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A Comparison of the β-Adrenoceptor Agonist Potency of Labetalol with Those of Sympathomimetic Drugs, and the Role of α-Receptors in Pharmacological Actions of These Drugs on Tracheal Smooth Muscles in Guinea Pigs

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The β-receptor agonist potency and α-receptor-blocking action of labetalol were investigated in tracheal smooth muscles of guinea pigs. With resting-tone preparations and methacholine-contracted preparations of trachea, labetalol produced relaxation and behaved as a partial agonist. The maximum relaxing response to labetalol of methacholine-contracted preparations was markedly smaller than that of the resting-tone preparations. The results suggest that the relaxing action of labetalol is weaker on the high tracheal tone induced by the cholinergic agonist, methacholine. With resting-tone preparations, norepinephrine and phenylephrine produced relaxation, but after the β-receptors were blocked, these drugs produced contraction. These findings confirm the existence of α-excitatory receptors and the predominance of β-receptors over α-receptors in the trachea. When labetalol was administered first, it showed β-receptor agonist action and inhibition of contraction of the resting-tone preparations due to α-receptors. When labetalol was administered secondly, it did not show β-receptor agonist action but exerted α-adrenoceptor-blocking action. These results suggest that labetalol produces relaxation of the tracheal preparations in guinea pigs through its β-receptor partial agonist action and α-receptor-blocking action. However, it is thought that this relaxing effect of labetalol might be small when the β-adrenoceptor reserve in the trachea is occluded or exhausted by pretreatment with a β-receptor blocker or a cholinergic agonist, methacholine.

Keywords——labetalol; trachea; alpha-receptor; guinea pig; beta-agonist action

Beta-adrenoceptors are considered to be dominant in tracheal muscles. However, it has been reported recently that α-adrenoceptors also exist in the trachea,[1,2] and that α-adrenoceptor-blocking agents prevent exercise-induced asthma.[3] On the other hand, β-adrenoceptor-blocking agents, such as propranolol, have been shown to enhance bronchoconstriction and to aggravate asthma.[4] Labetalol is considered to be an antihypertensive drug with α- and β-adrenoceptor-blocking and β-adrenoceptor partial agonist actions, and is more potent at β- than α-receptors.[5] We have previously reported that labetalol relieved experimental asthma in guinea pigs, induced by inhaling histamine aerosol, but that propranolol aggravated it.[6] One reason for these results was thought to be that labetalol can relax the bronchial tone by its β-receptor partial agonist action. Another reason was thought to be the α-adrenoceptor-blocking action of labetalol. In the present study, we confirmed the existence of α-receptors in the trachea and studied the effect of α-receptor-blocking action on the trachea in guinea pigs. As regards the β-receptor agonist action in tracheal smooth muscles, it has been demonstrated that there is a functional antagonism between β-receptor agonists and cholinergic agonists, and under conditions of high tracheal tone, the maximum of the dose–response curve to β-receptor partial agonists is depressed more than that to other agonists such as isoproterenol.[7] Therefore, we compared the β-receptor agonist potencies of labetalol

and sympathomimetics on the trachea of guinea pigs, by utilizing the functional antagonism between β-receptor agonists and cholinergic agonists.

Experimental

Materials——Drugs used were as follows: isoproterenol hydrochloride (Sigma Chemical Co.), norepinephrine (Sankyo Co., Ltd.), phenylephrine hydrochloride (Kowa Co., Ltd.), phentolamine mesylate (Ciba Geigy), propranolol hydrochloride (ICI Pharmaceutical Division), reserpine (Daiichi Seiyaku Co., Ltd.), cocaine hydrochloride (Dainippon Pharmaceutical Co., Ltd.) and tyramine hydrochloride (Wako Pure Chemical Industries, Ltd.). Labetalol hydrochloride was a gift from the Shin-Nippon Jitsugyo Co., Ltd. Drugs were dissolved in saline just before use and diluted with bathing solution.

