

Influence of epithelium on beta-adrenoceptor desensitization of guinea pig tracheal smooth muscle

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Abstract

The effect of tissue incubation with a β_2 -agonist of denuded and intact epithelium trachea on the responsiveness to isoprenaline and β -receptor blocked by propranolol (CR-1) was examined in this study. We examined the effect of epithelium removal on the β -adrenoceptor desensitization resulting from incubation of guinea pig trachea in the β -adrenergic agonist isoprenaline (10 μ M). Desensitization was measured as the change in EC_{50} , the concentration of β -agonist that produced 50% relaxation of tracheal rings contracted with methacholine. As a second measure of desensitization, we measured the shift in EC_{50} resulting from incubation of tracheal rings with the β -adrenoceptor antagonist propranolol (20 nM), expressed as CR-1 ($[\text{post-propranolol } EC_{50}/\text{baseline } EC_{50}] - 1$). Initially, we measured desensitization immediately after incubation in isoprenaline; subsequently, we repeated the protocol and allowed a 30 min rest between the end of incubation and the measurement. The sensitivity of denuded epithelium trachea to isoprenaline and (CR-1) was significantly higher than that of intact epithelium only in non-incubated preparations ($p < 0.05$ to $p < 0.001$). Incubation to isoprenaline caused a significant reduction in the tracheal response to isoprenaline in both the denuded groups ($p < 0.005$ for both cases) and intact epithelium groups ($p < 0.05$ for both cases). Incubation to isoprenaline also caused a significant reduction in (CR-1) value in both the denuded groups ($p < 0.005$ for group 2 and $p < 0.001$ for group 4) and intact epithelium only in group 1 ($p < 0.05$). However, the changes in EC_{50} due to tissue incubation with isoprenaline were significantly greater in denuded than intact epithelium trachea ($p < 0.05$ for all cases) and for CR-1 value only in groups 1 and 2 ($p < 0.05$). These results indicate decrease in both tracheal response to β -agonist (tolerance) and CR-1 (due to incubation of tissues with isoprenaline), which were greater in denuded epithelium groups.

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1. Introduction

There are controversies regarding bronchial responsiveness to β -adrenergic agonist in asthma. Previous studies have shown very similar dose-response relationships to β -agonists in normal and asthmatic subjects (Harvey and Tattersfield, 1982; Holgate et al., 1980; Harvey et al., 1981; Barnes and Pride, 1983). In addition, there are reports regarding desensitization of β_2 receptors in asthmatic patients which is mainly due to long time use of β_2 agonist, one of the most popular drugs used by these patients (Barnes, 1995). Reduction of beta-receptor numbers on lymphocytes from asthmatics is reported which is due to receptor down-regulation secondary to treatment with β -agonists (Harvey and

Tattersfield, 1982; Tashkin et al., 1982). Incubation of bovine (Hall and Chilves, 1989) and pig (Goldie et al., 1986) tracheal smooth muscle with isoproterenol also resulted in a reduction in β -receptor density, confirming that down-regulation of the receptor number is possible in this tissue over a relatively short time course. Administration of long-term albuterol to the guinea pig also leads to reduction of the relaxant effect of isoprenaline in the airways of these animals (Chong et al., 1998). Tolerance to β -agonist due to administration of this type of drug was also shown in asthmatic patients (Cockcroft and Swystun, 1996; Hancox et al., 1999).

However, our previous studies showed an increased bronchial responsiveness to isoprenaline (Boskabady and Snashall, 2000) and salbutamol (Boskabady and Saadatinejad, 2003) in asthmatic compared to normal subjects. The increased tracheal responsiveness to isoprenaline in sensitized guinea pigs (Boskabady and Zarei, 2004) and denuded epithelium trachea (Boskabady and Teymoory, 2003) was also observed. Therefore

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desensitization of β_2 receptors and airway responsiveness to β -agonists in asthma, even in asthmatic patients receiving long-term β -agonist therapy, are still questionable and require more explorations.

Epithelial shedding of airway is a pathological feature of asthma (Latinen et al., 1985). Airway epithelial damage is known to be associated with airway hyperreactivity, which is the most characteristic feature of asthma. In fact, several *in vitro* studies have shown that epithelial damage leads to increased bronchial responsiveness to different pharmacological agonists (Holroyde, 1986; Flavahan et al., 1985; Mapp et al., 1992). Serosal versus mucosal application of agonist ligands also lead to increased bronchial responsiveness (Fedan et al., 1990); but denudation of epithelium abolished this increased responsiveness (Munakata et al., 1989).

In the light of (1) controversies regarding bronchial responsiveness to β -adrenergic agonist, (2) reports regarding desensitization of β_2 receptors and (3) the existence of airway epithelial damage in asthma, in the present *in vitro* study, the effect of pre-exposure (incubation) of denuded and intact epithelium trachea with β -agonist, on tracheal responsiveness to isoprenaline was examined. In addition the β -receptor blockade by propranolol was also examined in non-incubated and incubated tissues with a β -agonist in denuded and intact epithelium trachea.

The mechanism of competitive antagonist blockade at the tracheobronchial tree is far simpler than the effect of an agonist. Competitive antagonist blockade is the degree of rightward shift in the concentration response curve and measured as dose ratio (DR) or concentration ratio (CR). It is determined only by concentration of antagonist at the receptor [I], which depends on dose and delivery and receptor affinity (K_a) (Arunlakshana and Schild, 1959). Therefore, if incubation of the tissues with isoprenaline affects competitive antagonism it will indicate the change of just one or both factors.

