Clinical Use of $\beta_2$-adrenergic Receptor Agonists Based on Their Intrinsic Efficacy

Hiroaki Kume

ABSTRACT

Clinical choice of $\beta_2$-adrenergic receptor agonists ($\beta_2$-agonists) is based on the parameter of receptor selectivity, potency, and duration of action. The guidelines for asthma management describe nothing about intrinsic efficacy concerning the use of $\beta_2$-agonists. Since intrinsic efficacy refers to the ability to activate $\beta_2$-adrenergic receptors independent of agonist concentration, $\beta_2$-adrenergic desensitization may be associated with intrinsic efficacy. However, little is currently known whether chronic administration of high intrinsic efficacy drugs interferes with the effects of $\beta_2$-agonists as a reliever medication. In this review, the causal relationship between intrinsic efficacy and desensitization to $\beta_2$-agonists is examined in tracheal smooth muscle using isometric tension records. Reasonable clinical use of these agonists based on these observations is discussed.

KEY WORDS

airway smooth muscle, bronchial asthma, desensitization, Gs, long-acting $\beta_2$-agonists, Rho, $\beta_2$-adrenergic receptors

INTRODUCTION

$\beta_2$-Adrenergic receptor agonists ($\beta_2$-agonists) are widely used clinically to relax airway smooth muscle and are the principal bronchodilator agents used to treat bronchial asthma. Regular administration of short-acting $\beta_2$-agonists may cause not only a deterioration of asthma control and an exacerbation of airway hyperreactivity, but also an accelerative decline in lung function in patients with this disease. These undesirable dysfunctions may be due to reduced responsiveness to short-acting $\beta_2$-agonists elicited by excessive exposure to these agents, a phenomenon referred to as desensitization. Moreover, a decline in $\beta_2$-agonist activity is observed after prolonged exposure to pro-inflammatory cytokines and lysophospholipid that participate in the airway inflammation of bronchial asthma. A postmortem study also has shown that the response to $\beta_2$-agonists in vitro is attenuated in airway smooth muscle harvested from patients with fatal asthma. The impaired $\beta_2$-adrenergic action is a characteristic feature of patients with this disease, and is an important problem in both the pathogenesis and therapy.

Rapid- and short-acting $\beta_2$-agonists suppress bronchoconstriction and related symptoms of acute
asthma exacerbations, but do not inhibit airway inflammation and airway hyperreactivity. Treatment with anti-inflammatory agents is more effective than that with bronchodilator agents for long-term management of bronchial asthma. Although the anti-inflammatory effects of long-acting β₂-agonists are still unclear in clinical use, the guidelines for asthma prevention and management recommend daily administration of long-acting β₂-agonists as a controller medication. The regular use of long-acting β₂-agonists in combination with inhaled glucocorticosteroid is considered to be beneficial to these patients according to the cross-talk between these two receptors. However, a few reports have demonstrated that regular use of inhaled long-acting β₂-agonists results in desensitization of β₂-adrenergic receptors even though these agonists are added to inhaled glucocorticosteroid therapy. Little is essentially known whether prolonged activation of β₂-adrenergic receptors is not harmful.

There are several parameters in characterizing the interaction of an agent with receptors, such as affinity, potency, and efficacy. However, β₂-agonists are just classified by onset and duration of action in the guideline for asthma management. This current classification may be insufficient to establish safe administration of β₂-agonists. Previously statistical correlation between regular use of β₂-agonists and asthma mortality or morbidity has been reported. This deterioration is considered to be due to desensitization of β₂-adrenergic receptors mediated by excessive administration of β₂-agonists of higher intrinsic efficacies, such as isoproterenol and fenoterol. We should carefully reconsider how to use these agonists. In this review, the relationship between intrinsic efficacy and desensitization to β₂-agonists is examined in detail using airway smooth muscle, and in addition, reasonable clinical use of these agents based on this observation is discussed.

