Original Article

Role of Relaxant Prostaglandins in the Effect of Nebivolol on Isolated Tracheal Muscle of Guinea Pig

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ABSTRACT

Aims and Objectives: The present study was therefore aimed to evaluate the role of relaxant prostaglandins in modulating the effect of Nebivolol on tracheal muscle of guinea pig since the exact mechanism underlying its effects on tracheal muscle has not been established yet.

Background: The use of beta blockers is limited by their ability to produce bronchospasm in asthmatics. Third generation β blockers like Nebivolol may show better tolerability since there may be involvement of relaxant prostaglandins in its effect. However the involvement of prostaglandins in the respiratory effects of Nebivolol remains unexplored. The present study, carried out on isolated tracheal muscle strips of guinea pigs was designed to explore this controversy.

Study Design: Experimental Study.

Place and Duration of Study: This study was conducted at the Department of Pharmacology, Army Medical College, Rawalpindi since April 2010 to November 2010.

Materials and Methods: Varying concentration of histamine ranging from 10^{-7}M to 10^{-3}M were used to plot a concentration response curve on the isolated tracheal muscle strips of guinea pig and was used as a control. The same concentration response curve was plotted in presence of a fixed concentration of Nebivolol 10^{-6}M and then again in presence of a fixed concentration of Indomethacin 10^{-6}M and Nebivolol 10^{-6}M together in a series of experiments using six sets of isolated tracheal muscle strips in each case.

Results: Nebivolol did not produce any significant shift in the concentration response curve in the presence and absence of Indomethacin.

Conclusion: Nebivolol does not augment the histamine induced contraction of respiratory smooth muscle of guinea pig in the presence of Indomethacin, prostaglandin synthesis inhibitor indicating no role of relaxant prostaglandins in the sparing of respiratory smooth muscle by Nebivolol.

Key Words: Beta blockers, Bronchospasm, Nebivolol, Isolated tracheal smooth muscle,Histamine, Indomethacin.

INTRODUCTION

Beta adrenoceptor antagonists are one of the most effective drugs in the treatment of cardiovascular diseases as well as non cardiovascular diseases.[1]However their therapeutic utility is limited by pulmonary adverse effects.[2] This is more commonly seen with non selective β blockers. So new molecules with a better respiratory tolerability were designed to overcome the narrow therapeutic window of first generation β blockers.[3]Nebivolol is such a newer beta blocker possessing ancillary properties due to which it may afford a greater margin of safety in patients with COAD.[2]Studies have reported that Nebivolol has sparing effect on airway smooth muscle.[4,5]However the mechanism underlying this effect is still controversial, Matera, 1998[6] has implicated modulation of nitric oxide release to be responsible for this sparing effect while Tilley *et al* 2003[7] have shown relaxant prostaglandins to be responsible. Therefore the proposed study was planned to evaluate the role of relaxant prostaglandins in the respiratory effects of Nebivolol.

MATERIALS AND METHODS

Animals: The present study has been conducted on the isolated tracheal smooth muscle of 24 guinea pigs of Dunkin Hartley variety weighing 500 to 600 grams.They were housed at room temperature and were given tap water *ad libitum* and fed with a standard diet consisting of carrots, choker and grams.

Drugs used for the study: Nebivolol was a generous gift from Menarini Recerche, Italy.Histamine was purchased from Sigma Chemical Co. USA while indomethacin was bought from Shanghai Chang Hua Industry, China.

Preparation of the Solutions: Krebs Henseleit solution was used as the nutrient solution the composition of which per 1000 mls is: NaCl-118.2mM, KCl-4.7mM, $MgSO_4.7H_2O$-1.2mM, $CaCl_2$-2.5mM, KH_2PO_4-1.3mM, $NaHCO_3$-25.0mM, Dextrose-11.7mM. Solutions of all drugs were prepared in the distilled water except for Nebivolol the solution of which was prepared in Dimethyl sulphoxide since Nebivolol is highly lipophilic and insoluble in water[8].