2. Material and methods

2.1. Tissue preparations

Guinea pigs (500–700 g) were killed by a blow on the neck, and tracheae were removed. Each trachea was cut into 10 rings (each containing two to three cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form tracheal chains (Holroyde, 1986). In the epithelium denuded group, the epithelium of trachea was removed by inserting a moistened cotton wire into lumen of trachea and gently rubbing with a corkscrew motion five to six times.

Tissue was then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, UK) containing Krebs-Henseliet solution of the following composition (mM): NaCl 120, NaHCO₃ 25, MgSO₄ 0.5, KH₂PO₄ 1.2, KCl 4.72, CaCl₂ 2.5 and dextrose 11. The Krebs solution was maintained at 37 °C and gassed with 95% O₂ and 5% CO₂. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

2.2. Measurement of tracheal responses

In each experiment two cumulative log concentration–response curves of isoprenaline sulphate (Sigma Chemical Ltd., UK) induced relaxation of precontracted tracheal chain with 10 μ M methacholine (Sigma Chemical Ltd., UK) were obtained, one 10 min after producing 20 nM concentration of propranolol hydrochloride (Sigma Chemical Ltd., UK) in organ bath (post-propranolol response curve), and the other 10 min after adding the same volume of saline (baseline isoprenaline response curve). To produce 20 nM concentration of propranolol in the organ bath 0.2 ml of a 1 μ M propranolol stock solution dissolved in Krebs was added to a 10 ml organ bath which was present throughout the construction of isoprenaline concentration response curve. The dose of propranolol was chosen according to previous studies (Boskabady and Teymoory, 2003; Boskabady and Zarei, 2004), which caused a shift in concentration response curve to isoprenaline. Consecutive concentrations (including; 0.1, 0.5, 1, 5, 10, 50 μ M, 0.1, 0.5 and 1 mM) were added every 2 min, and the percentage of relaxation due to each concentration of isoprenaline in proportion to the maximum contraction obtained due to 10 μ M methacholine in the presence of saline was plotted against log concentration of isoprenaline. The dose of methacholine was chosen according to previous studies with the same methodology, which produce 70% of maximum contractile response of tracheal chains to methacholine (Boskabady and Adel-Kardan, 1999). The relaxation due to each concentration was recorded at the end of 2 min, and the effect reached a plateau in all experiments.

The effective concentration of isoprenaline, causing 50% of maximum response (EC₅₀) of baseline and post-propranolol isoprenaline response curve in each experiment, was measured and expressed as EC₅₀ and post-propranolol EC₅₀, respectively. The EC₅₀ was taken as 50% of the maximum response observed under the condition being tested. In addition, the maximum relaxant effect due to isoprenaline was also measured in each experiment. The maximal response was the highest relaxation response observed to isoprenaline, in which in all tissues a plateau was obtained.

The effect of β -adrenergic receptor blockade by propranolol was assessed as concentration ratio minus one (CR-1), which was calculated by, (post-propranolol EC₅₀/baseline EC₅₀) – 1. The tracheal response to isoprenaline and β -adrenergic receptor blockade was studied on tracheal chains with four different groups of experiments as follows (Table 1):

- (1) Tracheal chains with intact epithelium (intact epithelium group, $n = 10$).
- (2) Tracheal chains with denuded epithelium (denuded epithelium group, $n = 10$).

All the pharmacological measurements in both groups were performed in two different conditions in random order as follows:

- a. Non-incubated tissues.
- b. Incubated tissues with 10 μ M isoprenaline during 1 h resting period. In incubated condition, the tissues were incubated for 1 h with 10 μ M isopre-

Table 1
Different groups of experiments of the study, two conditions in each group and exact time of pharmacological measurements in incubated condition

Groups	Definition	Conditions	Pharmacological measurements	<i>n</i>
1	Intact epithelium	Non-incubated Incubated	Immediately after 1 h incubation	10
2	Denuded epithelium	Non-incubated Incubated	Immediately after 1 h incubation	10
3	Intact epithelium	Non-incubated Incubated	30 min after 1 h incubation	5
4	Denuded epithelium	Non-incubated Incubated	30 min after 1 h incubation	5

The study was performed in four different groups as defined in Table 1, each group in two conditions of non-incubated and incubated of tissues with isoprenaline during 1 h resting period in random order. In groups 1 and 2 pharmacological measurements were made immediately after incubation period. In groups 3 pharmacological measurements were made after an additional 30 min resting period after 1 h incubation.

naline, and then washed three times before the dose response curve was constructed. Pharmacological measurements were performed immediately after the third washout of the tissue in incubated condition.

(3) and (4) Groups 3 and 4 were the same as groups 1 and 2, but in incubated conditions pharmacological measurements were performed after an additional 30 min resting period without incubation ($n=5$ for each group).

Therefore, isoprenaline played two roles in the protocol: (1) incubation of the tissues (pre-exposure) to study the effects of desensitization and (2) as an agonist to construct concentration–response curves.

The non-incubated measurements were made 1.5 h resting period after pre-exposure measurements, and vice versa, in each tracheal chain while washing the tissues every 15 min. The experiments for measuring baseline isoprenaline response curve and post-propranolol, isoprenaline response curve in each tracheal chain were performed randomly with 1 h resting period between each two experiments while washing the tissues every 15 min. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris) and measured after fixation. The study was approved by the ethical committee of our institution.

