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Commentary

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β-Agonists and asthma: too much of a good thing?

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In an unusual paradox, asthmatics who are chronically treated with bronchodilating β-agonists sometimes experience a worsening of their condition. A new study (see the related article beginning on page 619) describes one possible mechanism and reveals a potential new therapeutic target in the treatment of asthma.

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Inhaled selective β_2-agonists are the most widely used treatment for the acute relief of asthma symptoms. In patients with asthma, these agents cause bronchodilation (improvement in lung mechanics) and bronchoprotection (reduced responsiveness to nonspecific contractile stimuli). These actions result from binding to the β_2-adrenergic receptor (β_2AR), a heptahelical receptor that couples predominantly to the stimulatory G protein, G_s (Figure 1a). Once activated by receptor-ligand binding, the α subunit of G_s activates adenylyl cyclase (AC), resulting in cAMP formation. cAMP phosphorylates protein kinase A (PKA), which phosphorylates multiple proteins, leading to reductions in intracellular calcium, smooth muscle relaxation, and bronchodilation.

It is possible to have too much of a good thing. Despite the ability of β-agonists to effect immediate reversal of airway obstruction, there has been continuing concern that regular use of these drugs may be associated with adverse outcomes. In some, but not all, studies, regularly scheduled use (e.g., multiple times per day, every day) of inhaled β-agonists has resulted in loss of asthma control, declines in morning peak flow, longer durations of asthma exacerbations, and rebound airway hyperresponsiveness (1–6). These adverse effects appear to be particularly important with β-agonists of high intrinsic efficacy, like fenoterol, and in patients with certain β_2AR genotypes.

The common explanation for these observations is that the adverse effects of regular β-agonist therapy are related to desensitization of the β_2AR (2, 3, 5, 7). The data reported by McGraw et al. in this issue of the *JCI* (8) suggest an alternative explanation, namely that persistent high-level activation of the β_2AR leads to increased expression of phospholipase C-β (PLCβ) in airway smooth muscle. Since agonists such as acetylcholine, histamine, and leukotrienes that cause airway smooth muscle contraction do so by

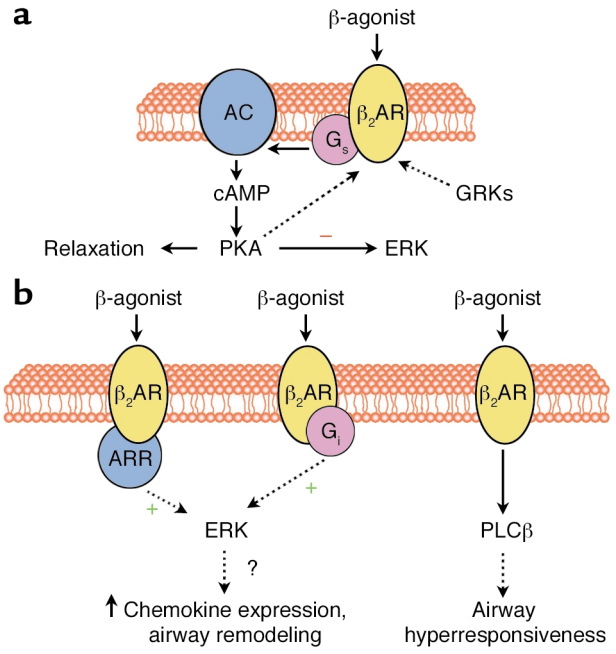

Figure 1
(**a**) Mechanism of β-agonist–induced airway smooth muscle relaxation. Ligand binding to the β_2AR activates G_s, leading to adenylyl cyclase (AC) activation, cAMP formation, and subsequent protein kinase A (PKA) activation. PKA phosphorylation of target proteins leads to smooth muscle relaxation and may also inhibit ERK activation. PKA also phosphorylates the β_2AR, leading to increased G_i coupling. In addition, ligand binding causes G protein receptor kinase (GRK) phosphorylation of the β_2AR, recruiting β-arrestin (ARR). (**b**) Inflammatory events in the asthmatic airway or regular β-agonist use may augment G_i coupling and/or increase β-arrestin binding. Under these circumstances, β-agonists may result in ERK activation, potentially amplifying production of inflammatory cytokines and leading to airway remodeling. Persistent activation of the β_2AR may also lead to phospholipase C-β (PLCβ) expression and consequent airway hyperresponsiveness (8).