Tracheal Preparation——The method employed was essentially similar to that of Takagi *et al.*[8] Guinea pigs (either sex, 450 to 700 g) were killed by means of a blow on the head, and the trachea was immediately excised and the cartilaginous region opposite the muscle was cut open longitudinally. The opened trachea was cut transversely along the ring cartilage into 13 to 15 strips (about 2 mm width). Four to five strips were tied to make sections about 2.5 cm long and three preparations were obtained from one animal. The tracheal strip preparation was suspended in a 30 ml organ bath containing (at 37 °C) Tyrode solution that had been aerated with a gas mixture of 95% O_2 and 5% CO_2. The tracheal tone was recorded with a force displacement transducer (Sanei Instrument, 45071) and a recorder (056, Hitachi Ltd.). A resting tension of 0.5 to 0.8 g was applied to the tracheal preparation, since an appropriate tension is usually applied to tracheal smooth muscles under physiological conditions. The preparation was left to equilibrate in the bath for 1 to 2 h before commencement of the experiment. The tone of the preparation in this steady state was regarded as the resting tone. The preparation loaded with this tension produced a consistent dose–response curve with isoproterenol and the maximum relaxation by isoproterenol was consistently produced at a concentration of about 10^{-7} M. Preparations with insufficient load produced inconsistent results. For cocaine treatment of the preparation, cocaine was added to the bathing solution at the concentration of 10 μg/ml in order to block the uptake of catecholamine. For methacholine treatment of the preparation, mechacholine was added to the bathing solution at the concentration of 3×10^{-6} M in addition to cocaine at the concentration of 10 μg/ml. To deplete catecholamine, the guinea pigs were pretreated with reserpine, 0.2 mg/kg/d, subcutaneously for 4 to 7 d, and it was confirmed that tyramine administration (10^{-5} g/ml) did not produce any effect due to endogenous catecholamine on the tracheal preparation obtained from reserpine-treated guinea pigs.

Results

Experiments were carried out to compare the relaxing effect of labetalol (Lab) with that of other sympathomimetics such as norepinephrine (NEp), phenylephrine (PhE) and

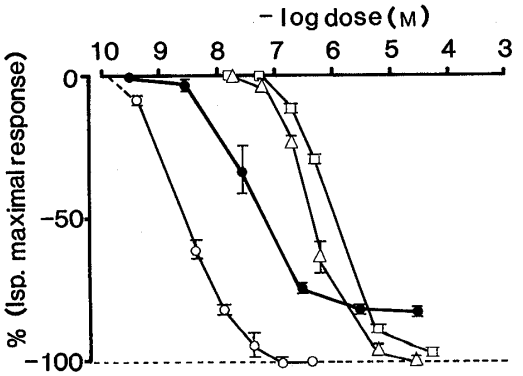

Fig. 1. The Relaxing Actions of Sympathomimetic Amines and Labetalol on the Resting Tone in Guinea Pig Tracheal Muscle Preparations

○, isoproterenol; △, norepinephrine; □, phenylephrine; ●, labetalol. Each point represents the mean ± S.E. of four experiments.

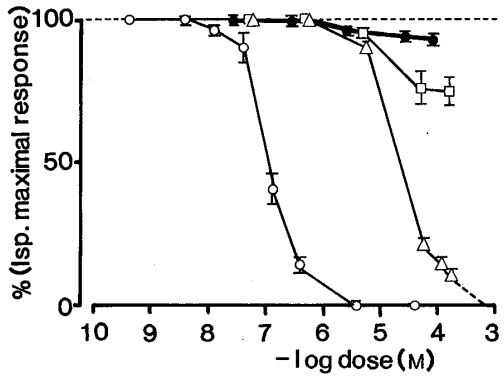

Fig. 2. The Relaxing Actions of Sympathomimetic Amines and Labetalol on Methacholine-Contracted Tone in Guinea Pig Tracheal Muscle Preparations

○, isoproterenol; △, norepinephrine; □, phenylephrine; ●, labetalol. Each point represents the mean ± S.E. of four experiments.