The tissues of some specimens were prepared for histological evaluations as follows:

- (1) Fixation of tissues with formaline 10%.
- (2) Drying of tissues using Autotecnicon apparatus (tissue passage apparatus) by passage of tissues through ethanol 70–100% and xylol to clear the tissues.
- (3) Preparing paraffin block of the tissues.
- (4) Preparing tissue slices with 3–5 μm thickness and putting them on a lam.
- (5) Tissues were stained using standard Hematixilline eosine method.

2.3. Statistical analysis

The data of tracheal response to isoprenaline (EC_{50}), β -adrenergic receptor blockade (CR-1), and maximum response

to isoprenaline were quoted as mean \pm S.E.M. According to the Kolmogorov Smirnov test these data had normal distribution. Therefore, the data of incubated and non-incubated tissues were compared using paired *t*-test. The data of intact epithelium trachea with those of denuded epithelium and the data of groups 3 and 4 with those of groups 1 and 2 were compared using unpaired *t*-test.

The change in EC_{50} or CR-1 due to incubation of the tissues was calculated as follows and expressed as percentage changes:

$$\frac{\text{EC}_{50} \text{ or CR-1 in incubated tissues} - \text{EC}_{50} \text{ or CR-1 in non-incubated tissues}}{\text{EC}_{50} \text{ or CR-1 in non-incubated tissues}} \times 100$$

The changes in EC_{50} or CR-1 due to incubation of the tissues between intact and denuded epithelium tracheal chains and between groups 1 and 2 with those of groups 3 and 4 were compared using both unpaired *t*-test and non-parametric Mann–Whitney *U*-test. Significance was accepted at $p < 0.05$.

3. Results

3.1. Histology

A few pairs of tissues were selected at random for histological evaluation. Among the rubbed epithelium trachea, more than 90% of epithelium had been removed without any damage to smooth muscle, while among the control trachea almost all of the epithelium was intact (Fig. 1).

3.2. Tracheal response to isoprenaline

In both sets of experiments (groups 1 and 2 and groups 3 and 4) the EC_{50} in the epithelium denuded group was significantly lower than in the intact epithelium group in non-incubated condition (Table 2). The incubation of the tissues with isoprenaline caused significant increase in EC_{50} in intact epithelium in both groups 1 and 3 (Figs. 2a and 3a) and denuded epithelium preparations in both groups 2 and 4 (Figs. 2b and 3b; Table 3). There was no significant difference between EC_{50} of the intact and denuded epithelium trachea in incubated preparations (Table 2). However, the increase in EC_{50} of denuded epithelium trachea (both groups 2 and 4) was significantly greater than those of

Table 2
Values of tracheal response to isoprenaline (EC₅₀), and β-adrenergic receptors blockade by propranolol (CR-1) in four groups of experiments

Tracheal response	Groups	Intact epithelium		Denuded epithelium	
		Non-incubated	Incubated	Non-incubated	Incubated
EC ₅₀	1 and 2	8.00 ± 0.53	33.10 ± 9.55	3.25 ± 0.49	25.6 ± 6.50
	St. Dif. inc. vs. non-inc.		<i>p</i> < 0.05		<i>p</i> < 0.005
	St. Dif. intact vs. denuded epi.			<i>p</i> < 0.001	NS
	3 and 4	10.20 ± 1.88	25.65 ± 4.9	2.54 ± 0.2	15.50 ± 0.80
	St. Dif. inc. vs. non-inc.		<i>p</i> < 0.05		<i>p</i> < 0.005
	St. Dif. intact vs. denuded epi.			<i>p</i> < 0.001	NS
	St. Dif. G. 3 and 4 vs. G. 1 and 2	NS	NS	NS	NS
CR-1	1 and 2	2.71 ± 0.59	1.16 ± 0.40	4.93 ± 0.81	1.30 ± 0.30
	St. Dif. inc. vs. non-inc.		<i>p</i> < 0.05		<i>p</i> < 0.001
	St. Dif. intact vs. denuded epi.			<i>p</i> < 0.05	NS
	3 and 4	1.69 ± 0.18	1.36 ± 0.54	7.56 ± 1.15	2.56 ± 0.54
	St. Dif. inc. vs. non-inc.		NS		<i>p</i> < 0.005
	St. Dif. intact vs. denuded epi.			<i>p</i> < 0.01	NS
	St. Dif. G. 3 and 4 vs. G. 1 and 2	NS	NS	NS	NS
Maximum response	1 and 2	89.82 ± 4.53	74.26 ± 5.63	100.72 ± 6.46	75.98 ± 4.88
	St. Dif. inc. vs. non-inc.		<i>p</i> < 0.05		<i>p</i> < 0.005
	St. Dif. intact vs. denuded epi.			NS	NS
	3 and 4	95.41 ± 2.61	86.17 ± 1.35	98.12 ± 0.79	84.65 ± 2.72
	St. Dif. inc. vs. non-inc.		<i>p</i> < 0.05		<i>p</i> < 0.01
	St. Dif. intact vs. denuded epi.			NS	NS
	St. Dif. G. 3 and 4 vs. G. 1 and 2	NS	NS	NS	NS

Values are presented as mean ± S.E.M.. St. Dif.: statistical differences, inc.: incubated, epi.: epithelium, G.: group, NS: non-significant difference. For groups 1 and 2, *n* = 10 and for groups 3 and 4, *n* = 5. The unit for EC₅₀ is μM isoprenaline, for CR-1 is the ratio of post-propranolol EC₅₀/baseline EC₅₀-1 and for maximum response is the percentage of highest relaxation response observed to isoprenaline relative to methacholine contraction. Comparison between two groups and two conditions (incubated and non-incubated) were done using unpaired and paired *t*-test, respectively.

intact epithelium (both groups 1 and 3) tissues (*p* < 0.05 for both cases), (Table 3).

