*Physiol. Res. 46: 79–87, 1997*

**MINIREVIEW**

# Coupling Cofactor – a Novel Regulatory Component in G Protein-Mediated Signalling?

C. NANOFF, M. FREISSMUTH

*Institute of Pharmacology, Vienna University, Vienna, Austria*

*Received June 14, 1996*
*Accepted December 20, 1996*

## Summary

The model for studying the mechanism of G protein-mediated signalling cannot account for the observation that high-affinity binding of agonists to many different receptors is not dissociated by the addition of high concentrations of guanine nucleotides. Using the cerebral $A_1$-adenosine receptor as a model system, we have recently identified a component which is responsible for this phenomenon. This protein, termed the coupling cofactor, can be solubilized from brain membranes and chromatographically resolved from both the G proteins and the receptor. Following reconstitution into appropriate acceptor membranes, the coupling cofactor confers resistance of high-affinity agonist binding to guanine nucleotides. The coupling cofactor acts as a brake and limits receptor-dependent signal amplification; in addition, it is a candidate for participating in the higher level organization of receptors and G proteins in membranes and in the membrane-delimited cross-talk between individual receptors. Here, we present a working hypothesis on the possible biological roles of the coupling cofactor.

**Key words**

G proteins – Adenosine receptors – Dopamine receptors – Guanine nucleotides – Signal transduction

## Introduction

G protein-coupled receptors are the most versatile among cell surface receptors which transmit signals from the exterior to the interior side of the cell membrane. The range of ligands which bind to and activate G protein-coupled receptors is highly diverse and comprises biogenic amines, nucleosides and nucleotides, amino acids, peptides, glycoproteins, odorants, photons etc. Receptor activation by agonists leads to the generation of a secondary cellular signal either by the modulation of enzymes controlling second messengers, or by changes of the membrane potential *via* alterations of ion channel conductance. Current research has repeatedly demonstrated that G protein-coupled receptors may in fact influence most of the signalling pathways available to the cell.

*Mechanism of G protein-mediated signal transduction*

Transfer and amplification of the receptor signal as well as termination of the signal input depends on the cycle of activation and deactivation of the G proteins involved. More than 20 G protein $\alpha$-subunits have been identified; these are classified in structurally and functionally related subfamilies. In addition, there are five $\beta$-subunits and more than seven $\gamma$-subunits thus allowing for a large array of different G protein oligomers (Hepler and Gilman 1992).

*Results were preliminary presented at "CNS – Advance in Research of Normal and Neoplastic Cells" which was held in Brno (April 25, 1996) as the satellite minisymposium of the 42nd International Congress of the European Tissue Culture Society (Freissmuth et al. 1996).*

Regardless of the nature of the individual G protein $\alpha$-subunits and $\beta$-oligomers, they all share several basic characteristics which are fundamental to the mechanism through which signal transduction is achieved (Freissmuth *et al.* 1989). In the basal state, the G protein exists as an $\alpha\beta\gamma$-heterotrimer with GDP bound to the $\alpha$-subunit. G protein activation comes about through exchanging prebound GDP for GTP. Deactivation is dependent on the intrinsic rate of GTP-hydrolysis of the $\alpha$-subunit. In the absence of an activated receptor, basal GDP release proceeds very slowly, while the rate of GTP hydrolysis is 10 to 1000 times faster. This kinetic feature suppresses the basal level of signalling. Upon binding of an agonist ligand, the receptor associates with the G protein oligomer and induces dissociation of GDP. This leads to the formation of a ternary complex (HRG) of agonist (H), receptor (R) and G protein (G), in which the agonist is bound with high affinity. In an intact cell, where GTP is present at high concentrations, the ternary complex is ephemeral. Binding of GTP is instantaneous and induces subunit dissociation; the $\alpha$- and $\beta\gamma$-subunit are set free to interact with effector molecules. Reciprocally, subunit dissociation reduces the affinity for the agonist ligand. Due to its catalytic activity, the G protein $\alpha$-subunit hydrolyzes GTP; in the GDP bound form, the $\alpha$-subunit reassociates with the $\beta\gamma$-dimer and returns to the resting state. Thus, the signal is switched off.

