Print ISSN 2319-2003 | Online ISSN 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.5455/2319-2003.ijbcp20141001

Research Article

Insulin causes airway hyper-reactivity

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Received: 17 June 2014
Accepted: 05 July 2014

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ABSTRACT

Background: We explored the acute effects of insulin and one possible mechanism underlying the acute contractile effects of insulin on isolated tracheal smooth muscle of guinea pig in vitro.
Methods: Effects of increasing concentrations of histamine (10^{-7}-10^{-3} M), insulin (10^{-7}-10^{-3} M), insulin pretreated with a fixed concentration of indomethacin (10^{-6} M) were studied on isolated tracheal tissue of guinea pig in vitro by constructing cumulative concentration response curves. The tracheal smooth muscle contractions were recorded with transducer on four channel oscillograph.
Results: Histamine and insulin produced a concentration-dependent reversible contraction of isolated tracheal muscle of guinea pig. The mean±standard error of the mean of maximum amplitudes of contraction with histamine, insulin and insulin pretreated with indomethacin were 92.5±1.20 mm, 35±1.13 mm and 14.55±0.62 mm respectively. Indomethacin shifted the concentration-response curve of insulin to the right and downwards.
Conclusions: Insulin has acute contractile effects on guinea pig airways, which were significantly inhibited by prostaglandin synthesis inhibitor indomethacin confirming the involvement of contractile prostaglandins in insulin-induced airway hyper-responsiveness.

Keywords: Histamine, Inhaled insulin, Indomethacin, Oscillograph, Tracheal muscle

INTRODUCTION

The pre-dominant mode of insulin delivery is through subcutaneous injections that are troublesome for diabetic patients. Several new delivery systems are now being developed for administration of insulin by non-subcutaneous route.[1] Inhalational formulation of insulin was available in the market from September 2006 to October 2007.[2] The large surface area, and high permeability of lungs make it an ideal delivery route with insulin being absorbed into the systemic circulation by alveoli.[3] In a randomized 1 year inhaled insulin study, it was demonstrated that its efficacy was similar to injectable insulin formulation. Pulmonary delivery of insulin alleviates the fear associated with insulin injection. Improved compliance leads to better glycemic control, which ultimately reduce the risk of long-term complication of diabetes.[4] Unfortunately inhaled insulin was withdrawn from the market due to its respiratory adverse effects such as increased airway hyper-reactivity, cough, dyspnea, bronchoconstriction.[5] The most likely mechanism of inhaled insulin-induced bronchoconstriction is that insulin promotes the mast cells degranulation and subsequently increased release of contractile prostaglandins are responsible for allergic inflammation of airways.[6] To evaluate the role of prostaglandins in mediating airway hyper-reactivity, prostaglandin synthesis inhibitor indomethacin is commonly used. It is a non-steroidal anti-

inflammatory drug, which inhibits cyclooxygenase enzyme that participate in prostaglandin synthesis from arachidonic acid.[7]

It is well-established from the review of literature that insulin-induced isolated tracheal muscle contraction in guinea pig model described in the present study closely resembles the bronchoconstriction induced by pulmonary delivery of inhaled insulin as high concentration of insulin gets deposited in airway smooth muscle compartment in both cases.[8] Aim of our study was to explore the magnitude of insulin-induced airway hyper-reactivity and to evaluate the potential role of prostaglandins in insulin-mediated tracheal tissue contraction of guinea pig in vitro. Histamine is commonly used for provocative studies on airway smooth muscles.[2] Histamine mediated enhanced myogenic tone of airways is taken as the gold standard for in vitro studies.[2] So to evaluate the extent of insulin-induced airway hyper-reactivity, histamine-mediated airway hyper-responsiveness was taken as standard (control) in our research study and was compared to insulin-mediated tracheal smooth muscle contraction.

METHODS

This experimental study was carried out in Pharmacology Department in collaboration with Centre for Research in Experimental and Applied Medicine Army Medical College Rawalpindi, from December 2011 to July 2012.

