The EMBO Journal vol.8 no.8 pp.2353 - 2357, 1989

Association of two pertussis toxin-sensitive G-proteins with the D_2-dopamine receptor from bovine striatum

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Communicated by S.Fuchs

The solubilized D_2-dopamine receptor from bovine striatum exhibits high and low affinity states for dopaminergic agonists. Guanine nucleotides and pertussis toxin convert the solubilized receptor from a high affinity state to a low one. A D_2-receptor preparation partially purified by affinity chromatography on a haloperidol adsorbent, exhibited agonist-stimulated GTPase activity. [^{32}P]ADP-ribosylation by pertussis toxin of this receptor preparation resulted in the specific labeling of two protein bands corresponding to mol. wts of 39 and 41 kd, in SDS-PAGE. Association of these G-proteins with the receptor was specifically inhibited by Gpp(NH)p. Immunoblot analysis of these G-proteins indicated that the 41- and 39-kd protein bands are analogous to brain Gi and Go respectively. These experiments demonstrate that two distinct pertussis toxin-sensitive G-proteins are functionally associated with bovine striatum D_2-dopamine receptor.

Key words: D_2-dopamine receptor/G-proteins/pertussis toxin

Introduction

Two different states of D_2-dopamine receptors were shown to exists in striatal and pituitary membranes and were found to be of a high and low affinity for dopaminergic agonists (Sibley and Creese, 1983; Simmonds *et al.*, 1986). Interconversion between these two states was demonstrated to be regulated by guanine nucleotides and also to be sensitive to pertussis toxin, suggesting coupling of the receptor with guanine nucleotide-binding proteins (G-proteins) (Cote *et al.*, 1983; Seeman *et al.*, 1985).

G-proteins which couple receptors to their effectors are heterotrimers composed of α, β and γ subunits (Spiegel, 1987; Gilman, 1987). The α subunit binds guanine nucleotides and undergoes ADP ribosylation catalyzed by different bacterial toxins. Several distinct pertussis toxin-sensitive G-proteins, with homologous structure, have been demonstrated by immunochemical analysis as well as cDNA cloning experiments (reviewed by Milligan, 1988).

D_2-dopamine receptors are known to be coupled to inhibition of adenylate cyclase activity both in pituitary and striatal membranes (Stoof and Kebabian 1984; Onali *et al.*, 1985; Cooper *et al.*, 1986). This inhibition is mediated by a Gi protein (Fujita *et al.*, 1985). Recently, alternative pathways for signal transduction mediated by D_2-receptor were postulated. It was shown that in anterior pituitary, dopaminergic inhibition of prolactin release is associated with Ca^{2+} mobilization (Delbeke and Dannies 1985; Judd *et al.*, 1985), an event possibly mediated by inhibition of phospholiphase C activity (Simmonds and Strange, 1985; Enjalbert *et al.*, 1986; Canonico *et al.*, 1982). This regulation of prolactin release is blocked by pertussis toxin (Cote *et al.*, 1984), indicating the involvement of a G-protein in this process. K^+ conductance was also shown to be regulated by D_2-dopamine receptors in bovine lactotroph cells as well as in neurons of rat substantia nigra (Israel *et al.*, 1985; Ingram *et al.*, 1986; Lacey *et al.*, 1987). Various pertussis toxin-sensitive G-proteins (Katada *et al.*, 1987) may mediate these events. Recently Senogles *et al.* (1987) have identified a Go-related protein to be associated with the D_2-receptor in the anterior pituitary.

In some neuronal systems D_2-receptor signal transduction has been shown to be cAMP independent (Stoof *et al.*, 1986) and to involve inhibition of PI turnover and Ca^{2+} mobilization (Pizzi *et al.*, 1987). In abdominal ganglia of Aplysia it was shown that a pertussis toxin-sensitive G-protein is involved in the regulation of K^+ channels by dopamine receptors (Sasaki and Sato, 1987). In another neuronal system, the D_2-receptor was shown to be associated with a decrease in voltage-dependent Ca^{2+} currents, and the involvement of a pertussis toxin-sensitive G-protein in this process has been proposed (Harris-Warrick *et al.*, 1988).