**MECHANISMS OF DESENSITIZATION OF β₂-ADREnergic RECEPTORS IN AIRWAY SMOOTH MUSCLE**

It is well known that response to β₂-agonists is markedly attenuated after continuous and repeated exposure to the agonists in airway smooth muscle of humans and other animals. Previous reports have demonstrated the molecular mechanisms of desensitization of β₂-adrenergic receptors in various tissues. After exposure to β₂-agonists for a short term (less than 30 min), the β₂-agonist activity is markedly attenuated via the phosphorylation of β₂-adrenergic receptors, resulting in an uncoupling from the stimulatory GTP-binding (G) protein of adenylyl cyclase, Gs. A longer-term exposure leads to down-regulation of surface receptor number via internalization of the receptor and its subsequent degradation. Two types of protein kinases are responsible for the receptor phosphorylation, i.e., 1) second messenger-activated kinases such as the adenosine 3', 5'-cyclic monophosphate (cAMP)-dependent kinase (PKA) and 2) the second messenger-independent G protein-coupled receptor kinases such as the β₂-adrenergic receptor kinase (βARK) and G protein-coupled receptor kinases (GRKs). Receptor phosphorylation mediated by PKA occurs with low concentrations of β₂-agonists, leading to heterologous desensitization. On the other hand, high concentrations of β₂-agonists are required for activation of βARK and GRKs, leading to homologous desensitization. The former refers to a reduced response to not only β₂-agonists but also other agents that elevate concentration of intracellular cAMP bypassing β₂-adrenergic receptors, such as forskolin, theophylline, db-cAMP, and prostaglandin (PG) E₂. The latter refers to a specific reduced response to β₂-agonists while response to these other agents remain intact.

In airway smooth muscle cells, intracellular cAMP formation in response to isoproterenol is attenuated after incubation with not only β₂-agonists but also other agents involving cAMP such as forskolin and PGE₂. An inhibition in cAMP formation is mediated by heterologous desensitization. However, in airway smooth muscle, relaxation induced by β₂-agonists is impaired after excessive exposure to these agonists, whereas the reduced relaxation by β₂-agonists does not occur after excessive exposure to these cAMP involving agents independent of β₂-adrenergic receptors. When desensitization of β₂-adrenergic receptors is examined by measuring a biochemical response (cAMP formulation), heterologous desensitization is observed. In contrast, when β₂-adrenergic desensitization is examined by measuring a physiological response (smooth muscle relaxation in vitro), heterologous desensitization is not observed. Interferon-γ inhibits the reduced relaxation by heterologous β₂-adrenergic desensitization after exposure to TGF-β₁, but does not recover the reduced cAMP formation by TGF-β₁. There is a discrepancy between cAMP formulation and relaxation concerning the impairment of β₂-adrenergic action. Hence, in airway smooth muscle measurement of relaxation needs to estimate accurately desensitization to β₂-agonists.

Forskolin needs to produce a large amount of cAMP to cause an equivalent relaxation that is achieved with isoproterenol by producing a small amount of cAMP in airway smooth muscle. cAMP formation in response to β₂-agonists is not always associated with relaxation. Recently, it is generally considered that β₂-agonists have the effects mediated by not only cAMP-dependent but also cAMP-independent processes. The discrepancy in causing heterologous desensitization may be due to these cAMP-independent pathways. Large conductance Ca²⁺-activated K⁺ (K₉Ca) channels, which are densely dis-
IN Volvement of Gs in β2-Adrenergic Desensitization in Airway Smooth Muscle

