Alterations in Detergent Solubility of Heterotrimeric G Proteins after Chronic Activation of Gi/o-Coupled Receptors: Changes in Detergent Solubility Are in Correlation with Onset of Adenylyl Cyclase Superactivation

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ABSTRACT

Prolonged Gi/o protein-coupled receptor activation has been shown to lead to receptor internalization and receptor desensitization. In addition, it is well established that although acute activation of these receptors leads to inhibition of adenylyl cyclase (AC), long-term activation results in increased AC activity (especially evident on removal of the inhibitory agonist), a phenomenon defined as AC superactivation or sensitization. Herein, we show that chronic exposure to agonists of Gi-coupled receptors also leads to a decrease in cholate detergent solubility of G protein subunits, and that antagonist treatment after such chronic agonist exposure leads to a time-dependent reversal of the cholate insolubility. With Chinese hamster ovary and COS cells transfected with several Gi/o-coupled receptors (i.e., μ- and κ-opioid, and mA2-muscarinic), we observed that although no overall change occurred in total content of Ga, and β1-subunits, chronic agonist treatment led to a marked reduction in the ability of 1% cholate to solubilize Ga as well as Gbg. This solubility shift is exclusively observed with Ga, and was not seen with Gbg. The disappearance and reappearance of Ga, and Gbg subunits from and to the detergent-soluble fractions occur with similar time courses as observed for the onset and disappearance of AC superactivation. Lastly, pertussis toxin, which blocks acute and chronic agonist-induced AC inhibition and superactivation, also blocks the shift in detergent solubility. These results suggest a correlation between the solubility shift of the heterotrimeric Gi protein and the generation of AC superactivation.

The heterotrimeric G proteins serve as central signaling molecules responsible for connecting cellular signals transduced from seven transmembrane domain receptors to their respective effectors. Early work focused on the Ga subunit in terms of its modulatory activity, but more recently, Gbg dimers have been shown to have important signaling properties of their own and to regulate the activity of some well characterized effectors, including several adenylyl cyclase (AC) isoforms, Ca2+ and K+ channels, phospholipase C-β2, and the extracellular signal receptor-activated/mitogen-activated protein kinase pathways (Federman et al., 1992; Wu et al., 1993; Creapo et al., 1994; Herlitze et al., 1996; Clapham and Neer, 1997).

Chronic G protein-coupled receptor activation has been shown to lead (with most receptors) to a reduction in the ability of the receptor to respond to its agonist. This process is due to receptor desensitization (mediated by receptor phosphorylation) and by agonist-induced receptor internalization (Krupnick and Benovic, 1998; Pitcher et al., 1998). However, it seems that with many (or all) Gi/o-coupled receptors, chronic agonist exposure has additional effects that are manifested at both G protein and effector levels. For example, acute activation of Gi/o-coupled receptors by the appropriate agonists has been shown to inhibit AC activity in a dose-dependent manner. Conversely, long-term activation of these

ABBREVIATIONS: AC, adenylyl cyclase; PTX, pertussis toxin; CHO, Chinese hamster ovary; IBMX, 1-methyl-3-isobutylxanthine; FS, forskolin; DMEM, Dulbecco’s modified Eagle’s medium; PAGE, polyacrylamide gel electrophoresis.
inhibitory receptors was found to lead to an increase in AC activity in a time- and dose-dependent manner. This phenomenon has been termed AC superactivation, or sensitization, and is especially prominent on removal of the inhibitory agonist (Sharma et al., 1975; Avidor-Reiss et al., 1995a, 1996; Thomas and Hoffman, 1996; Palmer et al., 1997; Nevo et al., 1998). Loss of the superactivated state is also a time-dependent process, and efficient wash or incubation with antagonist leads to a gradual decrease in AC superactivation until the normal level of AC activity is reached. AC superactivation has been shown to be dependent on sustained activation of heterotrimeric Gi/o proteins and is blocked by pertussis toxin (PTX) treatment (Avidor-Reiss et al., 1995a, 1996; Palmer et al., 1997). In addition, molecules that sequester Gi/o-dimers were found to block the superactivation of AC isoforms V and VI, indicating a role for Gi/o in the mediation of AC superactivation (Avidor-Reiss et al., 1996; Thomas and Hoffman, 1996).