In the present study we demonstrate that two pertussis toxin-sensitive G-proteins co-purify with the D_2-dopamine receptor from striatal membranes and are functionally associated with the receptor. These G-proteins are immunologically related to brain Gi and Go. Preliminary

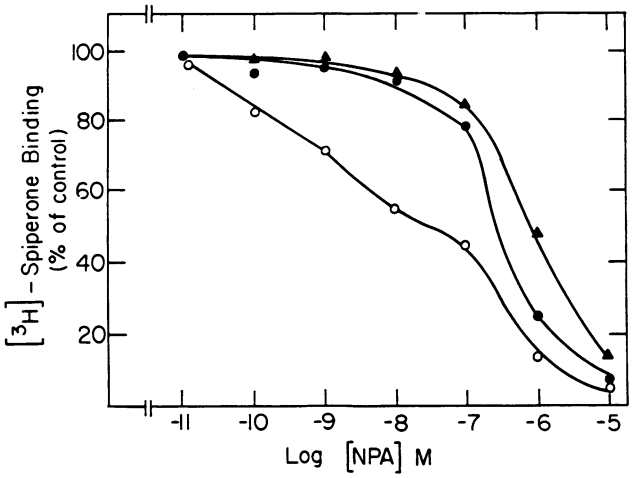

Fig. 1. Agonist competition of [^{3}H]spiperone binding to solubilized striatal membranes. Solubilized membranes preparation was incubated with 2 nM [^{3}H]spiperone and increasing concentrations of NPA in the absence (○) or presence (●) of 100 μ Gpp(NH)p, and upon treatment with pertussis toxin and NAD (unlabeled) (▲). The specific binding was determined in the presence of 1 mM (+)-butaclamol. The 100% [^{3}H]spiperone bound correspond to 150 fmol/mg protein.

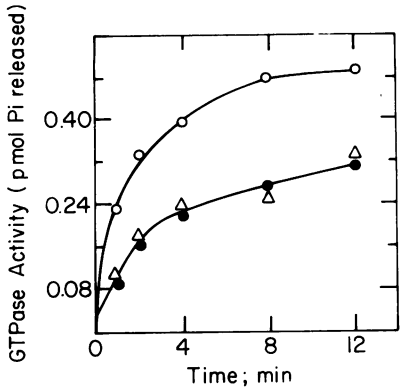

Fig. 2. GTPase activity of affinity purified D_2-receptor. Reconstituted affinity purified receptor preparation was assayed for GTPase activity. (●), control; (○), in the presence of 10 μM NPA; (△), in the presence of 10 μM NPA and treatment with pertussis toxin and NAD.

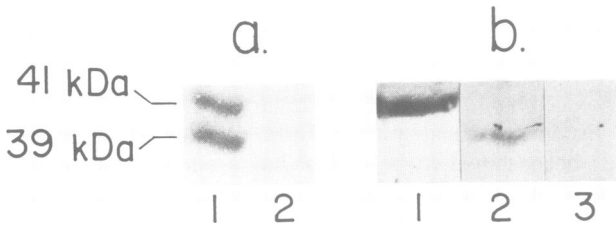

Fig. 3. ADP ribosylation of affinity purified receptor (**a**) and immunoblotting. (**b**). (**a**) Affinity purified receptor preparation was incubated with [^{32}P]NAD in the presence (1) or the absence (2) of active pertussis toxin. Samples were separated on SDS−PAGE and exposed to autoradiography. (**b**) Affinity purified receptor preparation was electrophoresed on SDS−polyacrylamide gel followed by Western blotting. The nitrocellulose membrane filters were overlayed with 1/100 diluted AS/7 antibody (1), RV/3 antibody (2) or with normal rabbit serum (3).

results of this study have been reported recently (Elazar *et al.*, 1988a).

