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ORIGINAL ARTICLE

Inhibitory Effect of Sodium Cromoglycate on Insulin Induced Airway Hyper-Reactivity

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ABSTRACT

Objective: To explore the acute effect of insulin on airway reactivity of guinea pigs and protective effects of sodium cromoglycate against insulin induced airway hyper-reactivity on isolated tracheal tissues of guinea pigs in vitro.

Subjects and Methods: Effects of insulin (10^{-7}- 10^{-3} M) and insulin pretreated with sodium cromoglycate (10^{-6} M) were observed on isolated tracheal strip of guinea pig (n=12) in vitro by constructing cumulative concentration response curves. The tracheal smooth muscle contractions were recorded with Transducer on Four Channel Oscillograph.

Results: Insulin produced a concentration dependent reversible contraction of isolated tracheal muscle of guinea pig. The mean ± SEM of maximum amplitudes of contraction with insulin and insulin pretreated with sodium cromoglycate were 35 ± 1.13 mm and 14.55 ± 0.62 mm respectively. Cromoglycate shifted the concentration response curve of insulin to the right and downwards.

Conclusion: Sodium cromoglycate significantly reduced the insulin mediated airway hyper-reactivity in guinea pigs. So we suggest that pretreatment of inhaled insulin with cromoglycate may have clinical implication in amelioration of its potential respiratory adverse effects such as bronchoconstriction.

Key words Bronchoconstriction, Inhaled insulin, Oscillograph, Sodium cromoglycate, Tracheal muscle.

Author`s Contribution
[1] Conception, synthesis, planning of research and manuscript writing
[2] Interpretation and discussion Data analysis,
[3,4] Interpretation, manuscript writing and Active participation in data collection

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Introduction

The predominant mode of insulin administration is by subcutaneous injection.[1] Injection related anxiety leads to poor compliance and suboptimal glycemic control.[2,3] Consequently alternative noninvasive inhalational route of insulin administration was approved in 2006.[4] Inhalational insulin causes similar reduction in HbAIc and fasting blood sugar, compared with regular insulin and less risk of hypoglycemic episodes and weight gain.[5] It increases patient's satisfaction and improved patients compliance leading to improved glycemic control [6,7] Unfortunately its use was limited due to its potential to produce respiratory adverse effects such as increased bronchial reactivity, cough, dyspnea and bronchoconstriction.[8] Insulin has long been recognized as pro-inflammatory and pro-contractile hormone.[9,10] The most likely mechanism of inhaled insulin induced bronchoconstriction is that insulin

increases the mast cells degranulation and subsequently increased release of histamine and contractile prostaglandins are responsible for allergic inflammation of airways.[11-13]

It is well established from the review of literature that cromoglycate sodium exerts its anti-allergic and anti-inflammatory effects due to its mast cell stabilizing activity. Prophylactic use of sodium cromoglycate decreases the symptoms of airway hyper-reactivity induced by variety of allergens and chemicals. Cromoglycate sodium has also shown to have weak bronchodilatory effects in guinea pig airway models.[14]

To our knowledge protective effects of sodium cromoglycate against inhalational insulin induced airway hyper-responsiveness have never been evaluated. Insulin induced tracheal muscle contraction in guinea pig model described in this study closely resembles the bronchoconstriction produced by pulmonary delivery of inhaled insulin as high concentration of insulin get deposited in airway smooth muscles in both cases.[15] Based on the pharmacological effects of sodium cromoglycate the present study was designed to evaluate the efficacy of sodium cromoglycate against insulin mediated airway hyper-reactivity of guinea pig in vitro.

Subjects and Methods

This experimental study was conducted in Pharmacology department in collaboration with Centre for Research in Experimental and Applied Medicine (CREAM) Army Medical College Rawalpindi from January 2012 to August 2012.

Total 12 healthy guinea pigs of either sex of Dunkin Hartely variety weighing 500-700g were used for current study.[16] These guinea pigs were randomly divided into two groups; each group consists of 6 guinea pigs. All the protocols described in this study were approved by Ethics commitee of Centre for Research in Experimental and Applied Medicine (CREAM). They were sacrificed by cervical dislocation.[17] The trachea was dissected out and tracheal chain was prepared with smooth muscle in the centre and cartilaginous portions on both sides. The tracheal strip was attached to the hook of oxygen tube of tissue bath containing oxygenated krebs-Henseleit solution at 37^{o} C.[18] Tracheal contractions were recorded with Research Grade Isometric Force Displacement Transducer Harvard Model No 72-4494 on four channel oscillograph Harvard Model No 50-9307.[19] This Research Grade Research Grade Isometric Force Displacement Transducer Harvard Model No 72-4494 was extremely sensitive to force applied with very short amplitude. It has a minimum displacement of 0.1mm for a force application of 25 grams. [20]

Group 1: Cumulative concentration response curve of insulin (10^{-7} to 10^{-3} M)

Cumulative dose response curves of insulin were constructed with varying concentrations (10^{-7} to 10^{-3}M). When maximum response with 10^{-7} M concentration was obtained then the subsequent doses were added without washing the previous dose. [21] Four channel oscillograph was used for recording tracheal muscle contraction. This group served as control group 1 and dose response curve of insulin pretreated with cromoglycate was compared with that of insulin alone.

