal ΔP prior to the second MCh concentration–response curve determination, which could influence reactivity to MCh independently of a treatment effect. However, we observed no effect of air or TDI exposure on the baseline ΔP values before delivering MCh (not shown).

2.5. Capsaicin pre-treatment

Some tracheal preparations were pretreated with capsaicin before the first concentration–response curve was generated to examine the potential role of tachykinins in TDI-induced alterations in reactivity. The preparations were incubated intraluminally for 30 min with 2 μM capsaicin, followed by washing at 15 min intervals for 1 h, at which time ΔP returned to resting values. The first MCh concentration–response curve was then obtained, and the remainder of the experiment proceeded as described above. There was no effect of incubation in ethanol vehicle in the control tracheas.

2.6. Generation of TDI vapor and delivery to the tracheal lumen

The effects of airborne levels of TDI in the ppb range were examined in this study. TDI vapor was generated by passing fresh, filtered air through a glass impinger containing TDI solution kept at 37°C. The vapor was led into a 3 l mixing chamber kept at 37°C and was diluted with fresh air to achieve the desired final TDI concentrations (5, 20, or 70 ppb). The concentrations actually achieved were (in ppb with range): 4.88 ± 0.21, 19.54 ± 1.71 and 68.92 ± 2.13, respectively. The vapor was pumped from the mixing chamber through the lumen of the trachea at a rate of 50 ml/min for 30 min.

The TDI level in the mixing chamber was monitored and regulated continuously by pumping vapor from the mixing chamber to the TDI detector, a fixed point ion mobility spectrometer (Environmental Technologies Group Inc., Baltimore, MD), and adjusting the flow rate of dilutant air to the mixing chamber to regulate the TDI concentration at the desired level. A display of the level was given by the instrument every second, providing the opportunity for moment-to-moment adjustments in dilutant air flow, which actually were not necessary once the detector became stabilized at the desired end point before delivery to the trachea. The detector measures both isomers of TDI, and has the range 0.5–102 ppb.

2.7. Histological examination

Tracheas were placed in 10% phosphate-buffered formalin at the conclusion of the concentration–response determinations. Paraffin sections (5 μm) were prepared from each end and the middle of the trachea and, after staining with modified Harris hematoxylin and eosin, were subjected to light microscopic examination of mucosal integrity.

2.8. Analysis of results

Responses of isolated, perfused tracheas were quantified as ΔP in cm H_2O. Geometric mean EC_{50} values for IL MCh concentration–response curves were derived from least squares analysis of logit curve fits (SigmaPlot®) and are presented along with 95% confidence intervals in parentheses. Other results are expressed as mean ± S.E.; n is the number of separate experiments. The results were analyzed for difference using Student's t-test for paired and unpaired data, as appropriate. Tests for differences in EC_{50} values were done using the normally-distributed $-\log EC_{50}$ values. $P < 0.05$ was considered significant.

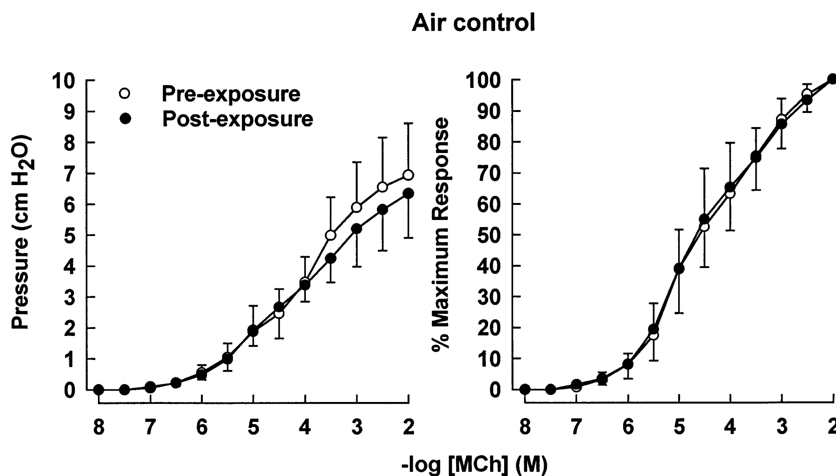

Fig. 1. Effects of air exposure on the intraluminal MCh concentration–response curve of isolated, perfused trachea. Two curves were obtained from each preparation, the first before (○) and the second after (●) exposure to air ($n = 6$).