Drugs/chemicals

Histamine dihydrochloride and indomethacin were purchased from Sigma Co A USA. Regular human insulin (100 IU/ml) was obtained from Lilly Pharma.

Experimental setup

Eighteen guinea pigs were randomly divided into three groups after the approval of Ethics Committee of CREAM. They were sacrificed by cervical dislocation.[9] The trachea was dissected out, and tracheal chain was prepared with smooth muscle in the center and cartilaginous portions on both sides. One end of the tracheal strip was attached to the hook of the oxygen tube of the tissue bath containing oxygenated Krebs–Henseleit solution at 37°C while the other end was connected to the Transducer (Harvard Model No 72-4494). Four channel oscillographs was used for recording tracheal muscle contraction.[10]

Group 1 Cumulative concentration response curve of histamine (n=6)

Cumulative dose-response curves of histamine were constructed with varying concentrations (10^{-7}-10^{-3}M). When maximum response with 10^{-7} M concentration was obtained then the subsequent doses were added without washing the previous dose. Four channel oscillographs was used for recording tracheal muscle contraction.[11] This group served as control Group 1 and dose response curve of histamine was compared with that of insulin to determine the magnitude of insulin-mediated contraction, because in research studies histamine-mediated airway smooth muscle contraction is commonly taken as a standard (100%).

Group 2 Cumulative concentration-response curve of insulin (n=6)

Cumulative concentration-response curve of insulin was obtained using varying concentrations ranging from 10^{-7} to 10^{-3} M and the same procedure was repeated as described for histamine.[2]

Group 3 Cumulative concentration-response curve of insulin in the presence of fixed concentration (1^{-6} M) of indomethacin (n=6)

Indomethacin (10^{-6} M) was added to the organ bath. After 15 mins, cumulative dose response curve was constructed by using varying concentrations of insulin (10^{-7}-10^{-3} M).

Statistical analysis

The results have been expressed as means±standard error of means. The arithmetic means of amplitudes of contractions and SEMs were calculated using SPSS version 16 (SPSS Inc. Chicago IL). In order to find the significance of the difference between two observations "student's t-test" was used.

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. However a significant enhancement of insulin-induced contractions was observed at 10^{-5} M, 10^{-4} M and 10^{-3} M concentrations (Table 1).

Histamine mediated airway contraction is commonly taken as standard in experimental studies, so to evaluate the magnitude of insulin-induced airway reactivity, acute effects of histamine on isolated tracheal smooth muscle were also studied and were compared with constrictor response of insulin (Table 1). Changes in tracheal smooth muscle contractions were measured by taking the amplitudes of tracheal smooth muscle contraction. Amplitudes of contraction with a maximum dose of histamine and insulin (10^{-3} M) were 92.5±1.20 mm and 35±1.13 mm respectively (Table 1). Hence, histamine and insulin significantly enhanced the myogenic airway smooth muscle tone. To evaluate the extent of insulin-induced airway hyperresponsiveness the percentage responses for Group 1 and 2 were also calculated. Maximum constrictor

response of insulin was 38% of maximal histamine response (Table 1).

This insulin-induced tracheal smooth muscle contraction was significantly reduced in indomethacin treated group from 35±1.13 mm to 14.55±0.62 mm. The means of amplitudes of contractions with varying doses of insulin when compared between Group 2 and 3 were found to be statistically significant (Table 2). Our data showed that maximum constrictor response of insulin in the presence of indomethacin (Group 3) was reduced by 41.57% as compared with insulin group (Table 2). Insulin concentration response curve in the presence of indomethacin was shifted to the right and downwards indicating a profound inhibitory effect of indomethacin on airway hyper-reactivity induced by insulin.

DISCUSSION

The current study was undertaken to observe the acute effects of insulin on guinea pig airways and explored one possible mechanism that may underlies the acute contractile effect of insulin on isolated tracheal muscle of guinea pig.