Results

Effect of guanine nucleotides and pertussis toxin on D_2-dopamine receptor in solubilized membranes

D_2-dopamine receptor in 3-[(3-cholamidopropyl)-dimethylaminiol]propanesulfonate (Chaps)-solubilized membranes from bovine striatum exhibits apparent high affinity for the dopaminergic agonist *N*-propylnorapomorphine (NPA). As shown in Figure 1 the NPA competition curve for [^{3}H]spiperone binding to the receptor is shallow and biphasic. The K_D^{high} and K_D^{low} for NPA were 0.08 ± 0.04 and 12.4 ± 3.2 nM, respectively. This indicates that the receptor exists in more than one affinity state for the agonist. Addition of 100 μM Gpp(NH)p steepened the curve and caused its shift from high to low potency (K_D 17.05 ± 4.3 nM) for NPA (Figure 1). It should be noted that Gpp(NH)p had no effect on the K_D (0.5 ± 0.15 nM) of [^{3}H]spiperone (data not shown). Treating the solubilized membrane preparation with pertussis toxin and NAD resulted in a similar reduction in the affinity of the agonist to the receptor (Figure 1). These results indicate that the D_2-receptor is functionally coupled to one or more pertussis toxin sensitive G-protein(s) and that this coupling is maintained following Chaps solubilization.

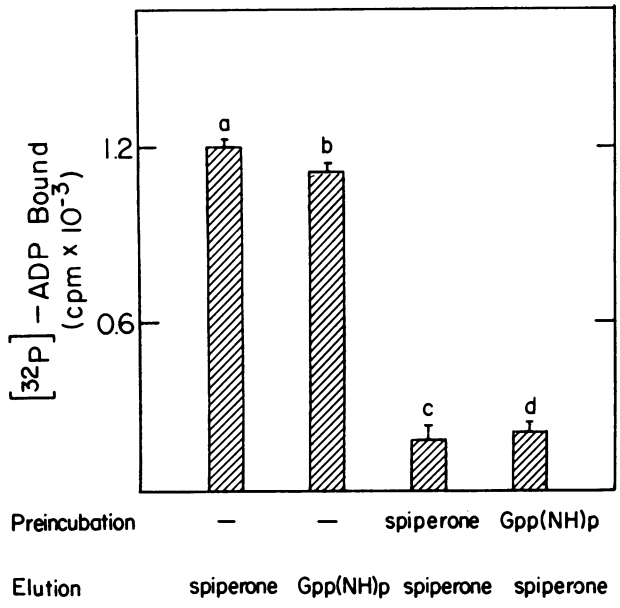

Fig. 4. Specific association of the D_2-receptor with G-proteins. 5 ml aliquots of solubilized membrane preparation were incubated with or without 10 mM spiperone or Gpp(NH)p as indicated and further exposed to affi-gel-HGE. The resins were washed followed by elution with 10 mM spiperone or 100 mM Gpp(NH)p. The eluted samples were labeled with [^{32}P]NAD and pertussis toxin and separated on SDS−PAGE. The labeled material was excised from the gel and the bound [^{32}P]ADP was analyzed.

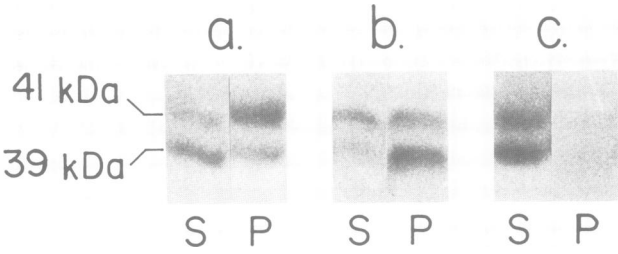

Fig. 5. Immunoprecipitation of G-proteins from the affinity purified D_2-receptor preparation. Affinity purified receptor preparation was ADP ribosylated using [^{32}P]NAD and pertussis toxin. Samples labeled with [^{32}P]ADP were incubated with AS/7 antibody (**a**), RV/3 antibody (**b**) or normal rabbit serum (**c**), immunoprecipitated with Sepharose protein A and separated on SDS−PAGE. S, represents the supernatant after the precipitation, and P represents the precipitate.