2.9. Solutions and reagents

MKH solution contained (mM): NaCl, 113.0; KCl, 4.8; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; and glucose 5.7 (pH 7.4, 37°C). Methacholine (acetyl-β-methylcholine chloride) and capsaicin were purchased from Sigma (St. Louis, MO). Toluene diisocyanate (2,4-2,6 isomer ratio 80:20) was purchased from Aldrich (Milwaukee, MI). Capsaicin (Sigma) was dissolved in 100% ethanol to give a stock solution of 300 mM; this was stored at 4°C and diluted in MKH solution as necessary for the experiment.

3. Results

3.1. General observations

It was observed at the end of the experiments, as it has been previously by others and us (Munakata et al., 1988; Fedan and Frazer, 1992), that every intact trachea relaxed in response to intraluminally-added KCl, and every epithelium-denuded trachea contracted in response to intraluminally-added KCl (not shown).

Histological examination of sections revealed that there were no overt morphological changes in the tracheas after exposure to air or TDI (not shown). As reported previously (Fedan and Frazer, 1992), after mechanical removal of the epithelium there was no apparent damage to the basement membrane, smooth muscle or other structures in the tracheal wall.

3.2. Effects of air exposure on reactivity to MCh

As shown in Fig. 1, a 30 min exposure of the trachea to air did not affect reactivity to MCh. The EC_{50} values were: pre-exposure, $0.47(0.12-2.72)\times 10^{-4}$ M; post-exposure, $0.50(0.08-3.34)\times 10^{-4}$ M, $P > 0.05$. The maximum response values were: pre-exposure, 6.9 ± 1.7 cm H_2O; post-exposure, 6.4 ± 1.4 cm H_2O, $P > 0.05$.

3.3. Effects of exposure to TDI on reactivity of epithelium-containing tracheas to MCh

Exposure to 5 ppb TDI did not affect reactivity to MCh (EC_{50} values were: pre-exposure, $0.43(0.08-2.26)\times 10^{-5}$ M; post-exposure, $0.59(0.12-3.13)\times 10^{-5}$ M, $P > 0.05$; maximum responses were: pre-exposure, 10.9 ± 2.7 cm H_2O; post-exposure, 11.0 ± 2.4 cm H_2O, $P > 0.05$) (Fig. 2).

On the other hand, the IL MCh concentration–response curve after exposure to 20 ppb TDI was shifted 2.36-fold to the left of control (EC_{50} values