Various groups have investigated whether chronic activation of Gi/o-coupled receptors leads to a change in the concentration of various Gi/o subunits in the exposed cells. For example, a reduction in Gi3 was found after chronic exposure of mixed cultures of dorsal root ganglion-spinal cord neurons to κ-opioid agonists (Attali and Vogel, 1989). A decrease in Gαq2, Gαq3, and Gβ subunits was reported after chronic A3-adenosine agonist treatment, although it was claimed that this reduction in Gi proteins was not responsible for the sensitization of AC (Palmer et al., 1997). In addition, a reduction in Gi1 and an increase in Go were reported on chronic morphine exposure in primary cultures of rat striatal neurons (van Vliet et al., 1991). In contrast, several other laboratories did not observe any changes in Gi or Gβ concentrations in cells treated chronically with opioids or with other Gi/o-coupled receptor agonists (Chen and Rasenick, 1995; Ammer and Schulz, 1997).

It was recently shown that agonist stimulation (e.g., bradykinin bound to B2BK receptors) promotes sequestration of Goq and Gai into the detergent-insoluble caveolin-rich fractions (de Weerd and Leeb-Lundberg, 1997). It was therefore of interest to investigate whether the changes in AC activity after chronic agonist exposure and after removal of the chronic agonist could be correlated with changes in detergent solubility.

In this article, we demonstrate with COS and Chinese hamster ovary (CHO) cells transfected with either μ-opioid, κ-opioid, or m1-muscarinic receptors that chronic receptor activation leads to a decrease in the cholate detergent solubility of Giα subunits and Gβ1 (probably present as Gβγ dimers), whereas it did not change the solubility of Go or the total content of Giα and Gβ1 in the cells. This detergent solubility shift occurs in a time-dependent manner that correlates with the onset of AC superactivation. In addition, the phenomenon is reversible and blocked by PTX. This data shows that chronic receptor activation leads to changes at the G protein level and allows us to present a model for the role of Gi/o heterotrimeric in AC superactivation.

**Experimental Procedures**

**Materials.** [3H-2]adenine (18.0 Ci/mmole) was purchased from American Radiolabeled Chemicals (St. Louis, MO). Morphine was obtained from the National Institute on Drug Abuse, Research Technology Branch (Rockville, MD). The phosphodiesterase inhibitors 1-methyl-3-isobutylxanthine (IBMX) and RO-20–1724 were from Calbiochem (La Jolla, CA). Forskolin (FS), BSA, cAMP, sodium cholate, and carbachol were purchased from Sigma Chemical Co. (St. Louis, MO). Tissue culture reagents were from Gibco-BRL (Bethesda, MD).

**Cell Cultures.** COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 5% fetal calf serum, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere consisting of 5% CO2 and 95% air, at 37°C. CHO cells expressing κ- (CHO-k) or μ- (CHO-μ) receptors have been described previously (Avidor-Reiss et al., 1995a,b), and were cultured in DMEM supplemented with 5% fetal calf serum, nonessential amino acids, 2 mM t-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere consisting of 5% CO2 and 95% air, at 37°C.

**Transfection of COS Cells.** COS-7 cells in 10-cm culture plates were transfected by the DEAE-dextran chloroquine method (Avidor-Reiss et al., 1996) with 2 μg/plate of either rat μ-opioid receptor cDNA in pCMV-neo (obtained from Dr. H. Akil, University of Michigan, Ann Arbor, MI), human m1-muscarinic receptor cDNA in pcD (provided by Dr. T. Bonner, National Institutes of Health, Bethesda, MD), or β-galactosidase cDNA in pcDNAIII. Transfection efficiency, determined by transfection with the cDNA for β-galactosidase and cell staining (Avidor-Reiss et al., 1997) was in the range of 60 to 80%.

