Most G protein–coupled receptors (GPCRs) probably exist as homodimers, but it is increasingly recognized that GPCRs may also dimerize with other types of GPCRs and that this physical interaction may affect the function of either receptor. A study in this issue of the JCI demonstrates how heterodimerization between prostaglandin E receptors and β2–adrenergic receptors (β2ARs) in airway smooth muscle cells results in uncoupling of β2ARs and a diminished bronchodilator response to β2AR agonists (see the related article beginning on page 1400). This illustrates what we believe to be a novel mechanism of receptor cross-talk and highlights the potential importance of GPCR heterodimerization in diseases such as asthma and how this could lead to the development of more specific therapies in the future.
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Cross-talk between different receptors has long been recognized as an important determinant of cellular response in health and disease. Traditionally this cross-talk has been explained by interaction of intracellular signal transduction pathways, phosphorylation of receptors and regulatory proteins by kinases, or effects on intracellular calcium release (1). Receptor cross-talk represents a means of fine-tuning the control of cellular function and is relevant to understanding disease and response to therapeutic agents that interact with cell-surface receptors. Recently there has been growing recognition that physical interaction between cell surface receptors may be a novel means of receptor cross-talk, and this has been studied in the greatest detail for G protein–coupled receptors (GPCRs).

Approximately 400 GPCRs are known to mediate the effects of endogenous ligands and are the targets for about half of currently used prescription drugs (2–4). The interaction of an agonist with the binding pocket of a GPCR induces a conformational change in the transmembrane-spanning segments. This results in its association with a G protein that leads to activation of a signal transduction pathway, resulting in the characteristic cellular response. GPCRs were conventionally thought to exist and act as monomers, but there is accumulating evidence that most GPCRs probably exist as dimers or even oligomers (5–7). Furthermore, different GPCRs may interact with each other, forming heterodimers. This has important implications for understanding cellular regulation and the action of agonists. Dimerization of GPCRs was first proposed by Agnati and colleagues in the 1980s (8), based on the finding of unexplained cooperativity between certain agonists and a larger-than-expected molecular size of receptor proteins observed by gel electrophoresis. However, this idea received little attention until the last decade.

Receptor homodimerization

Although the idea of receptor dimerization was at first resisted and then thought to be the exception or a feature of artificial overexpression systems, it is now clear that most GPCRs exist as homodimers for at least some period during their existence. This has most convincingly been demonstrated using fluorescence resonance energy transfer or bioluminescence resonance energy transfer (BRET), which directly demonstrate receptor protein dimerization in living cells. Indeed, studies using BRET indicate that more than 80% of β2-adrenergic receptors (β2ARs) exist as homodimers (9). This dimerization appears to occur during the synthesis of receptors in the endoplasmic reticulum and is necessary for the transport of the newly formed receptors to the cell surface (5–7).

Furthermore, interfering with receptor homodimerization affects the trafficking and function of the receptor (7). The sites of physical interaction between GPCRs appear to be the transmembrane α-helices. Using a peptide that corresponds to transmembrane helix VI of the β2AR in order to interfere with the presumed dimerization sequence, there was a significant reduction in the incidence of dimerization, and this was associated with reduced activation of adenyl cyclase by β2AR agonists (10). This suggests that high-affinity binding of receptor and G protein may require the GPCR dimer. Furthermore, mutation of the putative dimerization motif in helix VI prevented the movement of β2ARs from their site of synthesis in the endoplasmic reticulum to the cell surface, indicating that homodimerization is critical for cell-surface expression of β2ARs (11). There is debate about the effect of agonists on the extent of receptor dimerization, but most studies indicate that dimers are formed during receptor synthesis in the endoplasmic reticulum and are formed in the absence of agonist stimulation (7).

Receptor heterodimerization

There is increasing evidence that different GPCRs may form heterodimers and that this can affect the function of each agonist, resulting in significant functional interactions. Indeed, this phenomenon may account for some drug interactions that were unexpected or previously difficult to explain. Many different GPCR heterodimers have now been described (5–7), but the functional consequence of heterodimerization is not predictable. β2ARs may interact with both δ- and κ-opioid receptors. This does not appear to affect the binding or effects of agonists but has an effect on receptor trafficking (12). When β2ARs are coexpressed with δ-opioid receptors, both a β2AR-agonist and a δ-opioid agonist cause downregulation and internalization of the δ-opioid receptor, whereas when coexpressed with κ-opioid receptors, neither agonist causes receptor downregulation.
Airway smooth muscle tone is regulated by multiple GPCRs, and cross-talk between different classes of receptor, such as β2ARs and muscarinic M3 receptors, has previously been demonstrated (17).

