

Ca²⁺ dynamics and allosterism in synergistic action between Beta2-adrenergic agonists and muscarinic antagonists

G proteins (G_s , G_i)/large conductance Ca^{2+} -activated K^+ (K_{Ca}) channels/L-type voltage-dependent Ca^{2+} (VDC) channels linkage (Ca^{2+} dynamics) may play key roles in β_2 -adrenergic relaxation and muscarinic contraction in the airways. β_2 -Adrenergic receptor agonists and muscarinic receptor antagonists are used as bronchodilators in therapy for asthma and COPD. Clinical trials have indicated that combination of these two agents is beneficial to these diseases. This amelioration may be due to crosstalk between β_2 -adrenergic receptors and muscarinic receptors (G protein-coupled receptors: GPCRs). This study was designed to investigate mechanism of synergistic action between these two agents with focuses on GPCR-related modulation of ion channels and allosteric sites.

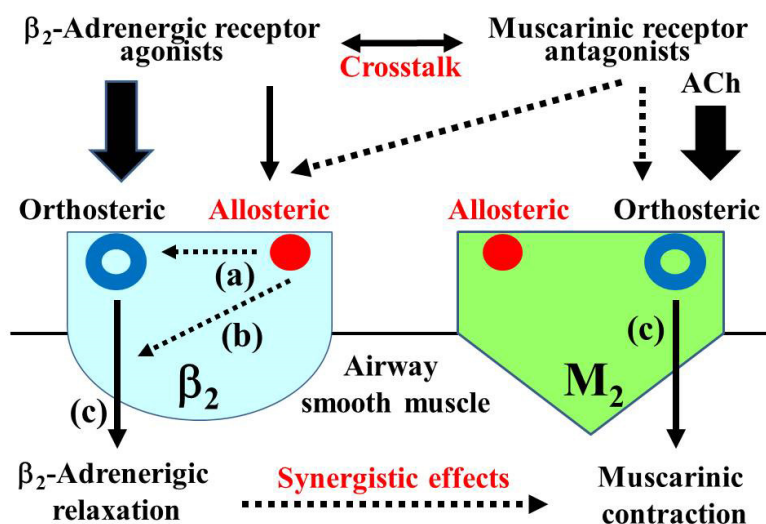


Fig. 1.

Isometric tension and F_{340}/F_{380} were recorded in the fura-2-loaded tracheal strips of guinea pigs. Procaterol, salbutamol, and formoterol were used as β_2 -adrenergic receptor agonists; tiotropium, glycopyrronium, and atropine were used as muscarinic receptor antagonists. Synergistic effects of these two agents were evaluated using the Bliss independence (BI) theory. To examine involvement of allosteric modulation (affinity, efficacy) in synergistic effects, concentration-inhibition curves for β_2 -adrenergic receptor agonists were analyzed in the presence and absence of these muscarinic receptor antagonists.

When β_2 -adrenergic receptor agonists was combined with muscarinic receptor antagonists, the values of percent inhibition of this combination of these two agents on methacholine-induced contraction was significantly greater than those values expected by the BI theory. This synergistic effect was reduced by iberiotoxin (a K_{Ca} channel inhibitor); in contrast was enhanced by verapamil (a VDC channel inhibitor). This combination of these two agents markedly reduced F_{340}/F_{380} ; iberiotoxin inhibited this phenomenon;

verapamil reversed this ibriotoxin-induced inhibition. This synergistic effect was augmented by incubation with cholera toxin that activates G_s and pertussis toxin that suppresses G_i . Therefore, synergistic effects induced by these two agents are associated with a reduction in Ca^{2+} dynamics mediated by the G proteins/ K_{Ca} channels/VDC channels coupling. In concentration-inhibition curves for these β_2 -adrenergic receptor agonists against methacholine, the complete inhibition was not observed, and the inhibitory effect was attenuated in the concentration-dependent manner at higher concentrations than the concentration that the maximal effect was produced (noncompetitive effects), indicating that these agents act not only on orthosteric sites but also on allosteric sites (partial agonists), different from isoproterenol (a full agonist). In contrast, in concentration-inhibition curves for these muscarinic receptor antagonists against methacholine, the complete inhibition was observed, and the inhibitory effect was not attenuated at higher concentrations (competitive effects), indicating that these antagonists act on only orthosteric sites, independent of allosteric sites, similar to isoproterenol. When these β_2 -adrenergic receptor agonists were cumulatively applied with lower concentrations of these muscarinic receptor antagonists, the concentration-inhibition curves for these β_2 -adrenergic receptor agonists were markedly dissociated from the curves without muscarinic receptor antagonists. Values of the concentration of these β_2 -adrenergic receptor agonists that produced a 50% response of contraction (EC_{50}) were significantly reduced in the presence of these muscarinic receptor antagonists (affinity modulation). Moreover, the maximal effects of these β_2 -adrenergic receptor agonists were augmented to the complete inhibition in the presence of these muscarinic receptor antagonists (efficacy modulation); the complete inhibition were not attenuated at higher concentrations, unlike the effects of these β_2 -adrenergic receptor agonists without these muscarinic receptor antagonists. Therefore, muscarinic receptor antagonists may act on allosteric sites on β_2 -adrenergic receptors; these antagonists suppress allosteric modulation induced by partial β_2 -adrenergic receptor agonists, leading to synergistic effects by these two agents (Fig. 1).

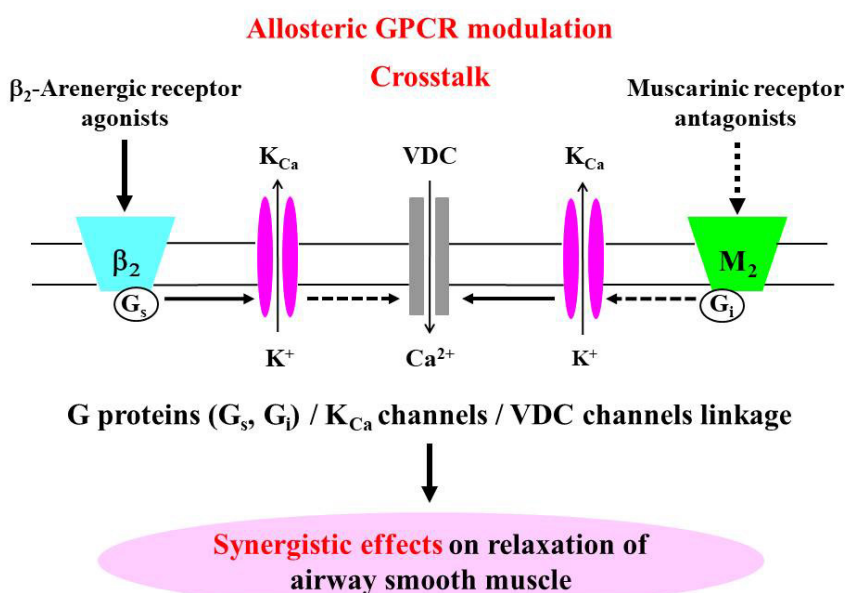


Fig. 2.

The K_{Ca} channels/VDC channels coupling and allosteric sites on β_2 -adrenergic receptors are involved in synergistic effects between β_2 -adrenergic receptor agonists and muscarinic receptor antagonists. The G protein-related modulation of Ca^{2+} dynamics and allosteric effects may be novel target molecules in research and development of bronchodilators for these diseases (Fig. 2).

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Publication

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