3.3. Propranolol blockade (CR-1)

In non-incubated conditions, the rightward shift of post-propranolol, isoprenaline response curves in both sets of exper-

iments (groups 1 and 2 and groups 3 and 4), compared to baseline isoprenaline response curves in the epithelium denuded groups, was greater than that of the intact epithelium groups (Figs. 4 and 5). In both sets of experiments (groups 1 and 2 and groups 3 and 4) the mean (CR-1) in the epithelium denuded groups were significantly higher than that of intact epithelium groups in non-incubated condition (Table 2). The incubation of

Table 3
Changes in tracheal response to isoprenaline (EC₅₀), and β-adrenergic receptors blockade by propranolol (CR-1) in four groups of experiments due to incubation of tissue to β-agonist, isoprenaline

Tracheal response	Groups		Intact epithelium	Denuded epithelium	St. Dif. int. vs. den.	
					<i>t</i> -test	<i>U</i> -test
EC ₅₀	1 and 2		302.14 ± 105.19	703.06 ± 147.85	<i>p</i> < 0.05	<i>p</i> < 0.05
	3 and 4		174.44 ± 77.43	546.32 ± 127.54	<i>p</i> < 0.05	<i>p</i> < 0.05
	St. Dif. G. 3 and 4	<i>t</i> -test	NS	NS		
	vs. G. 1 and 2	<i>U</i> -test	NS	NS		
CR-1	1 and 2		107.24 ± 53.72	437.39 ± 118.00	<i>p</i> < 0.05	<i>p</i> < 0.005
	3 and 4		128.70 ± 79.67	242.60 ± 92.85	NS	NS
	St. Dif. G. 3 and 4	<i>t</i> -test	NS	NS		
	vs. G. 1 and 2	<i>U</i> -test	NS	NS		
Maximum response	1 and 2		24.82 ± 7.83	34.63 ± 8.07	NS	NS
	3 and 4		7.54 ± 1.85	13.74 ± 2.18	NS	NS
	St. Dif. G. 3 and 4	<i>t</i> -test	NS	NS		
	vs. G. 1 and 2	<i>U</i> -test	NS	NS		

Values are presented as mean ± S.E.M. of percent change. St. Dif.: statistical differences, den.: denuded epithelium, int.: intact epithelium trachea, G.: group, NS: non-significant difference. For groups 1 and 2, *n* = 10 and for groups 3 and 4, *n* = 5. The change in tracheal response to isoprenaline due to incubation of the tissues was calculated as follows: [(EC₅₀ or CR-1 in incubated tissues – EC₅₀ or CR-1 in non-incubated tissues)/EC₅₀ or CR-1 in non-incubated tissues × 100]. Statistical comparisons between groups were made using both unpaired *t*-test and non-parametric Mann–Whitney *U*-test.

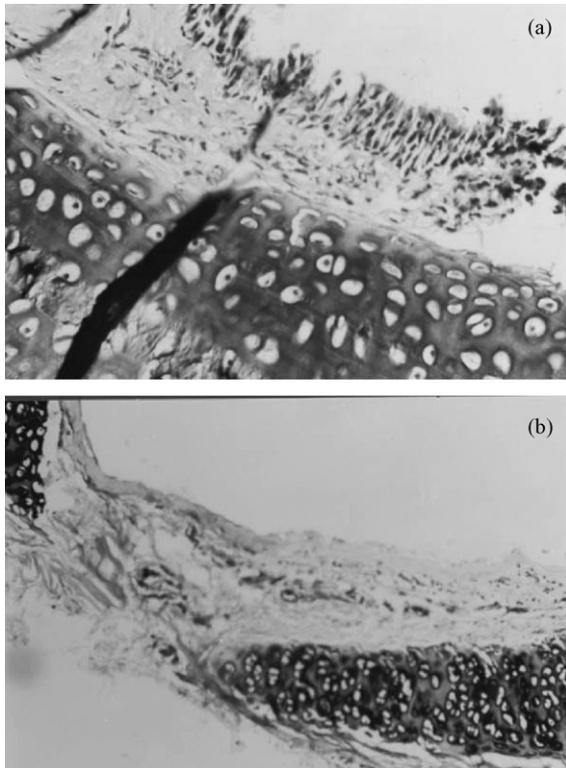


Fig. 1. Photographs of intact (a) and denuded epithelium thrachea (b).

the tissues with isoprenaline caused significant decrease in (CR-1) in intact epithelium trachea in group 1 (Fig. 2c) and denuded epithelium preparations in both groups 2 and 4 (Figs. 2d and 3d). There was no significant difference between (CR-1) of the intact and denuded epithelium trachea in incubated preparations (Table 2). The decrease in (CR-1) of denuded epithelium trachea was significantly greater than that of intact epithelium tissues only in groups 1 and 2 ($p < 0.05$), (Table 3).

3.4. Maximum response to isoprenaline

In both sets of experiments (groups 1 and 2 and groups 3 and 4) in non-incubated conditions, the mean value of maximum response in tracheal chains of the epithelium denuded groups was not significantly greater than that of intact epithelium groups (Table 2). The incubation of the tissues with isoprenaline caused significant decrease in maximum response in both intact and denuded epithelium preparations. There was no significant difference between maximum response of the intact and denuded epithelium trachea in the incubated preparations (Table 2).