*Additional proteins which modulate receptor-G protein coupling*

Signalling *via* G proteins thus requires a set of limited basic components, namely the agonist, receptor, the G protein subunits ($\alpha$-subunit and $\beta\gamma$-dimer) and the effector. The signal sorting process is determined in part by the affinities of the reaction partners for mutual interaction. Structural components of the cell membrane may provide an additional level of specificity in organizing R/G coupling (Neubig 1994). Several proteins are known to interfere with receptor-G protein coupling and are stably localized on or translocate to the inner side of the cell membrane for action. Phosducin, arrestin, and their related homologues, as well as the cytoskeletal protein tubulin, are among the best characterized proteins which modulate the interaction between receptors and G proteins. (i) Phosducin (and phosducin-like proteins) has been identified in the cytosol of the cerebral cortex; G protein $\beta\gamma$-subunits bind to phosducin and are supposed to target phosducin to the cell membrane (Lee *et al.* 1987, Bauer *et al.* 1992, Miles *et al.* 1993). The association of phosducin with G proteins inhibits the receptor-dependent activation of effectors. (ii) The arrestins are cytosolic cofactors which mediate receptor desensitization. These proteins bind with a high affinity to receptors phosphorylated by the G protein-coupled receptor kinase and compete directly with G proteins (for review see Hausdorff *et al.* 1991). (iii) Tubulin was demonstrated to bind to G proteins and augment effector stimulation indicating that it may even enhance subunit dissociation (Wang and Rasenick 1991, Popova *et al.* 1994). (iv) Likewise, reports on the stimulation of leukocytes indicate that G proteins adhere to the actin filament network and are released upon fMLP receptor or direct guanine nucleotide-induced activation (Särndahl *et al.* 1993). (v) Other structural proteins like spectrin, dynamin and caveolin are thought to participate in the organization of receptor-G protein coupling. Recent evidence indicates that in smooth muscle cells the endothelin receptor is clustered in caveolae together with G proteins, $IP_3$-receptor and $Ca^{2+}$-channel by aggregating with caveolin (Chun *et al.* 1994).

*"Tight" R/G coupling mode – Resistance of the HRG-ternary complex to guanine nucleotide-induced destabilization*

As outlined above, in the ternary complex HRG, the agonist, is bound with high-affinity. This complex is destabilized by guanine nucleotides resulting in the dissociated $\alpha$-subunit and $\beta\gamma$-dimer as well as the low affinity complex HR. A phenomenon repeatedly observed in radioligand binding studies on membranes from cells and various tissues is the inability of guanine nucleotides to destabilize high affinity agonist ligand binding. This peculiar feature has been referred to as "tight coupling mode" but its molecular basis has remained enigmatic. GTP-resistance of the ternary complex (HRG) can neither be explained by the current model of G protein-mediated signal transduction nor can it be accounted for by the additional components known to impinge on the signalling cascade (see above).

In a hallmark work, Levitzki and coworkers studied the rate of adenylyl cyclase activation in response to receptor activation in red blood cells revealing slower activation rates through the $A_2$-adenosine than the $\beta$-adrenergic receptor (Rimon *et al.* 1978, Tolkovsky and Levitzki 1978). The observations on coupling of the $A_2$-adenosine receptor to the effector did not fit into a model where the molecular reaction partners diffuse freely in the cell membrane. This phenomenon was referred to as the "tight coupling mode" and would be opposed to the "collision coupling model" which describes signalling through a receptor whose mobility is unrestricted. Collision coupling permits activation of the entire pool of effector molecules by an individual receptor whereas the number of effector moieties in the tight coupling mode is limited down to a 1:1 signal transfer from receptor to effector. In the past, we have studied G protein coupling of the $A_{2A}$-adenosine receptor subtype in the brain (Nanoff *et al.* 1991). We found that

the interaction of agonists with these receptors deviates from the classical, ternary complex model since the agonist/receptor/G protein complex cannot be dissociated even by high concentrations (≥100 $\mu$M) of the hydrolysis-resistant GTP analogue GTP$\gamma$S. However, after detergent solubilization the $A_2$-adenosine receptor-G protein complex can be recovered in the supernatant; in this solubilized complex, the sensitivity to GTP analogues is enhanced suggesting that an inhibitory constraint has been removed (Nanoff and Stiles 1993).

This phenomenon, i.e. the resistance of high-affinity agonist binding to guanine nucleotides is not unique to the $A_{2A}$-adenosine receptor; analogous findings have been obtained with the 5-$HT_2$ receptor, the $PgI_2$ receptor in platelets, the bovine muscarinic receptor (Florio and Sternweis 1985, Szele and Pritchett 1993) and with various species homologues of the $A_1$-adenosine receptor (Ströher *et al.* 1989, Nanoff *et al.* 1995).