**AC Assay.** The assay was performed as described previously (Avidor-Reiss et al., 1995a; Bayewitch et al., 1998a). In brief, CHO-μ cells cultured in 24-well plates were incubated for 2 h with 0.25 ml/well of fresh growth medium containing 5 μCi/ml of [3H]adenine. This medium was replaced with DMEM containing 20 mM HEPES (pH 7.4), 1 mg/ml BSA, 0.5 mM IBMX, and 0.5 mM RO-20–1724. FS at 1 μM final concentration was then added and the cells incubated at 37°C for 10 min. The reactions were terminated by adding to the cell layer 1 ml of 2.5% perchloric acid containing 0.1 mM unlabeled cAMP. Aliquots of 0.9 ml were then neutralized with 100 μl of 3.8 M KOH and 0.16 M K2CO3 and applied to a two-step column separation procedure. The [3H]cAMP was eluted into scintillation vials and counted.

**Preparation of Crude Membrane Fraction and Cholate Detergent Extraction.** CHO-μ, CHO-k, as well as Gi/o-muscarinic transfect COS-7 cells, were grown to 70 to 80% confluence on 10-cm plates and exposed to the appropriate agonists as indicated. Cells were then scraped in 1 ml/pate of lysis buffer (10 mM Tris, pH 7.4, 150 mM NaCl, 50 mM KCl, and 1 mM EDTA) containing the protease inhibitors aprotinin (2 μg/ml), pepstatin (2 μg/ml), phenylmethylsulfonyl fluoride (100 μM), and benzamidine (100 μM), and lysed by transferring the suspension 10 times through a 21-gauge needle. Nuclei were cleared from the lysates by centrifugation in Eppendorf tubes at 5,000 rpm (2,000g) for 5 min at 4°C. Supernatants (1 ml, corresponding to one culture plate) were then transferred to fresh tubes and centrifuged at 14,000 rpm (16,000g) for 45 min at 4°C. After centrifugation we solubility shift occurs under these centrifugation conditions, > 98% of the G protein βγ-subunits were recovered in the pellet fraction compared with airfuge centrifugation (40 min at 100,000g). The resulting pellets containing the crude membrane fraction (~125 μg of protein) were then resuspended in 20 μl of 50 mM Tris, pH 8.0, 10 mM EDTA, and 1% sodium cholate, and the mixture was allowed to stand on ice for 30 min. All samples were then centrifuged at 14,000 rpm (16,000g) for 10 min at 4°C. The supernatants containing the cholate-soluble membrane proteins and the pellets containing the cholate-insoluble proteins were separately mixed with final concentration of 1X Laemmli sample buffer containing 0.1 M dithiothreitol and boiled for 5 min, and equivalent fractions (each originating from one quarter of a culture dish containing ~22 μg of cholate-soluble and 8 μg of cholate-insoluble protein) were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE). In a few control experiments, we have used airfuge centrifugation (40 min at 100,000g) to pellet the cholate-insoluble fraction. We found that this change in proce-
dure did not appreciably affect the ratio of cholate-soluble to cholate-insoluble G protein subunits. More than 90% of the Gi1, pelleted at 100,000g after cholate treatment could be sedimented by a 10-min spin at 16,000g. The 16,000g centrifugation had the advantage of easier handling of the pellets, which could be treated with Laemmlı sample buffer in the same tube used for the centrifugation.

**SDS-PAGE and Western Blotting.** Proteins were separated on 10% polyacrylamide gel and transferred to nitrocellulose. The nitrocellulose was blocked in PBS containing 5% w/v fat-free powdered milk and 0.5% Tween 20 for 1 h followed by 1.5-h incubation with the appropriate antibodies at room temperature in blocking buffer. The following antibody preparations, all at dilutions of 1:1000, were used: RA-polyclonal against Ga (Bayewitch et al., 1998a), AS-polyclonal against Gb1 (Goldsmith et al., 1987), and RM-polyclonal against Gs (Simonds et al., 1989). The blots were then washed three times with 1× PBS containing 0.3% Tween 20 for 15 min each. Secondary antibody was horseradish peroxidase-coupled goat anti-rabbit (Jackson ImmunoResearch, West Grove, PA), diluted 1:20,000 in blocking buffer. The secondary antibody was incubated with the blot for 1 h and the blot extensively washed (>2 h) with PBS containing 0.3% Tween 20. The peroxidase activity on the blots was visualized by the enhanced chemiluminescence technique (Amerham, Arlington Heights, IL).

