Induction of non-specific airway hyperreactivity by potassium channel blockade in rat isolated trachea

N. Chand, W. Diamantis & R.D. Sofia

Wallace Laboratories, Division of Carter-Wallace, Inc., Cranbury, New Jersey 08512 U.S.A.

- 1 The exposure of rat isolated tracheal segment to the K⁺-channel inhibitor tetraethylammonium (TEA, 10 mM) for a period of 10–15 min generally produced little or no contractile response.
- 2 Cooling (10°C) provocation alone usually produced small (10 ± 3% acetylcholine maximum) contractile responses.
- 3 In the presence of TEA (10 mM, 10–15 min exposure), rat trachea exhibited airway hyperreactivity to acetylcholine, 5-hydroxytryptamine (5-HT) and cooling. It also increased the peak tension induced by 5-HT.
- 4 TEA-induced airway hyperreactivity to cooling was significantly inhibited in Ca²⁺-free Krebs solution suggesting an important role for extracellular Ca²⁺ influx.
- 5 We conclude that the blockade of potassium channels with TEA induces non-specific airway hyperreactivity to cooling, 5-HT and acetylcholine in rat isolated tracheal segments.

Introduction

Non-specific airway hyperreactivity (AH) to a variety of pharmacological and physical stimuli such as histamine, methacholine, KCl, prostaglandin F_{2α} (PGF_{2α}) and exercise is a hallmark of asthma (Bleeker, 1986; Dixon *et al.*, 1989). There are at least two hypotheses (cooling and/or drying of the airways and transient increase in the osmolarity/osmolality of tracheal mucosal fluid) which have been put forward to explain the underlying cause of exercise-induced asthma (Anderson, 1984). We have recently developed an *in vitro* model of AH to cooling in rat trachea, in which cooling (10°C) itself causes weak (5–10% acetylcholine maximum) contractile responses. However, tracheal segments exposed to threshold/subthreshold concentrations of potassium chloride, chemical mediators such as platelet activating factor (PAF), acetylcholine, adenosine, phospholipase A₂, and phospholipase C, or following recovery from allergic responses, exhibit marked AH to cooling (Chand *et al.*, 1986; 1987; 1988). In the present study we now demonstrate that tetraethylammonium (TEA; a potassium channel inhibitor; a depolarizing agent) induces non-specific airway hyperreactivity to acetylcholine, 5-hydroxytryptamine (5-HT) and cooling in rat trachea.

Methods

Adult male Sprague-Dawley rats, weighing between 241–689 g were killed by CO₂ exposure. A terminal segment of the trachea, 8 to 10 mm long, was dissected from each rat and kept in warm Krebs-Henseleit solution (37°C). Each tissue was cut into two equal segments and set up 'in pairs' in isolated tissue baths containing Krebs-Henseleit solution, maintained at 37°C and gassed with a mixture of 95% O₂ and 5% CO₂. The composition of Krebs-Henseleit solution was (in mM): NaCl 118, KCl 4.7, CaCl₂ · 2H₂O 2.5, MgSO₄ · 7H₂O 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0 and glucose 10.0 (pH 7.4). The tracheal segments were attached to force-displacement transducers (Grass type FT.03C) by two L-shaped stainless steel hooks. Tissues were allowed to equilibrate for 1 to 3 h with washings at 30–60 min intervals. An optimal resting force of about 1 g was readjusted and maintained during the stabilization period.

Following the equilibration period, tissues were exposed to tetraethylammonium (TEA, 10 mM) for 10–15 min and the circulating water in the outer jackets of the isolated tissue baths was switched to a cold water supply (10°C, refrigerated con-

stant temperature circulator, Polyscience Series 9000). The cold-induced contractile responses were recorded isometrically for a period of 15 to 30 min or until a plateau was established. The circulation of heated (37°C) water in the outer jacket of the tissue baths produced immediate relaxation of cold-induced contractions. Fifteen min later, the resting force (if lowered beyond resting level) was readjusted and then tissues were exposed to acetylcholine (ACh, 10⁻³ M) to determine contractility (peak developed tension) in mg. This response was termed the ACh maximum contractile response (ACh max.). The cold-induced responses were expressed as % of ACh max. About 20% of the tracheal segments exhibited contractions to TEA and were not included in this study.