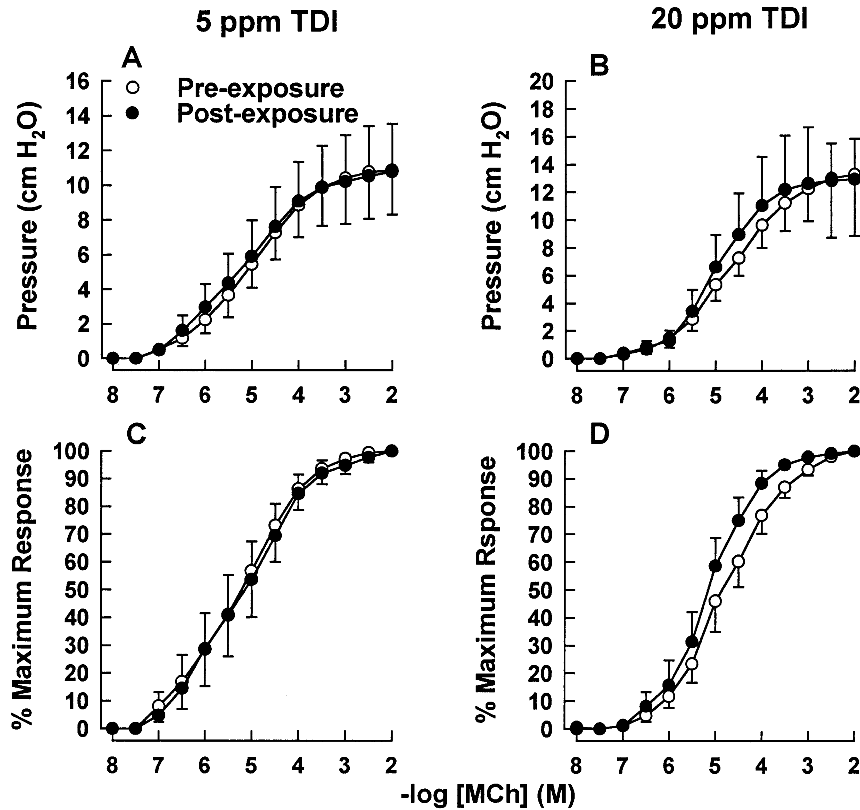

Fig. 2. Effects of 5 and 20 ppb TDI exposure on the intraluminal MCh concentration–response curve of isolated, perfused trachea. Two curves were obtained from each preparation, the first before (○) exposure and the second (●) after exposure of the tracheal lumen to 5 ppb (A and C) and 20 ppb (B and D) TDI. Separate animals were used for each TDI concentration, $n = 6$ for 5 and 20 ppb TDI.

were: pre-exposure, $1.32(0.39-4.46)\times 10^{-5}$ M; post-exposure, $0.56(0.24-1.33)\times 10^{-5}$ M, $P<0.05$) (Fig. 2). The maximum response was not affected (maximum responses were: pre-exposure, 13.3 ± 2.6 cm H_2O; post-exposure, 12.8 ± 4.2 cm H_2O, $P>0.05$).

Exposure to 70 ppb TDI also increased (2.88-fold) sensitivity to MCh (EC_{50} values were: pre-exposure, $1.41(0.65-3.04)\times 10^{-4}$ M; post-exposure, $0.48(0.18-1.32)\times 10^{-4}$ M, $P<0.01$) (Fig. 3). The maximum response was not affected (maximum responses were: pre-exposure, 10.2 ± 2.7 cm H_2O; post-exposure, 9.0 ± 1.9 cm H_2O, $P>0.05$).

3.4. Effect of TDI exposure on reactivity of epithelium-denuded tracheas to MCh

To determine whether hyperreactivity to MCh after TDI-treatment was due to an effect of the vapor on the epithelium, the effect of TDI was evaluated in epithelium-free tracheas. This experiment was performed using 70 ppb TDI, which had produced the largest increase in reactivity of the three TDI concentrations. As is widely known (Munakata et al., 1989; Fedan and Frazer, 1992), epithelium removal by itself increased reactivity to intraluminally-applied MCh (compare the pre-exposure curves in panels A and B in Fig. 3); this

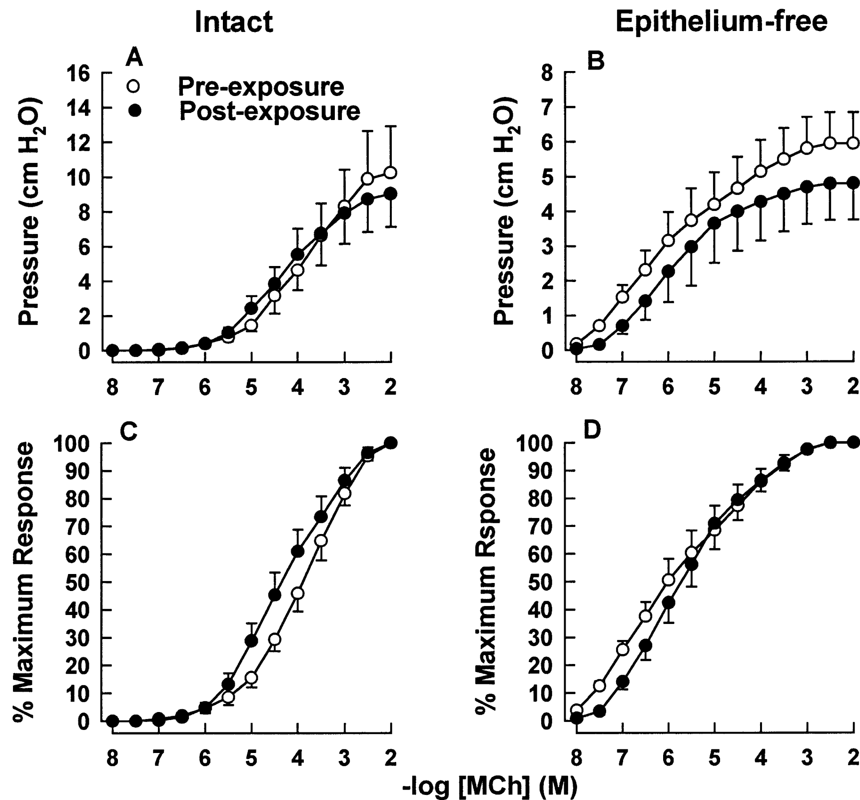

Fig. 3. Effects of 70 ppb exposure on the intraluminal MCh concentration–response curve of intact, epithelium-containing (A and C), or epithelium-free (B and D) perfused trachea. Two curves were obtained from each preparation, the first before (○) and the second (●) after exposure of the tracheal lumen to 70 ppb TDI. Separate animals were used to study intact and epithelium-free tracheas, $n = 7$ for intact and epithelium-free tracheas.