**Results**

**Chronic Opioid Treatment Leads to Reduction in Cholate Solubility of Heterotrimeric Gi Subunits and Does Not Affect Solubility of Gs**. In search of long-term effects of agonist treatments on the heterotrimeric G proteins, we investigated the membrane content of Gi proteins after chronic opioid agonist exposure. It was shown previously that heterotrimeric G proteins can be readily extracted from crude cell membrane preparations with sodium cholate (Northup et al., 1980). Figure 1 shows that chronic morphine treatment (18 h with 1 μM morphine) of CHO-μ cells leads to a marked decrease (~70%) in the amount of Gi1 in the cholate-soluble membrane fraction. Conversely, an increase in Gi2 levels was observed in the particulate fraction that was resistant to solubilization by cholate. Moreover, with antibodies that selectively bind to Gi1-subunits, we found that the same pattern of solubility shift occurs for the Gi1-subunit on chronic exposure to morphine. Moreover, with antibodies that selectively bind to Gi1-subunits, we found that the same pattern of solubility shift occurs for the Gi1-subunit on chronic exposure to morphine, indicating a translocation of Gi1 and Gβ1 (and probably of the heterotrimeric Gi protein) from the detergent-soluble to the insoluble fraction on chronic morphine exposure. As a control, we investigated the pattern of solubility of Gs, both the long and short isoforms of Gs are present in CHO cells, although the long form predominates (Fig. 1; Newman-Tancredi et al., 1999). We did not observe any changes in the solubility of either form when comparing nontreated and morphine-treated cells. This difference between Gs and Gi1 is in agreement with the lack of coupling between opioid receptors and Gi1 subunits.

To show that this finding is not limited to CHO cells and the μ-opioid receptor, we have investigated transiently transfected COS-7 cells expressing the μ-opioid or m4-muscarinic receptors, as well as stably transfected CHO cells expressing the k-opioid receptor (Fig. 2). Initial observations with μ-transfected COS cells indicated that the total amount of membrane-associated G proteins (with respect to both Gi1 and Gi2 content in crude membrane fractions directly solubilized in 1× Laemmlı sample buffer) was not altered by the chronic exposure to morphine (Fig. 2a). Conversely, cholate-soluble extracts from crude membrane preparations con-

**Fig. 2.** Reduction in cholate solubility of Gi subunits after chronic activation of Gi1-coupled receptors. A, Western blot analysis of Gi1 and Gab in crude membrane fractions (originating from one quarter of a 10-cm culture dish) from μ-transfected COS cells with and without chronic morphine (1 μM; 18 h) treatment. B, transfected COS cells or CHO cells were treated where indicated for 18 h with the appropriate receptor agonist. The figure shows the results of separate experiments showing Western blot analysis of Gi1 in aliquots (see Experimental Procedures) of 1% cholate-solubilized membrane fractions obtained from control COS cells (transfected with β-galactosidase) and from COS cells transfected with either μ-opioid or m4-muscarinic receptor, as well as from CHO cell lines expressing either μ- or k-opioid receptors. Agonist concentrations were 1 μM morphine (for μ-opioid receptor and β-gal transfected cells), 0.1 μM carbachol for m4-muscarinic, and 1 μM U69593 for k-opioid receptors. C, CHO-μ cells were pretreated with 100 ng/ml PTX for 18 h. During the last 8 h, the cells also were exposed to 1 μM morphine. The cholate-soluble membrane extracts were analyzed for Gb1. The results shown with COS cells represent one of four repetitions, and the results with CHO-μ and -k are representatives of two repetitions with similar results.