These interactions may be of clinical relevance in asthma, where airway smooth muscle tone is increased. In this issue of the JCI, McGraw and colleagues demonstrate a novel type of cross-talk between GPCRs involving receptor heterodimerization and demonstrate its functional consequences on murine airway smooth muscle contraction (18). PGE2 activated the PGE2 receptor, EP2 subtype (EP2R) on airway smooth muscle cells, which is coupled to Gs and calcium ion release and yet did not elicit contraction as expected. However, PGE2 reduced the bronchodilator response to a β2AR agonist by attenuating the increase in cAMP. Forskolin, a direct activator of adenylyl cyclase, was unaffected, indicating an upstream interaction between PGE2 and β2AR agonist. Using fluorescence microscopy in airway smooth muscle cells, and BRET and coimmunoprecipitation in a cell line, heterodimerization between EP2Rs and β2ARs was demonstrated. EP2R agonists bind to the heterodimer and uncouple β2ARs from Gs, thus diminishing the bronchodilator response of the β2AR agonist (Figure 1). This represents a novel level of functional antagonism between bronchodilator and bronchoconstrictor mechanisms and may contribute to the reduced response to β2AR agonists that may occur in severe asthma, when endogenous concentrations of PGE2 may be elevated. Since airway smooth muscle cells express more than 30 different GPCRs, many other possible GPCR heterodimerizations remain to be explored (19).

**Clinical implications**

At present there is relatively little information about the functional consequences of receptor heterodimerization, but the study by McGraw et al. (18) demonstrates that such receptor interactions may have important functional consequences. Receptor heterodimerization may affect the surface expression of receptors, the rate of receptor desensitization, and the effect of agonists on signal transduction, resulting in several different and so-far unpredictable functional consequences. This allows for the possibility of finding unexpected drug interactions or novel therapeutic agents that selectively activate certain heterodimer pairs. Since the relative expression of different GPCRs in various cell types differs, this makes it potentially possible to develop more selective drugs in the future. The role of receptor heterodimerization in disease has hardly been explored, but genetic polymorphisms in areas of the receptor that affect dimerization with other receptors may alter the function of the receptor, as has already been demonstrated for chemokine receptors (6, 20). GPCR heterodimerization appears to be a novel means of cell regulation that is likely to have clinical and therapeutic significance in the future.

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Autoimmune diseases such as the diabetes that develops in NOD mice depend on immunologic recognition of specific autoantigens, but recognition can result in a pathogenic or protective T cell response. A study by Du et al. in this issue of the JCI demonstrates that TGF-β signaling by T cells recognizing the insulin peptide B9–23 is essential for such protection and that this inhibitory cytokine functions in both a paracrine and an autocrine manner (see the related article beginning on page 1360). We propose that the insulin peptide B9–23 and a conserved TCR motif form an “immunologic homunculus” underlying the relatively common targeting of insulin by T cells that, as demonstrated by the study of Du and coworkers, results in a protective T cell response, or diabetes, as shown by other investigators, for related T cell receptors.

There are a limited number of common autoimmune disorders, and some diseases, such as type 1A diabetes (immune-mediated type 1 diabetes), both are common and have increased dramatically in incidence over the past 50 years (1). Irun Cohen has advanced the hypothesis that the normal immune repertoire includes a high frequency of lymphocytes that recognize key self-antigens. The relative distribution of lymphocytes specific for various antigens, termed the “immunologic homunculus,” therefore defines the spectrum of potential autoimmune immune responses (2). The common lack of clinical immunopathology in the presence of a relevant autoreactive lymphocyte repertoire is attributed to active “immune regulation.” Conversely, breakdown of such regulatory mechanisms, presciently postulated almost 100 years ago (3), will lead to autoimmune disease. As such tolerance mechanisms are, in part, based on the activity of autoreactive lymphocytes, the increased prevalence of certain autoimmune disorders may be the direct consequence of a biased immunologic repertoire (preferential recognition of selected autoantigens), and its particular susceptibility to functional modulation by environmental and genetic factors. A fundamental question about the pathogenesis of type 1A diabetes is: What is the range of T cell specificities for islet antigens that modulate induction of pathogenic (destructive) and/or regulatory (protective) T cell activities; and in particular, can both destructive and regulatory T cells recognize the same antigenic determinant?

T cell specificity, pathogenesis, and prevention of diabetes

Research over the past few years has identified insulin as a central autoantigen for both the generation of destructive T cell responses and the therapeutic induction of protective T cell immunity. Until now, the field has lacked T cell receptor transgenic mice targeting insulin, an important tool in exploring autoimmune pathogenesis. In the current issue of the JCI, Du and coworkers describe the generation of a novel TCR transgenic (Tg) mouse in which the transgenic TCR reacts with a well-defined epitope within the insulin B chain (aa 9–23 or aa 12–25) (4). Although this epitope is recognized by a majority of pathogenic CD4+ T cells (5) and may, according to our recent work, constitute the primary autoantigen in the NOD model for type 1A diabetes (6), the TCR Tg mouse characterizes...