increase in mucosal reactivity occurred because the modulatory effect on airway smooth muscle reactivity that is exerted by the epithelium had been removed upon denudation. In contrast to what had been observed when the epithelium was present, exposure to 70 ppb TDI did not increase reactivity to MCh in epithelium-free tracheas; reactivity appeared to be inhibited by TDI, but this effect was not significant (EC_{50} values were: pre-exposure, $0.15(0.05-0.47)\times 10^{-5}$ M; post-exposure, $0.22(0.08-0.59)\times 10^{-5}$ M, $P > 0.05$; maximum responses were: pre-exposure, 5.99 ± 0.88 cm H_2O; post-exposure, 4.82 ± 1.90 cm H_2O; $P > 0.05$; $n = 6$) (Fig. 3). Therefore, the TDI-induced hyperreactivity to MCh occurred only in the presence of epithelium. These experiments suggest that the hyperreactivity of intact tracheas caused by TDI vapor probably did not originate from an effect of the vapor on the smooth muscle.

3.5. Effects of exposure to TDI on reactivity of capsaicin-pretreated epithelium-containing tracheas to MCh

A strong, long-lasting contraction of the trachea was induced upon the intraluminal addition of 2 μM capsaicin (not shown). After washout, the re-addition of intraluminal capsaicin did not induce a second contraction of the trachea, indicating that the first challenge with capsaicin had effectively depleted tachykinins from the preparation.

As shown in Fig. 4, exposure to 70 ppb TDI caused a leftward shift of the MCh concentration–response curve of capsaicin-pretreated tracheas

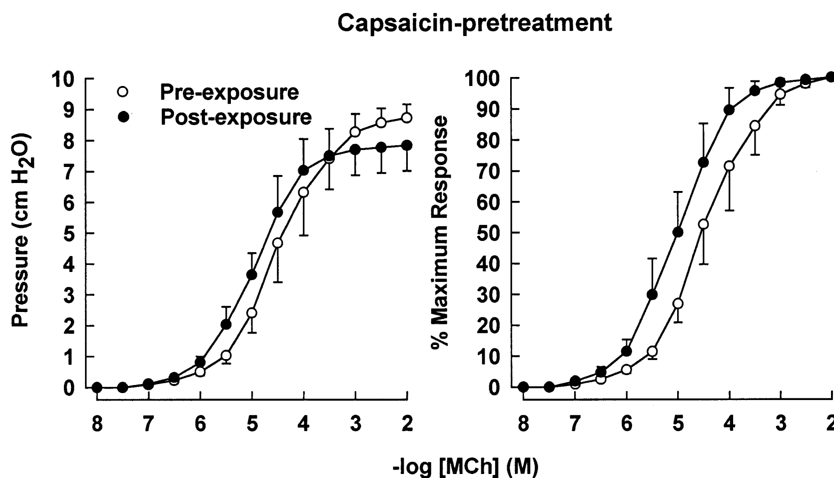

Fig. 4. Effects of 70 ppb exposure on the intraluminal MCh concentration–response curve of capsaicin-pretreated intact, epithelium-containing tracheas. Two curves were obtained from each preparation, the first before (○) and the second (●) after exposure of the tracheal lumen to 70 ppb TDI, $n = 5$.

(EC_{50} values pre-exposure, 4.36 (2.34 – 10.00) $\times 10^{-4}$ M; post-exposure, 1.09 (0.65 – 2.04) $\times 10^{-4}$ M). Thus, the effect of TDI was not attenuated by capsaicin-pretreatment. The 4-fold shift of the curve was of comparable magnitude to that seen in tracheas that were not treated with capsaicin (Fig. 3). There was no effect of TDI-treatment on the maximum responses of capsaicin-pretreated preparations.

