The Effect of Forskolin on Isoproterenol-Induced Relaxation in Rat and Guinea-Pig Tracheal Preparations

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\textbf{Abstract}

\textbf{Objective:} In this study, we have examined the relaxant effects of forskolin and isoproterenol on isolated rings of the rat and guinea-pig trachea. The objective of the study was to compare the potency and efficacy of both agents on the tracheal preparations from different species and to assess any possible synergistic bronchodilator effect of forskolin and isoproterenol. \textbf{Method:} The preparations were mounted in organ baths containing Krebs-Henseleit solution and changes in isometric tension were recorded. \textbf{Results:} Forskolin, an activator of adenylyl cyclase, and the \(\beta\)-adrenergic stimulator isoproterenol produced concentration-dependent (10\(^{-9}\) to 3 \(\times\) 10\(^{-5}\) \(M\)) relaxations in rat and guinea-pig tracheal rings pre-contracted by carbachol (10\(^{-6}\) and 3 \(\times\) 10\(^{-7}\) \(M\), respectively). Forskolin was less potent than isoproterenol but is had a higher relaxing efficacy. It increased isoproterenol-elicited maximal relaxation in rat tracheal preparations. Forskolin significantly potentiated the bronchodilator response to isoproterenol in guinea-pig preparations (lower IC\(_{50}\) values). Moreover, relaxation to standard concentrations of isoproterenol (10\(^{-8}\) and 3 \(\times\) 10\(^{-8}\) \(M\)) were significantly enhanced in the presence of forskolin (10\(^{-6}\) and 3 \(\times\) 10\(^{-6}\) \(M\)) in both rat and guinea-pig tracheal rings. \textbf{Conclusion:} These results suggest that in guinea-pig tracheal ring preparations, forskolin produces an apparent potentiation of isoproterenol-induced relaxation, while in rat tracheal preparations, forskolin increases the efficacy of isoproterenol. This response could provide an alternative to increasing the dosage of \(\beta\)-adrenergic bronchodilator drugs in patients with asthma. Therefore, a combined therapy with forskolin may be considered.
Introduction

Forskolin, a diterpene derivative from the roots of *Coleus forskohlii*, has been shown to effectively stimulate adenylate cyclase probably via a direct action on the catalytic unit of the enzyme [1]. Forskolin has been reported to relax a variety of smooth muscles [2–4].

The activation of β-adrenoceptors causes smooth muscle relaxation due to increased cyclic AMP (cAMP) formation [5]. Synergistic interaction of forskolin and hormones were first observed in intact brain slices [6, 7], where forskolin increased cAMP accumulation elicited by neurohormones in guinea-pig and rat cerebral cortical slices. Since then, there have been many reports describing synergistic increases in cAMP levels by combinations of forskolin with stimulatory agents in many cell types and tissues [8]. However, in bovine coronary arteries, low concentrations of forskolin (≤10⁻⁶ M), which themselves markedly elevated cAMP levels in the arteries, did not potentiating the effects of isoproterenol on cAMP levels or relaxation in these preparations [9]. We have already demonstrated that forskolin was capable of enhancing the bronchodilator effects and also to reverse tachyphyaxis development to salbutamol on guinea-pig tracheal preparations [10].

In the present study, we compared the relaxant effects of forskolin and isoproterenol on isolated rings of the rat and guinea-pig trachea. The objective of the study was to compare the potency and efficacy of both agents on the tracheal preparations from different species. In addition we wanted to assess a possible synergistic bronchodilator effect of forskolin and isoproterenol.