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Nonstandard abbreviations used: β_2-adrenergic receptor (β_2AR); protein kinase A (PKA); phospholipase C-β (PLCβ); inositol 1,4,5 trisphosphate (IP_3); extracellular signal–regulated kinase (ERK); G protein receptor kinase (GRK).

acting on receptors that couple to G_q and activate PLCβ, chronic β-agonists might augment the effects of these bronchoconstrictors. How could this occur? PLCβ hydrolyzes phosphatidylinositol 4,5 biphosphate, resulting in inositol 1,4,5 trisphosphate (IP_3) production. IP_3 increases intracellular calcium, leading to activation of myosin light chain kinase, myosin phosphorylation, and consequent muscle contraction. If the observations of McGraw et al. are borne out, this mechanism could explain the adverse effects of chronic stimulation of the β_2AR. However, before we embrace this hypothesis, which is based on data from mouse airways, it will be important to confirm the observations of McGraw et al. in human airway smooth muscle stimulated with β-agonists rather than by β_2AR overexpression.

We think that there may be additional explanations for the bronchoconstrictor effects of chronic β_2AR stimulation. For example, in some cell types the β_2AR can couple to G_i as well as G_s (9–12). The switch from G_s to G_i coupling appears to be regulated by PKA phosphorylation of the β_2AR (13). β_2AR-induced G_i activation leads to activation of the extracellular signal–regulated kinase (ERK) MAPKs (Figure 1b) through a pathway dependent on the G_i βγ subunits and activation of Ras (12). β_2AR activation also leads to G protein receptor kinase–induced (GRK-induced) phosphorylation of the β_2AR, resulting in its interaction with β-arrestin. β-arrestin can serve as a scaffolding protein linking the β_2AR to both the ERK and JNK MAPK pathways (9, 14). To complicate the matter, G_s-dependent formation of cAMP by β_2AR activation can also inhibit ERK activation (10), apparently as a result of Raf-1 phosphorylation by PKA (15). The ultimate effect of β_2AR activation on ERK phosphorylation is likely to reflect a balance of these various events.

Although these pathways have been described in other cell types, none of these events has been examined in airway smooth muscle cells. Thus it is interesting to consider the potential functional implications of β-agonist–induced ERK activation in these cells. Persistent ERK activation is known to be required for mitogenesis in airway smooth muscle cells (16). Activation of ERK is also required for the full expression by airway smooth muscle of the eosinophil chemotactic factors eotaxin and RANTES, as well as other chemokines (17). β-agonists normally inhibit mitogenesis (18) and inhibit expression of eotaxin (19) in airway smooth muscle. However, there may be conditions in which this is not the case. For example, G_s-to-G_i switching could be exaggerated by inflammatory cytokines, which have been shown to increase G_i expression (20). Inflammatory cytokines have also been shown to increase GRK expression in these cells (20). Increased GRK activation could be expected to enhance ERK activation through effects on β-arrestin binding to the β_2AR. It is also possible that the balance of ERK-activating and ERK-inhibiting effects of β_2AR activation may be affected by β_2AR desensitization under conditions of regular β-agonist use.

It is interesting to note that the adverse effects of regular β-agonist use can be observed even weeks after their withdrawal, at a time when β_2AR desensitization should be resolved (2). If regular use of β-agonists negatively impacts the balance of factors contributing to airway smooth muscle mitogenesis or airway remodeling in asthma, it could have long-term consequences. Importantly, studies of regular use of β-agonist have reported adverse effects only in subjects homozygous for the Arg16 variant of the β_2AR (2, 3, 5). The impact of β_2AR polymorphisms on non–G_s-mediated events such as ERK phosphorylation has not been examined in any cell type.

β-agonists are currently one of the most important forms of therapy for asthma. Little is known about non–G_s-mediated effects of β_2AR activation and other events that could negatively impact the function of airway smooth muscle in asthma. In this issue of the *JCI*, McGraw et al. (8) report one such event, induction of PLCβ, but it is possible that this is just the tip of the iceberg. Understanding the panoply of β_2AR-mediated events in airway smooth muscle might lead to the design of new agonists that avoid negative effects of β_2AR activation while enhancing events that lead to relaxation.