Association of two G-proteins with the D_2-dopamine receptor

Affinity purification of a solubilized receptor preparation on a affi-gel-haloperidol glycine ester (affi-gel-HGE) column, results in a partially purified receptor preparation (designated eluate I) which exhibits a typical D_2 dopaminergic binding profile upon reconstitution (Elazar *et al.*, 1988b). In order to find out whether the receptor at this stage of purification is still coupled to G-proteins, we tested whether it displays D_2 agonist-stimulated GTPase activity. As shown in Figure 2, reconstituted eluate I preparation exhibits GTPase activity which is stimulated ~2-fold by 10 μM NPA. This NPA-induced GTPase activity was inhibited by pertussis toxin and NAD. Similarly, GTPγS binding activity of the affinity purified D_2-receptor was also stimulated by NPA (data not shown). By using [^{35}S]GTPγS and [^{3}H]spiperone binding assays we have found that ~1% of the G proteins

and 40% of the [^{3}H]spiperone binding sites, present in the solubilized membrane preparation, were eluted from the affi-gel-HGE column. The ratio of [^{35}S]GTPγS to [^{3}H]spiperone binding in the reconstituted affinity purified D_2-receptor corresponded to a ratio of 1−1.3 mol G-protein/mol D_2-dopamine receptor.

In order to identify the G-proteins which co-purify with the D_2-receptor upon affinity chromatography on affi-gel-HGE, eluate I was [^{32}P]ADP ribosylated by pertussis toxin. Chromatography on SDS−polyacrylamide gel resulted in two labeled polypeptide bands of 39 and 41 kd (Figure 3a). To further verify that these pertussis toxin-sensitive G-proteins are specifically associated with the D_2-receptor, affinity chromatography of solubilized membranes was performed under conditions which inhibit specifically the adsorption of D_2-receptor to affi-gel-HGE, or others which induce the dissociation of the G-proteins from the receptor. Pre-incubation of the solubilized membranes with spiperone, prior to affinity chromatography, a treatment which has been shown to inhibit the binding of the receptor to the affinity column (Elazar *et al.*, 1988b), resulted in a dramatic decrease in the amount of G-proteins eluted from the column, as was domonstrated by ADP-ribosylation, following the affinity chromatography (Figure 4c). Both the 39 and the 41 kd bands were equally reduced (data not shown). Similarly, pre-treatment of the solubilized membranes with Gpp(NH)p also reduced the amount of bound [^{32}P]ADP (Figure 4d), but not of spiperone binding. In addition, the pertussis toxin-sensitive G-proteins were equally eluted from the affi-gel-HGE column when Gpp(NH)p was used for elution instead of spiperone (Figure 4b). It should be noted that pre-incubation with Gpp(NH)p, prior to affinity chromatography and reconstitution, resulted in a 90−95% decrease in [^{35}S]GTPγS binding, when compared with the binding to control, affinity purified receptor. These data strongly suggest that the two G-proteins are specifically associated with the D_2-receptor and that the receptor−G-protein complex can be dissociated by guanine nucleotides.

Characterization of the G-proteins

As demonstrated above (Figure 3a), pertussis toxin [^{32}P]ADP ribosylated elutate I exhibits two distinct protein bands of 41 and 39 kd on SDS−PAGE. In order to further characterize these two G-proteins, two polyclonal antibody preparations, AS/7 and RV/3 specific for Gi and Go respectively (Goldsmith *et al.*, 1987; Gierschik *et al.*, 1986), were used. Immunoblotting experiments with these antibodies demonstrated that AS/7 recognizes exclusively the 41 kd band and RV/3 recognizes only the 39 kd band (Figure 3b), indicating that the two G-proteins (41 and 39 kd) which co-purify with the D_2-receptor are immunologically related to Gi and Go proteins respectively. In addition, peptide maps obtained from the endogenous 39 and 41 kd proteins were similar to those obtained from purified brain Gi and Go, respectively (Elazar *et al.*, 1989). Although the anti-G-protein antibodies employed were highly specific for Gi and Go proteins respectively, in immunoprecipitation experiments they demonstrated some cross-precipitation. Thus, AS/7 precipitated the 41 kd as well as some of the 39 kd protein band whereas RV/3 precipitated the 39 kd and also part of the 41 kd band (Figure 5). This cross-precipitation suggests that some heterogeneous, and possibly aggregated receptor−G-protein complexes may exist in the affinity purified receptor preparation.

