J. Smooth Muscle Res. 2014; 50: 29–38
Published online: May 14, 2014; doi: 10.1540/jsmr.50.29

Original

# Onset of diabetes modulates the airway smooth muscle reactivity of guinea pigs: role of epithelial mediators

Bano Saidullah[1, 2], Kambadur Muralidhar[3] and Mohammad Fahim[4]

[1]*Department of Physiology, VP Chest Institute, University of Delhi, Delhi , India*
[2]*School of Sciences, Indira Gandhi Nation Open University, New Delhi, India*
[3]*Department of Zoology, University of Delhi, Delhi, India*
[4]*Hamdard Institute of Medical Science and Research, Hamdard University, New Delhi, India*

Submitted October 15, 2013; accepted in final form February 12, 2014

## Abstract

Background: Diabetes induces lung dysfunction, leading to alteration in the pulmonary functions. Our aim was to investigate whether the early stage of diabetes alters the epithelium-dependent bronchial responses and whether nitric oxide (NO), $K_{ATP}$ channels and cyclooxygenase (COX) pathways contribute in this effect. Methods: Guinea pigs were treated with a single injection of streptozotocin (180 mg/kg, ip) for induction of diabetes. Airway conductivity was assessed by inhaled histamine, using a non-invasive body plethysmography. The contractile responses of tracheal rings induced by acetylcholine (ACh) and relaxant responses of precontracted rings, induced by isoproterenol (IP) were compared in the presence and absence of the epithelium. Effects of $N^{\omega}$-Nitro-L-arginine methyl ester (L-NAME, a nitric oxide synthase inhibitor), glybenclamide (a $K_{ATP}$ channel inhibitor) and indomethacin (a COX inhibitor) were also assessed in diabetic guinea pigs. Results: Early stage diabetes did not alter the airway conductivity. ACh-induced bronchoconstriction in epithelium intact tracheal rings was not affected by the onset of diabetes, however a reduction in the increased ACh responses due to epithelium removal, to L-NAME or to indomethacin was observed. The relaxation response to IP was impaired in trachea from guinea pigs in which diabetes had just developed. Early diabetes significantly reduced the IP response to glybenclamide and to indomethacin. Conclusion: Our results demonstrate that the early stage of diabetes, modulate the bronchial reactivity to both ACh and IP by disrupting the NO, $K_{ATP}$ channels and COX pathways, without affecting the airway conductivity in guinea pigs.

**Key words:** guinea pig trachea, diabetes, epithelium, NO, $K_{ATP}$ channel, COX pathway

Corresponding author: Dr. Bano Saidullah, Faculty of Life Sciences, School of Sciences, Block –D, Indira Gandhi National Open University (IGNOU), Maidangarhi-68, Delhi, India
Phone: 29572818 e-mail: bano.saidullah@gmail.com

## Introduction

The foundation for curiosity whether lungs are affected by diabetes is based on a few studies: the incidence of asthma in diabetic patients is less than in the residual population, the increased frequency of pulmonary disorders in children born to diabetic mothers and the necessity to understand the pathophysiology of this organ for inhalant anti-diabetic drugs (1–5). A growing mass of data indicates a correlation of diabetes mellitus with lung dysfunction (6–8). Previous experimental studies have reported that diabetes attenuates the bronchomotor response as a result of decrease in sensory neuropeptide release (9). Contrary to this, some groups have reported an increased or decreased sensitivity to ACh in trachea from diabetic animals. This contradiction may be attributed to the duration and severity of the disease (10, 11, 12).

Some studies as mentioned before have indicated that the incidence of asthma in diabetic patients was less than in the residual population (13, 14). Another study showed, however that diabetes did develop in a few asthmatic patients, though the symptoms of asthma became less violent or even vanished (15). Interestingly, another study has shown a strong positive association between the occurrence of type 1 diabetes and symptoms of asthma suggesting that diabetes modulates airway reactivity (16–18). Diabetes is associated with increased glucose in airway surface liquid (ASL) which affects the respiratory epithelium or *vice versa* (19). The respiratory epithelium plays an important role in the regulation of airway reactivity as it releases brochoactive factors, which modulate the bronchial muscle tone and regulate the airway diameter. Damage to the respiratory epithelium may contribute to abnormal responses of the airway smooth muscle resulting in respiratory disorders. An analogous situation exists in the vascular system, where endothelial cells release relaxing and contracting factors that modulate the tone of the underlying smooth muscle. The bioavailability of nitric oxide (NO), a potent vasorelaxant is diminished in diabetes which results in endothelial dysfunction and cardiovascular complications (20). When diabetes occurs with cardiovascular complications, COX upregulation is associated with impaired β cell function and overactive $K_{ATP}$ channel which is associated with decreased glucose sensitive insulin secretion (21, 22). Hyperreactivity to vasoconstrictors has been reported in diabetes. This hyperreactivity has been attributed to reduced NO, decreased expression of $K_{ATP}$ channel and enhanced release of COX-2 derived prostaglandins in aortic tissue (21–23).