Methacholine was added to the bathing solution at the concentration of 3×10^{-6} M in addition to cocaine at the concentration of 10 μg/ml.

isoproterenol (Isp) by using isolated tracheal preparations of guinea pigs. The cumulative dose–responses of these drugs on the resting-tone preparation are shown in Fig. 1; the dose–response curve of isoproterenol represents the stimulating efficacy on β-receptors, and the maximum effect is shown as minus 100% response with respect to the resting tone. As can be seen in Fig. 1, NEp and PhE produced the same type of relaxation as isoproterenol, even though their dose–response curves were located to the right as compared to that of isoproterenol. This result shows that β-receptors are dominant over α-receptors in the trachea. Labetalol also caused relaxation of the tone by its β-receptor partial agonist action, which was confirmed in our previous experiments.[6] The maximum relaxation due to labetalol was about 80% compared to that of isoproterenol, and the difference in relaxing effect between labetalol and other sympathomimetics indicates a complicated interference of β- and α-receptor blocking actions against the intrinsic sympathomimetic action of labetalol.

The following experiments were done in order to compare the relaxing efficacies of Isp, NEp, PhE and labetalol on the tone increased by methacholine (3×10^{-6} M). The results obtained are shown in Fig. 2. The pD_2 values and the intrinsic activities (i.a., ratio of maximum relaxation to that in the case of isoproterenol) obtained from Figs. 1 and 2 are given in Table I. As shown in Fig. 2, isoproterenol produced complete relaxation of methacholine (3×10^{-6} M)-contracted tracheal preparations, but the relaxing efficacies of PhE and labetalol on methacholine (3×10^{-6} M)-contracted preparations were markedly smaller than that of isoproterenol. As shown in Table I, the i.a. value of PhE was approximately one-fourth of that of isoproterenol on methacholine-contracted preparations. The i.a. value of labetalol obtained from methacholine-contracted preparations was only 7% of that of isoproterenol, so that no precise value of pD_2 could be obtained.

With resting-tone preparations, Isp and NEp behaved as full agonists, since the maximum response to NEp was equal to that to Isp. PhE also behaved as a full agonist on the resting-tone preparations, since the extrapolated maximum response was equal to that to Isp. However, labetalol behaved as a partial agonist on the resting-tone preparations, *i.e.* the maximum response was less than that to Isp. With methacholine (3×10^{-6} M)-contracted tracheal preparations, PhE and labetalol behaved as partial agonists, and their i.a. were markedly smaller than those on the resting-tone preparations. Isp behaved as a full agonist. NEp was also regarded as a full agonist with methacholine-contracted preparations, since the extrapolated maximum response was equal to that in the case of Isp. The difference in the pD_2 values between Isp and NEp on methacholine-contracted preparations (300-fold) was larger than that on resting-tone preparations (120-fold). PhE and labetalol showed a marked difference of i.a. on the two types of tracheal preparations.

TABLE I. Comparison of Relaxing Action of Isoproterenol, Norepinephrine, Phenylephrine and Labetalol in Isolated Tracheal Preparation of Guinea Pig

Drug	Resting tone			Methacholine-contracted tone		
	$n^{a)}$	$pD_2{}^{b)}$	i.a.[c]	$n^{a)}$	$pD_2{}^{b)}$	i.a.[c]
Isoproterenol	4	8.55±0.06	1.00	4	7.15±0.04	1.00
Norepinephrine	4	6.47±0.09	1.00	4	4.68±0.05	0.87±0.02
Phenylephrine	4	5.98±0.03	0.96±0.002	4	4.98±0.03	0.25±0.06
Labetalol	4	7.29±0.25	0.83±0.02	4	5—7.5[d]	0.07±0.02

a) Number of experiments. *b*) Negative log molar ED_{50}. *c*) i.a.: intrinsic activity is expressed as the ratio of the maximum relaxation of each drug to that of isoproterenol. *d*) pD_2 value of labetalol in methacholine-contracted preparations could not be obtained precisely. Each value represents the mean±S.E.