General procedure for the experiments: The guinea pigs were killed by cervical dislocation. Chest was

opened through midline incision. The whole of trachea, from larynx to bronchi, was dissected out and was transferred to a dissecting dish containing Kreb's Henseleit solution at room temperature. The tracheal tube was cut into rings, two to three mm wide, each containing about two cartilages. Each ring was opened by a longitudinal cut on the ventral side opposite to the smooth muscle,forming a tracheal chain with smooth muscle in the centre and cartilagenous portion on the edges. The tissue preparation was then transferred to an isolated tissue bath of 50 ml capacity, containing Kreb's Henseleit solution at 37ºC and was aerated with oxygen continuously.[9] One end of the tracheal strip was attached to the oxygen tube inside the tissue bath while the other end was connected to an Isometric Force Displacement Transducer by means of a thread. The tissue was allowed a period of equilibration for 45 minutes against an imposed tension of two grams. During this period, the physiological solution in the organ bath was changed three to four times. A tension of one gram was applied to the tracheal strip continuously throughout the experiment after the initial equilibration period. The trachealis muscle activity was recorded through the transducer on Four Channel Oscillograph.

Method for the individual Experiment

Concentration response curve of histamine in normal isolated tracheal muscle of guinea pig (Group 1): After the initial equilibration period, the baseline tension was adjusted. Histamine in a concentration 10^{-3}M (18500 micrograms/100 mls) was added to the organ bath and its effect was recorded on the oscillograph, through an isometric force displacement transducer. When the effect reached a plateau, the drug was washed away from the tissue bath and the tissue was allowed to relax passively. After the baseline tension had been restored, the same procedure was repeated with other concentrations of histamine, i.e., 10^{-4}M, 10^{-5}M, 10^{-6}M and 10^{-7}M in a random order. An interval of at least ten minutes was allowed before the addition of next concentration. Six experiments were performed in the same way and the mean response for each concentration was worked out. A concentration response curve was obtained by plotting the percent contraction against the logarithm of concentrations.

Concentration response curve of histamine in the presence of fixed dose of Nebivolol (10^{-6}m) in normal (unsensitized) isolated tracheal muscle of guinea pig (Group 2): In this set of experiments, the effect of histamine on normal tracheal muscle was studied in the presence of a fixed dose of Nebivolol, i.e.,10^{-6}M.[10] After adjusting the baseline tension, Nebivolol 10^{-6}M was added to the organ bath. It was then left in contact with the tissue for 15 minutes. Then the same procedure as described for the above mentioned experiment was followed.

Concentration response curve of histamine in the presence of fixed dose of indomethacin (10^{-6}m) and Nebivolol (10^{-6}m) in normal (unsensitized) isolated tracheal muscle of guinea pig (Group 3): After adjusting the baseline tension, indomethacin at a concentration 10^{-6}M[11] was added to the organ bath to block the synthesis of endogenous prostaglandins followed by Nebivolol 10^{-6}M.The drugs were left in contact with the tissue for 15 minutes. Then histamine 10^{-3}M was poured into the organ bath in the presence of indomethacin and Nebivolol and contraction of the tissue was recorded on the oscillograph, through an isometric transducer and the same procedure as described previously was repeated. Six experiments were performed.

Statistical evaluation::The results have been expressed as Means ± Standard Error of Means. The arithmetic means and SEMs were calculated using Microsoft Office Excel 2007.In order to find the significance of the difference between two observations Student's'*t*' test was used.The difference between two observations was considered as significant if the *p* value was less than 0.05.

RESULTS

Comparison of Group 1 (Histamine alone) with Group 2 (Histamine after pretreatment with Nebivolol 10^{-6}M): In a series of six experiments, the mean $\pm$ SEM values of the responses and the percent responses to the different concentrations of histamine in the two groups are shown in the Table 1. Group 1 was taken as the control group and percent response with 10^{-3} M in group 1 was taken as 100% and responses with other concentrations were compared with it The mean values of responses produced by different concentrations of histamine when compared between Group I and Group 2 were found statistically insignificant ($P > 0.05$) (Table 1 and Figure 1). The mean percent deviations were calculated for each dose of histamine and found statistically insignificant. The mean deviation was 2.42 percent (Figure 1).

Comparison of Group 2 (Histamine on normal tissue pretreated with fixed dose of Nebivolol 10^{-6}M) with Group 3 (Histamine on normal tissue pretreated with fixed concentration of Nebivolol 10^{-6}M and Indomethacin 10^{-6}M): The mean values of responses and mean percent responses in these groups were statistically insignificant ($P > 0.05$) (Table 2 and Figure 2 & 3). The mean percent deviations calculated for each dose of histamine used in Group 2 and Group 4 were 4.04, 3.5, 1.56, 1.5 and 0.64 percent respectively. The mean deviation was 2.24 percent.