*Tight-coupling is mediated by a distinct membrane component – the coupling cofactor*

The following lines of evidence support the hypothesis that an additional factor is responsible for inducing the tight coupling mode. (i) With the 5-$HT_2$ receptor, the degree of guanine nucleotide refractoriness is variable with the type of cell chosen to express a particular receptor clone. This was demonstrated by Szele and Pritchett (1993) and suggests that the GTP-sensitivity of the 5-$HT_2$ receptor depends on the cell line used to express the cloned receptor; in the 293 human embryonic kidney cell line, the entire receptor population was resistant to GTP analogues, whereas 70 % and 30 % of the receptor population did not respond in a mouse cell line (NIH 3T3-fibroblasts) and in rat brain membranes, respectively. (ii) As described above for the $A_2$-adenosine receptor, the binding of agonists to the $A_1$-adenosine receptor in rodent brain membranes is resistant to guanine nucleotide modulation but the sensitivity of agonist binding increases dramatically in the detergent solubilized receptor-G protein complex (Nanoff *et al.* 1995). (iii) Most importantly, the fraction of $A_1$-adenosine receptors which remain in the membrane following solubilization are also highly sensitive to the dissociating effect of guanine nucleotides. This indicated that an ancillary component had been removed which was responsible for the "tight-coupling mode" (Nanoff *et al.* 1995).

We have therefore used a biochemical approach to prove that this resistance to guanine nucleotides was indeed mediated by a distinct membrane component. The $A_1$-adenosine receptor was chosen as a model system because the coupling of the purified receptor as well as the recombinant receptor with defined G proteins of the $G_o/G_i$-family has been characterized extensively (Freissmuth *et al.* 1991a,b, Jockers *et al.* 1994). Detergent-solubilized rat brain membranes, in which the high-affinity binding of the agonist [$^{125}$I]HPIA to the $A_1$-adenosine receptor-G protein complex was dissociated to GTP$\gamma$N, were used as acceptor membranes. "Tight coupling", i.e. resistance of [$^{125}$I]HPIA binding to GTP$\gamma$S, was restored in these acceptor membranes by readdition of the solubilized extract. The activity responsible for "tight coupling" is attributable to a distinct membrane protein, which we termed the "coupling cofactor". The following observations support this interpretation: coupling cofactor activity can only be extracted from the membranes by detergent treatment but is not found in the cytosolic fraction, it is heat-labile and trypsin-sensitive and it can be chromatograpically resolved from both the receptor and the G proteins (Nanoff *et al.* 1995). However, the activity of the coupling cofactor obviously requires the presence of G proteins as it combines with the ternary HRG-complex. Additional evidence for the conclusion that GTP$\gamma$S-resistance of agonist binding is not a property specified by the receptor comes from heterologous expression: transfection of the cDNA coding for the rat brain $A_1$-adenosine receptor into mammalian cells results in the expression of a receptor which is fully sensitive to modulation of agonist binding by GTP$\gamma$S (Nanoff *et al.*, unpublished observation). A partially purified coupling cofactor severely decreases the catalytic efficiency with which one receptor can activate several molecules of G proteins. This indicates that the coupling cofactor acts as a brake on signal amplification by trapping the agonist-liganded receptor at the level of the ternary HRG complex.

*Possible biological roles for the coupling cofactor – working hypotheses*

The biological role of the coupling cofactor is not clear at present and purification to homogeneity is a necessary step to reach firm conclusions; however, several potential biological roles can at present be inferred from preliminary experiments which we have carried out; (i) the coupling cofactor is a candidate for participating in the higher level organization of receptors and G proteins in membranes, since it discriminates among $A_1$-adenosine receptor-G protein complexes. (ii) Association between the $A_1$-adenosine receptor and coupling cofactor is not a unique phenomenon; a similar phenomenon can be observed with the $D_2$-dopamine receptor. (iii) The coupling cofactor may participate in membrane-delimited cross-talk between receptors.