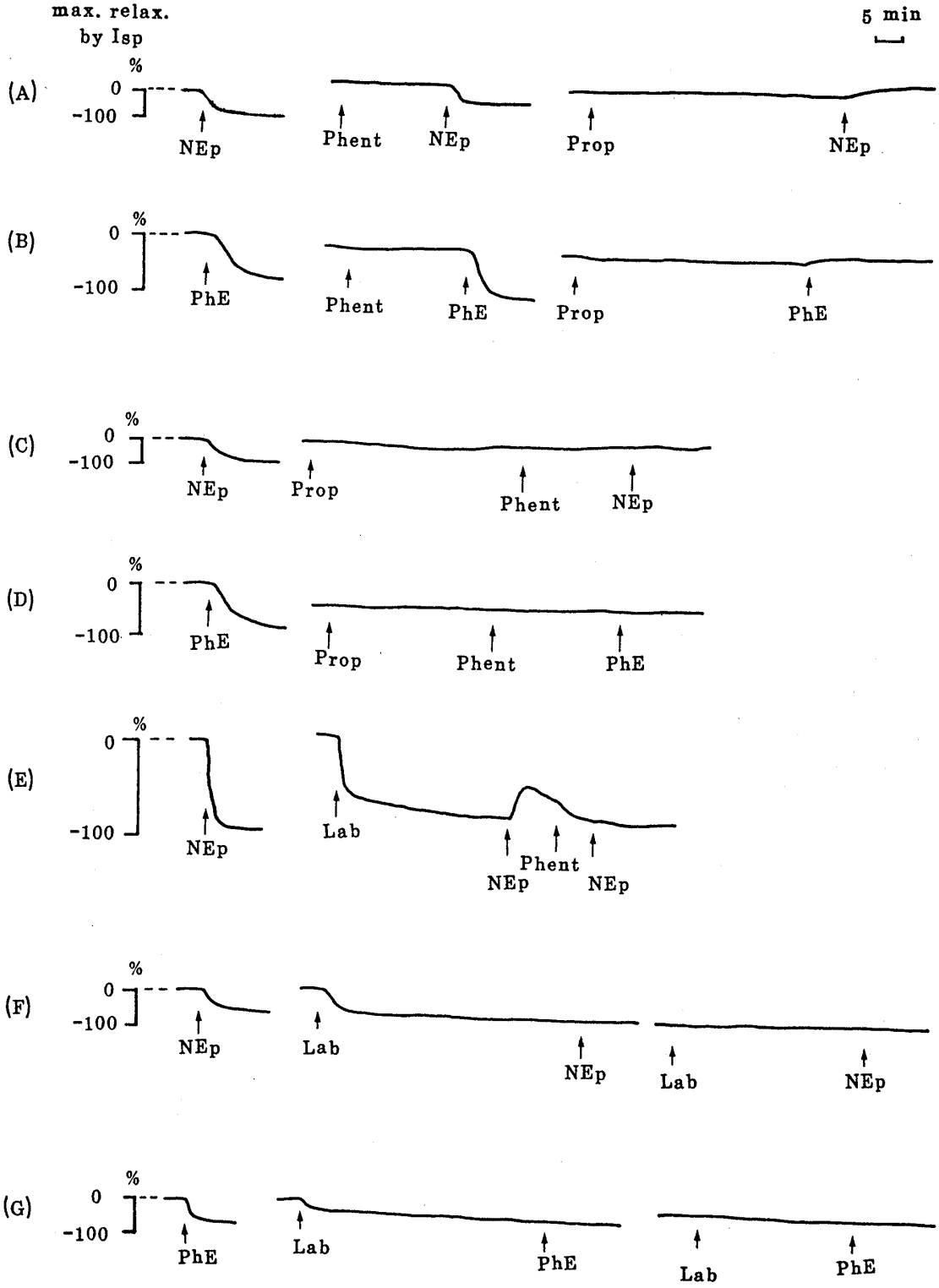

Fig. 3. Relationships of α- and β-Adrenoceptors in Tracheal Muscles in Guinea Pigs

NEp, norepinephrine 5.91×10^{-6} M; PhE, phenylephrine 4.91×10^{-6} M; Prop, propranolol 3.38×10^{-6} M; Phent, phentolamine 2.65×10^{-6} M; Lab, labetalol (E) and (G): 1.37×10^{-5} M, (F): 2.74×10^{-5} M.

Verticals: maximum relaxation by isoproterenol (max. relax. by Isp) is expressed as minus 100% with respect to the resting tone, which is taken as 0%.