Role of calcium in TEA-induced airway hyperreactivity

After the equilibration period in normal Krebs solution, one tracheal segment of each pair was incubated in Ca²⁺-free Krebs solution for a period of 120 min with two or three washes. The second tissue was maintained in normal (Ca²⁺-containing) Krebs solution. Both tissues were exposed to TEA (10 mM) and then to cold (10°C) by changing circulation fluid in the outer jacket of the tissue baths. Fifteen min later, tissues were exposed to ACh (10⁻³ M) at 37°C. The cold responses were expressed as % of ACh max. and compared by paired *t* test.

Induction of non-specific airway hyperreactivity to acetylcholine and 5-HT

After the equilibration period one tissue of each pair was exposed to TEA (10 mM) and another received vehicle (Krebs solution 0.1 ml) for a period of 15 min. The cumulative concentration-effect curves to either ACh or 5-HT were established with effect expressed in mg and also as a % of control ACh max. responses and compared by paired *t* test. The EC₅₀s (μM), as a measure of sensitivity, were calculated from the linear part of the concentration-effect curves; the 95% confidence limits were also determined and compared by the Bliss method (Bliss, 1967).

Drugs and statistics

Tetraethylammonium chloride (TEA), acetylcholine chloride (ACh) and 5-hydroxytryptamine creatinine sulphate (5-HT) were obtained from Sigma Chemical Co., St. Louis, MO,

U.S.A. These drugs were dissolved in normal Krebs solution immediately before use. Drug concentration is expressed as that occurring in the bath fluid.

Statistical significance was determined by comparing cold-induced responses (% ACh max.) as well as peak developed tension (mg) in control (vehicle)-treated and TEA-treated segments by Student's *t* test for paired observations. Statistical significance was indicated by a *P* value of 0.05 or less.

Results

The exposure of rat tracheal segments to TEA (10 mM) generally produced little (50–200 mg) or no contractile response in about 80% of the preparations. However, this treatment produced airway hyperreactivity to ACh and 5-HT (Figures 1 and 2; Table 1). In the case of ACh, TEA produced a significant change in sensitivity (a six fold decline in EC_{50}). With 5-HT, it increased both the sensitivity (a seven fold decline of EC_{50}) as well as increasing the peak tension developed to 238% of the control value (Table 1).

Rat isolated tracheal segments responded with poor contractile responses ($10 \pm 3\%$ of ACh max.) to cold provocation. However, cold provocation produced strong and rapid contractile responses ($35.3 \pm 4.2\%$ ACh max.; an increase to 264% of the control) in segments exposed to TEA for a period of 10–15 min (Figures 3 and 4).

The exposure of rat tracheal segments to Ca^{2+} -free Krebs solution for a period of two hours produced a significant inhibition of TEA-induced airway hyperreactivity as well as peak developed tension to acetylcholine (Figure 5).

Figure 1 Cumulative concentration-effect curves for 5-hydroxytryptamine (5-HT) in the presence ($\diamond$) and absence ($\square$) of tetraethylammonium (TEA, 10 mM, 15–30 min exposure) in rat isolated tracheal segments. Each point represents the mean of values from 12 tissues; vertical lines show s.e.mean. Asterisks indicate significant difference (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$) compared with corresponding response in the absence of TEA.

Figure 2 Cumulative concentration-response curves for acetylcholine in the presence ($\diamond$) and absence ($\square$) of tetraethylammonium (TEA, 10 mM, 15–30 min exposure) in rat isolated tracheal segments. Each point represents the mean of values from 8 tissues; vertical lines show s.e.mean. Asterisks indicate significant difference (* $P < 0.05$ and ** $P < 0.01$) compared with corresponding response in the absence of TEA.

Figure 3 Typical tracings of tetraethylammonium (TEA)-induced airway hyperreactivity to cooling (10°C) in rat tracheal segments.

Table 1 Effect of tetraethylammonium (TEA) on sensitivity and contractility to acetylcholine and 5-hydroxytryptamine (5-HT) in rat isolated tracheal segments

Pretreatment	Acetylcholine (n = 8)		5-HT (n = 12)	
	Sensitivity EC_{50} (μM)	Peak developed tension (mg)	Sensitivity EC_{50} (μM)	Peak developed tension (mg)
Krebs solution 0.1 ml	19.5 (16.9–22.6) $r = 0.99$; slope = 31	2693 $\pm$ 357	2.7 (2.1–3.3) $r = 0.96$; slope = 36	648 $\pm$ 141
TEA, 10 mM (15–30 min)	3.4 (1.8–6.4) $\dagger$ $r = 0.97$; slope = 53	2760 $\pm$ 238	0.36 (0.13–0.99) $\dagger$ $r = 0.97$; slope = 124	1341 $\pm$ 138***

Values are means $\pm$ s.e.mean.