Material and Methods

Mature male Sprague-Dawley rats and guinea-pigs were used in this study. The animals were sacrificed by decapitation under light ether anaesthesia. The tracheas were isolated and excess connective tissue was removed and cut into rings of about 5 mm. The preparations were mounted in organ baths containing 25 ml Krebs-Henseleit solution at pH 7.4. The tissue bath solution was maintained at 37°C and was aerated with 95% O₂ and 5% CO₂ mixture. Isometric tension was recorded on a Lectromed UFI-dynamometer and recorder system. The preparations were left for 45 min, changing Krebs’ solution at 15-min intervals, thereafter a pretension of 1.0 or 1.5 g was applied to the rat and guinea-pig preparations. The rings were left for a stabilization period of about 45 min until a stable baseline tone was obtained.

Protocol

The tracheal preparations from the rat and guinea-pig were pre-contracted with carbachol 10⁻⁶ or 3 × 10⁻⁷ M, respectively, before cumulative dose-response curves for isoproterenol, and forskolin (10⁻⁹ to 3 × 10⁻⁵ M) were established. When the carbachol-induced contraction had reached a plateau, ascending concentrations of the agonists were added cumulative-ly to the organ baths to generate concentration-response curves. The response to any concentration of the agonist was allowed to plateau before the next concentration was added. The maximal concentration to carbachol was considered as 100% response and from this level the amount of relaxation was calculated.

The possible potentiation effect of forskolin on isoproterenol-induced relaxation was examined by pretreatment of the tissues with forskolin as described by Satake and Shibata [11]. In our study, the preparations were pre-contracted with carbachol (3 × 10⁻⁷ or 10⁻⁶ M) and followed by the addition of forskolin (10⁻⁷, 3 × 10⁻⁷, 10⁻⁶ or 3 × 10⁻⁶ M) to the tissue bath for 20 min before a cumulative dose-response curve for isoproterenol (10⁻⁹ to 3 × 10⁻⁵ M) was established. Since forskolin produces a relaxant response, the maximal contraction just before the addition of isoproterenol was taken as 100% to avoid any additive effect of forskolin [10].

Statistics

Results are presented as mean ± SEM of (n) experiments (n = 6–8). Dose-response curves were analysed using Graph-Pad Prism® software. Where necessary, differences between two mean values were compared using Student’s t test or paired as appropriate. The dif-
Fig. 1. Cumulative dose-response curves for forskolin (■) and isoproterenol (▲) (10⁻⁹ to 3 × 10⁻⁵ M) on isolated rat (a) and guinea-pig (b) tracheal rings pre-contracted by carbachol (10⁻⁶ and 3 × 10⁻⁷ M, respectively, n = 8).

Results

In rat and guinea-pig tracheal rings, pre-contracted with carbachol (10⁻⁶ and 3 × 10⁻⁷ M, respectively), relaxation induced by forskolin and isoproterenol (10⁻⁹ to 3 × 10⁻⁵ M) was concentration-dependent (fig. 1). The degree of relaxation elicited by the β-agonist isoproterenol was much more pronounced in the guinea-pig preparations. Forskolin produced higher maximal responses in both specimens (significant in the rat) but potency to isoproterenol was higher in the rat and guinea-pig tracheal preparations.

Drugs and Chemicals

The composition of the Krebs-Henseleit solution was as follows (mM): NaCl (118.3), KCl (4.7), CaCl₂ (2.5), MgSO₄ (1.2), NaHCO₃ (25), KH₂PO₄ (1.2) and glucose (11.2). Isoproterenol hydrochloride and carbachol were obtained from Sigma Chemicals (St. Louis, Mo., USA), salbutamol from RBI Natik. Forskolin was a gift from Hoechst AG, Germany. Forskolin was dissolved in DMSO, while isoproterenol in 0.1 N HCl. Dilutions were made in distilled water. The final concentration of the solvents in the organ baths in all experiments did not exceed 0.1%, which did not have any effect on tissue responses as tested in preliminary experiments.
Forskolin Potentiates Isoprenaline-Induced Relaxation

