0026-895X/03/6305-959–960$7.00
MOLECULAR PHARMACOLOGY
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*Mol Pharmacol* 63:959–960, 2003

Vol. 63, No. 5
2460/1062747
*Printed in U.S.A.*



*PERSPECTIVE*

# An Array of Details on G-Protein Coupled Receptor Signaling: Differential Effects of $\alpha_1$-Adrenergic Receptor Subtypes on Gene Expression and Cytokine Receptor Signaling

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Received February 12, 2003; accepted February 14, 2003

This article is available online at http://molpharm.aspetjournals.org







Knowledge of the factors responsible for both the similarities and differences in the actions mediated by the individual members of a multi-gene family are important both to understand the molecular and cellular bases for their physiological responses and for the development of specific pharmacological interventions. The application of molecular cloning in the 1980s led to the realization that there were many more subtypes of G-protein-coupled receptors than were apparent from pharmacological analyses. The expression of individual cloned receptors in transfected cell systems has allowed the analysis of details of the functional coupling, regulation, and pharmacological properties of the various subtypes of a given family of receptors, such as the adrenergic or muscarinic receptors. Gene targeting technology allows the role of individual receptors to be studied in vivo. Although each of these approaches has caveats (e.g., effects of overexpression or oddities of individual cell lines in transfection experiments or developmental or compensatory effects in knockout animals), suitable controls and multiple types of analyses allow these types of experiments to yield valuable information.

The $\alpha_1$-adrenergic receptors represent a typical example of this paradigm. Although pharmacological analyses provided good evidence for the existence of two subtypes of $\alpha_1$ receptor, genes encoding three subtypes of $\alpha_1$ receptor were subsequently identified and are now called $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ (Zhong and Minneman, 1999). Although all three subtypes couple to the $G_q$ family of G-proteins to mediate activation of phospholipase C, they differ in their apparent efficacies for eliciting this response. For example, when expressed in PC-12 cells, $\alpha_{1A}$ and $\alpha_{1B}$ receptors induced similar levels of inositol phosphate formation that were much greater than that produced by the $\alpha_{1D}$ receptor. The three receptors also differed in their abilities to activate extracellular signal-regulated kinase, c-Jun $NH_2$-terminal kinase, and p38 mitogen-activated protein kinase pathways and to induce transcription of reporter genes driven by a variety of *cis*-acting regulatory elements (Zhong et al., 2001). Gene targeting studies have begun to identify specific roles for the individual receptor subtypes in vivo (Cavalli et al., 1997; Tanoue et al., 2002).

Analyses of reporter gene expression or induction of specific individual gene products can provide valuable information on the continuing changes than can occur after activation of a receptor. However, the amount of information such analyses provide about the potentially complex regulation at both the transcriptional and post-transcriptional levels that can occur after activation of a receptor that couples to multiple signaling pathways is limited. A significant advance in this type of analysis of G-protein coupled receptor action was provided by Wurmbach et al. (2001), who used a cDNA array of 956 selected genes to identify a network of genes regulated by the gonadotropin-releasing hormone receptor endogenously expressed in a gonadotroph cell line. The article by Gonzalez-Cabrera et al. (2003) in this issue provides a new dimension to the comparative analyses of functional responses of the $\alpha_1$-adrenergic receptor family. These authors used oligonucleotide arrays to examine the regulation of 7000 genes in Rat-1 fibroblasts stably expressing the $\alpha_{1A}$, $\alpha_{1B}$, and $\alpha_{1D}$ receptors. Twenty-nine genes were induced by all three subtypes and included cytokines and growth factors, transcription factors, signaling enzymes, and extracellular matrix proteins. Nine genes were inhibited by all three receptors. Although the three receptors activated phospholipase C to very different extents, these genes exhibited similar magnitudes of induction and repression. Most interestingly, there were a significant number of genes whose expression was modified by only a single receptor subtype: 17 by $\alpha_{1B}$, 12 by $\alpha_{1D}$, and 6 by $\alpha_{1A}$. There were also genes regulated by only two of the three receptor subtypes. These results imply, not surprisingly, that multiple signaling cascades differentially

**ABBREVIATIONS:** IL, interleukin; JAK, Janus tyrosine kinase; STAT, signal transducer and activator of transcription.

activated by the three receptors are responsible for these distinct effects on gene expression.