Group 2: Cumulative concentration response curve of insulin in the presence of fixed concentration (10^{-6} M) of sodium cromoglycate. Cromoglycate sodium was added to the organ bath in a concentration of 10^{-6} M.[14] After 15 minutes, the successive doses of insulin ranging from10^{-7} to 10^{-3} M were added into the organ bath in the presence of cromoglycate sodium. Cumulative concentration response curves pretreated with cromoglycate sodium were constructed.

Statistical analysis

The means of amplitudes of contractions and SEMs were calculated using SPSS version 16. Student t test was applied to determine the significant difference between two observations.

p-value of less than 0.05 was considered as statistically significant.

Results

Acute effects of insulin were studied on isolated tracheal smooth muscles of guinea pig by adding the successive doses of insulin ranging from 10^{-7} to 10^{-3} M. Insulin induced contraction of tracheal smooth muscle was evident at a concentration of 10^{-7} M concentration. However, a significant enhancement of insulin-induced contractions was observed at 10^{-5} M, 10^{-4} M and 10^{-3} M concentration. (Figure 1).

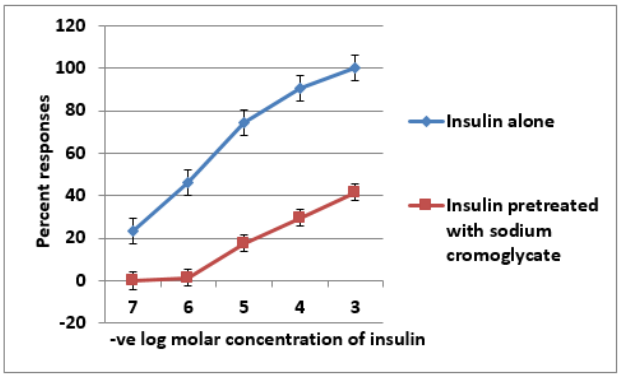

Figure 1: Comparison of semi log concentration response curve of group 1 and group 2 on isolated tracheal smooth muscle of guinea pig.

- Results are average of six separate experiments.
- Data is represented as mean ± standard error of means (SEM)

Changes in tracheal smooth muscle contractions were measured by taking the amplitudes of tracheal smooth muscle contraction. Amplitudes of contraction with maximum dose of insulin (10^{-3} M) was 35 ± 1.13mm (Table 1).

So insulin significantly enhanced the myogenic airway smooth muscle tone. This insulin induced tracheal smooth muscle contraction was significantly reduced in sodium cromoglycate treated group from 35 ± 1.13mm to 14.55 ± 0.62 mm. The means of amplitudes of contractions with varying doses of insulin when compared between group 1 and 2 were found to be statistically significant (Table 1). Our data showed that maximum constrictor response of insulin in the presence of cromoglycate was reduced by 41.57 percent as compared with insulin group (Table 1). Insulin concentration response curve in the presence of cromoglycate was shifted to the right and downwards indicating a profound inhibitory effect of cromoglycate sodium on airway hyper-reactivity induced by insulin (Figure 1).

Discussion

The present study demonstrated that insulin induced airway smooth muscle contraction of guinea pigs in a concentration range of 10^{-7} M to 10^{-3} M. These contractions were reversible and sustained in nature. Schaafsma et al also reported the acute contractile effect of insulin on isolated tracheal preparation of guinea pig but the concentration of insulin was in the range of 10^{-10} to 10^{-5} M. [21] Our observations are also supported by in vivo studies in which treatment of diabetic rats with insulin resulted in airway hyper-reactivity and inflammation. This enhanced airway reactivity was due to the release of inflammatory mediators from mast cells under the influence of insulin. [22]

When isolated tracheal muscle was pretreated with sodium cromoglycate, the maximum percent response of insulin in the presence of cromoglycate sodium was reduced to 41.57 percent of insulin control. So cromoglycate sodium significantly ameliorated the insulin mediated airway hyper-reactivity. The protection offered by cromoglycate is presumably through the inhibition of release of contractile prostaglandins and histamine from mast cells of isolated tracheal strip. Our results are in accordance with other studies in which sodium cromoglycate has been shown to inhibit the bronchoconstrictor response to several kinds of challenges.[23]

Table 1: Comparison of responses of isolated tracheal muscle of guinea pig between two groups

Concentration of insulin (M)	Group 1 (n=6) Amplitude of contraction (mm) (mean ± SD)	Group 2 (n=6) Amplitude of contraction (mm) (mean ± SD)	p-value	Percent response in group 1 from baseline to 10-3 M dose	Percent response in group 2 from baseline to 10-3 M dose (group 1)
10^{-7}	8.167 ± 2.14	0 ± 0	.004*	23.34	0
10^{-6}	16.16 ± 2.48	0.5 ± 0.837	.007*	46.17	1.43
10^{-5}	26.1 ± 2.78	6.17 ± 1.169	0.000*	74.58	17.62
10^{-4}	31.8 ± 2.04	10.33 ± 1.63	.003*	90.86	29.5
10^{-3}	35 ± 2.76	14.55 ±1.52	.004*	100	41.57

Conclusion

This study provides us a clue that cromoglycate can attenuate the pro-contractile effect of insulin. So we suggest that pretreatment with cromoglycate may ameliorate respiratory adverse effects of inhaled insulin therapy in diabetic patients. Further clinical trials are warranted to confirm whether the protection offered by cromoglycate in guinea pig model can translate to human airways.

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