Discussion

The data presented in this work demonstrate the existence of two G-proteins which co-purify with the bovine striatal D_2-dopamine receptor on a haloperidol affinity column. These G-proteins seem to be functionally associated with the D_2-receptor. Several lines of experimental evidence support the notion that the two G-proteins present in the affinity purified receptor preparation (eluate I) are specifically associated with the D_2-receptor. (i) The G-proteins which co-purify with the D_2-dopamine receptor do not adsorb to the haloperidol column under conditions (pre-incubation with spiperone) which prevent the adsorption of the D_2-receptor (Figure 4). Although several proteins, other than the D_2-receptor, bind to the haloperidol resin (Elazar *et al.*, 1988b), only a few, including the G-proteins, are depleted by pre-incubation with spiperone. (ii) Treating the solubilized membranes with guanine nucleotides, known to functionally dissociate receptor−G-protein complexes, also resulted in a depletion of the two G-proteins from the material eluted off the affinity column. It should be noted that these conditions do not affect the interaction of the receptor with the D_2-antagonist affinity column. (iii) The affinity-purified receptor preparation exhibits GTPase activity which is stimulated by a D_2-specific agonist. Futhermore, this stimulation is mediated by a pertussis toxin-sensitive G-protein(s) (Figure 2).

Pertussis toxin-mediated ADP ribosylation of the affinity purified receptor (eluate I), resulted in the labeling of two G-protein bands of 41 and 39 kd. These bands co-migrated on SDS−PAGE with brain Gi and Go respectively (unpublished data). Immunoblotting with two specific antibodies directed against Gi and Go proteins, indicates that the 41 kd polypeptide is related to Gi whereas the 39 kd band is related to Go protein. Since the anti-Gi antibody employed in this study does not discriminate between the various Gi subtypes (Gi1, Gi2 or others) we cannot conclude at this point which of these is associated with the D_2-receptor. The interaction of a Go-related protein (with a mol. wt of 40 kd) with the D_2-receptor both in neurons (Harris-Warrick *et al.*, 1988) and in the anterior pituitary (Senogles *et al.*, 1987) has been previously reported.

The data presented in this study demonstrate that at least two different G-proteins are coupled to the D_2-receptor purified from bovine striatum. These results are consistent with the recent report of Ohara *et al.* (1988), demonstrating that D_2-dopamine receptor from porcine striatal membranes can interact with isolated brain Gi and Go, upon reconstitution. We do not know whether the same species of the D_2-receptor can interact with more than one G-protein or whether there are various subtypes of the receptor, each specific for a particular G-protein. The immunoprecipitation experiment with G-specific antibodies indicated that the anti-Gi-antibody precipitated in addition to the 41 kd protein, also the 39 kd protein and vice versa, anti-Go antibody also precipitated some of the 41 kd protein (Figure 5). A possible explanation for these results would be the presence of aggregates of several receptor−G-protein complexes in the preparation. Further experiments will be needed in order to clarify this issue.

We have shown that the two G-proteins that interact with the D_2-receptor can be eluted from the haloperidol affinity column by Gpp(NH)p (Figure 4). The individual G-proteins can be separated further (Mumby *et al.*, 1988). The availability of the isolated endogenous G proteins and of the purified receptor (Elazar *et al.*, 1988b) should permit us to perform reconstitution experiments and to elucidate the role of the various G-proteins in D_2-receptor functions.

Materials

Materials

[^{3}H]spiperone, [γ-^{32}P]GTP and [^{32}P]NAD were purchased from New England Nuclear. App(NH)p, Gpp(NH)p, Chaps, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (Hepes), dithiothreitol (DTT) were from Sigma. Butaclamol (+) and (−), and NPA were from Research Biochemicals Inc. Pertussis toxin (islet-activated protein) was purchased from List Laboratories (Campbell, CA). Spiperone was a gift from Janssen pharmaceuticals. Specific anti-Gi and anti-Go proteins antibodies (AS/7 and RV/3) were a generous gift from Dr Allen Spiegel from the National Institutes of Health, Bethesda, MD. The AS/7 antibody was elicited against the carboxy-terminal decapeptide of the α subunit of transducin, whereas the RV/3 antibody was elicited against purified Go protein from bovine brain (Goldsmith *et al.*, 1987; Giershik *et al.*, 1986).