One of the most interesting aspects of the work of Gonzalez-Cabrera et al. (2003) is the effect on expression of proteins involved in signaling by IL-6. IL-6 is member of a family of cytokines that includes leukemia inhibitory factor and cardiotropin-1, which use gp130 as a component of their receptors and can activate multiple signal transduction cascades, including the JAK/STAT and the Ras/mitogen-activated protein kinase pathways (Wollert and Chien, 1997) Activation of the gp130 receptor system can mediate cardiac cell survival and induce cardiac hypertrophy (Wollert and Chien, 1997), and alterations in gp130/JAK/STAT have been found in patients with end-stage dilated cardiomyopathy (Podewski et al., 2003). Increased circulating levels of IL-6 family cytokines are found in patients with heart failure (Kanda et al., 2000), and genetic disruption of gp130 causes hypoplastic development of the ventricular myocardium (Yoshida et al., 1996). Gonzalez-Cabrera et al. (2003) found that all three $\alpha_1$ receptors increased IL-6 mRNA expression and stimulated IL-6 secretion into the medium. However, the $\alpha_{1A}$ and $\alpha_{1D}$ receptors increased expression of mRNAs for STAT3, gp130, and p21-Ras, and also stimulated both serine- and tyrosine-phosphorylation of STAT3, while $\alpha_{1B}$ did not change the mRNAs levels of the three signaling proteins and only stimulated the tyrosine but not serine phosphorylation of STAT3. There were additional differences in the effects of the three $\alpha_1$-adrenergic receptors on the expression of the gp130 *protein*. Stimulation of the $\alpha_{1A}$ and $\alpha_{1D}$ receptors decreased gp130 protein levels; despite the lack of change in gp130 mRNA in $\alpha_{1B}$ expressing cells, the basal level of gp130 polypeptide was greatly reduced in $\alpha_{1B}$ expressing cells, but was not further regulated by norepinephrine. Several lines of evidence, including the use of IL-6 neutralizing antibodies, led to the surprising conclusion that the effects on gp130 protein levels and STAT3 phosphorylation were not caused by activation of the cytokine receptor by secreted IL-6 and thus were an apparent direct effect of $\alpha_1$-adrenergic receptor stimulation. These results thus demonstrate that there are very dramatic differences in the regulation of cytokine receptor signaling by the various subtypes of $\alpha_1$-adrenergic receptor.

As the authors themselves point out, there are limitations in their study. The authors used non–receptor-expressing cells as the control for their gene array analyses, rather than unstimulated cells expressing the individual receptor subtypes. (However, their analyses of protein levels of the IL-6 signaling system compared stimulated and unstimulated cells expressing the three subtypes.) In addition, it is impossible to exclude potential artifactual results due to ectopic coupling because of high receptor expression levels or differences in signaling that can occur between different clonal isolates of the same cell line (Lefkowitz et al., 2002). Interestingly, the authors have shown that increased cardiac expression of the $\alpha_{1B}$ receptor in transgenic mice also results in decreased gp130 expression (Yun et al., 2003), providing an important partial in vivo confirmation of the results described by Gonzalez-Cabrera (2003).

This study by Gonzalez-Cabrera et al. (2003) provides a dramatic demonstration that the three subtypes of $\alpha_1$-adrenergic receptors, despite their similarities in functional coupling to G-proteins, differ dramatically in the details of their regulation of gene expression. The application of gene array analyses will provide new insights into the similarities and differences in action of G-protein coupled receptor family members.

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