Histamine and insulin increased the airway reactivity in a dose-dependent manner. The magnitude of insulin-mediated airway hyper-reactivity was evaluated by comparing it with histamine-mediated tracheal tissue contraction as histamine provoked airway hyper-responsiveness is taken as a standard (control) for experimental studies.[11] The maximum insulin-induced tracheal tissue contraction was 38% of histamine-mediated contraction (control) in contrast to 33% contraction which was reported in an in vitro study using the same experimental setup.[2] Our results are also in accordance with in vivo studies in which airway reactivity of diabetic rats was increased after administration of insulin due to increase the release of prostaglandins and histamine from mast cells.[12]

Tracheal muscle when pretreated with cyclo-oxygenase inhibitor indomethacin, concentration response curve of insulin was shifted downwards and to the right with percent response of 41.57% of the insulin control suggesting a pivotal role of contractile prostaglandins in insulin mediated airway hyper-reactivity. Our findings are in accordance with results of a study in which insulin-induced tracheal muscle contraction was strongly inhibited in the presence of indomethacin (prostaglandin synthesis inhibitor), FP and EP_1 receptor blockers.[2] Since insulin is a pro-inflammatory hormone[13] the potential beneficial effects of indomethacin may also be exerted through its anti-inflammatory effects.[14]

Inhibitory effects of indomethacin provide us a clue that insulin mediated airway-hyper-responsiveness is prostaglandin mediated.

Table 1: Comparison of responses of isolated tracheal muscle of guinea pig between histamine (Group 1) and insulin (Group 2).

Concentration (M) of histamine/insulin	Amplitude of contraction with histamine (mean±SD) (mm) (n=6)	Amplitude of contraction with insulin (mean±SD) (mm) (n=6)	p value between Group 1 and 2	Percent response with histamine	Percent response with insulin
10^{-7}	19.67±2.65	8.167±2.14	0.000*	21.26	8.87
10^{-6}	44.8±4.12	16.16±2.48	0.000*	48.43	17.55
10^{-5}	68.67±5.16	26.1±2.78	0.000*	74.24	28.34
10^{-4}	87.3±3.266	31.8±2.04	0.001*	94.37	34.53
10^{-3}	92.5±2.949	35±2.76	0.001*	100	38

p<0.05=Significant (*). SD: Standard deviation

Table 2: Comparison of responses of isolated tracheal muscle of guinea pig between insulin (Group 2) and insulin pretreated with indomethacin (Group 3).

Concentration of insulin (M)	Amplitude of contraction with insulin (mean±SD) (mm) n=6	Amplitude of contraction with insulin pretreated with indomethacin (mean±SD) (mm) n=6	p value between Group 2 and 3	Percent response with insulin	Percent response with insulin pretreated with indomethacin
10^{-7}	8.167±2.14	0±0	0.004*	23.34	0
10^{-6}	16.16±2.48	0.5±0.837	0.007*	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	0.003*	90.86	29.50
10^{-3}	35±2.76	14.55±1.52	0.004*	100	41.57

p<0.05=Significant (*). SD: Standard deviation

CONCLUSION

Insulin induces airway smooth muscle contraction presumably through the production of prostaglandins that were significantly inhibited in the presence of indomethacin. So indomethacin can become useful therapeutic agent for the attenuation of airway hyper-reactivity mediated by inhaled insulin therapy in diabetic patients**.**

ACKNOWLEDGMENTS

We are grateful to National University of Sciences and Technology (NUST) Islamabad for providing financial support for this research study.

Funding: From National University of Sciences and Technology Islamabad (NUST)
Competing interest: None
Ethical approval: From institutional ethical Committee

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doi: 10.5455/2319-2003.ijbcp20141001
Cite this article as: Sharif M, Khan BT, Afzal A, Anwar MA. Insulin causes airway hyper-reactivity. Int J Basic Clin Pharmacol. 2014;3:789-92.