After excessive exposure to β2-agonists, the inhibitory effects of agents involving cAMP independently of β2-adrenergic receptors are not attenuated; on the contrary those effects are significantly augmented. Moreover, β2-agonist activity is still intact after exposure to PGE2, which activates Gs via its own receptor which is different from β2-adrenergic receptors. These results indicate that after excessive exposure of airway smooth muscle to β2-agonists, relaxation induced by these agonists is markedly diminished mediated by homologous desensitization, not by heterologous desensitization (Fig. 1). The reduced responsiveness to β2-agonists is not affected even when the tissues are exposed to β2-agonists for an extended period in the presence of Rp-cAMP, a permeable inhibitor of PKA, also indicating that the cAMP/PKA processes are not involved in β2-adrenergic desensitization measured as a physiologic response. The receptor/Gs processes play an important functional role in not only the β2-adrenergic action but also the β2-adrenergic desensitization in airway smooth muscle. β2-agonists are more potent in causing both relaxation and desensitization than other agents involving cAMP bypassing β2-adrenergic receptors. Hence, measurement of cAMP formation without examination of relaxation response is not appropriate to an assessment of β2-adrenergic action and desensitization in airway smooth muscle.

As described above, the phosphorylation of β2-adrenergic receptors elicits a functional uncoupling between the receptors and the Gs proteins. It is well known that cholera toxin (CTX) irreversibly activates Gs mediated by ADP-rybosylation of α-subunit with a remarkable reduction of guanosinetriphosphatase activity. When airway smooth muscle is treated with...
CTX before continuous or repeated exposure to β2-agonists, CTX causes an inhibition in a subsequent reduction in the relaxation action by β2-agonists and lysophosphatidylcholine in a concentration- and time-dependent manner. This inhibitory action of CTX is not affected in the presence of Rp-cAMP. The reduced responsiveness to β2-agonists after repeated exposure to the agonists is enhanced in the presence of a selective inhibitor of KCa channels, and is associated with an elevation in intracellular Ca²⁺ concentration. On the other hand, the reduced responsiveness to β2-agonists after continuous exposure to the agonists and lysophosphatidylcholine is not associated with an increase in intracellular Ca²⁺ concentration, and is antagonized by an inhibition in Rho, a small G protein, in the presence of Y-27632, an inhibitor of Rho-kinase, in a concentration-dependent manner. These results indicate that in airway smooth muscle β2-adrenergic desensitization is due to Ca²⁺ mobilization mediated by suppression of the Gα/KCa channel stimulatory linkage and to Ca²⁺ sensitization induced by Rho/Rho-kinase pathways (Fig. 1). Moreover, an irreversible activation of Gα leads to prevention of β2-adrenergic desensitization, independent of cAMP/PKA processes. If a complete dissociation between the receptors and G proteins occurs in the reduced responsiveness to β2-agonists, the activation of Gα may have no effects on prevention of the desensitization of β2-adrenergic receptors. Impairment of Gα activity may be involved in this phenomenon.

### Measurement of β2-Adrenergic Intrinsic Efficacy

An agonist’s potency depends on its affinity for a receptor and on its intrinsic efficacy. Affinity refers to the attraction between an agent and its receptors, and intrinsic efficacy refers to the ability of an agent to activate its receptors. Since the limited dose of β2-agonists is inhaled as a bronchodilator therapy against acute asthma exacerbations, affinity for the receptors may be a relatively unimportant parameter in taking the clinical effects of these agonists. In contrast, intrinsic efficacy may reflect the capability of β2-agonists to activate the receptors in the clinical administration. If the values of intrinsic efficacy are accurately measured, it would be an important parameter in a rational clinical use of β2-agonists. However, international guidelines for asthma management have never described anything about it.

Some agonists completely activate receptors, however others partially activate them. The former are referred to as a full agonist, and the latter are as a partial agonist. Moreover, partial agonists are classified into two subtypes, i.e. 1) its efficacy is lower (weak partial agonists) and 2) its efficacy is higher (strong agonists). Activation of β2-adrenergic receptors is directly measured as a conformational change using receptor fluorescence. Intrinsic efficacy is also measured indirectly as a response to activation of the post-receptor signal transduction pathways (changing in cAMP formation), and as a physiological response (changing in smooth muscle relaxation in vitro and airway resistance in vivo). Measurement of agonist efficacy markedly depends on variable factors in the target cells, such as receptor number and presence of functional antagonism. Intrinsic efficacy of each agonist is expressed by difference between Kd (the dissociation constant: the dependency of receptor occupancy on agonist concentration) and EC₅₀ (agonist concentration that produces 50% inhibition of the maximal contraction). The values of Kd and EC₅₀ are common parameters in affinity and potency of an agonist, respectively. More efficacious agonists have greater difference of these two parameters. Partial agonists need to occupy a large fraction of receptors to produce an equivalent effect that full agonists achieve by occupying much less receptors. The ratio of the intrinsic efficacy of any two β2-agonists is expressed as a fraction between 0 and 1, taking that of adrenaline as 1.