1. Vathenen, A.S., Knox, A.J., Higgins, B.G., Britton, J.R., and Tattersfield, A.E. 1988. Rebound increase in bronchial responsiveness after treatment with inhaled terbutaline. *Lancet.* **1**:554–558.
2. Israel, E., et al. 2000. The effect of polymorphisms of the beta(2)-adrenergic receptor on the response to regular use of albuterol in asthma. *Am. J. Respir. Crit. Care Med.* **162**:75–80.
3. Taylor, D.R., et al. 2000. Asthma exacerbations during long term beta agonist use: influence of beta(2) adrenoceptor polymorphism. *Thorax.* **55**:762–767.
4. Taylor, D.R., et al. 1998. Asthma control during long-term treatment with regular inhaled salbutamol and salmeterol. *Thorax.* **53**:744–752.
5. Hancox, R.J., Sears, M.R., and Taylor, D.R. 1998. Polymorphism of the beta2-adrenoceptor and the response to long-term beta2-agonist therapy in asthma. *Eur. Respir. J.* **11**:589–593.
6. Sears, M.R., et al. 1990. Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet.* **336**:1391–1396.
7. Hanania, N.A., Sharafkhaneh, A., Barber, R., and Dickey, B.F. 2002. Beta-agonist intrinsic efficacy: measurement and clinical significance. *Am. J. Respir. Crit. Care Med.* **165**:1353–1358.
8. McGraw, D.W., Almoosa, K.F., Paul, R.J., Kobilka, B.K., and Liggett, S.B. 2003. Antithetic regulation by β-adrenergic receptors of G_q receptor signaling via phospholipase C underlies the airway β-agonist paradox. *J. Clin. Invest.* **112**:619–626. doi:10.1172/JCI200318193.
9. Tohgo, A., et al. 2003. The stability of the G protein-coupled receptor-beta-arrestin interaction determines the mechanism and functional consequence of ERK activation. *J. Biol. Chem.* **278**:6258–6267.
10. Crespo, P., Cachero, T.G., Xu, N., and Gutkind, J.S. 1995. Dual effect of beta-adrenergic receptors on mitogen-activated protein kinase. Evidence for a beta gamma-dependent activation and a G alpha s-cAMP-mediated inhibition. *J. Biol. Chem.* **270**:25259–25265.
11. Zhu, W.Z., et al. 2001. Dual modulation of cell survival and cell death by beta(2)-adrenergic signaling in adult mouse cardiac myocytes. *Proc. Natl. Acad. Sci. U. S. A.* **98**:1607–1612.
12. Zou, Y., et al. 1999. Both Gs and Gi proteins are critically involved in isoproterenol-induced cardiomyocyte hypertrophy. *J. Biol. Chem.* **274**:9760–9770.
13. Daaka, Y., Luttrell, L.M., and Lefkowitz, R.J. 1997. Switching of the coupling of the beta2-adrenergic receptor to different G proteins by protein kinase A. *Nature.* **390**:88–91.
14. Hall, R.A., and Lefkowitz, R.J. 2002. Regulation of G protein-coupled receptor signaling by scaffold proteins. *Circ. Res.* **91**:672–680.
15. Cook, S.J., and McCormick, F. 1993. Inhibition by cAMP of Ras-dependent activation of Raf. *Science.* **262**:1069–1072.
16. Orsini, M.J., et al. 1999. MAPK superfamily activation in human airway smooth muscle: mitogenesis requires prolonged p42/p44 activation. *Am. J. Physiol.* **277**:L479–L488.
17. Hallsworth, M.P., Moir, L.M., Lai, D., and Hirst, S.J. 2001. Inhibitors of mitogen-activated pro-

tein kinases differentially regulate eosinophil-activating cytokine release from human airway smooth muscle. *Am. J. Respir. Crit. Care Med.* **164**:688–697.
18. Roth, M., et al. 2002. Interaction between glucocorticoids and beta2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. *Lancet.* **360**:1293–1299.
19. Pang, L., and Knox, A.J. 2001. Regulation of TNF-alpha-induced eotaxin release from cultured human airway smooth muscle cells by beta2-agonists and corticosteroids. *FASEB J.* **15**:261–269.
20. Mak, J.C., Hisada, T., Salmon, M., Barnes, P.J., and Chung, K.F. 2002. Glucocorticoids reverse IL-1beta-induced impairment of beta-adrenoceptor-mediated relaxation and up-regulation of G-protein-coupled receptor kinases. *Br. J. Pharmacol.* **135**:987–996.