Table No.1: Comparison of responses of tracheal muscle to histamine in group 1 and 2

	Group 1	Group 2	Group 1	Group 2	Group 1	GROUP 2	GROUP 1	GROUP 2	GROUP 1	GROUP 2
Serial No.	10^{-3}M	10^{-3}M	10^{-4}M	10^{-4}M	10^{-5}M	10^{-5}M	10^{-6}M	10^{-6}M	10^{-7}M	10^{-7}M
1	79 mm	80 mm	70 mm	70 mm	50 mm	52 mm	34 mm	32 mm	14 mm	10 mm
2	75	76	63	64	51	57	32	38	12	14
3	80	84	68	76	59	62	39	40	11	15
4	79	76	60	62	50	50	28	26	10	8
5	82	80	71	69	49	50	33	31	10	13
6	81	78	68	67	49	48	30	30	12	13
Mean	79.33	79	66.66	68	51.33	53.16	32.66	32.83	11.5	12.16
SD	2.42	3.03	4.27	4.93	3.82	5.30	3.77	5.23	1.51	2.63
SEM	0.98	1.23	1.74	2.01	1.56	2.16	1.54	2.13	0.61	1.07
P VALUE	0.782^{Ns}		0.400^{Ns}		0.130^{Ns}		0.901^{Ns}		0.618^{Ns}	

p value > 0.05 = Not significant (Ns) *p* value < 0.05 = Significant (*)

Table No.2: Comparison of responses of tracheal muscle to histamine in group 2 and 4

	Group 2	Group 4	Group 2	Group 4	Group 2	Group 4	Group 2	Group 4	Group 2	Group 4
Serial no.	10^{-3}M	10^{-3}M	10^{-4}M	10^{-4}M	10^{-5}M	10^{-5}M	10^{-6}M	10^{-6}M	10^{-7}M	10^{-7}M
1	80	81	70	70	52	55	32	36	10	10
2	76	85	64	74	57	57	38	40	14	20
3	84	72	76	64	62	47	40	30	15	8
4	76	84	62	71	50	59	26	33	8	12
5	80	75	69	63	50	54	31	30	13	12
6	78	80	67	72	48	52	30	35	13	14
Mean	79	79.5	68	69	53.16	54	32.83	34	12.16	12.66
SD	3.03	5.08	4.93	4.47	5.30	4.19	5.23	3.84	2.63	4.13
SEM	1.23	2.07	2.01	1.82	2.16	1.71	2.13	1.57	1.07	1.68
P VALUE	0.88^{Ns}		0.78^{Ns}		0.81^{Ns}		0.65^{Ns}		0.79^{Ns}	

p value > 0.05 = Not significant (Ns), *p* value < 0.05 = Significant (*)

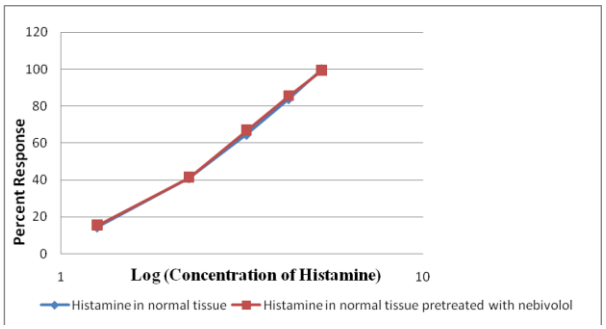

Figure No. 1: Log concentration-response curves of histamine in guinea pig tracheal smooth muscle in the absence (Group 1) and presence of fixed dose (10^{-6}M) of Nebivolol (Group 2).

DISCUSSION

In the first set of experiments, effects of different concentrations of histamine were studied on normal isolated tracheal muscle of guinea pig. When isolated tracheal muscle was pretreated with a fixed concentration of Nebivolol (10^{-6}M) it did not produce statistically significant effect. From these findings, it is inferred that Nebivolol has a sparing effect on histamine-induced contractions of tracheal smooth muscle. These findings support the results of *in vivo* study by Agostino *et al.*, (2001)[5] whereby Nebivolol did not affect airway responsiveness to inhaled histamine in rabbits. Similar findings have been reported in other *in vivo* studies.[12]

Some aspects concerned with the mechanisms that may be responsible for the lack of bronchoconstrictor effect of Nebivolol on tracheal muscle were also explored. There may be many possible mechanisms which can explain its sparing effect. First mechanism is related to its β_1 selectivity. It is the most selective β_1-adrenoceptor antagonist currently available for clinical

use.[13,14,15]Beta 1 receptor selectivity is an important determinant of less incidence of bronchoconstriction seen with cardioselective blockers such as Metoprolol and Atenolol as compared to non selective antagonists[16].De Clerck *et al*., (1989)[4] compared the bronchoconstrictor effects of atenolol, Nebivolol and propranolol in guinea pigs and they reported that bronchoconstriction was greatest with Propranolol followed by atenolol while Nebivolol had sparing effect. Van Zyl *et al*., (1989)[17]and Fogari *et al*. (1990)[16]