Though a lot of data regarding the effect of diabetes on vascular reactivity and how it modulates the endothelial mediators are available, there is sparse information on its effect on bronchial reactivity and how it modulates the epithelial mediators. Therefore, the aim of this study was to assess the effect of diabetes particularly early diabetes on (a) the epithelium-dependent bronchoconstrictor and bronchodilator responses in guinea pig trachea; (b) participation of epithelial mediators: NO, $K_{ATP}$ channel and COX pathway in these bronchial responses.

## Materials and Methods

### *Animals*

The present study was conducted on twenty adult, healthy guinea pigs of either sex weighing between 550–750 grams. The animals were maintained according to the recommendations by National Accreditation Board of Testing and Calibration Laboratories (NABL) and the study was approved by the VP Chest institute's animal ethical committee. During treatment, the guinea pigs were housed at a constant room temperature,

humidity, and light cycle (12:12 h light-dark), with free access to tap water and were fed with standard chow *ad libitum*. The guinea pigs were divided into two groups and treated as follows: (a) Control ($n$=10, given a single intraperitoneal (ip) injection of citrate buffer); (b) Diabetic ($n$=10, given a single ip injection of streptozotocin 180 mg/kg) (9). The experiments were performed 4 weeks after the streptozotocin treatment.

### *Oral glucose tolerance test*

Both the groups were subjected to oral glucose tolerance test (OGTT) (24). The guinea pigs were fasted overnight for 18h and subsequently challenged with a glucose load of 1.75 gm/kg body weight. Blood glucose levels were determined at 0 min (pre-glucose treatment) and at 60, 120, 180 and 240 min (post-glucose treatment). The glucose levels were measured using a complete blood glucose monitoring system (ACCU-CHEK Glucose Meter, Roche, India). OGTT was done on all animals before treatment and then before sacrifice. Animals with impaired glucose tolerance after treatment were considered early diabetic.

### *Assessment of bronchial hyperresponsiveness to histamine*

Bronchial hyperresponsiveness was accessed by the measurements of specific airway conductance (SGaw) carried out in all animals 4 days before induction as well as before sacrificing the animal. Measurement of SGaw to inhaled histamine was carried out using a non-invasive body plethysmographic technique as described by previous studies (25). It was assessed by plotting a log dose response curve and the concentration of histamine producing 35% fall in SGaw was calculated ($ED_{35}$ histamine).

### *Bronchoreactivity studies*

Trachea from guinea pigs of both control and exprimental groups was carefully dissected and cleaned of adhering connective tissue. For reactivity experiments, the trachea was cut into rings (approximately 2–3 mm wide). The tracheal rings were mounted isometrically, under a resting tension of 2 g in an organ bath, between a stationary stainless steel hook and an isometric force tension transducer (Grass FT-03, USA). Changes in isometric tension were recorded by a Power Lab data-acquisition system (8SP 20B, AD Instruments, Australia) provided with a computerized analysis programme (Chart 5.4.2, AD Instruments, Australia). Tracheal rings were maintained at 37ºC in an organ bath containing, 10 mL of modified Krebs buffer solution of the following composition (in mM): NaCl 118; KCl 4.8; $MgSO_4$ 1.2; $KH_2PO_4$ 1.2; $NaHCO_3$ 2.5; $CaCl_2$ 2.5; and glucose 11.0; pH, 7.4, bubbled with 95% $O_2$ and 5% $CO_2$.

The tracheal rings were allowed to equilibrate for 2 h under resting tension, before the experiments were started. After the equilibration period, the tracheal rings were exposed to 10 µM acetylcholine (ACh), in order to check their functional integrity.

In order to evaluate bronchial reactivity, dependent and independent of the epithelium, concentration-response curves were obtained from epithelium denuded as well epithelium intact tracheal rings of normal and early diabetic guinea pig separately using bronchoconstrictor ACh ($10^{-12}$ to $10^{-4}$ M) and bronchodilator isoproterenol (IP, $10^{-12}$ to $10^{-4}$ M). Removal of epithelium was achieved by rubbing the tracheal lumen with forceps. Epithelial denudation was confirmed by histology (data not shown).