**Fig. 1.** Chronic activation of μ-opioid receptor leads to a loss of Gi and Gab from the cholate-soluble crude membrane fraction and to their increase in the particulate fraction. CHO-μ cells were either treated or not treated with morphine (1 μM; 18 h). Both cholate-soluble extracts and nonsoluble (particulate) fractions (see Experimental Procedures) were analyzed for Gi1, Gb1, and Gs with the appropriate antibodies. A representative gel is shown of three experiments that yielded similar results.
tained less G\(_{\alpha 1}\) after chronic agonist treatment compared with control, untreated cells (Fig. 2b). This was shown herein for COS-7 and CHO cells expressing the \(\mu\)-opioid receptor after 18 h morphine treatment, as well as for CHO cells expressing the \(\kappa\)-opioid receptor chronically treated with the \(\kappa\)-agonist U-69593, and for COS cells transfected with the m\(_2\)-muscarnic receptor after treatment with the muscarinic agonist carbachol (1 \(\mu M\); 18 h). On average, the decrease in detergent solubility of G\(_{\beta 1}\) was between 50 and 80% (based on density quantitation of the developed Western blots). In addition, Fig. 2b shows that COS-7 cells that were control transfected with \(\beta\)-galactosidase cDNA lacked the sensitivity to chronic morphine treatment, and the G\(_{\beta 1}\) subunit’s cholate solubility did not differ in morphine-treated compared with control cells. These results demonstrate that the solubility shift is dependent on receptor activation and appears to be a general phenomenon associated with chronic activation of G\(_{\alpha i}\)-coupled receptor signaling.

**PTX Pretreatment Blocks G Protein Solubility Shift.** To further correlate G protein activation and the G protein solubility shift, we studied whether treatments that block G protein signaling could affect the change in G protein cholate solubility. Herein, we show (Fig. 2c) that PTX pretreatment, which ADP-ribosylates the G\(_{\alpha i}\) subunit and thus interferes with the agonist-induced activation of G\(_{\alpha i}\), and release of G\(_{\beta\gamma}\), also blocks the decrease in G\(_{\beta 1}\) cholate solubility observed in CHO-\(\mu\) cells treated chronically with morphine. This demonstrates that G protein signaling is required for the G protein detergent solubility shift.

**Reduction of G\(_{\alpha i}\) and G\(_{\beta}\) Subunit Cholate Solubility by Chronic Morphine Is Time-Dependent and Correlates with AC Superactivation.** We and others have previously shown that chronic agonist activation of G\(_{\alpha i}\)-coupled receptors can lead to AC superactivation (Sharma et al., 1975; Avidor-Reiss et al., 1995a,b, 1997; Thomas and Hoffman, 1996; Ammer and Schulz, 1997; Palmer et al., 1997). The kinetics of the onset of AC superactivation for both transiently transfected COS-7 cells expressing \(\mu\)-opioid or D\(_2\)-dopaminergic receptors and CHO cells that stably express the \(\mu\)-opioid or \(A_2\)-adenosine receptor were previously explored (Avidor-Reiss et al., 1995a, 1996; Palmer et al., 1997; Nevo et al., 1998). For example, with CHO-\(\mu\) cells, we reported that AC superactivation reached half-maximal effect after ~2 h of exposure to 0.32 \(\mu M\) morphine, with maximal activity observed 4 to 6 h after the start of chronic treatment (Avidor-Reiss et al., 1995a). In addition, we have shown that the shift of AC to the superactivated state is dependent on sustained activation of opioid receptors (Avidor-Reiss et al., 1995a, 1996). To determine whether the shift observed herein in G protein solubility could be related to the phenomenon of AC superactivation, we have investigated if the kinetics of the solubility shift observed on chronic morphine treatment parallels the kinetics of the induction of AC superactivation. Indeed, as shown in Fig. 3, CHO-\(\mu\) cells that were treated with 1 \(\mu M\) morphine for increasing periods of time showed a time-dependent decrease in the cholate solubility of both G\(_{\alpha i}\) and G\(_{\beta 1}\) subunits. Quantitative densitometric analysis of Western blots for G\(_{\alpha i}\) and G\(_{\beta 1}\) shows that maximal decrease of these subunits in the cholate-soluble fraction was observed to occur at ~4 h of morphine treatment. Half-maximal decrease in the intensity of the bands was observed at 1.5 h for both G\(_{\alpha i}\) and G\(_{\beta 1}\). These kinetics follow very closely those previously observed for the development of AC superactivation in cells on exposure to 0.32 \(\mu M\) morphine (Fig. 3b; Avidor-Reiss et al., 1995a).