Purification, reconstitution and binding assay of D_2-dopamine receptor

Affinity purification of D_2-dopamine receptor on affi-gel-HGE, reconstitution and [^{3}H]spiperone binding assay were performed essentially as described previously (Elazar *et al.*, 1988b,c). Briefly, bovine striatal membranes were solubilized in 50 mM Hepes pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA and 1 mM phenylmethylsulfonyl fluoride (PMSF) (HMEP buffer), supplemented with 10 mM Chaps, 1 M NaCl, 2 mM DTT and leupeptin (10 μg/ml). Following stirring for 1 h at 4°C the suspension was centrifuged at 100 000 *g* for 1 h at 4°C. The supernatant was diluted with 1 vol of HMEP buffer and mixed with affi-gel-HGE (5:1, v/v). The mixture was shaken gently for 2 h at 22°C. Unbound material was removed and the gel was washed three times, each with 10 bed volumes of HMEP buffer supplemented with 1 mM Chaps, 100 mM NaCl and 1 mM DTT (wash buffer). Elution was carried out with 10 μM spiperone in wash buffer for 40 min at 22°C. The eluted material, designated eluate I, was chromatographed on Sephadex G-50 mini-column in order to remove the free ligand, prior to assaying.

For reconstitution, desalted elute I (200 μl) was incubated with HMEP buffer containing 100 mM NaCl, bovine serum albumin (BSA, 2 mg/ml), sonicated phosphatidylcholine (0.7 mg/ml) and octyl-β-D-glucoside (0.8%) in a final volume of 0.5 ml, for 15 min at 4°C and then dialyzed against 1000 vols of HMEP buffer containing 100 mM NaCl and Bio-Beads SM-2 resin (2 g/l) for 16 h at 4°C.

[^{35}S]GTPγS binding

Samples of D_2-dopamine receptor were incubated for 30 min at 30°C with [^{35}S]GTPγS (5−1000 nM) in a final volume of 50 μl of HMEP buffer containing 100 mM NaCl, 1 mM App(NH)p and 1 mM DTT. The bound [^{35}S]GTPγS was separated on nitrocellulose filter. The filters were washed three times each with 5 ml of cold HMEP buffer containing 100 mM NaCl. Non-specific binding was determined in the presence of 10 μM GTPγS.

GTPase activity

GTPase activity of the affinity purified receptor preparation, eluate I (Elazar *et al.*, 1988b), was measured as described by Brandt and Ross (1985) with some modifications. Prior to measuring GTPase activity, eluate I was concentrated and reconstituted (Elazar *et al.*, 1988b). Reconstituted eluate I (0.02 ml) was incubated at 30°C with the same volume of reaction mixture containing: 40 mM Hepes, pH 7.2, 10 mM $MgCl_2$, 0.2 mM ATP, 1 mM EDTA, 2 mM DTT, 0.4 mg/ml BSA, 0.5 mM App(NH)p and 2 μM [γ-^{32}P]GTP. Creatine phosphate (10 mM) and creatine phosphokinase (0.4 mg/ml) were added for the regeneration of ATP. At the indicated time points the reaction mixture was quenched with 0.5 ml charcoal suspension (5% w/v) in 50 mM NaH_2PO_4. The mixture was centrifuged and the radioactivity in 0.2 ml aliquots of the charcoal suspension was determined.

Pertussis toxin ADP ribosylation

ADP ribosylation of eluate I was performed by modification of the procedure of Neer *et al.* (1984). Briefly, eluate I (0.3 μg protein) was incubated at 30°C with reactivated pertussis toxin (0.5 μg pre-incubated in 10 mM DTT for 15 min at 30°C) in a reaction mixture containing: 75 mM Tris pH 8.0, 10 mM thymidine, 1 mM ATP, 2.7 mM $MgCl_2$, 1 mM DTT, 1 mM EDTA, 2.5 mM [^{32}P]NAD, 5 mM L-α-dimyristoylphosphatidylcholine, 7.5 mM phosphoenolpyruvate and 25 μg/ml pyruvate kinase. The reaction was stopped by addition of 0.01 ml of 10% SDS and heating to 90°C for 10 min. The samples were further treated with 1 mM *N*-ethylmaleimide for 10 min and mixed with sample buffer (Laemmli, 1970) followed by SDS−PAGE on slab gels (10% acrylamide, 0.13% *N,N'*-methylene-bis-acrylamide, 28 cm long). For immunoprecipitation of the labeled G-proteins from eluate I, ADP ribosylation was stopped under non-denaturing conditions by addition of 2 vol of buffer containing 1 mM NAD.