In order to clarify the roles which α- and β-adrenoceptors play in the relaxing effects of NEp, PhE and labetalol on tracheal smooth muscles, adrenergic blockers were used in combination with these drugs on the preparations in the resting state, and the results obtained are shown in Fig. 3. As shown in (A) and (B) in Fig. 3, the relaxing actions of NEp and PhE were not affected by pretreatment with phentolamine, but the muscle tone was slightly elevated by NEp and PhE after propranolol treatment. The results indicated that the role of β-adrenoceptors was not altered by the blockade of α-adrenoceptors in the present study, while the blockade of β-adrenoceptors revealed the involvement of α-adrenoceptors in the action of the sympathomimetics, producing a tone elevation of tracheal muscles. This was demonstrated by the results shown in (C) and (D) in Fig. 3: when phentolamine and propranolol were preadministered, the effects of NEp and PhE disappeared completely. As shown in (E) in Fig. 3, administration of labetalol first at the concentration of 1.37×10^{-5} M produced a marked relaxation of the muscle tone and induced contraction of the muscle tone after the administration of NEp. When phentolamine at the concentration of 2.65×10^{-6} M was administered, the contraction by NEp was removed. This result indicated that the α-adrenoceptor-blocking action of labetalol at the concentration of 1.37×10^{-5} M was not sufficient to block the α-agonist action of NEp at the concentration of 5.91×10^{-6} M. Part (F)

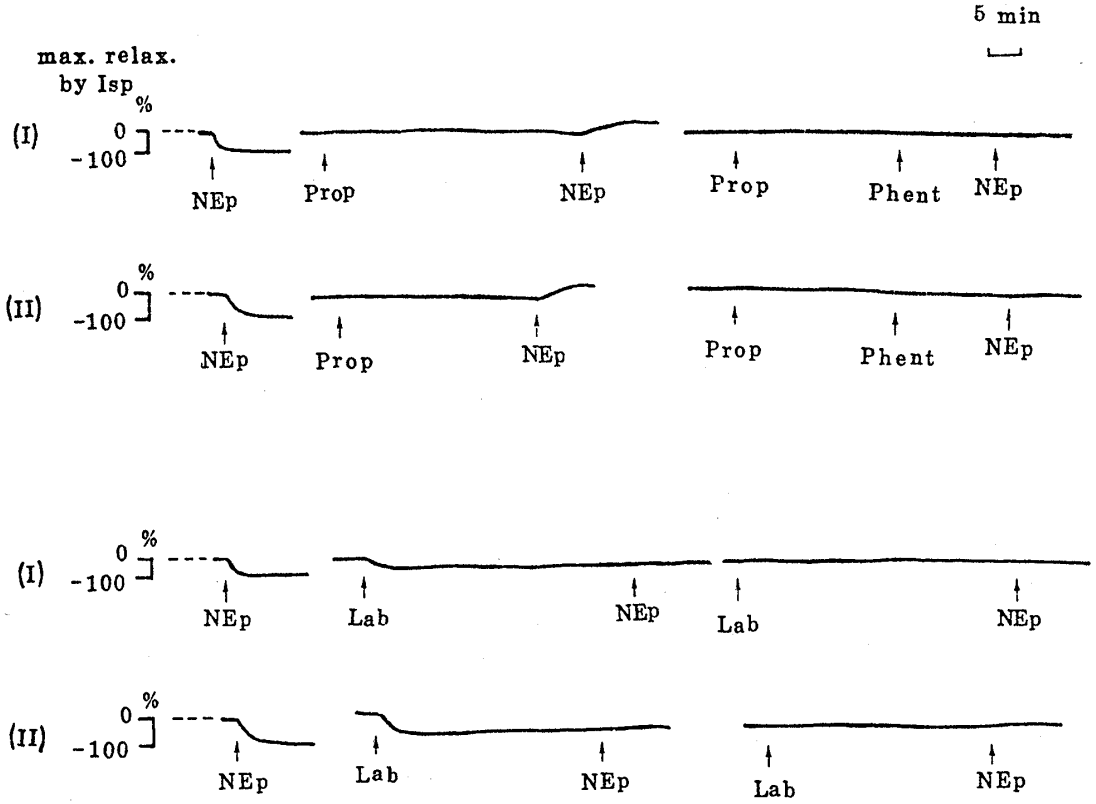

Fig. 4. Relationships of α- and β-Adrenoceptors in Tracheal Muscles Treated with Cocaine or Pretreated with Reserpine in Guinea Pigs