We have previously shown that the superactivated state of AC is gradually lost after the withdrawal of the chronically applied agonist (Avidor-Reiss et al., 1995a, 1996). We have therefore studied whether the chronic agonist-induced decrease in cholate solubility of G\(_{\alpha i}\) and G\(_{\beta 1}\) could be reversed after agonist withdrawal. CHO-\(\mu\) cells that were chronically treated (for 18 h) with 1 \(\mu M\) morphine were extensively washed (to remove the morphine) and were allowed to incubate for increasing periods of time in the absence of morphine. Subsequently, the cholate-soluble amounts of G\(_{\alpha i}\) and G\(_{\beta 1}\) were determined. The results show (Fig. 4) that after agonist withdrawal, the G\(_{\alpha i}\) along with the G\(_{\beta 1}\) subunits returned to the cholate detergent-soluble fraction in a time-dependent manner. This return to the cholate-soluble fraction achieved a plateau level after 1.5 to 2 h of antagonist treatment. The half-life of this recovery for both G\(_{\alpha i}\) and G\(_{\beta 1}\) was ~1 h. Again, this time course resembles the kinetics of the disappearance of AC superactivation after withdrawal in chronic morphine-treated cells (Avidor-Reiss et al., 1995a, 1996). In addition, withdrawal conditions induced by the addition of an opioid antagonist (e.g., naloxone, 1 \(\mu M\)) yielded similar results as those obtained after wash-induced withdrawal (data not shown). As a control, the levels of the G\(_{\alpha a}\)
isoforms were examined and it was found that their cholate solubility was not affected by the withdrawal process, indicating that the changes occurring during the chronic treatment and their reversal after withdrawal are specific to the G protein subtypes that are coupled to the activated receptor.

**Discussion**

The molecular mechanism underlying opiate drug addiction relies on the ability of opioid agonists to activate both short- and long-term signal transduction events, with the latter leading to alterations in the state of the signaling complex. One of the changes observed to occur on chronic exposure to morphine is the generation of AC superactivation. The observation that increased levels of cAMP are found in mammalian cells and tissues chronically exposed to opioid agonists is not new (Sharma et al., 1975; Nestler et al., 1993), but it is only recently that some of the molecular events that could lead to such changes in AC regulation have begun to be revealed (Avidor-Reiss et al., 1996; Ammer and Schulz, 1997; Bayewitch et al., 1998b). Herein, we present evidence for an intrinsic change in the biochemical characteristics of the heterotrimeric G proteins based on their ability to be solubilized in the anionic detergent cholate, and suggest that this alteration in solubility could represent a change in cellular signaling, including the regulation of AC activity in response to long-term activation of Gxo-coupled receptors.

Long-term agonist exposure has been shown to lead to changes in the intensity of signaling. Most of the mechanisms reported so far have been concerned with alterations of signaling at the receptor level. For example, it was shown that the receptor can be uncoupled from the G protein due to 1) agonist-induced receptor phosphorylation (Krupnick and Benovic, 1998; Pitcher et al., 1998), 2) receptor sequestration (Raynor et al., 1994), and 3) receptor down-regulation (Campbell et al., 1990). Herein, we would like to suggest that the chronic treatment also leads to a change at the G protein (Campbell et al., 1990). Herein, we would like to suggest that the chronic treatment also leads to a change at the G protein level, whereby the G protein undergoes a biochemical or compartmental alteration that is manifested by a change in its detergent solubility. We have shown herein that chronic activation of the μ-opioid receptor leads to a time-dependent shift in the detergent solubility of both G1 and Gβ1 subunits. The change in Gβ1 solubility very likely signifies a change in detergent solubility of Gαi dimers because most, if not all, of Gαi is known to be tightly bound to γ-subunits (Simonds et al., 1991). Moreover, because this detergent solubility shift has been found with all three Gαo-coupled receptors tested (i.e., μ, κ, and m), this phenomenon is likely to be common to all, or most, Gαo-coupled receptors. This conclusion is in agreement with the finding that Gαi is changing its detergent solubility due to Gxo-coupled receptor activation, whereas Gαs is not affected.