*(i) G protein-selectivity of coupling cofactor*

Several receptors have previously been tested in reconstitution experiments with defined components for their ability to discriminate between closely related G protein subunits (Senogles *et al.* 1990, Kurose *et al.* 1991, Freissmuth *et al.* 1991b,c, Bertin *et al.* 1992). In

general, modest selectivities have been observed. This is in contrast with the stringent requirement obtained in intact cells injected with specific antisense oligonucleotides to deplete individual $\alpha$-, $\beta$- and $\gamma$-subunits (Kleuss *et al.* 1991, 1992, 1993). For example, in various neuroendorine cells, the activation of both muscarinic and somatostatin receptors inhibits $Ca^{2+}$-channel conductance to modulate hormone release. Whereas the muscarinic receptor requires exclusively the $\alpha_{o1}\beta_3\gamma_4$ trimer, the somatostatin receptor relies on a distinctly different composition ($\alpha_{o2}\beta_1\gamma_4$); this observation was independent of the cell type (for review see Offermanns and Schultz 1994). Clearly, the requirements of receptor/effector coupling for G protein specificity appears much more stringent in intact cells compared with the evidence provided by reconstitution experiments. This discrepancy suggests additional interaction sites present in cell membranes and a higher level of organization that cannot be accounted for in reconstitution systems. We believe that the coupling cofactor may also participate in scaffolding receptors and specific G proteins to a higher level of organization in the membrane; this hypothesis is based on the experiment depicted in Figure 1. As mentioned above, the coupling cofactor can be chromatographically resolved from G proteins but requires G proteins to detect its activity. In the reconstitution experiment (Fig. 1), partially purified coupling cofactor, which was depleted of G proteins and therefore *per se* inactive, was recombined with detergent-treated rat brain membranes (i.e. acceptor membranes) in the absence (none) and presence of G proteins (recombinant $G_{i\alpha\text{-}1}$, recombinant $G_o$ and a mixture of $G_o$ and $G_i$ purified from the bovine brain). The bars represent IHPIA bound specifically in the absence (hatched) or presence of GTP$\gamma$S (3 $\mu$M, cross-hatched). In the absence of exogenously added G proteins, the coupling cofactor is essentially inactive (none). Each of the G protein species enhances formation of the high affinity complex to a similar degree, whereas they vary in their ability to restore GTP$\gamma$S-refractoriness. In contrast to recombinant $G_{i\alpha\text{-}1}$, the addition of equivalent amounts of recombinant $G_{o\alpha}$ does not essentially increase resistance to GTP$\gamma$S. This is also true for the bovine brain $G_o$ (bb); the modest effect observed with this preparation is probably due to contamination with $G_i$.