3.5. Differences in different parameters between groups 1 and 2 with those of groups 3 and 4

There was no significant difference between the results of groups 3 and 4 with those of groups 1 and 2 (Table 2).

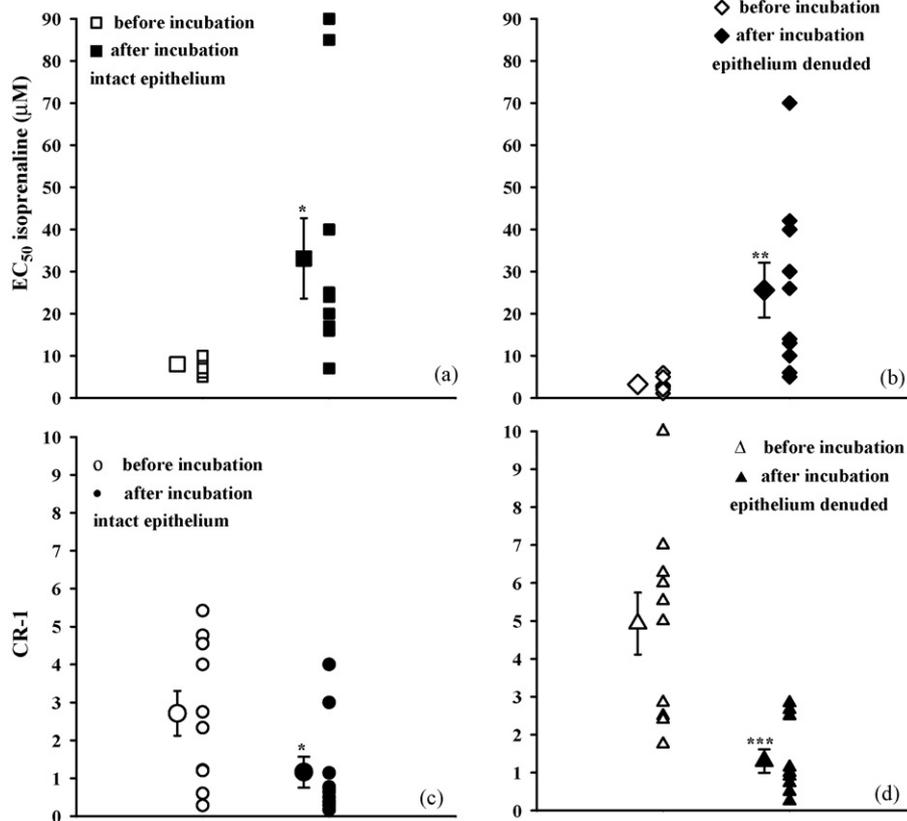


Fig. 2. Individual values and mean ± S.E.M. (big symbols with bars) of tracheal response to isoprenaline (EC₅₀) and β-adrenergic receptor blockade by propranolol (CR-1) in non-incubated (open symbols) and in incubated (filled symbols) intact epithelium and denuded epithelium trachea in groups 1 and 2 (n = 10 for each group).