I: Cocaine-treated preparation. Cocaine was added to the bathing solution at the concentration of 10 μg/ml in order to block the uptake of catecholamines. II: Reserpine-pretreated preparation. The guinea pigs were pretreated with reserpine, 0.2 mg/kg/d, subcutaneously for 4 to 7 d and it was confirmed repeatedly throughout the experiment that tyramine 10^{-5} g/ml did not produce any effect due to endogenous catecholamine on the preparation.

Verticals: maximum relaxation by isoproterenol (max. relax. by Isp) is expressed as minus 100% with respect to the resting tone, which is taken as 0%.

NEp, norepinephrine 5.91×10^{-6} M; PhE, phenylephrine 4.91×10^{-6} M; Phent, phentolamine 2.65×10^{-6} M; Prop, propranolol I: 1.35×10^{-5} M, II: 3.38×10^{-6} M; Lab, labetalol I: 2.74×10^{-5} M, II: 1.37×10^{-5} M.

in Fig. 3 shows that administration of labetalol first at the concentration of 2.74×10^{-5} M produced a marked relaxation of the muscle tone and inhibited the contraction by NEp. It was found that labetalol at the concentration of 2.74×10^{-5} M blocked the α-agonist action of NEp completely. Part (G) in Fig. 3 shows that administration of labetalol first at the concentration of 1.37×10^{-5} M relaxed the muscle tone and did not allow the contraction by phenylephrine. From a comparison of (E) and (G) in Fig. 3, it was indicated that the contractile response to PhE at the concentration of 4.91×10^{-6} M was less than that to NEp at the concentration of 5.91×10^{-6} M. Administration of labetalol secondly, as shown in (F) and (G) in Fig. 3, did not produce relaxation of the muscle tone but inhibited the contraction by NEp completely. This suggests that labetalol at first presents the intrinsic β-receptor agonist activity and then produces the α- and β-adrenoceptor-blocking effect.

Since the question remained as to whether labetalol exerted some effect on the storage of catecholamines in the terminals of sympathomimetic nerves or not, experiments were carried out by using tracheal preparations treated with cocaine to block the uptake of catecholamines, and preparations pretreated with reserpine to deplete catecholamines. As can be seen in Fig. 4, the treated preparations showed the same patterns of responses to NEp and labetalol as those shown in Fig. 3, which were obtained in the non-treated preparations. It was concluded, therefore, that the action of labetalol did not require the libration of catecholamines from the termination of sympathetic nerves.

Discussion

As shown in Fig. 1, Isp, NEp and PhE behaved as full agonists, but labetalol behaved as a partial agonist on the resting tone of guinea pig traches. With methacholine (3×10^{-6} M)-contracted preparations, as shown in Fig. 2, Isp and NEp behaved as full agonists, but PhE and Lab behaved as partial agonists. The i.a. values of PhE and Lab on methacholine-contracted preparations were found to be much smaller than those on the resting-tone preparations. The difference in the i.a. values of β-agonists between the resting tone and the tone contracted by cholinergic agonists in guinea pig trachea has already been reported by other investigators.[9,10]

O'Donnell and Wanstall[9] in 1978 reported that β-receptor agonists such as fenoterol and salbutamol behaved as full agonists on the resting tone of guinea pig trachea, but their maximum relaxing responses on the carbachol-contracted tone were different, and salbutamol behaved as a partial agonist. Buckner and Saini in 1975[10] also demonstrated that there was a difference between the maximum relaxations of isoproterenol and soterenol on guinea pig tracheal tone when the β-receptor reserve was 'exhausted' by carbachol.