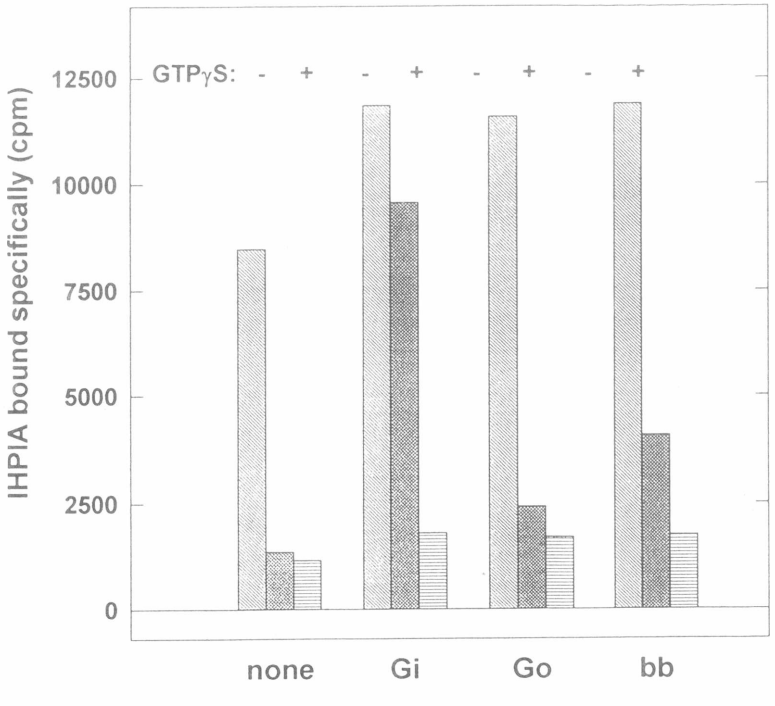


**Fig. 1.** *Coreconstitution of G protein heterotrimers and coupling cofactor differentially affects the GTPγS-refractoriness of [$^{125}$I] HPIA binding. Reconstitution of detergent extracted brain membranes (acceptor membranes; 25 μg) was carried out in a final volume of 40 μl containing 0.1 μM $rG_{i\alpha\text{-}1}$ ($G_i$), 0.1 μM $rG_{o\alpha}$ ($G_o$) or 0.1 μM of a purified bovine brain $G_i/G_o$ preparation (bb) consisting predominantly of $G_o$; each sample contained 0.3 μM purified bovine brain βγ and partially purified coupling cofactor (for details see Nanoff et al. 1995). The control reaction (none) was carried out in the absence of added $G_\alpha$-subunits, a condition under which coupling cofactor activity is not detectable in a preparation where coupling cofactor has been resolved from the G proteins. [$^{125}$I] HPIA binding (5 nM) was determined at a final concentration of 1 mM CHAPS. Bars indicate specific binding in the absence (hatched) and presence (cross-hatched) of 3 μM GTPγS; striped bars indicate binding in the presence of 3 μM GTPγS without addition of partially purified cofactor.*