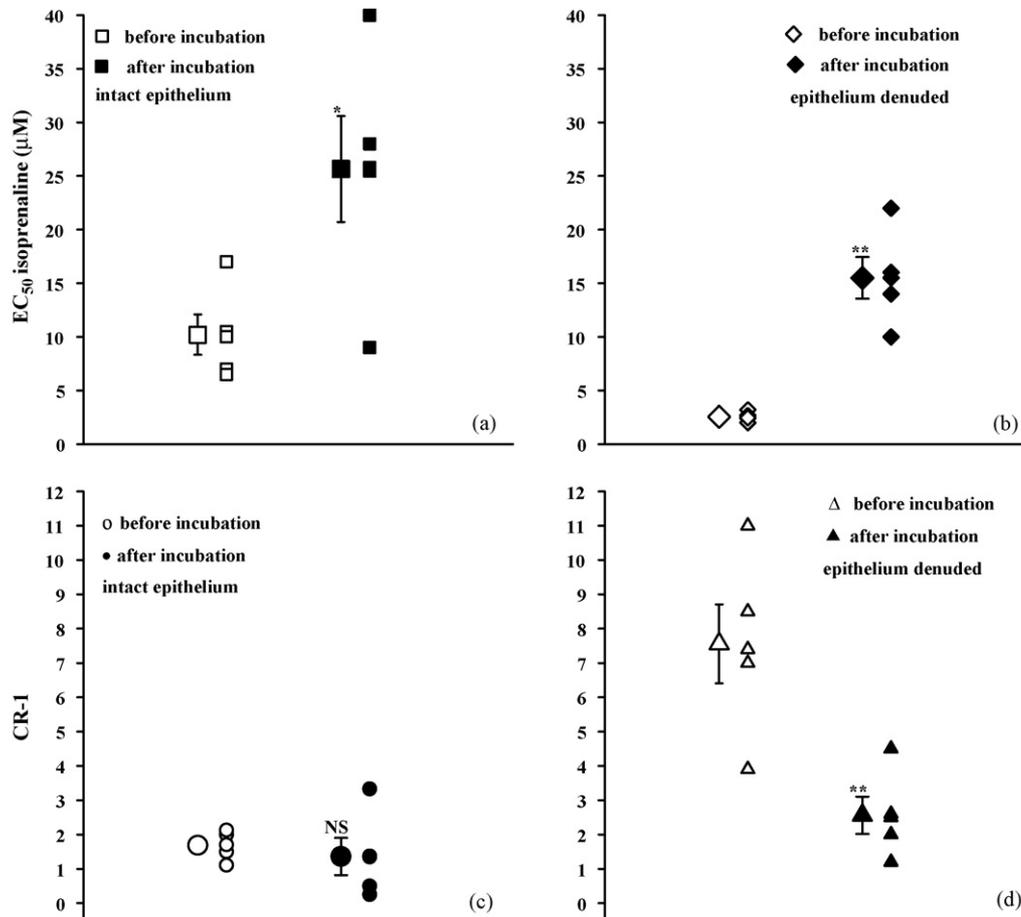


Fig. 3. Individual values and mean \pm S.E.M. (big symbols with bars) of tracheal response to isoprenaline (EC₅₀) and β -adrenergic receptor blockade by propranolol (CR-1) in non-incubated (open symbols) and in incubated (filled symbols) in intact epithelium and denuded epithelium trachea in groups group 3 and 4 ($n=5$ for each group).

4. Discussion

This study showed increased tracheal responsiveness to isoprenaline in epithelium denuded tracheal chains of guinea pig compared to intact epithelium trachea which is supported by the results of our previous study (Boskabady and Teymoory, 2003), that of Holroyde (1986) and Fedan et al. (1990) studies. The maximum response to isoprenaline was also significantly greater in denuded epithelium compared to intact epithelium tracheal chains. In addition, β -adrenoceptor blocked by propranolol in denuded epithelium trachea was greater than that of intact epithelium trachea, which was also supported by our previous study (Holroyde, 1986). Histological evaluation confirmed the removal of epithelium in the denuded epithelium trachea.

The incubation of tissue with 10 μ M isoprenaline for 1 h caused significant decrease in both tracheal responsiveness to isoprenaline and β -receptor blocked. However, the changes of tracheal responsiveness to isoprenaline and β -receptor blocked in denuded epithelium trachea were greater than those of intact epithelium trachea. In fact, both parameter and maximum response to isoprenaline in denuded tracheal chains were at the similar level with intact epithelium trachea in incubated conditions.

The response to methacholine in the intact epithelium trachea was less than denuded epithelial groups and tissues pre-exposed to isoprenaline caused reduction in response to methacholine. However, because the relaxant response to isoprenaline was measured as percentage of methacholine contraction, the differences of methacholine contraction did not affect isoprenaline response (Boskabady et al., 2006). Although half of the chains were first pre-exposed to isoprenaline to desensitized beta-receptors, and were then measured in the non-incubated state, the significant and high differences between incubated and non-incubated with relatively small S.E.M. of the data in non-incubated conditions in all groups indicate that the desensitized beta-receptors pre-exposed to isoprenaline trachea were removed. In addition, the significant and high differences between post-propranolol and pre-propranolol with relatively small S.E.M. of the data in pre-propranolol conditions in all groups indicate beta-adrenoceptor blockade was completely reversed before the “pre-propranolol” measurements.

Although there are some controversies regarding the desensitization of β -adrenoceptors in the asthmatic airways (Harvey and Tattersfield, 1982), most studies showed desensitization of β -receptors in airway tissues incubated with β -agonist (Hall and Chilves, 1989; Goldie et al., 1986) in both sensitized animals (Chong et al., 1998) and asthmatic patients (Cockcroft