The basal tone of the smooth muscle prior to the addition of IP was important, since the potency and even direction (contraction or relaxation) of IP effect may depend on the basal tone present. Trachea from diabetic guinea pigs when compared with the control tracheal rings showed no significant difference in the contraction or sensitivity to 0.1 µM-ACh. Thus, relaxation responses induced by IP were studied at equal levels of pre-contraction in tissues from control and diabetic animals.

$N^{\omega}$-Nitro-L-arginine methyl ester (L-NAME, 100 μM), glybenclamide (10 μM ) and indomethacin (10 μM ) were used in order to evaluate the participations of epithelial mediators NO, $K_{ATP}$ channels and Cyclo-oxygenase (COX), respectively, in the responses to ACh or IP in normal and onset of diabetic conditions.

### *Data analysis and statistics*

All values are expressed as mean ± S.E.M. of the number of observations (*n*) in each experiment. In the bronchial reactivity experiments, bronchoconstrictor responses were expressed as absolute values in gram tension while bronchodilator responses were expressed as the % change of the previous contraction to ACh. The individual effect of L-NAME, glybenclamide and indomethacin on the response to ACh (10 μM) and IP (10 μM) were expressed as % change in response to ACh and IP. The results for comparison between groups were analyzed using Student's *t* test. Differences were considered statistically significant at $P < 0.05$.

## Results

The mean body weight significantly decreased in guinea pigs treated with streptozotocin (Fig. 1). The blood glucose levels were higher at 60, 120, 180, 240 min after glucose load challenge in early diabetic guinea pigs as compared to control guinea pigs data (Fig. 2a). The postprandial blood glucose levels were higher in early diabetic guinea pigs (Fig. 2b).

### *Effect of early diabetes on SGaw*

There was no significant change in SGaw in response to histamine in animals with early diabetes as compared to control animals (Fig. 2c).

### *Effect of early diabetes on sensitivity and responsiveness of the tracheal rings to ACh and IP*

ACh produced a concentration-dependent contraction in control guinea pig trachea (Fig. 3). In animals with early stages of diabetes, ACh induced a similar concentration dependent contraction as well. The effects of epithelium removal in the ACh dose-response curves were then compared between control and diabetic animals. The epithelium removal significantly increased ACh-induced contraction in control animals indicating that the epithelium plays a modulatory role in ACh-induced contractions. When the same experiment was performed in the diabetic animals, no significant increase in the contractile response to ACh was observed indicating loss of modulatory role of epithelium in the early diabetes.

Additional confirmation for the impairment of epithelium was obtained by observing the responses to IP in tracheal rings precontracted by ACh (Fig. 4). IP produced a concentration-dependent relaxation in control guinea pig trachea (Fig. 4). In animals with early stages of diabetes IP induced significantly less relaxation compared to the control. In control guinea pigs, the relaxant response to IP was blunted by the removal of epithelium, suggesting that the relaxant response to IP was mediated in part through the epithelium. The response produced by IP in denuded trachea from diabetic animals was similar to that observed in epithelium intact tracheal rings from diabetic guinea pigs confirming loss of epithelium-mediated response in diabetes.

### *Effect of early diabetes on epithelium-dependent bronchial responses via NO pathway, $K_{ATP}$ channel and COX pathways*

In order to test whether altered release or action of epithelium-derived relaxing/contracting factors might contribute to the loss of responsiveness to ACh and IP in guinea pigs with early diabetes, the effects of L-

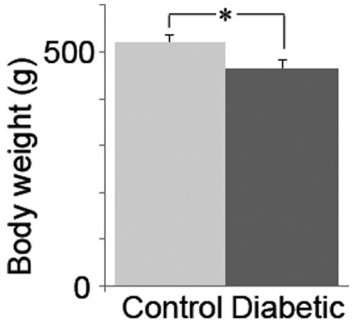


Fig. 1. Body weight in grams in healthy and diabetic guinea pigs. Data represents mean ± S.E.M. ($n$=10). *$P$ < 0.05.