4. Discussion

These experiments have demonstrated that a brief exposure of guinea-pig airways in vitro to low concentrations of airborne TDI vapor induces hyperreactivity to MCh applied to the mucosal surface of the isolated, perfused trachea preparation. The development of the hyperreactivity appears to involve a direct action of TDI on the respiratory epithelium. The increase in reactivity is not attributable to an interaction between TDI and the airway smooth muscle, and it does not seem to be dependent on the action of endogenous tachykinins. Our results suggest that the direct effect of TDI may contribute to the development of airway hyperreactivity in occupational asthma.

The tracheal hyperreactivity induced by TDI was completely independent of systemic events which operate in vivo in response to TDI exposure, such as TDI-specific antibody-mediated immunological mechanisms, inflammation, and sensory neural reflexes involving central pathways (Cartier et al., 1989; Baur et al., 1994), because tracheas from naive non- sensitized, non-inflamed animals were used in this study, and removal of the organ from the animal severed central neural connections.

The guinea-pig isolated, perfused tracheal preparation provided a novel paradigm of intact airways that is very representative of the behavior of airways in vivo, especially in the context of the ways in which the epithelium regulates airway reactivity. It has the additional advantage over tracheal strip preparations of being able to restrict delivery of TDI vapor and other agents such as MCh to the mucosal surface of the airway. This preparation has allowed an unambiguous examination of the direct, local effects of TDI on the airway epithelium and on airway smooth muscle. To our knowledge this is the first study to examine the effects of low concentrations of TDI vapor in vitro, in which the agent was delivered in air to only the mucosal surface. It is reasonable to conclude that the effects observed in the perfused trachea mimic very closely the direct effects of TDI in human airways following brief inhalation exposure to low concentrations of the chemical.

The induction of tracheal hyperreactivity was clearly dependent on the exposure concentration of TDI vapor. While 5 ppb TDI had no effect on MCh concentration–response curves, 20 and 70 ppb TDI elevated reactivity to MCh. The concentration-dependence of these changes in vitro are consistent with the results of in vivo animal models (Marek et al., 1996; Gagnaire et al., 1997).

TDI-induced tracheal hyperreactivity occurred as a consequence of alterations in the epithelium and did not result from a direct effect of TDI on the airway smooth muscle. This conclusion is derived from the evidence that the reactivity of epithelium-free trachea was not enhanced by 70 ppb TDI as had occurred in the epithelium-containing trachea. This finding contrasts with the results obtained in guinea-pig in vitro bronchial ring preparations by Mapp et al. (1992). They observed in the absence of the epithelium an increase in the response to TDI. However, the bronchial rings were bathed in physiological salt solution, and the TDI was added directly to the organ bath. Under these conditions, TDI was no longer in a vapor phase, and it was not possible to restrict delivery of the TDI to the mucosal or serosal surfaces of the bronchial rings.

Several mechanisms involving the epithelium could contribute to the increase in reactivity to MCh caused by TDI vapor. There is evidence for a contribution of capsaicin-sensitive nerves to the development of airway hyperresponsiveness and asthma (Barnes et al., 1991a,b). TDI vapor could have stimulated the release of contractile tachykinins from sensory nerves in the mucosa. In addition, TDI inhibits epithelial neutral endopeptidase which is involved in breakdown of tachykinins (Thompson et al., 1987; Mapp et al., 1991; Marek et al., 1996; Gagnaire et al., 1997); this effect could accentuate the potentiating effect of small amounts of released tachykinin. However, our study found that capsaicin did not eliminate the onset of hyperreactivity to MCh in intact trachea. Therefore, the increase in reactivity to MCh did not result from the effects of tachykinins released upon TDI exposure.

TDI may also interact with airway epithelium to cause the generation of arachidonic acid metabolites, which could cause contraction of the airway mediated presumably through the activation of the efferent function of sensory nerves (Fabbri et al., 1994). However, the finding that the preparations were refractory to capsaicin at the time MCh concentration–response curves were obtained argues against this mechanism as being a predominant one in our in vitro experiments.

It is possible that TDI increased epithelial permeability to MCh. A change such as this, by itself, would cause a leftward shift of the intraluminal MCh concentration–response curve (Fedan and Frazer, 1992). If disruption of the diffusion barrier afforded by the epithelium occurred, the change was not accompanied by histological evidence of epithelial damage or disruption. In addition, TDI-exposed tracheas containing epithelium also relaxed in response to intraluminally-applied KCl, which indicates that the epithelium had not received a severe toxic insult, was functioning and was capable of releasing EpDRF.