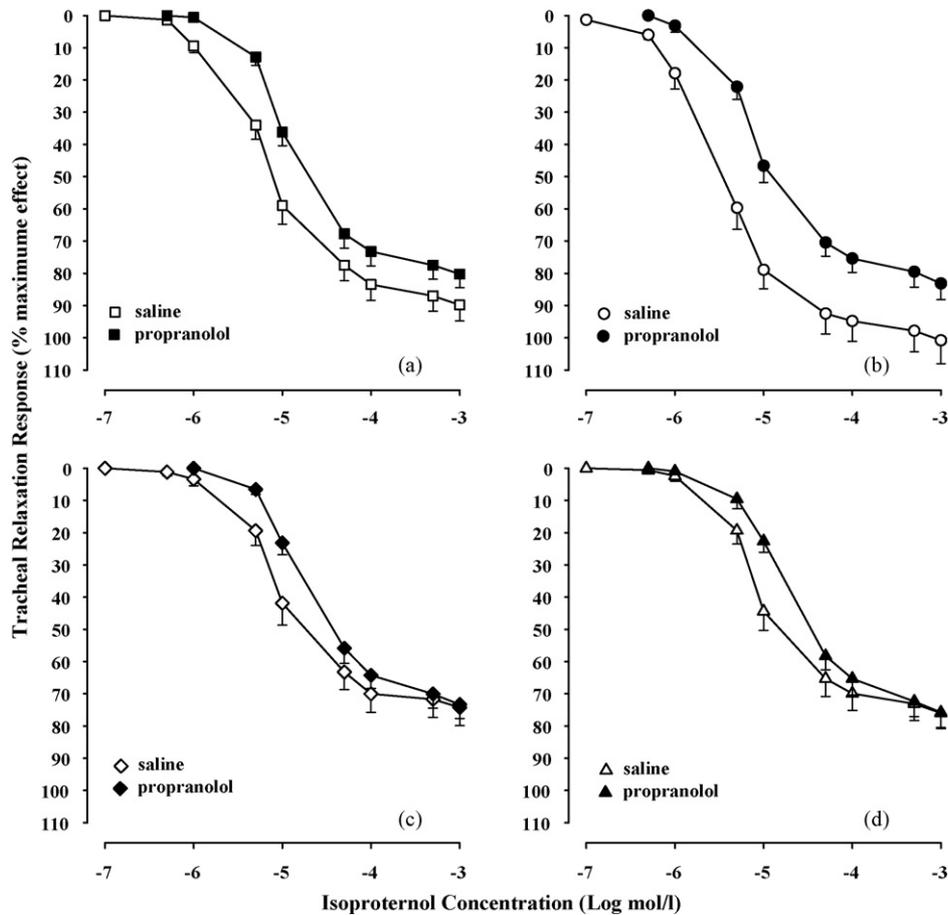


Fig. 4. Cumulative log concentration–response curves of isoprenaline induced relaxation of precontracted tracheal chains of guinea pig in the presence of saline (open symbols) and propranolol (filled symbols) in intact (a) and denuded (b) epithelium in non-incubated and incubated (c and d) conditions in groups 1 and 2 ($n = 10$ for each group).

and Swystun, 1996; Hancox et al., 1999) due to administration of β -agonist drugs which supports the results of the present study.

Epithelial damage is one of the pathological features of asthmatic airways (Latinen et al., 1985; Jeffery et al., 1989; Beasley et al., 1989; Padrid et al., 1995). Therefore, in the present study, the effect of epithelium denudation was examined on the phenomenon of desensitization. The results showed significantly greater desensitization due to incubation of the tissues with isoprenaline in the denuded epithelium trachea, which is one of the novel findings of the present study. The possible mechanism for greater desensitization of denuded tracheal chains is due to different physiological properties of epithelial cells including:

(1) Release of relaxant factors by epithelial cells such as epDRF (Fernandes et al., 1990) and PGE2 (Flavahan et al., 1985) in intact epithelium trachea causing greater relaxant effect in this group. In addition, membrane bound peptidase (natural endopeptidase) produced by epithelial cells which degraded substance P to inactive metabolites (Fine et al., 1989) can also lead to increased relaxant response in intact epithelium trachea. Incubation of intact epithelium trachea may also lead to decrease in secretion of spasmogen mediators by epithelial cells such endotheline (Howarth et al., 1995)

which can in turn cause increased relaxant response to isoprenaline in this group.

(2) Removal of barrier function of epithelium (Holroyde, 1986; Hogg, 1981) in denuded epithelium tracheal chains, which can lead to better access of isoprenaline to the receptor sites during incubation time. This, in turn, can cause greater desensitization in denuded epithelium trachea.

Enhanced block of different receptors by their corresponding antagonists have been shown in our previous studies both in asthmatic patients (Boskabady and Snashall, 1992, 1997, 2000) and in sanitized animals (Boskabady et al., 1998; Boskabady and Adel-Kardan, 1999; Boskabady and Zarei, 2004) as well as in denuded tracheal chains (Boskabady and Teymoory, 2003) and in another study (Chung and Snashal, 1984). Therefore, in the present study the effect of incubation of tracheal tissues with isoprenaline was also examined on β -receptor blockade (CR-1). The results showed significant reduction in (CR-1) due to incubation both in intact and denuded epithelium tracheal chains. However, the reduction of (CR-1) in denuded epithelium preparation was significantly greater than that of intact epithelium trachea.

Receptor blockade depends only on receptor affinity (K_a) and concentration $[I]$ of antagonist at the receptor sites