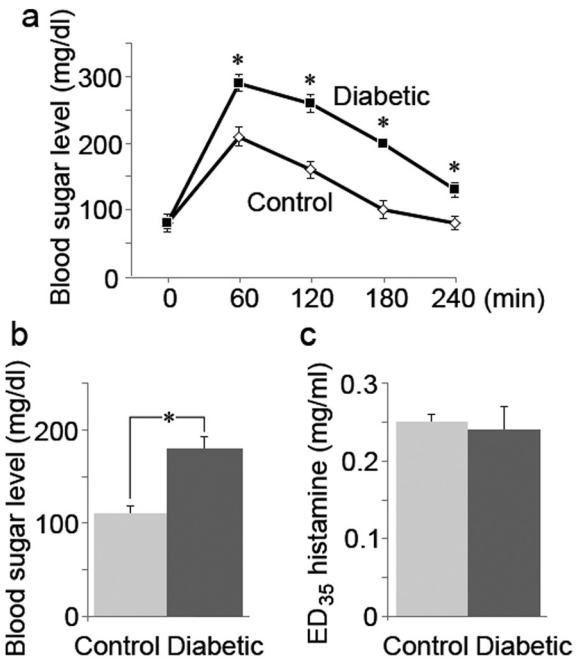


Fig. 2. a: Blood glucose level after 0, 60, 120, 180 min by means of oral glucose tolerance test done. Data represents mean ± S.E.M. ($n$=10). *$P$ < 0.05. b: Postprandial blood glucose level. Data represents mean ± S.E.M. ($n$=10). *$P$ < 0.05. c: Fall of $ED_{35}$ by histamine (mg/ml) in SGaw ($sec^{-1}$ cm $H_2O^{-1}$). Data represents mean ± S.E.M. ($n$=10).

NAME, glybenclamide and indomethacin, that interfere with epithelium-dependent pathways were separately evaluated in tracheal rings with intact epithelium from control and diabetic guinea pigs. The same set of experiments was performed on epithelium denuded tracheal rings, neither L-NAME, glybenclamide nor indomethacin did produce any change in the ACh and IP responses.

When L-NAME was added to inhibit the synthesis of NO, the responses to ACh in epithelium-intact trachea from control and from animals with early diabetes were increased, indicating NO was involved. However, the % change produced by L-NAME (100 μM) was significantly larger in trachea from control animals when compared to trachea from diabetic animals (Fig. 5). To assess the potential contribution of COX pathway to the ACh-induced constriction, we replaced L-NAME with the COX pathway inhibitor, indomethacin (10 μM). It increased the ACh responses in control tracheal rings suggesting the role of COX (Fig. 6). Augmentation of the constrictor response of ACh in the presence of indomethacin was significantly smaller in diabetic trachea as

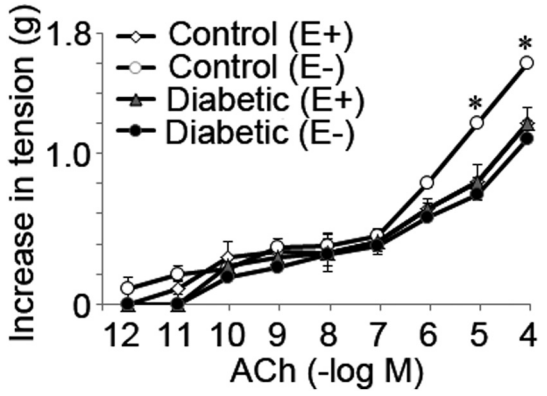


Fig. 3. Comparison of responses to ACh in control and diabetic tracheal rings with (E+) or without (E−) intact epithelium. Data represent mean±S.E.M. ($n$=12). *$P$ < 0.05.

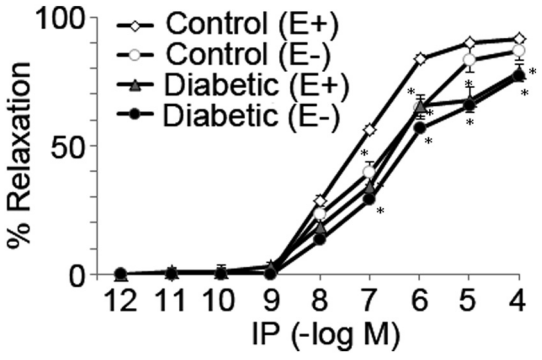


Fig. 4. Comparison of responses to cumulative concentrations of IP ($10^{-12}$–$10^{-4}$ M) in control and diabetic tracheal rings precontracted by ACh (10 μM) with (E+) or without (E−) intact epithelium. Data represent mean ± S.E.M. ($n$=12). *$P$<0.05, compared to corresponding value in the control group.

compared to control trachea. Glybenclamide (10 μM) did not change the contractile response of ACh in healthy and diabetic guinea pigs (Data not shown).

When indomethacin (10 μM) was added, the relaxing responses to IP in epithelium-intact trachea precontracted by ACh from control and from animals with early diabetes were increased, indicating the COX pathway was involved. However, the % change produced by indomethacin was significantly larger in trachea from control animals when compared to trachea from diabetic animals (Fig. 7).