Immunoblotting

Samples for immunoblotting were separated by SDS−PAGE as described above. Electrophoretic transfer of the resolved proteins from the polyacrylamide gel onto nitrocellulose membrane filters (0.45 μM) was performed according to Wilson *et al.* (1984). The filters were then quenched in 1% (w/v) hemoglobin in 10 mM phosphate-buffered saline (PBS), pH 7.4 quenching buffer at 22°C. The membrane filters were incubated for 3 h at 22°C with a 1:100 dilution of the different antibodies in the quenching buffer. After five washes with PBS containing 0.03% (v/v) Tween 20, the membrane filters were incubated with goat anti-rabbit immunoglobulin conjugated to peroxidase and subsequently developed using diaminobenzidine as a substrate.

Immunoprecipitation

Pertussis toxin [^{32}P]ADP ribosylated eluate I was used for immunoprecipitation of the different G-proteins. The ^{32}P-labeled material was incubated with a 1:50 dilution of the appropriate serum for 3 h at 22°C.The antibody−antigen complex was precipitated with Sepharose protein A (Pharmacia). Following centrifugation the pellet was washed, dissolved in Laemmli sample buffer and boiled for 5 min. The different samples were analyzed by SDS−PAGE as described above.

Acknowledgements

We thank Dr A.Spiegel from the National Institutes of Health, Bethesda, MD for fruitful discussions and for the generous gift of specific anti-Gi and anti-Go antibodies, and Janssen Pharmaceuticals for spiperone. This research was supported by grants from Enosh and Harry Stern Foundations, from the Henry and Francoise Glasberg Foundation for Research on Cellular Diseases, and from the Rockefeller-Weizmann Collaboration Trust Fund.

References

Brandt,D.R. and Ross,E.M. (1985) *J. Biol. Chem.*, **260**, 266−272.

Canonico,P.L., Valdenergo,C.A. and MacLeod,R.M. (1982) *Endocrinology*, **111**, 347−349.

Cooper,D.M.F., Bier-Laning,C.M., Holford,M.K., Ahlijanian,M.K. and Zahniser,N.R. (1986) *Mol. Pharmacol.*, **29**, 113−119.

Cote,T.F., Frey,E.A., Grewe,C.W. and Kebabian,J.W. (1983) *J. Neurol. Trans. Suppl.*, **18**, 139−147.

Cote,T.E., Frey,E.A. and Sekura,R.D. (1984) *J. Biol. Chem.*, **259**, 8693−8698.

Delbeke,D. and Dannies,P.S. (1985) *Endocrinology*, **117**, 439−446.

Elazar,Z., Siegel,G., David,C., Kanety,H. and Fuchs,S. (1988a) *Soc. Neurosci. Abstr.*, **14**, 933.

Elazar,Z., Kanety,H., David,C. and Fuchs,S. (1988b) *Biochem. Biophys. Res. Commun.*, **156**, 602−609.

Elazar,Z., Kanety,H., Schreiber,M. and Fuchs,S. (1988c) *Life Sci.*, **42**, 1987−1993.

Elazar,Z., Siegel,G., Kanety,H. and Fuchs,S. (1989) In Evangelopoulos,A.E. (ed.), *Receptors Membrane Transport and Signal Transduction*. Springer-Verlag. Nato ASI Series H29, pp. 242−250.

Enjalbert,A., Sladeczek,F., Guillon,G., Bertrand,P., Shu,C., Epelbaum,J., Garcia-Sainz,A., Jard,S., Lombard,C., Kordon,C. and Bockaert,J. (1986) *J. Biol. Chem.*, **261**, 4071−4075.