Barber et al. have expressed values of intrinsic efficacy by measuring receptor activation as a biochemical response to activation of cAMP/PKA processes using human embryonic renal and cultured murine lymphoma cells other than airway smooth muscle cells. Under this experimental condition, the rank of order of intrinsic efficacies is: isoproterenol > fenoterol > procaterol > formoterol > albuterol > salmeterol > tuloberterol. These results indicate that procaterol can activate approximately 15 times more β2-adrenergic receptors than tuloberterol when these two agonists occupy the same number of receptors.
Fig. 2 Pre exposure to long-acting $\beta_2$-agonists results in a reduction in the inhibitory action of short-action $\beta_2$-agonists against 1 μM methacholine-induced contraction in guinea pig tracheal smooth muscle. The relaxation responses were expressed as a percentage of the maximal relaxation produced by free Ca$^{2+}$ solution.

* : $p < 0.05$, ** : $p < 0.01$.

most advantageous route of administration of $\beta_2$-agonists is thought to deliver directly into airways via inhalation. This route leads to the direct and rapid onset of action, and to avoidance of the systemic side effects. It is currently unclear whether $\beta$-agonists inhibit clinically the airway inflammation elicited by activated eosinophils. Moreover, as described above, $\beta_2$-agonists have cAMP-independent pathways and there are discrepancies between cAMP formation and relaxation induced by these agonists. Hence, in this study $\beta_2$-adrenergic intrinsic efficacy was expressed as receptor activation based on smooth muscle relaxation. Isometric tension records demonstrate the direct action of $\beta_2$-agonists on airway smooth muscle independent of indirect action via inflammatory cells, similar to inhalation, because these agonists are directly applied to the strips in the organ bath. Receptor number and functional antagonism have an affect on measurement of agonist efficacy. However, these two factors are probably not affected when the experiments are performed under the condition that the intact tissues are used and that an equivalent concentration of antagonists is applied. According to the methods described previously, isometric tension was recorded using guinea pig tracheal smooth muscle. $\beta_2$-agonists were cumulatively applied to the intact tissues pre contracted by 1 μM methacholine. The values of EC$_{50}$ and the maximal effects in the concentration-inhibition curves for a $\beta_2$-agonist express its potency and efficacy, respectively. The values of EC$_{50}$ and the maximum percent inhibition for these $\beta_2$-agonists in the curves are shown in Table 1. The order of potency (EC$_{50}$) was:

formoterol > procaterol = isoproterenol = salbutamol > salmeterol >> tulobuterol. Isoproterenol, formoterol, and procaterol caused the complete inhibition against 1 μM methacholine-induced contraction (100% inhibition), indicating that these agents are full agonists. In contrast salmeterol, salbutamol, and tulobuterol did not cause complete inhibition under this experimental condition, indicating that these agents are partial agonists. The order of efficacy (the minimum percent inhibition) was:

isoproterenol = formoterol = procaterol > salbutamol > salmeterol > tulobuterol. When the functional antagonism was intensified by application of 10-fold higher concentration of methacholine, isoproterenol caused complete relaxation against this contraction, but the maximal effects in the curves for formoterol and procaterol were attenuated. The values of the maximum percent inhibition for isoproterenol, formoterol, procaterol were 100, 59.4 ± 9.2, 71.9 ± 10.9%, respectively (data not shown). Under these experimental conditions, formoterol and procaterol behave as partial agonists for relaxation in comparison with isoproterenol. Therefore, it is considered that isoproterenol is a full agonist, that procaterol and formoterol are strong agonists. Our data are roughly consistent with the rank order of intrinsic efficacy measured as a biochemical response to the adenylyl cyclase/cAMP pathway.