When glybenclamide (10 μM) was added, the relaxing responses to IP in epithelium-intact trachea precontracted by ACh from control and from animals with early diabetes were increased, indicating $K_{ATP}$ channels were as involved. However, the % change produced by glybenclamide was significantly larger in trachea from control animals when compared to trachea from diabetic animals (Fig. 8). L-NAME (100 μM) did not change the relaxing response of IP in healthy and diabetic guinea pigs (Data not shown).

## Discussion

The present study demonstrates that diabetes, even at initial stages, modulates the reactivity of tracheal airway smooth muscles to ACh and IP but does not affect airway conductance. This change in reactivity of tracheal airway is due to the disruption in the functionality of epithelial mediators: NO, $K_{ATP}$ channels and COX pathways. The dysfunction of the respiratory epithelium begins in the initial stage of diabetes and may be one of the factors, involved in the pathophysiology of diabetes-induced lung dysfunction.

Four weeks streptozotocin treatment (180 mg/kg) induced a decreased tolerance to glucose along with increased post prandial blood glucose and a significant decrease in weight in guinea pigs suggesting the onset of type 1 diabetes. Previous studies have shown an increase in fasting blood glucose levels along with weight loss in streptozotocin treated guinea pigs after 8 weeks of treatment (9). Insulin implant were given to diabetic guinea pigs 4 weeks post streptozotocin treatment (9). Hence, the present experiments were performed at four weeks to study the effect of diabetes on respiratory epithelium at the very onset of the disease. Guinea pig was chosen as an animal model due to high sensitivity of its respiratory system to bronchoactive agents. Epithelial

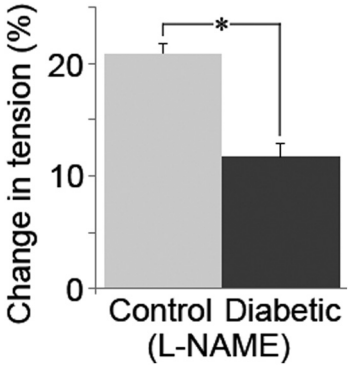


Fig. 5. Effect of diabetes on NO modulation of the bronchoconstrictor response of ACh. % change in ACh (10 μM)-induced contraction of tracheal rings with intact epithelium in the presence of L-NAME (100 μM). *$P$ < 0.05 ($n$=12).

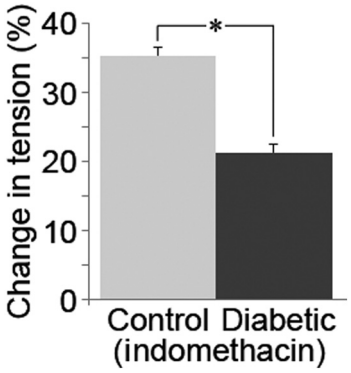


Fig. 6. Effect of diabetes on COX modulation of the bronchoconstrictor response to ACh. % change in ACh (10 μM)-induced contraction of tracheal rings with intact epithelium in the presence of indomethacin (10 μM). *$P$ < 0.05 ($n$=12).

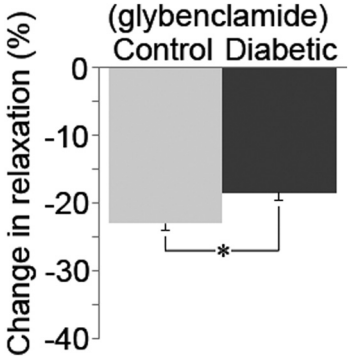


Fig. 7. Effect of diabetes on $K_{ATP}$ channel modulation of the bronchodilator response to IP. % change in IP (10 μM)-induced relaxation of tracheal rings with intact epithelium precontracted by ACh (10 μM) in the presence of glybenclamide (10 μM). *$P$ < 0.05 ($n$=12).

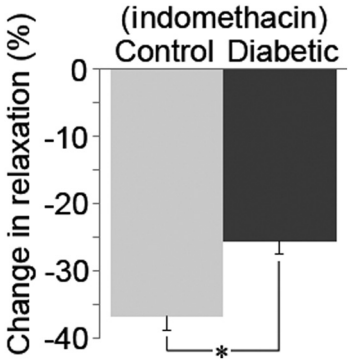


Fig. 8. Effect of diabetes on COX modulation of the bronchodilator response to IP. % change in IP (10 μM) -induced relaxation of tracheal rings with intact epithelium precontracted by ACh (10 μM) in the presence of indomethacin. *$P$ < 0.05 ($n$=12).

lining of the lungs is a key disease mediator and target for therapeutic interventions. The epithelial composition changes according to the local functional needs such as mucociliary clearance, hydration, host defense, and gas exchange. Epithelial cells also play a vital role in the modulation of airway tone by working as a physical barrier that protects sensory nerves and smooth muscle cells from inhaled irritants (26). In addition, the epithelial layer has the ability to release smooth muscle relaxant factors, such as prostaglandin $E_2$ ($PGE_2$), EDHF and NO, protecting the airway from excessive bronchoconstriction (27).