The exact nature of the mechanism of the detergent solubility shift in heterotrimeric G proteins after chronic treatment is not clear, but there is room for speculation among a number of possibilities. The protein may undergo a time-dependent physical or chemical modification induced by the chronic agonist exposure that alters its ability to be solubilized, or the G protein may interact with other protein partners that prevent its solubilization. For example, it may translocate to detergent-insoluble microdomains that are rich in glycosphingolipids, cholesterol, and glycosylphosphatidylinositol-anchored proteins (Varma and Mayor, 1998), or to caveolin-rich domains, termed caveolae, which have been previously described (Kurzhalia et al., 1995). Indeed, high concentrations of G proteins have been found in detergent-insoluble caveolin-rich domains, and various Gα proteins (but not Gβγ; Carman et al., 1999) were shown to bind to the N-terminal domain (residues 61–101) of caveolin 1 (Li et al., 1995). Moreover, it was reported that receptor activation (e.g., bradykinin activation of the B2BK receptor) promotes the recruitment and sequestration of the occupied receptors and of the receptor-coupled Gα proteins (e.g., Gαq and Gαi) into caveolae (de Weerd and Leeb-Lundberg, 1997). It should however be noted that the results obtained by several other laboratories suggested that G proteins are not enriched in caveolae (Stan et al., 1996), and that there are no obvious interactions between G proteins (α or βγ) and caveolin (Huang et al., 1997).

Alternatively, the G proteins may bind to cytoskeletal elements of cells, such as actin or microtubules. In this regard, it is of interest to note that there are reports suggesting that Gβγ (Roychowdhury and Rasenick, 1997), as well as Gαi subunits (Aronin and DiFiglia, 1992), interact with the microtubule cytoskeleton. Although further studies are necessary to clarify the exact cause of the G protein solubility shift, there is no doubt that these changes are specific because Gαs is affected by chronic opioid treatment, whereas Gαas is not. Moreover, the phenomenon is reversible with both Gαs and Gβγ, because these subunits return to the cholate-soluble fraction after removal of the chronically applied agonist.

As described in Results, the level of the decrease in G protein cholate solubility after chronic agonist exposure was very high (amounting in several cases to 50–70% of the total soluble G protein fraction). This recruitment of G proteins into the insoluble fraction has rather slow kinetics. This would tend to suggest that the receptor chronic activation leads to a continuous turnover of G proteins into the cholate insoluble pool. At this stage, we cannot distinguish whether the large fraction of G proteins mobilized represents those previously directly coupled to the overexpressed receptor, or whether receptor coupling of only a small pool of the total G heterotrimer is sufficient to induce a wider G protein mobilization due to cyclic recruiting of “new” G proteins.

The kinetics of both solubility shifts (out of the soluble fraction and back into the soluble fraction) are similar to the kinetics of the onset and loss of the superactivated state of AC. Thus, the shift in solubility of the G protein indicates a
change whose main consequence could be an alteration of the activity of the effector system (in this case AC). In addition, CHO-μ cells pretreated with PTX failed to show the characteristic regulatory pattern described in non-PTX-treated cells (i.e., inhibition of AC activity by acute agonist exposure and AC superactivation on withdrawal from chronic agonist treatment) (Avidor-Reiss et al., 1995b, 1996; Palmer et al., 1997). These results lend support to the hypothesis that the phenomenon of AC superactivation and the G protein solubility shift are correlated.

References