Fujita,N., Nakahiro,M., Fukuchi,I., Saito,K. and Yoshida,H. (1985) *Brain Res.*, **333**, 231−236.

Gierschik,P., Milligan,G., Pines,M., Goldsmith,P., Codina,J., Klee,W. and Spiegel,A.M. (1986) *Proc. Natl. Acad. Sci. USA*, **88**, 2258−2262.

Gilman,A.G. (1987) *Annu. Rev. Biochem.*, **56**, 615−649.

Goldsmith,P., Gierschik,P., Milligan,G., Unson, Vinitsky,R., Molech,H.L. and Spiegel,A.M. (1987) *J. Biol. Chem.*, **262**, 14683−14688.

Harris-Warrick,R.M., Hammond,C., Paupardin-Tritsch,D., Homburger,V., Rout,B., Bockaert,J. and Gerschenfeld,H.M. (1988) *Neuron*, **1**, 27−32.

Ingram,C.D., Bicknell,R.J. and Mason,W.T. (1986) *Endocrinology*, **119**, 2508–2518.
Israel,J.M., Jaquet,P. and Vincent,J.D. (1985) *Endocrinology*, **117**, 1448–1455.
Judd,A.M., Koide,K., Schettini,G., Login,I.S., Hewelett,E.L., Yosumoto,T. and MacLeod,R.M. (1985) *Endocrinology*, **117**, 1215–1221.
Katada,T., Oinuma,M., Kusakabe,K. and Ui,M. (1987) *FEBS Lett.*, **213**, 353–358.
Lacey,M.G., Mercuri,N.B. and North,R.A. (1987) *J. Physiol. Lond.*, **392**, 397–416.
Laemmli,U.K. (1970) *Nature*, **227**, 680–685.
Milligan,G. (1988) *Biochem. J.*, **255**, 1–13.
Mumby,S., Pang,I.H., Gilman,A.G. and Sternweis,P.C. (1988) *J. Biol. Chem.*, **263**, 2020–2026.
Neer,E.J., Lok,J.M. and Wolf,L.G. (1984) *J. Biol. Chem.*, **259**, 14222–14229.
Ohara,K., Haga,K., Berstein,G., Haga,T. and Ichiyama,A. and Ohara,K. (1988) *Mol. Pharmacol.*, **33**, 290–296.
Onali,P., Olianas,M.C. and Gessa,G.L. (1985) *Mol. Pharmacol.*, **28**, 138–145.
Pizzi,M., D'Agostini,F., DaPrada,M., Spano,P.F. and Haefely,W.E. (1987) *Eur. J. Pharmacol.*, **136**, 263–264.
Sasaki,K. and Sato,M. (1987) *Nature*, **325**, 259–262.
Seeman,P., Watanabe,M., Grigoriadis,D., Tedesco,J.L., George,S.R., Svensson,U., Nilsson,J.L.G. and Neumeyer,J.L. (1985) *Mol. Pharmacol.*, **28**, 391–399.
Senogles,S.E., Benovic,J.L., Amlaiky,N., Unson,C., Milligan,G., Vinitsky,R., Spiegel,A.M. and Caron,M.G. (1987) *J. Biol. Chem.*, **262**, 4860–4867.
Sibley,D.R. and Creese,I. (1983) *J. Biol. Chem.*, **258**, 4957–4965.
Simmonds,S.H. and Strange,P.G. (1985) *Neurosci. Lett.*, **60**, 267–272.
Simmonds,S.H., Strange,P.G., Hall,A.W. and Taylor,R.J.K. (1986) *Biochem. Pharmacol.*, **35**, 731–735.
Spiegel,A.M. (1987) *Mol. Cell. Endocrinol.*, **49**, 1–16.
Stoof,J.C. and Kebabian,J.W. (1984) *Life Sci.*, **35**, 2281–2296.
Stoof,J.C., Werkman,T.R., Lodder,J.C. and De Vlieger,Th.A. (1986) *Trends Pharmacol. Sci.*, **7**, 7–9.
Wilson,P.T., Gershoni,J.M., Hawrot,E. and Lentz,T.L. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 2553–2557.

Received on January 30, 1989; revised on April 19, 1989