Responses to ACh in trachea with intact epithelium of guinea pigs with early diabetes and control guinea pigs were similar. In healthy tracheal rings, the bronchoconstriction induced by ACh was augmented by removal of epithelium indicating a reductive role of epithelium in ACh induced bronchoconstriction. This effect was not observed in early stages of diabetes, suggesting that early diabetes induces dysfunction of the respiratory epithelium.

To validate whether early diabetes also produces changes in the relaxation at the epithelial levels, the re-

sponse of $\beta_2$ agonist, IP was studied. IP is a bronchodilator and a therapeutic agent for asthma. Previous studies have shown a decreased relaxant response to IP in epithelium-denuded preparations (28). In our study, removal of epithelium from trachea in the control guinea pigs caused a statistically significant decrease to IP induced relaxation at higher concentrations. This indicates that activation of epithelial $\beta_2$-adrenoceptors releases some relaxing factor(s), or that the presence of a background secretion of these factor(s) facilitates the action of the bronchodilator (28, 29). In our study, there was a significant decrease in bronchodilation induced by IP in epithelium intact tracheal rings in animals with early diabetes and the responses to IP in epithelium-intact and epithelium-denuded tracheal rings from diabetic guinea pigs were similar to the epithelium-intact tracheal rings from diabetic guinea pigs, reiterating our conclusion that there is an impairment in the epithelium-dependent response.

Increase/decrease in the responsiveness of airway smooth muscle as a result of removal of epithelium in trachea may be due to: (i) absence of diffusion /permeability barrier, although the epithelial cells form tight junctions between each other thereby impeding access to underlying structures and acting as a physical barrier to foreign insults; (ii) synthesizing and releasing a number of biologically active contractile and relaxant substances such as NO, EDHF and $PGE_2$ and removal of epithelium causes the loss of such factors; (iii) loss of metabolic activity (such as neural peptidases) (26, 30–32).

The bronchoconstriction induced by ACh is depressed by both L-NAME and indomethacin in the intact tracheal tissues having epithelium, suggesting that the bronchoconstriction induced by ACh is separately blunted by NO and COX pathways. Other studies have also found that high concentrations of L-NAME ($10^{-4}$ M) were able to partially increase the contractile effect of ACh (30). NO acts as a "braking" mechanism to cholinergic bronchoconstriction (27). $PGE_2$ is a dominant cyclooxygenase product of airway epithelium and smooth muscle and is thought to be predominately bronchoprotective (33). Support for this latter statement rests in part on the observations that $PGE_2$ inhibits exercise-induced bronchoconstriction (34) and allergen-induced early and late asthmatic responses (31).

L-NAME and indomethacin did not affect the bronchoconstriction response to ACh in epithelium-intact trachea from guinea pigs with early stage of diabetes implying that the NO-mediated and COX-mediated component of the response were already impaired. Incubation of epithelium-intact tracheal tissues with indomethacin and glybenclamide, separately showed significantly reduced relaxant response of IP in healthy animals, specifying that $PGE_2$ and $K_{ATP}$ channels play a significant role in modulating the airways. The IP-induced epithelium-dependent relaxation is probably due to $PGE_2$ and $K_{ATP}$ channels which contribute significantly to the total relaxation in guinea pig trachea. Thus the results of our study on IP response in epithelium intact trachea of healthy guinea pigs are similar to the earlier reports where prostanoids-mediated relaxation contributes to about one third of total epithelium-dependant relaxation. Furthermore our results showing attenuation of IP-induced relaxation by both indomethacin or glybenclamide is in agreement with the earlier findings on guinea pig, where indomethacin slightly reduced IP induced relaxation and opening of $K_{ATP}$ channel mediated the relaxation of the tracheal tissue induced by IP (32, 35). In contrast to the effects observed in healthy tracheal rings, indomethacin and glybenclamide did not affect the bronchorelaxation response to IP in epithelium intact tracheal rings from guinea pigs with early diabetes, indicating that COX and $K_{ATP}$ channel mediated components of the response were already impaired.