Thyroid hormone action: a binding contract

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Thyroid hormones are critical for differentiation, growth, and metabolism. A new study (see the related article beginning on page 588) investigating the biological role of the TH receptor TR-β has demonstrated that DNA binding is critical for most of its functions, but also suggests that novel mechanisms independent of DNA binding may contribute to regulation of auditory function by TR-β.

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Thyroid hormone (TH) plays a critical role in the development and adult functions of many organs and tissues. Many of the effects of TH are mediated by a family of high-affinity receptor proteins, called TH receptors (TRs). Three functional TRs, TR-β1, TR-β2, and TR-α1, are encoded on two mammalian genes (1). The *TR-α* gene also encodes variant proteins that do not bind TH and whose function may be to inhibit the action of other TRs (2). The TRs are members of a larger family of nuclear receptors (NRs) for lipophilic signaling molecules that includes steroid hormones, vitamin derivatives such as retinoic acid and vitamin D_3, fatty acid and cholesterol metabolites, and xenobiotics (3). As the family name suggests, a key aspect of TR function involves nuclear regulation of gene transcription. In this issue of the *JCI*, Shibusawa and colleagues use mice whose *TR-β* gene products cannot bind DNA in order to shed light on the mechanisms by which TR functions in development and physiology (4). The TR-β mutant mice have a phenotype that is similar but not identical to that of mice lacking TR-β, suggesting that most but not all TR functions involve direct DNA binding.

Positive regulation of gene expression by TH: a simple model

Like nearly all NRs, the central portion of TR contains a zinc-ordered domain that binds to double-stranded DNA with a well-characterized sequence specificity. TR recognizes the sequence AGGTCA, to which it can bind as a monomer. This sequence also serves as a half-site for TR homodimers as well as heterodimers with the retinoid X receptor (RXR) (5). RXR increases the DNA-binding affinity of TR and also restricts binding to a subset of half-site arrangements whose binding by the TR/RXR heterodimer is energetically favorable. The most stable binding occurs on the classical DR4 thyroid response element (TRE), in which two half-sites are directly repeated with a spacing of 4 bp (6). The structural basis of this binding preference has been determined (7).

On a TRE-containing target gene, TH binding acts as a switch between repressed and activated states (Figure 1) (8). Genes that are bound by TR/RXR heterodimers, and potentially TR homodimers, are actively repressed in the absence of TH. The repression function is located in the C-terminal ligand-binding domain, which binds to the corepressor molecules nuclear receptor corepressor (N-CoR) and silencing mediator of retinoid and thyroid receptors (SMRT) (9), which anchor large multiprotein complexes containing histone deacetylase (HDAC) activity and mediate ligand-independent repression. TH binding induces a conformational change that destabilizes corepressor binding and that favors binding of transcriptional coactiva-

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Nonstandard abbreviations used: thyroid hormone (TH); TH receptor (TR); nuclear receptor (NR); retinoid X receptor (RXR); thyroid hormone response element (TRE); NR corepressor (N-CoR); silencing mediator of retinoid and thyroid receptors (SMRT); histone deacetylase (HDAC); thyroid-stimulating hormone (TSH); negative TRE (nTRE).

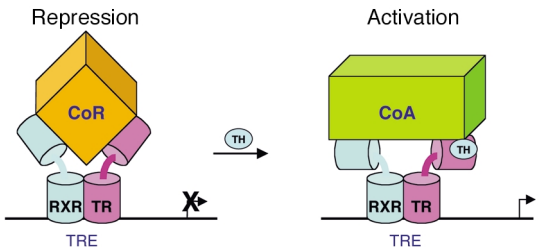

Figure 1
Paradigm for positive regulation of gene expression by TH. TH binding to TR triggers a switch from corepressor (CoR) to coactivator (CoA) binding on a TRE on DNA.