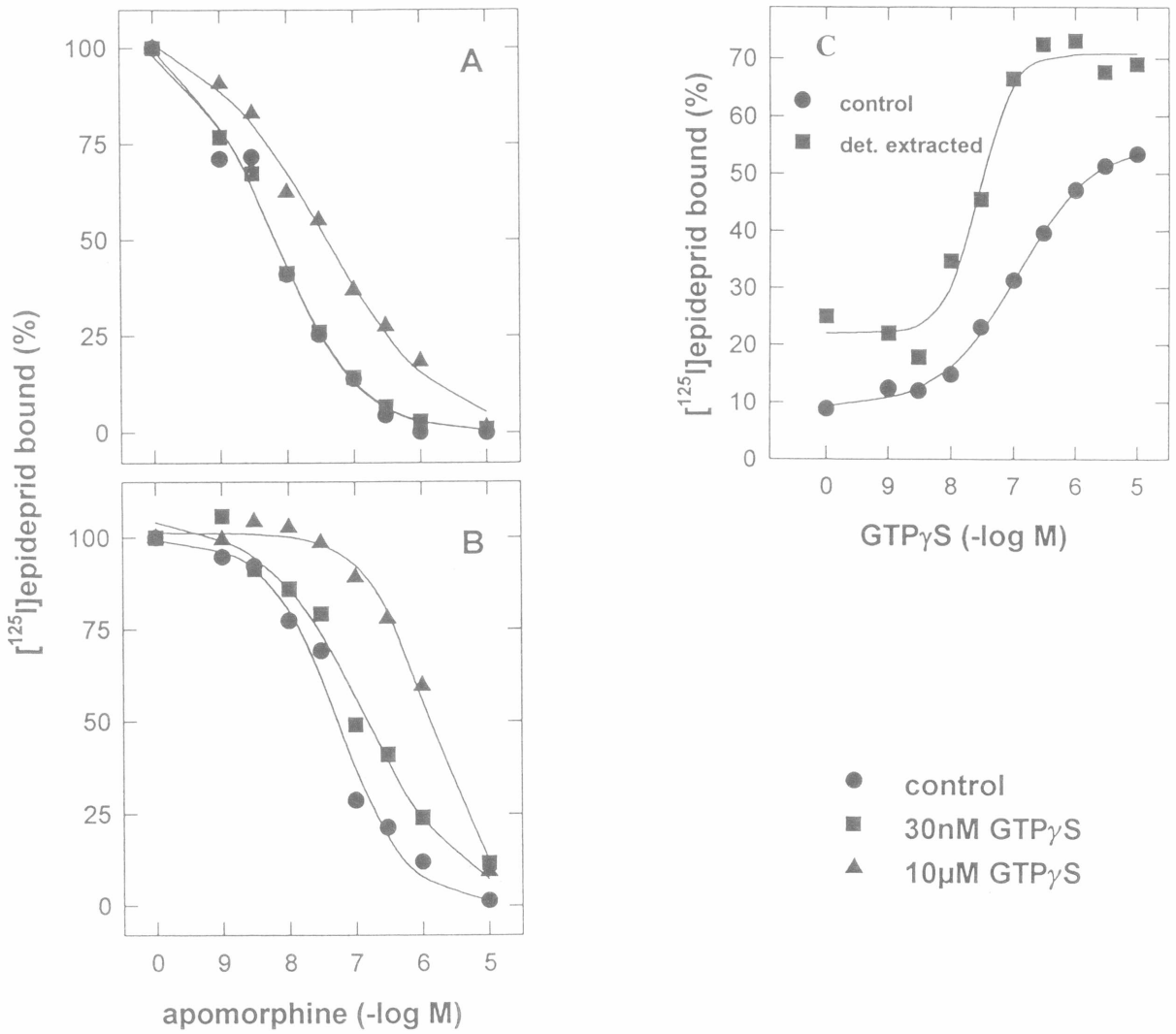


**Fig. 2.** *Striatal dopamine $D_2$ receptors labelled by [$^{125}$I]epideprid: Competition of apomorphine for [$^{125}$I]epideprid binding to native (A) and CHAPS extracted rat striatal membranes (B). [$^{125}$I]epideprid binding (≈ 0.2 nM) was displaced by increasing concentrations of apomorphine in the absence or presence of GTPγS (at two different concentrations as indicated). Competition curves were obtained in native (A, ≈10 μg membrane protein) and detergent extracted striatal membranes (B, ≈20 μg membrane protein). Binding values are given as percentage of specific binding in the absence of apomorphine. For details on the binding methodology, see Beindl et al. (1996). Panel C: GTPγS is more potent in reversing the displacement by apomorphine (100 nM) after detergent extraction. Competition of [$^{125}$I]epideprid binding to native (full circles, ≈10 μg membrane protein) and to detergent extracted membranes (full squares ≈20 μg membrane protein) by apomorphine (100 nM) was done at increasing concentrations of GTPγS. Binding values are given in percentage of specific binding for each GTPγS concentration but in the absence of apomorphine.*

*(ii) Release of tight-coupling in the striatal dopamine $D_2$-receptor*

A constrained mode of receptor G protein coupling is not unique to the brain $A_1$-adenosine receptor but appears common to several types of receptors (see above). In order to obtain direct evidence for a physical constraint imposed on a different receptor, we have subjected striatal membranes to an analogous detergent extraction protocol and examined the guanine nucleotide sensitivity of the dopamine $D_2$-receptor in striatal membranes from the rat brain. For the $D_2$ receptor competition, binding experiments were performed using the $D_2$-selective benzamide antagonist radioligand [$^{125}$I]epideprid; [$^{125}$I]epideprid binding to striatal membranes is displaced by increasing concentrations of the agonist apomorphine. Sensitivity of the $D_2$-receptor to GTPγS was estimated from a

shift in the displacement curve when the binding assay was carried out in the presence of 30 nM or 10 $\mu$M GTP$\gamma$S. Figure 2 shows a typical experiment where panel A depicts binding to native, panel B binding to CHAPS-extracted membranes. Similar to the $A_1$-adenosine receptor in cortical membranes, the effect of GTP$\gamma$S on agonist affinity is markedly increased in extracted versus control membranes; the $IC_{50}$ for apomorphine in the absence of GTP$\gamma$S were 13.9±3.7 nM and 66.7±27.8 nM in native and detergent extracted membranes, respectively, n=3). The corresponding shift in $IC_{50}$ in control membranes was 1.6 (0.6–4.4, 95 % confidence interval) at 30 nM GTP$\gamma$S and 6.1 (3.9–9.4) at 10 $\mu$M GTP$\gamma$S, while in extracted membranes the shifts were 7.4 (1.3–43.9) at 30 nM and 24.5 (8.9 and 67.4) at 10 $\mu$M GTP$\gamma$S. The effect of detergent extraction on coupling of the striatal dopamine $D_2$-receptor is illustrated in an additional experiment which allows to assess the GTP$\gamma$S effect on agonist binding in a concentration-dependent manner (Fig. 2 C); here, [$^{125}$I]epideprid binding ($\approx$ 0.2 nM) was carried out in the presence of a near maximum concentration of apomorphine (100 nM, see Fig. 2, Panels A,B). The sensitivity to GTP$\gamma$S was assayed through reversing the displacement of [$^{125}$I]epideprid binding by 100 nM apomorphine. As expected, the $D_2$-receptor is more sensitive to guanine nucleotide modulation after detergent extraction of membranes; $EC_{50}$ for GTP$\gamma$S in reversing the inhibition by apomorphine was 127±53 nM and 20±3 nM in control and extracted membranes, respectively, resulting in a mean shift of 6.4±1.9. Readdition of partially purified coupling cofactor decreased GTP$\gamma$S-sensitivity; however, interpretation of this finding is limited by the fact that the partially purified material contains activity which depresses the binding of [$^{125}$I]epideprid.