Pretreatment of the rat tracheal preparations with forskolin (10⁻⁷ to 3 × 10⁻⁶ M) increased the relaxation induced by isoprenaline (10⁻⁹ to 3 × 10⁻⁵ M) in a concentration-dependent manner (fig. 2a). Forskolin, at 10⁻⁶ and 3 × 10⁻⁶ M, produced a marked increase in the relaxant response both at lower and higher concentrations of isoprenaline apparently without obvious changes of EC₅₀ value. In the guinea-pig tracheal preparations, pretreatment of the tissues with forskolin (10⁻⁶ and 3 × 10⁻⁶ M) at the lower concentrations of isoprenaline induced a significant potentiation (p < 0.001) in the relaxant response without increasing the maximal effect of isoprenaline (fig. 2b).

**Discussion**

The diterpene forskolin from the roots of _Coleus forskohlii_ possesses positive inotropic and vasodilating properties [12] and has been reported to activate cardiac [13] and brain adenylate cyclase [6, 14]. Activation of adenylate cyclase by forskolin does not appear to be related to an interaction with any of the major classes of cell surface receptors, as a
variety of receptor blockers do not inhibit forskolin-elicited accumulations of cAMP in brain slices. Forskolin has been shown to have no effect on the activity of phosphodiesterases [12] and thus it appears to act directly by activating the catalytic subunit of the adenylate cyclase and increases cAMP in airway smooth muscle [15].

Obviously our study reveals a marked species difference in the tracheal relaxant responses to the non-specific β-agonist isoproterenol which was much smaller in rat specimens. This can be due to a low β₂ receptor density in the rat, since a series of experiments with the β₂-agonist salbutamol failed to elicit any tracheal relaxation. In this study, the potency of isoproterenol was increased while the efficacy was not increased in the guinea-pig tracheal preparations. In rat preparations, the efficacy was increased while the potency was apparently not increased (or exhibited only minor changes). The two species seem to have different limiting steps in the regulation of relaxation. Our results are similar to those of Waldeck and Widmark [16], who showed that both isoproterenol and forskolin were effective bronchodilators in guinea-pig preparations. These authors, however, did not report a significantly increased relaxation when combining isoproterenol with forskolin. This can be explained by a difference in technique and the small number of experiments (n = 4). In this study, we have determined the degree of relaxation according to the reduction of the carbachol-induced tone, which was taken as 100%. In the cumulative concentration-response curves for isoproterenol, this also included abolition of pre-existing basal tone, which in the guinea-pig tracheas was considerable and amounted to 130% for isoproterenol and 200% for forskolin. When this technique was applied, we could clearly see a significant increase in potency (lower EC₅₀ values) in the combination experiments and a higher degree of relaxation at standard concentrations of isoproterenol. These results are in line with our previous experience when we tested the antithrombotic effect of forskolin on platelet deposition in vascular grafts [17]. In further in vitro studies on platelet aggregation, we could demonstrate that a combination of forskolin with prostaglandin I₂ or iloprost at concentrations which produced half-maximal inhibition of aggregation on their own caused complete inhibition of ADP-induced platelet aggregation when combined [18]. Tachyphylaxis to β₂-agonists occurs in some asthmatics. It has been hypothesized that this is due to down-regulation or desensitization of β₂-receptor affinity to the agonist or a decrease in the number of β₂-receptors [19]. Recently, we have shown that forskolin shares with salbutamol the ability to relax airway smooth muscle and produces an apparent reversal of tachyphylaxis to the bronchodilator effects of salbutamol [10]. Therefore, a combination therapy of a β₂-agonist plus forskolin might reduce the likelihood of tachyphylaxis development, since the effect of forskolin is not restricted by down-regulation or desensitization of β-adrenoceptors.

Conclusion

We have shown in this study that forskolin produces an apparent increase in isoproterenol-induced relaxation in guinea-pig tracheal preparations and in the efficacy of isoproterenol in rat preparations. Combined therapy with forskolin may provide an alternative to increasing the dosage of β₂-bronchodilator agonists in asthmatics.
References