TDI has been found to be a strong inhibitor of cholinesterase, which is consistent with the TDI-induced airway hyperreactivity to acetylcholine that was reported by Brown et al. (1982). Inasmuch as we used MCh in this study, and this agonist is not rapidly degraded by acetylcholinesterase, inhibition of this enzyme by TDI cannot explain our findings.

Another epithelial mechanism of TDI-induced tracheal hyperreactivity can be postulated based on evidence obtained in our laboratory with ozone exposure. It is possible that the release of EpDRF from epithelium, which downregulates smooth muscle reactivity, might have been decreased by TDI exposure. In experiments in progress, ozone-exposure of guinea pigs induced airway hyperreactivity to inhaled MCh in vivo (Fedan et al., 1996) and hyperreactivity of the isolated, perfused tracheas obtained from the animals to intraluminally-applied MCh. The in vitro hyperreactivity to MCh in the tracheas from ozone-exposed animals was accompanied by an inhibition of EpDRF release. The possibility exists that inhaled agents which produce irritant effects on the airways, i.e. TDI and ozone, may share the common mechanism of interfering with the epithelium's ability to regulate airway smooth muscle responsiveness via EpDRF.

In summary, brief exposure to low levels of TDI vapor increased the reactivity of guinea-pig isolated trachea to MCh. The development of the hyperreactivity was dependent on the TDI concentration and on the presence of epithelium, but was independent of tachykinins. This effect may play a role in the appearance of airway hyperreactivity in vivo after TDI inhalation.

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References

Barnes, P.J., Baraniuk, N., Belvisi, M., 1991a. Neuropeptides in the respiratory tract (Part I). Am. Rev. Respir. Dis. 144, 1187–1198.

Barnes, P.J., Baraniuk, N., Belvisi, M., 1991b. Neuropeptides in the respiratory tract (Part II). Am. Rev. Respir. Dis. 144, 1391–1399.

Baur, X., 1990. New aspects of isocyanate asthma. Lung 168, 606–613.

Baur, X., Marek, M., Ammon, J., Czuppon, A.B., Marczynki, B., Raulf-Heimsoth, M., Roemmel, H., Fruhmann, G., 1994. Respiratory and other hazards of isocyanate. Int. Arch. Occup. Environ. Health 66, 141–152.

Brown, W.E., Green, A.L., Karol, M.H., Alarie, Y.C.E., 1982. Inhibition of cholinesterase activity by isocyanates. Toxicol. Appl. Pharmacol. 63, 45–52.

Burge, P.S., 1991. New developments in occupational asthma. Br. Med. Bull. 48, 221–230.

Cartier, A., Grammar, L., Malo, J.L., Lagier, F., Ghezzo, H., Harris, R., Patterson, R., 1989. Specific serum antibodies against isocyanate: association with occupational asthma. J. Allergy Clin. Immunol. 84, 507–551.

Cibulas, W., Brooks, S.M., Murlas, C.G., Miller, M.L., Mckay, R.T., 1988. Toluene-induced airway hyperreactivity in guinea-pigs depleted of granulocytes. J. Appl. Physiol. 67, 1773–1778.

Fabbri, L.M., Maestrelli, P., Saetta, M., Mapp, C.E., 1994. Mechanism of occupational asthma. Clin. Exp. Allergy 24, 628–635.

Fedan, J.S., Hay, D.W.P., Farmer, S.G., Raeburn, D., 1988. Modulation of airway smooth muscle reactivity by epithelium cells. In: Rodger, I.W., Barnes, P.J., Thomson, N.C. (Eds.), Asthma: Basic Mechanisms and Clinical Management. Academic Press, New York, pp. 143–162.

Fedan, J.S., Nutt, M.E., Frazer, D.G., 1990. Reactivity of guinea-pig isolated trachea to methacholine, histamine and isoproterenol applied serosally vs mucosally. Eur. J. Pharmacol. 190, 337–345.

Fedan, J.S., Frazer, D.G., 1992. Influence of epithelium on the reactivity of guinea-pig isolated, perfused trachea to bronchoactive drugs. J. Pharmacol. Exp. Ther. 262, 741–750.

Fedan, J.S., Warner, T.E., Yuan, L.-X., Robinson, V.A., Frazer, D.G., 1995. Nitric oxide synthase inhibitor and lipopolysaccharide effects on reactivity of guinea-pig airways. J. Pharmacol. Exp. Ther. 272, 1141–1150.