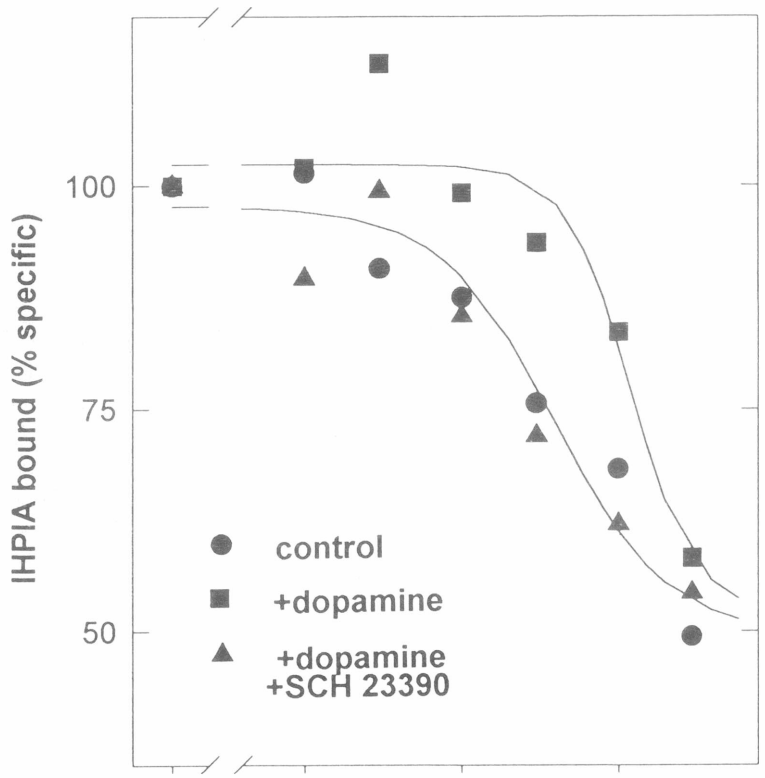


**Fig. 3.** *Stimulation of the dopamine $D_1$-receptor enhances the GTP$\gamma$S-refractoriness of the $A_1$-adenosine receptor. Rat striatal membranes (15 $\mu$g/assay) were subjected to [$^{125}$I] HPIA equilibrium binding (1 nM) in the presence of increasing concentrations of GTP$\gamma$S. A batch of membrane had been split in three; dopamine was added to yield a final assay concentration of 10 $\mu$M (full squares to one aliquot; to the second aliquot dopamine was added together with 100 nM SCH23390 (full triangles), a $D_1$-selective dopamine antagonist; the third aliquot received vehicle alone (control, full circles). The incubation lasted for 90 min at 25 °C. Binding values are given as % of specific binding determined in the absence of GTP$\gamma$S. The fitted curves for the control reaction and [dopamine +SCH 23390]-treated membranes are superimposable and only one curve is drawn. The experiment shown is representative for a total of 5 experiments preformed in rat and porcine striatal membranes.*

*(iii) Receptor cross-talk*: The dopamine $D_2$-receptor and the $A_{2A}$-adenosine receptor are colocalized in the same neurones of the brain striatum (Ferriet *et al.* 1993, 1994a). The locomotor effects mediated by the dopamine $D_2$-receptor (either through receptor stimulation or through receptor sensitization) in Parkinson's disease are inversely modulated by agents acting *via* the $A_2$-adenosine receptor; antagonists enhance while receptor agonists reduce locomotor activity. Ferré *et al.* (1991, 1993, 1994a) suggested that this interaction occurs – not *via* presynaptic modulation of dopamine release but – on the basis of receptor cross-talk in individual neurones. A direct receptor-receptor interaction on the level of the plasma

