Hormonal regulation of $G_{i\alpha}$ level and adenylyl cyclase responsiveness

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Prolonged exposure of cells to adenylyl cyclase stimulatory hormonal factors can cause an increase in the level of membrane inhibitory G protein (G_i) α -subunits, while inhibitory receptor agonists have been reported to induce the opposite response. As studied in cultured rat cardiomyocytes, the β -adrenoceptor-induced increase in the level of $G_{i\alpha}$ proteins is protein synthesis-dependent, is apparently not accompanied by an increase in G protein β -subunits and results in a decreased adenylyl cyclase responsiveness. On the other hand, a decrease in $G_{i\alpha}$ level apparently results in sensitization of adenylyl cyclase stimulation. These data suggest that the up- or down-regulation of the level and activity of G_i protein α -subunits is a rather general cellular response, providing an intracellular negative feedback control against prolonged receptor activation.

Keywords adenylyl cyclase guanine nucleotide-binding protein noradrenaline heart cells

Introduction

Hormonal regulation of adenylyl cyclase activity involves two distinct guanine nucleotide-binding regulatory proteins, G_s and G_i , mediating receptor-dependent stimulation and inhibition of the enzyme, respectively. As other signal-transducing G proteins, G_s and G_i are heterotrimeric proteins, composed of distinct α -subunits and very similar or even identical β - and γ -subunits. Prolonged treatment of cells with agonists has been shown in many cell types to cause a decrease in the number of the agonist-specific membrane receptors. This down-regulation, which is well documented for various stimulatory and inhibitory receptors, induces a loss of the specific hormone response. In addition to the decrease in the specific hormone response, prolonged stimulation or inhibition of adenylyl cyclase by agonists has been shown to induce also alterations in receptor-independent adenylyl cyclase regulation in several cell types. Evidence has been accumulated that changes in the level or activity

of other adenylyl cyclase system components, i.e. the G_i proteins, are involved in this heterologous desensitization of adenylyl cyclase regulation (Jones & Bylund, 1988; Parsons & Stiles, 1987; Reithmann *et al.*, 1989; Rich *et al.*, 1984). This short communication summarizes our recent findings on hormonal regulation of $G_{i\alpha}$ proteins in rat cardiomyocytes.

Methods

The methods used have been described in detail (Reithmann *et al.*, 1989) including cultivation of heart muscle cells from neonatal rats in defined serum-free media, preparation of crude and purified membranes of these cells, adenylyl cyclase assay, pertussis toxin-catalyzed [³²P]ADP-ribosylation and immunoblotting of G protein subunits.

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Results

Prolonged treatment of rat heart muscle cells with noradrenaline (1 μ M, 48 h) caused about a two-fold increase in the level of membrane G_i protein α -subunits, as analyzed by pertussis toxin-catalyzed [32 P]ADP-ribosylation and immunoblotting with specific anti- $G_{i\alpha}$ antisera. Both $G_{i\alpha}$ proteins present in rat heart muscle cell membranes, $G_{i\alpha40}$ and $G_{i\alpha41}$, were up-regulated by the noradrenaline treatment. In contrast, the level of G protein β -subunits (β_{36}) was not altered by the noradrenaline exposure. The increase in $G_{i\alpha}$ proteins induced by noradrenaline was accompanied by a decrease in receptor-independent (forskolin) adenylyl cyclase stimulation by about 30%. Inactivation of membrane G_i proteins by pertussis toxin treatment of the cells prevented this hyporesponsiveness of the enzyme (Reithmann *et al.*, 1989). Since rat heart muscle cells contain in addition to β_1 -adrenoceptors equal or even higher amounts of α_1 -adrenoceptors, it was of interest to study by activation of which type of adrenoceptors the non-selective agonist noradrenaline regulates the level of $G_{i\alpha}$ proteins and, in addition, whether the up-regulation of $G_{i\alpha}$ proteins and the concomitant down-regulation of adenylyl cyclase responsiveness are mediated by the same receptor type. Blockade of myocyte β_1 -adrenoceptors (by timolol) but not of α_1 -adrenoceptors (by prazosin) prevented the noradrenaline-induced increase in $G_{i\alpha}$ proteins. Additionally, the β -adrenoceptor-selective agonist, isoprenaline, mimicked the effect of noradrenaline. Most important, myocyte β_1 - but not α_1 -adrenoceptor blockade prevented the noradrenaline-induced decrease in receptor-independent adenylyl cyclase stimulation (Reithmann *et al.*, 1990). These data suggested that prolonged occupancy of cardiomyocyte β_1 -adrenoceptors induced not only a down-regulation of β -adrenoceptor number and response but also an increase in $G_{i\alpha}$ proteins, leading to a decrease in β -adrenoceptor-independent adenylyl cyclase stimulation. The concomitant activation of myocyte α_1 -adrenoceptors by noradrenaline is apparently without functional consequence for the parameters studied.