Therefore, the β-receptor partial agonist action of labetalol was found to be small in high tracheal tone where the β-receptor reserve was occluded or exhausted by pretreatment with a cholinergic agonist (*i.e.* methacholine) or β-adrenoceptor blocker. The authors and colleagues previously reported that labetalol possessed β-agonist action in guinea pig tracheal muscles,[6] whereas on guinea pig atria,[11] labetalol had only a slight β-agonist action. Therefore, we previously suggested that the β-agonist action of labetalol on trachea is mainly due to β_2-receptor agonist action.[6] As for the existence of α-adrenoceptors in trachea, *in vitro* evidence has been obtained in guinea pig trachea[1] and in human trachea.[2] In 1963, Takagi *et al.*[1] demonstrated that α-excitatory receptors exist in tracheal muscle of guinea pig. They reported that in the presence of β-adrenoceptor blockers such as pronethalol and propranolol, α-stimulants such as norepinephrine, epinephrine and phenylephrine caused contraction of the tracheal muscle which was inhibited by pretreatment with α-adrenoceptor blockers, such as tolazoline and dibenamine. Furthermore, in clinical tests, Prime *et al.*[12] in 1972 and Patel and Kerr[13] in 1973 reported that phenylephrine caused the constriction of airways after

preadministration of propranolol in normal subjects and in exercise-induced asthma.

The methods used for demonstrating the existence of α-receptors were as follows: (I) in the presence of β-blocker, administration of an α-stimulant caused contraction of the tracheal muscles,[1,2,12,13] (II) the effects of β-stimulants on the respiratory systems were enhanced by administration of α-blockers,[14,15] (III) α-blockers prevented exercise-induced asthma,[3] *etc.* Palmer *et al.*[14] showed that an α-blocker enhanced the effect of a β-stimulant in normal subjects. Ishihara *et al.*[15] demonstrated that an α-blocker stimulated the bronchodilation induced by norepinephrine or epinephrine. Furthermore, Bianco *et al.*[3] reported that indoramin prevented the appearance of exercise-induced asthma and that an α-blocker enhanced the effect of a β-stimulant in normal subjects.

Alpha-adrenoceptors, like β-receptors, have been classified into α_1- and α_2-subtypes.[16-18] In 1974, Langer[16] suggested that α_1-receptors were localized at the effector cells (postsynaptic) and α_2-receptors exist at the nerve endings (presynaptic). Presynaptic α-receptors inhibit the release of norepinephrine from the nerve endings, and when α_2-receptors are blocked, norepinephrine release continues. However, it has recently been demonstrated that α_2-receptors may also exist postsynaptically in some tissues[19] and that both α_1- and α_2-subtypes may be present in canine tracheal smooth muscles.[20,21] It has been reported that in canine tracheal smooth muscle, α-receptor contractile responses are mediated predominantly by α_2-receptors, not by α_1-receptors.[21] On the other hand, it has been demonstrated that peripheral airways have a high density of α_1-receptors but in smooth muscles of large airways, α_1-receptors are sparse.[22] Therefore, it has been considered that α_1-receptor antagonist might have less effect on large airway response than on small airway response.[23]

From the results shown in Figs. 3 and 4, the authors conclude that in the presence of a β-adrenoceptor blocker such as propranolol, stimulation of α-receptors by α-stimulants such as norepinephrine (both α_1- and α_2-receptor agonist) and phenylephrine (α_1-selective agonist) enhanced the tone of tracheal muscles, and that the resting tone was hardly changed by sympathomimetic drugs when both α- and β-adrenoceptors were blocked. These results are in agreement with those of Takagi *et al.*[1] It was confirmed that the contraction by NEp and PhE was inhibited by labetalol, which is known to have α_1-receptor-blocking action with no apparent α_2-receptor-blocking activity.[24] However, it was suggested that since the contraction induced by NEp was greater than that by PhE, the contractile response due to α_2-receptors might also exist in guinea pig tracheal smooth muscle, as found in canine tracheal muscle by Leff and Munoz,[20] and Barnes *et al.*[21]

In conclusion, the authors confirmed that labetalol exerted relaxing effects on guinea pig trachea by two mechanisms: a β-receptor partial agonist action and an α-receptor-blocking action. However, these actions of labetalol were not so marked as to nullify its β-adrenoceptor-blocking action. Therefore, it is thought that the administration of labetalol might induce aggravation in subjects in whom high tracheal tone is easily evoked, or it might cause a decrease of tracheal tone in some subjects in whom the β-receptor reserve remains intact in the trachea.

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