membrane was proposed to account at least in part for the adenosine mediated inhibition of dopamine $D_2$-receptor activity. These two receptor types are not thought to share a common pool of G proteins, the dopamine $D_2$-receptor and the $A_{2\alpha}$-adenosine receptor being prototypical $G_i/G_o$- and $G_s$-coupled receptors, respectively. Hence, a clear picture of the mechanism of action has not evolved. Since both the $D_2$- and the $A_2$-receptor in striatal membranes appear to be under control of a coupling cofactor-like component, it seems intriguing to consider the coupling cofactor as a "go-between" regulator of receptor efficacy. Thus, activation of one receptor type may dissociate the coupling cofactor which binds to a resting receptor within a short distance. A pronounced $A_2/D_2$ cross-talk was reported in the ventral striatopallidal system, which is presumed to represent an important site of action for neuroleptic agents in the treatment of schizophrenia. In striatal neurones, a similar membrane-delineated receptor cross-talk has been suggested to occur in neuronal membranes between $A_1$-adenosine and $D_1$-dopamine receptors (Ferré *et al.* 1994b). In the work by Ferré *et al.* (1991, 1994a), this interaction was approached from the dopamine receptor point of view, i.e. the modulation of dopamine effects by adenosine receptor activation was studied. Cross-talk, however, requires mutual interference. We have therefore examined the adenosine/dopamine cross-talk by assessing the dopamine modulation of $A_1$-adenosine receptor agonist binding. If the coupling cofactor is involved, dopamine ought to have an influence on guanine nucleotide sensitivity of the $A_1$-adenosine receptor. Figure 3 demonstrates that dopamine increases the GTP$\gamma$S-refractoriness of IHPIA-binding shifting the GTP$\gamma$S-concentration curve to the right. The effect is reversed by the dopamine $D_1$-receptor antagonist SCH 23390. $A_1$-adenosine receptor ($G_i/G_o$-coupled) and $D_1$-receptor ($G_s$-coupled) do not interact with the same class of G proteins. In addition, if the receptors had shared a common G protein pool, activation of the $D_1$-dopamine receptor would have increased rather than decreased the sensitivity of the $A_1$-adenosine receptor to GTP$\gamma$S. This phenomenon may, however, be accounted for by the coupling cofactor or related activity. We have therefore tested whether receptor cross-talk is similarly disrupted by detergent extraction. This is in fact the case. However, the evidence is still not conclusive since detergent solubilization of membranes lowers the concentration of reaction partners by more than half. Thus, reconstitution of receptor cross-talk by addition of the purified coupling cofactor will be needed for compelling evidence.

*Future perspective*

Several clinical consequences of unrestrained activity in G protein-linked signalling pathways have been discovered recently (for review see Clapham 1994). Distinct diseases and neoplasias were related to mutational alterations which lead to the constitutive activation of the $\alpha$-subunit, i.e. signalling in the absence of receptor stimulation (familial precocious puberty, the McCune-Albright Syndrome, pituitary, adrenal and thyroid tumours). Enhanced signal generation may similarly arise from constitutively active receptors. Here, the concept is increasingly being appreciated that constitutive activity is not exclusively caused by mutations of the receptor molecule but that native receptor homologues may possess some level of constitutive activity even without activating mutations (Schütz and Freissmuth 1992, Lefkowitz *et al.* 1993). Although there is no evidence for constitutive activity of the $A_{2A}$-adenosine receptor in its native environment, e.g. platelet membranes or renal tubules (Freissmuth *et al.* 1987, Nanoff *et al.* 1994), it was observed after heterologous expression of the cloned canine $A_{2A}$-adenosine receptor cDNA in various cell types; this resulted in constitutive activation of adenylyl cyclase regardless of the cell type used (dog and mouse thyroid cells, Y1-adrenal cells and *Xenopus* oocytes; Maenhaut *et al.* 1990). A striking example for the biological consequences of unrestrained signalling was provided in transgenic mice whose thyroids expressed the $A_{2A}$-adenosine receptor (Ledent *et al.* 1992). Thyroid cells normally do not possess this receptor type but proliferate in response to cAMP accumulation. These transgenic mice developed impressive hyperactive goiters, reflecting a non-physiological condition due to extensive adenylyl cyclase activation. Conversely, the $A_{2A}$-adenosine receptor in native membranes stimulates adenylyl cyclase at a much slower rate than activation of other stimulatory receptors such as the $\beta$-adrenergic or the $PgI_2$-receptor (Tolkovsky and Levitzki 1978, Gross and Lohse 1991, Nanoff *et al.* 1994). Our hypothetical explanation is that the cell lines used for heterologous expression of the $A_{2A}$-receptor clone lack an endogenous constraint which impedes the receptor-G protein interaction. Testing this speculation and other hypothetical roles of the coupling cofactor outlined above requires its purification to homogeneity and molecular cloning. This is currently being attempted.

**Acknowledgements**

Work from the authors— laboratory was supported by a grant from the Austrian Science Foundation (FWF P10871-MOB) and by a concerted action "Adenosine receptors in the brain" (Adeuro) within the EC Biomedical Programme.

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**Reprint requests**

C. Nanoff, Institute of Pharmacology, Vienna University, Währinger Str. 13a, A-1090 Vienna, Austria.