In conclusion, the data indicates that at the onset of diabetes epithelial function is impaired in trachea as a consequence of the loss of NO, COX and $K_{ATP}$ channels, mediated relaxation and contraction while no change was observed in airway conductivity. Therefore, epithelium mediated mechanisms are more likely to be important in the development of the respiratory disorders as seen in diabetic individuals in the populations.

## Conflict of interest

The authors declare that they have no conflict of interest.

## References


1) Treviño-Alanís M, Ventura-Juárez J, Hernández-Piñero J, Nevárez-Garza A, Quintanar-Stephano A, González- Piña A. Delayed lung maturation of foetus of diabetic mother rats develop with a diminish, but without changes in the proportion of type I and II pneumocytes, and decreased expression of protein D-associated surfactant factor. Anat Histol Embryol. 2009; 38(3): 169–76.
2) Tosca MA, Silòvestri M, D'Annunzio G, Lorini R, Rossi GA, Ciprandi G. May T1 diabetes mellitus protect from asthma? Allergol Immunopathol (Madr). 2013; 41(5): 288–91.
3) Klingler C, Müller BW, Steckel H. Insulin-micro- and nanoparticles for pulmonary delivery. Int J Pharm. 2009; 377(1–2): 173–9.
4) Zheng J, Zhang G, Lu Y, Fang F, He J, Li N, Talbi A, Zhang Y, Tang Y, Zhu J, Chen X. Effect of pulmonary surfactant and phospholipid hexadecanol tyloxapol on recombinant human-insulin absorption from intratracheally administered dry powders in diabetic rats. Chem Pharm Bull (Tokyo). 2010; 58(12): 1612–6.
5) Raskin P, Heller S, Honka M, Chang PC, Boss AH, Richardson PC, Amin N. Pulmonary function over 2 years in diabetic patients treated with prandial inhaled Technosphere Insulin or usual antidiabetes treatment: a randomized trial. Diabetes Obes Metab. 2012; 14(2): 163–73.
6) Shah SH, Sonawane P, Nahar P, Vaidya S, Salvi S. Pulmonary function tests in type 2 diabetes mellitus and their association with glycemic control and duration of the disease. Lung India. 2013 Apr; 30(2): 108–12.
7) Davis WA, Knuiman M, Kendall P, Grange V, Davis TM. Glycemic exposure is associated with reduced pulmonary function in type 2 diabetes: the Fremantle Diabetes Study. Diabetes Care. 2004; 27(3): 752–7.
8) Meo SA, Al-Drees AM, Arif M, Al-Rubean K. Lung function in type 2 Saudi diabetic patients. Saudi Med J. 2006; 27(3): 338–43.
9) Szilvássy J, Sziklai I, Horvath P, Szilasi M, Németh J, Kovács P, Szilvássy Z. Feeble bronchomotor responses in diabetic rats in association with decreased sensory neuropeptide release. Am J Physiol Lung Cell Mol Physiol. 2002 May; 282(5): L1023–30.
10) Ozansoy G, Karasu C, Altan VM. The effects of acetylcholine on insulin-dependent and non-insulin-dependent diabetic rat tracheal segments. Gen Pharmacol. 1993 Jan; 24(1): 159–64.
11) Ozdem SS, Sadan G, Usta C, Taşatargil A. Effect of experimental diabetes on GABA-mediated inhibition of neurally induced contractions in rat isolated trachea. Clin Exp Pharmacol Physiol. 2000 Apr; 27(4): 299–305.
12) Ozdem SS, Sadan G, Usta C, Taşatargil A. The effect of experimental diabetes on cholinergic neurotransmission in rat trachea: role of nitric oxide. Eur J Pharmacol. 2000 Jan 17; 387(3): 321–7.
13) Douek IF, Leech NJ, Gillmor HA, Bingley PJ, Gale EA. Children with type-1 diabetes and their unaffected siblings have fewer symptoms of asthma. Lancet. 1999; 353(9167): 1850.
14) Decreased prevalence of atopic diseases in children with diabetes. The EURODIAB Substudy 2 Study Group. J Pediatr. 2000 Oct; 137(4): 470–4.
15) Stene LC, Nafstad P. Relation between occurrence of type 1 diabetes and asthma. Lancet. 2001 Feb 24; 357(9256): 607–8.
16) Kolahian S, Asadi F, Nassiri SM. Airway inflammatory events in diabetic-antigen sensitized guinea pigs. Eur J Pharmacol. 2011 Jun 1; 659(2–3): 252–8.