Fedan, J.S., Yuan, L.-X., Frazer, D.G., 1996. Inhibited release of epithelium-derived relaxing factor accompanies increased reactivity to mucosal application of methacholine in guinea-pig trachea in vitro after ozone inhalation. Am. J. Respir. Crit. Care Med. 153, A168.

Flavahan, N.A., Aarhus, L.L., Rimele, T.J., Vanhoutte, P.M., 1985. Respiratory epithelium inhibits bronchial smooth tone. J. Appl. Physiol. 58, 834–838.

Frossard, N., Rhoden, K.J., Barnes, P.J., 1989. Influence of epithelium on guinea pig airway responses to tachykinins: role of endopeptidase and cyclooxygenase. J. Pharmacol. Exp. Ther. 248, 292–298.

Gagnaire, F., Ban, M., Cour, C., Micillino, J.C., Bonnet, P., Hettich, D., 1997. Role of tachykinins and neutral endopeptidase in toluene diisocyanate-induced bronchial hyperresponsiveness in guinea pigs. Toxicology 116, 17–26.

Goldie, R.G., Hay, D.P.W., 1997. Epithelium-dependent responsiveness or airway smooth muscle. In: Barnes, P.J., Leff, A.R., Woolcock, A.J. (Eds.), Asthma. Lippincott-Raven, Philadelphia, PA, pp. 901–915.

Hay, D.W.P., Farmer, S.G., Raeburn, D., Robinson, V.A., Fleming, W.W., Fedan, J.S., 1986. Airway epithelium modulates the reactivity of guinea pig respiratory smooth muscle. Eur. J. Pharmacol. 129, 11–18.

Karol, M.H., Jin, R., 1991. Mechanism of immunotoxicity to isocyanates. Chem. Res. Toxicol. 4, 503–509.

Lamport, S.J., Fedan, J.S., 1990. Modulation of the reactivity of the guinea-pig isolated trachealis by respiratory epithelium: Effects of cooling. Br. J. Pharmacol. 99, 369–373.

Mapp, C.E., Graf, P.D., Boniotti, A., Nadel, J.A., 1991. Toluene diisocyanate contracts guinea pig bronchial smooth muscle by activating capsaicin-sensitive sensory nerves. J. Pharmacol. Exp. Ther. 256, 1082–1085.

Mapp, C.E., Boniotti, A., Papi, A., Chitano, P., Saetta, M., Di Stefano, A., Ciaccia, A., Fabbri, L.M., 1992. The effect of phosphoramidon and epithelium removal on toluene diisocyanate-induced contractions in guinea pig bronchi. Eur. Respir. J. 5, 331–333.

Marek, W., Potthast, J.J.W., Marczynski, B., Baur, X., 1996. Role of substance P and neurokinin A in toluene diisocyanate-induced increased airway responsiveness in rabbits. Lung 174, 83–97.

Munakata, M., Mitzner, W., Menkes, H., 1988. Osmotic stimuli induce epithelial-dependent relaxation in the guinea pig trachea. J. Appl. Physiol. 64, 466–471.

Munakata, M., Huang, I., Mitzner, W., Menkes, H., 1989. Protective role of epithelium in the guinea pig airway. J. Appl. Physiol. 66, 1547–1552.

Nijkamp, F.P., Van der Linde, H.J., Folkerts, G., 1993. Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Am. Rev. Respir. Dis. 148, 727–734.

Pavlovic, D., Fournier, M., Aubier, M., Pariente, R., 1989. Epithelial vs. serosal stimulation of tracheal muscle: Role of epithelium. J. Appl. Physiol. 67, 2522–2526.

Smith, J.A., Frazer, D.G., Fedan, J.S., 1993. Alteration in the modulatory role of respiratory epithelium after exposure of guinea pigs to respirable cotton dust. J. Pharmacol. Exp. Ther. 264, 683–688.

Thompson, J.E., Scypinsky, L.A., Gordon, T., Sheppard, D., 1987. Tachykinins mediate the acute increase in airway responsiveness caused by toluene diisocyanate in guinea pig. Am. Rev. Respir. Dis. 136, 43–49.

Tschirhart, E., Bertrand, C., Theodorsson, E., Landry, Y., 1990. Evidence for the involvement of calcitonin gene-related peptide in the epithelium-dependent contraction of guinea-pig trachea in response to capsaicin. Naunyn-Schmiedeberg's Arch. Pharmacol. 342, 177–181.