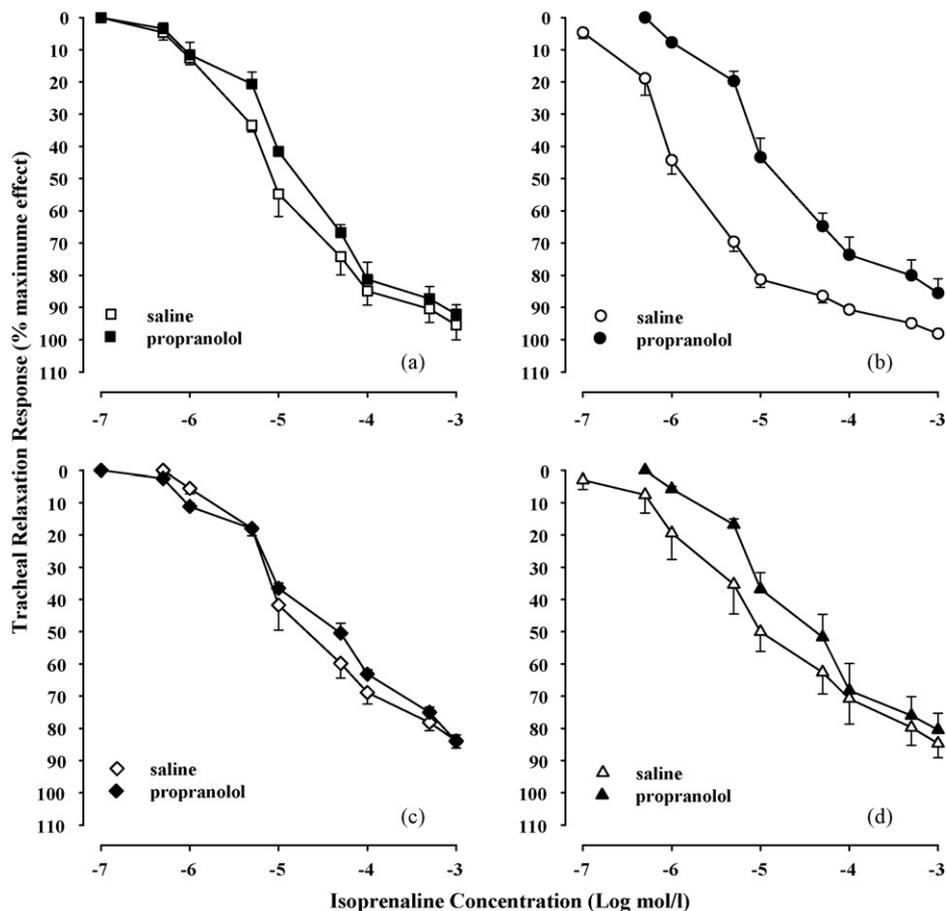


Fig. 5. Cumulative log concentration–response curves of isoprenaline induced relaxation of precontracted tracheal chains of guinea pig in the presence of saline (open symbols) and propranolol (filled symbols) in intact (a) and denuded (b) epithelium in non-incubated and incubated (c and d) conditions in groups 3 and 4 ($n = 5$ for each group).

(Arunlakshana and Schild, 1959). In addition, there is little or no variation in receptor affinity (K_a) in different tissues (Brown and Rand, 1980). Therefore, the possible cause for greater reduction of (CR-1) in denuded epithelium trachea is better access of isoprenaline to the receptor sites during incubation. This mechanism could also be the cause of greater reduction of EC_{50} in denuded epithelium trachea due to incubation. These results may indicate that the desensitization of β -adrenoceptors in asthma is also due to greater access of β -agonists to the receptor sites. Therefore epithelial damage in asthma (Latinen et al., 1985; Jeffery et al., 1989; Beasley et al., 1989; Padrid et al., 1995) could lead to greater access of β -agonists to receptor sites during long time administration of this type of drugs and cause desensitization of β -receptors.

In groups 1 and 2, post-incubation measurement of tracheal responsiveness to isoprenaline and β -receptor blockade by propranolol was performed immediately after three consecutive washes of incubated tissue with Krebs solution. Therefore, the reduction in both tracheal response to isoprenaline and (CR-1) seems to be due to the existence of incubated isoprenaline at the receptor sites. So, the effect of tissue incubation with isoprenaline in tracheal response to β -agonist and β -receptor blockade by propranolol was re-examined in groups 3 and 4. In these two groups tissues were incubated with isoprenaline

for 1 h following a 30 min rest at which tissues were washed with Krebs solution every 15 min. The post-incubation measurements of tracheal response to isoprenaline and β -receptor blockade were performed after the 30 min rest period. Therefore, with this design, the tracheal response to β -agonist and (CR-1) would not be affected or at least, would be less affected by the presence of incubated isoprenaline at the receptor sites. However, the reduction of tracheal response to isoprenaline and (CR-1) in the incubated tissues was very similar to that of groups 1 and 2.

Previous studies showed increased airway responsiveness to β -agonist drugs in asthmatic patients (Boskabady and Snashall, 2000; Boskabady and Saadatinejad, 2003), sensitized animals (Boskabady and Zarei, 2004) and in tracheal chains of guinea pig with denuded compared to intact epithelium (Holroyde, 1986; Boskabady and Teymoory, 2003). Bai et al. (1992) also showed increased airway smooth muscle relaxation response to β -agonist. Based on the result of the above studies it seems the phenomenon of airway hyper responsiveness in asthma should be extended to β -agonist drugs. In addition our previous studies showed enhanced receptor blocked due to propranolol in asthmatic patients (Boskabady and Snashall, 2000), in sensitized animal (Boskabady and Zarei, 2004) and in denuded compared to intact epithelium trachea (Boskabady and Teymoory, 2003).

Therefore, the important question raised by the results of the present study is whether greater decrease in trachea response to isoprenaline and (CR-1) in denuded trachea due to incubation to a β -agonist is an indication of beneficial or harmful effect of long term administration of β -agonist therapy in asthma. In fact the results of our previous study with similar methodology to the present study showed that incubation of tissue with 10 μ M isoprenaline for 1 h caused significant decrease in tracheal responsiveness to methacholine which was more pronounced in denuded epithelium compared to intact epithelium trachea (Boskabady et al., 2006).

The greater reduction in tracheal response to isoprenaline in denuded epithelium tissue may indicate that incubation of tissue with β -agonist as well as long-term administration of β -agonist in asthmatic patients may lead to decreased airway responsiveness in asthma. In fact sustained increase in pulmonary function tests, improvement of asthma symptom and no significant effect on airway responsiveness due to regular and long-term use of β_2 -agonists have been shown (Rosenthal et al., 1999; Yates et al., 1995). However, the magnitude of importance of the findings of the present study and hypothesis raised should be examined in future and specially *in vivo* studies.

In conclusion, the results of the present study showed desensitization of β -adrenoceptors, which was greater in denuded epithelium tracheal. The incubation of the tissues to isoprenaline also leads to reduction in β -receptor blockade by propranolol, which was also greater in denuded epithelium preparation. Therefore, the results indicate that greater access of β -agonist due to epithelial damage in asthma may play an important role in desensitization of β -receptors seen in this disease. However, incubation of denuded epithelium trachea with β agonists as well as long-term administration of this type of drug in asthmatic patients may lead to decrease in airway responsiveness in asthma.

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