$\dagger P < 0.05$ as compared to respective controls (Bliss method).

*** $P < 0.001$ as compared to respective controls (paired *t* test).

Figure 4 Induction of airway hyperreactivity to cooling provocation (10°C) by tetraethylammonium (TEA, 10 mM, 10–15 min exposure) in rat isolated tracheal segments. Values are means and bars show s.e.mean ($n = 12$). ** $P < 0.01$, *** $P < 0.001$. (▨) Normal Krebs solution, (■) Krebs solution containing TEA (10 mM).

Figure 5 Inhibition of tetraethylammonium (TEA)-induced airway hyperreactivity to cooling and acetylcholine max. (contractility) in Ca^{2+} -free Krebs solution (2 h exposure). All responses to cooling were obtained after 15 min treatment with, and in the presence of, 10 mM TEA. Values are means and bars show s.e.mean ($n = 12$). ** $P < 0.01$, *** $P < 0.001$. (▨) Normal Krebs solution, (■) Ca^{2+} -free Krebs solution.

Discussion

The data obtained in this study clearly demonstrate that the potassium channel inhibitor, TEA, induces non-specific airway hyperreactivity to cooling, ACh and 5-HT in rat tracheal segments. Antigen- and phospholipase A_2 (Chand *et al.*, 1987; 1988)- and TEA (this study)- induced airway hyperreactivity to cooling in this tissue is predominantly mediated via the influx of extracellular Ca^{2+} . The maximum response to ACh (contractility; peak developed tension) utilized 40% intracellular calcium (Figure 5).

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TEA increased sensitivity to 5-HT and ACh and also increased peak tension (contractility) to 5-HT. The exact explanation for this differential effect of TEA on maximal responses to ACh and 5-HT in rat trachea is not clear. However, it should be noted that 5-HT exerts only ~25% of the intrinsic activity (contractility or peak tension) of ACh, a full agonist in rat trachea.

Airway hyperresponsiveness is defined as an exaggerated airway narrowing in response to a wide variety of stimuli such as exercise, methacholine, $\text{PGF}_{2\alpha}$, histamine, fog, distilled water etc., that have little or no effect in normal subjects. The exact underlying mechanism of bronchial hyperresponsiveness is not yet known. However, bronchial inflammation or inflammatory mediators may exert a priming effect (depolarization) on airway smooth muscle and thus produce a synergistic or exaggerated response to a combination of two or more stimuli, which are individually weak or inert in normal subjects. The data obtained in this study suggest that airway depolarization by TEA simulates a situation in asthmatics, i.e., stimuli (e.g. 5-HT and cooling) with weak activity induce marked constriction after potassium channel blockade by TEA. In guinea-pig trachealis, TEA (0.1–10 mM) failed to produce any leftward shift in the concentration-effect curves for ACh, histamine, KCl and PGE_2 (Boyle *et al.*, 1988). Therefore, due to species-related variations, caution must be exercised when extrapolating results of *in vitro* studies to a disease state(s) in man.

Rat tracheal segments generally did not contract in response to TEA. However, TEA induced slow waves of contraction, occurring at 5–10 min intervals, or produced a sustained contractile response (500–1000 g) in about 20% of the tracheal segments examined. Such tissues were extremely hyperresponsive to low concentrations of ACh (10^{-7} – 10^{-6} M). TEA has been shown to depolarize and to induce action potentials and contractions in guinea-pig, bovine and canine trachealis (Kirkpatrick, 1975; Kroeger & Stephens, 1975; Suzuki *et al.*, 1976; Foster *et al.*, 1983). Repeated antigen challenge (repeat sensitization) has been shown to produce depolarization of guinea-pig trachealis (McCaig, 1987; Souhrada & Souhrada, 1981). These data and the findings of the present study suggest that the basic fundamental physiological defect in the induction of airway hyperreactivity/hyperresponsiveness in asthmatics may be depolarization (blockade of potassium channels) of the airway smooth muscle cells.

TEA, KCl, subthreshold concentrations of chemical mediators, added exogenously (ACh, 5-HT, PAF, adenosine, etc.) or released endogenously (neuropeptides, PAF, ACh, etc.) during repeated antigen challenge, could produce slight depolarization of airway smooth muscle cells and thus induce non-specific airway hyperresponsiveness, a cardinal sign of asthma (this study, Chand *et al.*, 1986; 1987; 1988; McCaig 1987).

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