The specific protein synthesis inhibitor, *Pseudomonas* exotoxin A, was then used to study whether the up-regulation of $G_{i\alpha}$ proteins induced by prolonged β -adrenoceptor activation is due to increased synthesis of these proteins. When the cardiomyocytes were treated with this toxin at a concentration causing about a 30% reduction in total protein synthesis, as analyzed by [3 H]-leucine incorporation, the noradrenaline-

induced increase in $G_{i\alpha}$ proteins was completely suppressed. Most important, the decrease in adenylyl cyclase stimulation by forskolin caused by noradrenaline exposure was also abolished by *Pseudomonas* exotoxin A treatment. Furthermore, although the down-regulation of β_1 -adrenoceptors was not affected, treatment with the toxin partially (about 30%) attenuated the noradrenaline-induced decrease in β -adrenoceptor-induced adenylyl cyclase stimulation (Reithmann *et al.*, 1990).

Discussion

Prolonged treatment of rat heart muscle cells with noradrenaline not only causes a down-regulation of β -adrenoceptor number and response but also increases the level of membrane G_i protein α -subunits without alteration in the level of G protein β -subunits. In addition, β -adrenoceptor-independent adenylyl cyclase stimulation is decreased by this treatment. All of these effects of the non-selective agonist noradrenaline are due to the activation of β_1 -adrenoceptors but not of α_1 -adrenoceptors, both being present in rat cardiomyocytes. The decrease in β -adrenoceptor-independent adenylyl cyclase stimulation is apparently a $G_{i\alpha}$ protein-mediated event. First, treatment of the cells with pertussis toxin, which functionally inactivates G_i proteins by ADP-ribosylating α_i -subunits, abolished the desensitization to the receptor-independent adenylyl cyclase stimulation. Second, protein synthesis inhibition by *Pseudomonas* exotoxin A not only prevented the noradrenaline-induced $G_{i\alpha}$ up-regulation but also abolished the decrease in receptor-independent adenylyl cyclase stimulation. Interestingly, the noradrenaline-induced decrease in β -adrenoceptor adenylyl cyclase stimulation was partially attenuated by the toxin, although having no effect on the β -adrenoceptor down-regulation. The data thus indicate that treatment of cardiomyocytes with β -adrenoceptor agonists primarily leads to stimulation of adenylyl cyclase with subsequent increase in intracellular cyclic AMP levels. As a consequence of prolonged agonist occupancy, the number of membrane β -adrenoceptors is down-regulated and the level of membrane $G_{i\alpha}$ proteins is up-regulated. The latter event apparently depends on *de novo* synthesis of the proteins. The increase in membrane $G_{i\alpha}$ proteins, occurring without concomitant increase in G protein β -subunits, then leads to a decreased adenylyl cyclase responsiveness. Similar data, i.e. apparent increase in $G_{i\alpha}$ proteins and decreased adenylyl cyclase responsive-

Figure 1 Hormonal regulation of the level of adenylyl cyclase system components. A hypothetical model. R_s , R_i , stimulatory and inhibitory receptor, respectively; G_s , G_i , stimulatory and inhibitory G protein; C, catalytic subunit of adenylyl cyclase. For further explanations see text.

ness, have been reported for long-term glucagon-treated MDCK cells (Rich *et al.*, 1984). On the other hand, prolonged exposure of intact cells to adenylyl cyclase inhibitory agonists (A_1 adenosine receptor and α_2 -adrenoceptor agonists) can apparently cause a decrease in the level of $G_{i\alpha}$ proteins, as analyzed by pertussis toxin-catalyzed ADP-ribosylation (Jones & Bylund, 1988; Parsons & Stiles, 1987). Con-

comitantly, a sensitization of adenylyl cyclase stimulation was observed. Therefore, we would like to propose (Figure 1) that the up- and down-regulation of the level of membrane $G_{i\alpha}$ proteins is a rather general cellular response to long-term adenylyl cyclase stimulation and inhibition, respectively, serving as an intracellular negative feedback control against prolonged receptor activation.

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