17) Garnett JP, Baker EH, Naik S, Lindsay JA, Knight GM, Gill S, Tregoning JS, Baines DL. Metformin reduces airway glucose permeability and hyperglycaemia-induced Staphylococcus aureus load independently of effects on blood glucose. Thorax. 2013 Sep; 68(9): 835–45.

18) Wood DM, Brennan AL, Philips BJ, Baker EH. Effect of hyperglycaemia on glucose concentration of human nasal secretions. Clin Sci (Lond). 2004 May; 106(5): 527–33.

19) Philips BJ, Meguer JX, Redman J, Baker EH. Factors determining the appearance of glucose in upper and lower respiratory tract secretions. Intensive Care Med. 2003; 29(12): 2204–10.

20) Lopez-Lopez JG, Moral-Sanz J, Frazziano G, Gomez-Villalobos MJ, Flores-Hernandez J, Monjaraz E, Cogolludo A, Perez-Vizcaino F. Diabetes induces pulmonary artery endothelial dysfunction by NADPH oxidase induction. Am J Physiol Lung Cell Mol Physiol. 2008 Nov; 295(5): L727–32.

21) Xavier FE, Davel AP, Rossoni LV, Vassallo DV. Time-dependent hyperreactivity to phenylephrine in aorta from untreated diabetic rats: role of prostanoids and calcium mobilization. Vascul Pharmacol. 2003 Jan; 40(1): 67–76.

22) Ren Y, Xu X, Wang X. Altered mRNA expression of ATP-sensitive and inward rectifier potassium channel subunits in streptozotocin-induced diabetic rat heart and aorta. J Pharmacol Sci. 2003 Dec; 93(4): 478–83.

23) Bagi Z, Erdei N, Toth A, Li W, Hintze TH, Koller A, Kaley G. Type 2 diabetic mice have increased arteriolar tone and blood pressure: enhanced release of COX-2-derived constrictor prostaglandins. Arterioscler Thromb Vasc Biol. 2005 Aug; 25(8): 1610–6.

24) Lang CM, Munger BL. Diabetes mellitus in the guinea pig. Diabetes. 1976; 25(5): 434–43.

25) Agrawal KP. Specific airway conductance in guinea pigs: normal values and histamine induced fall. Resp Physiol. 1981; 43(1): 23–30.

26) Folkerts G, Nijkamp FP. Airway epithelium: more than just a barrier! Trends Pharmacol Sci. 1998; 19(8): 334–41.

27) Folkerts G, Kloek J, Geppetti P, Van der Linde HJ, Nijkamp FP. Factors that determine acetylcholine responsiveness of guinea pig tracheal tubes. Eur J Pharmacol. 2001 May 25; 420(2–3): 151–7.

28) Shore SA, Moore PE. Regulation of beta-adrenergic responses in airway smooth muscle. Respir Physiol Neurobiol. 2003 Sep 16; 137(2–3): 179–95.

29) Boskabady MH, Teymoory S. The influence of epithelium on the responsiveness of guinea-pig trachea to -adrenergic agonist and antagonist. Med Sci Monit. 2003 Sep; 9(9): BR336–42.

30) Spicuzza L, Basile L, Belvisi MG, Bellofiore S, Matera MG, Cazzola M, Di Maria GU. The protective role of epithelium-derived nitric oxide in isolated bovine trachea. Pulm Pharmacol Ther. 2002; 15(4): 357–62.

31) Pavord ID, Wong CS, Williams J, Tattersfield AE. Effect of inhaled prostaglandin E2 on allergen-induced asthma. Am Rev Respir Dis. 1993; 148(1): 87–90.

32) Gauvreau GM, Watson RM, O'Byrne PM. Protective effects of inhaled PGE2 on allergen-induced airway responses and airway inflammation. Am J Respir Crit Care Med. 1999 Jan; 159(1): 31–6.

33) Melillo E, Woolley KL, Manning PJ, Watson RM, O'Byrne PM. Effect of inhaled PGE2 on exercise-induced bronchoconstriction in asthmatic subjects. Am J Respir Crit Care Med. 1994; 149(5): 1138–41.

34) Omini C, Folco G, Sautebin L, Nava G, Mandelli V, Berti F. beta-Adrenoceptor activation induces prostaglandin E2 generation in guinea-pig trachea. Eur J Pharmacol. 1981 Jun 19; 72(2–3): 227–31.

35) Cook SJ, Small RC, Berry JL, Chiu P, Downing SJ, Foster RW. Beta-adrenoceptor subtypes and the opening of plasmalemmal K(+)-channels in trachealis muscle: electrophysiological and mechanical studies in guinea-pig tissue. Br J Pharmacol. 1993 Aug; 109(4): 1140–8.