**RELATIONSHIP BETWEEN $\beta_2$-ADRENERGIC DESSENSITIZATION AND AGONIST INTRINSIC EFFICACY IN AIRWAY SMOOTH MUSCLE**

Inhalation of procaterol or salbutamol, which has a
rapid onset and short duration of action, is used clinically as a reliever medication to suppress bronchoconstriction elicited by acute asthma attacks. Inhalation of formoterol or salmeterol, which have duration of action lasting for approximately 12 hour, is given twice daily as a controller medication in addition to inhaled glucocorticosteroid therapy. A patch formulation of tulobuterol, a sustained-release β2-agonist, also is used as a controller medication because of a 24-hour duration of action.36 To determine whether a controller medication using β2-agonists leads to a reduction in the inhibitory effects of β2-agonists for a reliever medication against acute asthma exacerbations, subsequent response to these rapid- and short-acting β2-agonists was examined after exposure to these long-acting β2-agonists in guinea pig tracheal smooth muscle using isometric tension recording (Fig. 2). When 0.1 μM formoterol, the intrinsic efficacy of which is strong, was applied to the intact strips for 10 min, subsequent inhibition by 0.03 μM procaterol and 0.3 μM salbutamol against contraction induced by 1 μM methacholine was markedly attenuated. The values of percent inhibition for procaterol were decreased from 87.4 ± 4.4 to 38.0 ± 10.7% (n = 8, p < 0.05). Those values for salbutamol were decreased from 77.4 ± 7.9 to 2.6 ± 0.9% (n = 8, p < 0.01). After exposure to equi-molar salmeterol, the intrinsic efficacy of which is moderate, for an equivalent time, the inhibitory effects of salbutamol on 1 μM methacholine-induced contraction were attenuated. The value of percent inhibition for salbutamol was decreased to 48.0 ± 8.2% (n = 8, p < 0.05). In contrast, those effects of procaterol were not significantly attenuated. Those values for procaterol before and after exposure to salmeterol were 87.4 ± 4.4 and 81.7 ± 4.1%, respectively (n = 8, not significant). After exposure to equi-molar tulobuterol, intrinsic efficacy of which is weak, for an equivalent time, the inhibitory effects of both procaterol and salbutamol on methacholine-induced contraction were not attenuated. Those values for procaterol and salbutamol after exposure to tulobuterol were 83.0 ± 5.4 and 73.0 ± 6.6, respectively (n = 8, each not significant). The rank order of causing β2-adrenergic desensitization is: formoterol > salmeterol > tulobuterol. This rank order was identical with that of intrinsic efficacy of β2-agonists.

When receptor activation is measured as a relaxation response, β2-adrenergic desensitization occurs in proportion to the strength of intrinsic efficacy of agonists. In airway smooth muscle full agonists cause more desensitization of the receptors, whereas partial agonists cause less desensitization. These results are consistent with those obtained by measurements of biochemical responses to activation of the postreceptor signal transduction pathways.30,34 Although PKA-mediated phosphorylation by partial agonists is similar to that by strong and full agonists, βARK- and GRKs-mediated phosphorylation by partial agonists are less than that by strong and full agonists.33 Strong and full agonists cause both homologous and heterologous desensitization, in contrast, partial agonists primarily cause heterologous desensitization. Therefore, it is generally considered that partial agonists are less potent in causing desensitization.37 Our results also indicate that cAMP/PKA processes are not involved in a reduction in the relaxant action of β-agonists after excessive exposure to the agonists, and that the reduced responsiveness to β2-agonists is mediated by homologous desensitization, not by heterologous desensitization in tracheal smooth muscle (Fig. 1).