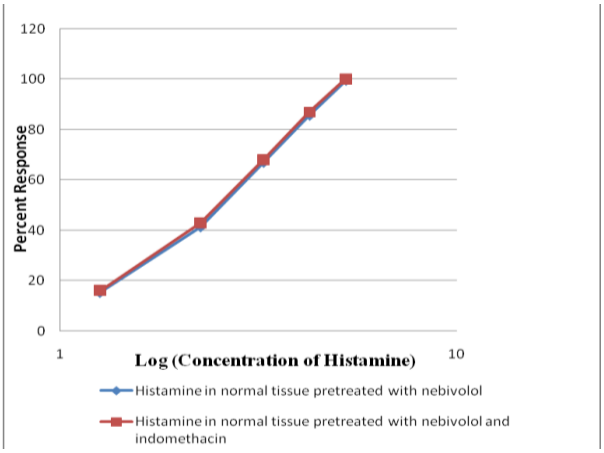

Figure 2: Log concentration-response curves of histamine in normal (unsensitized) isolated guinea pig tracheal muscle in Group 2 and Group 3.

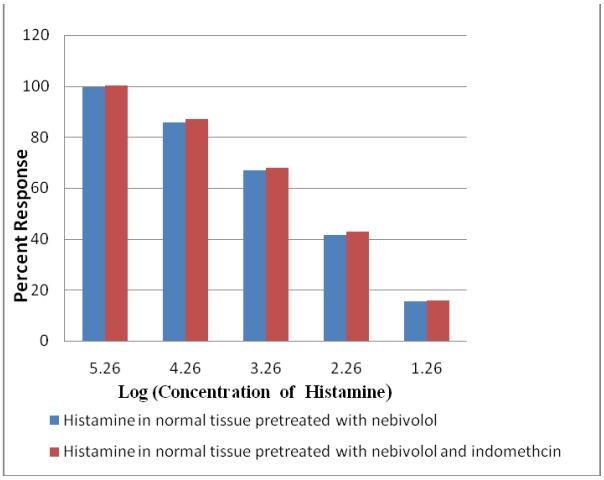

Figure 3: Bar diagram showing histamine induced contractions in normal isolated guinea pig tracheal smooth muscle in Group 2 and Group3.

carried out studies in asthmatic patients and had same results. So the different effect of Nebivolol can not be fully explained by its β_1 selectivity.Also Nebivolol lacks partial agonist activity at β_2 receptors.[18,19] So this mechanism is not plausible.

Other mechanisms apart from β_1 receptor selectivity i.e. relaxant prostaglandins may be involved in its sparing effect.Their involvement was therefore evaluated and a set of experiments was designed. The tracheal muscle was pretreated with indomethacin in a concentration of 10^{-6}M and Nebivolol 10^{-6}M. The concentration response curve was then constructed using different concentrations of histamine.This concentration response curve and its parameters were compared with the curve obtained with histamine in tracheal muscle pretreated with Nebivolol 10^{-6}M alone. Though mean $\pm$ SEM of maximum contraction increased from 79 $\pm$ 1.23mm to 79.5 $\pm$ 2.07mm, the difference was statistically insignificant.Similarly percent responses for other concentrations and percent deviation was statistically insignificant.

Prostaglandins of the E series have been shown to mediate inhibition of the respiratory smooth muscle in rabbit, guinea pig, sheep and pig and it has been suggested that PGEs play an important role in maintaining bronchial tone in asthmatic patients.[7] It has also been demonstrated that drugs which inhibit the cyclooxygenase pathway of arachidonic acid metabolism can reduce the effect of a relaxant prostaglandin such as PGE_2.[20] Had there been involvement of relaxant prostaglandins in the effect of Nebivolol the effect should have been blocked by inhibition of synthesis of prostaglandins by indomethacin and curve would have shifted upwards and to the left, but there was no significant change in this study, so the above mentioned possibility of involvement of relaxant prostaglandins could not be substantiated experimentally.

CONCLUSION

The involvement of relaxant prostaglandins in the sparing effect of Nebivolol could not be substantiated experimentally indicating the involvement of some other mechanism.

RECOMMENDATIONS: Further exploratory work is recommended to elucidate the exact mechanism underlying the sparing effect of Nebivolol against histamine-induced contraction of isolated guinea pig trachea.

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