On the other hand, in inverse proportion to the rank order of intrinsic efficacy, the reduced respon-
siveness to salbutamol was intensified more than that to procaterol after exposure to each of these long-acting β-agonists (Fig. 2). The effects of full agonists are not affected under the condition of either high or low receptor density in the cell surface. In contrast, the effects of partial agonists may be intact with high receptor density, but the effects of these agonists are attenuated as receptor number is lowered. When the uncoupling of the receptors from Gs and the receptor internalization occurs in airway smooth muscle, full agonists can yield a full relaxation response, whereas partial agonists cannot do so. In the presence of functional antagonism (contractile agents), the maximal effects of β2-agonists are antagonized by signal transduction pathways of contractile agents, such as methacholine, histamine, prostaglandins, and leukotrienes. Airway contraction leads to a reduction in β2-adrenergic intrinsic efficacy. Hence, the effects of partial agonists can be attenuated when an acute asthma attack occurs.

RATIONAL CHOICE OF INHALED β2-AGONISTS IN THE MANAGEMENT OF BRONCHIAL ASTHMA

Since the induced desensitization to β2-agonists increases as the values of intrinsic efficacy of agonists are elevated, daily administration of strong and full agonists may be harmful for long-term asthma management. Weak partial agonists are appropriate for controller medications. On the other hand, as the values of intrinsic efficacy are lowered, the reduced responsiveness to β2-agonists increases by decreased receptor density after excessive exposure to these agonists and by functional antagonism. Inhalation of weak partial agonists, taken as needed, may not be beneficial for the management of acute asthma. Strong and full agonists are appropriate for reliever medications. Previous clinical trials have demonstrated that regular inhalation of salmeterol and tulobuterol (weak partial agonists) as controller medication results in sustained bronchodilutional effects throughout the observation periods,36,38,39 and that, in contrast, regular inhalation of formoterol (a strong agonist) results in reduced bronchodilation effect.40,41 These results support the idea that excessive administration of strong and full agonists causes more desensitization of β2-adrenergic receptors in airway smooth muscle than that of weak partial agonists. When the inhibitory effects of β2-agonists on bronchial hyperresponsiveness are evaluated using the methacholine challenge test, inhalation of formoterol protects against methacholine-induced bronchoconstriction in a dose-dependent manner, whereas salmeterol has a weaker protective effect.38,42 Regular inhalation of salmeterol (a weak partial agonist) does not antagonize the response to rescue short-acting β2-agonists, such as albuterol.43,44 These clinical data also demonstrated that strong and full agonists should be used as needed to relieve asthma symptoms and weak partial agonists should be used regularly to keep persistent asthma under control (Fig. 3).

CONCLUSIONS

The membrane-delimited, cAMP-independent Gs activation plays an important functional role in both the physiologic response and desensitization to β2-agonists. The intrinsic efficacy of β2-agonists, which is involved in an increase in Gs activity, is responsible for causing desensitization to these agonists. In airway smooth muscle excessive exposure to β2-agonists of high intrinsic efficacy gives rise to greater desensitization of the receptors than that to these agonists of low intrinsic efficacy. When the β2-adrenergic receptor function declines under the conditions of uncoupling from Gs, lowered receptor density, and functional antagonisms, β2-agonists of lower intrinsic efficacy do not generate a full intracellular signal, leading to a reduction in the relaxation response to partial agonists. Although the clinical relevance of our data is still unclear, they may provide the evidence that β2-agonists with a long duration of action and low intrinsic efficacy are optimal for the maintenance therapy of bronchial asthma, and that those agonists with rapid onset of action and high intrinsic efficacy are optimal for rescue therapy against acute asthma attacks.

ACKNOWLEDGEMENTS

I would like to thank Dr. Kaoru Shimokata (Division of Respiratory Medicine, Department of Medicine, Nagoya University Graduate School of Medicine) for helpful comments.

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