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Original paper

INTERACTION OF INTRACELLULAR LOOPS OF DOPAMINE D_1 RECEPTOR WITH G PROTEIN SUBUNITS

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Summary: A simple and rapid method for qualitative and quantitative estimation of Gα subunit interactions with the second and the third intracellular loop, as well as with C-terminal part of human D_1 dopamine receptor has been developed. For this purpose, D_1-ICL_2 and D_1-ICL_3 were cloned in pGEX-2T vector and expressed in *E. coli* BL21 as fusion proteins with glutathione-S-transferase (D_1-ICL_2-GST and D_1-ICL_3-GST). C-terminal part was cleaved into two fragments which were cloned in pGEX-2T and expressed in *E. coli* BL21 DE3 as fusion proteins with glutathione-S-transferase (D1-CTSF-GST and D1-CTLF-GST). The resulting soluble constructs were purified by affinity chromatography on glutathione-Sepharose. Gα subunits were expressed and purified as His-tagged proteins (Gαo and $Gαi_1$ in *E. coli* BL21 DE3 and Gαs in *E. coli* JM 109). For quantitative assay, varying concentrations of pure His-tagged Gα subunits were immobilized on His-Bind resin and titrated with fusion proteins and the interactions were estimated by a colorimetric assay for GST activity determination. Similar assay was employed to qualitatively demonstrate the interactions. For this purpose pure fusion proteins were immobilized on glutathione-Sepharose in known concentrations and treated with known concentrations of pure His-tagged Gα subunits. Thus created complexes were eluted from glutathione-Sepharose and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). It was shown that D_1-CTSF interacts specifically with Gαs subunit, and D_1-CTLF with Gαo. No other interactions were observed. Based on saturation binding analyses, Kd values in nanomolar range of concentrations demonstrated the highest binding affinity of His-Gαs for D_1-CTSF-GST and of His-Gαo for D_1-CTLF-GST.

Key words: human D_1 dopamine receptor, intracellular loops, G proteins, Gα subunits, interactions.

Introduction

Dopamine is a neurotransmitter which plays an important role in both central and peripheral nervous system. There are at least five different dopamine receptor subtypes involved in dopaminergic signal transduction across cellular membranes via coupling to G proteins (1 3). D_1-like dopamine receptors D_1A (or D_1) and D_1B (or D_5) couple to the activation of adenylyl cyclase (3). These receptors are also coupled to other signalling systems such as stimulation of phospholipase C (4), translocation of protein kinase C (5), activation of arachidonic acid cascade system (6), inhibition of Na/K-ATPase activity (7) and stimulation of K^+ ion efflux (8). Some of these effects are mediated *via* coupling of D1 receptors to G proteins especially Gαo and Gαs (9, 10), but also to Gαq protein (11). Interactions of D_1 dopamine receptors with α-subunits of G proteins were studied by several authors. Some authors (12, 13) indicated that amino acid sequence of ICL2 and ICL3 of the D_1 receptor mediate the interaction with Gα proteins. Studies with synthetic peptides (14) suggested that the peptides with amino acid sequence corresponding to the parts of the second and the third cytoplasmic loop, as well as the part of C-terminus of rat D_1 receptor play a role in coupling of this receptor to Gαs protein.

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Divergence in the third cytoplasmic loop and also in C-terminal part between D_1 and D_5 dopamine receptors (15), studies on other GPC-receptors (16 18) and the effect of these receptors on multiple signalling systems led to the conclusion that they are able to couple differentially to G proteins.

These facts prompted us to examine which part(s) of human D_1 receptor is responsible for interactions with Gα subunits. In this study we report the results on qualitative and quantitative estimation of interactions between the parts of the D_1 receptor and Gαs, Gαo and $G\alpha i_1$ proteins.

Materials and methods

Materials

Plasmid DNA encoding human D_1 dopamine receptor was a generous gift of Dr. H.H.M. Van Tol (Dept. Psychiat. Pharmacol., Univ. Toronto). Plasmids NPT7-5, encoding $G\alpha i_1$-His and Gαo-His, were kindly provided by Dr. M. Linder (Washington Univ. Sch. Med., St. Louis, MO, USA).

The other chemicals used in this work were from the following sources: expression vector pGEX-2T, restriction endonucleases, T4 DNA ligase and alkaline phosphatase BioLabs New England; *Pfu* DNA polymerase Stratagene; DNA-se Boehringer; lysosyme Serva; ATP, GDP, DNTPs, PMSF, ampicillin - Sigma; glutathione (GSH) and Na-deoxycholate Merck; His-Bind Resin Qiagen; CDNB Squib Bristol Labs; GSH-Sepharose Pharmacia LKB; Bacto peptone, yeast extract and bacto agar Difco.

Cloning of the second and third intracellular loops and C-terminal fragments of the D_1 receptor into pGEX-2T. Standard procedures were used for DNA manipulations (19). Fragments of human D_1 dopamine receptor were amplified by the polymerase chain reaction (PCR). To obtain these fragments, the following pairs of primers containing plasmid DNA encoding human D_1 dopamine receptor were used:

a) for D1- ICL2 (amino acids 120-138)
N-terminus 5'-CGCGGATCCGACAGGTATTGGG-CTATCTCCAGC-3'
C-terminus 5'-CCGGAATTCCTTGGGGGTCATC-TTTCTCTCATACC-3'

b) for D1- ICL3 (amino acids 221-277)
N-terminus 5'-GCGCGGATCCAGGATCTACAGGA-TTGCTCAG-3'
C-terminus 5'-GCCGGAATTCCTTCAGGACTTTA-GTTTCTCTGAA-3'

c) for D1- CTSF (amino acids 338-359)
N-terminus 5'-CGCGGATCCCGCAAGGCATTTT-CAACCCTGTTAGGA TGCTACAGACTCTGCCC- 3'
C-terminus 5'-CCGGAATTCGTCTCTCTATGGCA-TTATTCGTC-3'

d) for D1- CTLF (amino acids 352-446)
N-terminus 5'-CGCGGATCCCGCCTTGCGACGA-ATAATGCCATAGA GA-3'
C-terminus 5'-CCGGAATTCGGTGAGGTGCTGAC-CGTTTTGTGTGATG GG-3'

To avoid introduction of errors during subcloning, all PCR products were cut with BamHI and EcoRI and cloned into BamHI-EcoRI sites of the prokaryotic expression vector pGEX-2T. The resulting clones were cut with BamHI and EcoRI and tested by DNA agarose gel electrophoresis for length of the fragments.

Expression and purification of fusion proteins

E. coli BL21 and BL21 DE3 cells were maintained and transformed with:

BL21	pGEX-2T-D_1-ICL_2
	pGEX-2T-D_1-ICL_3
BL21 DE3	pGEX-2T-D_1-CTSF
	pGEX-2T-D_1-CTLF

using $CaCl_2$ method (19). The cells were grown in Luria-Bertani broth supplemented with ampicillin (final conc. 100 mg/mL) at 37 °C until A_{600} reached 0.5 0.7, then the expression was induced with isopropylthiogalactoside (IPTG; final conc. 0.1 mmol/L) and glucose was added (final conc. 20 mmol/L). Temperature was decreased to 25 °C, for D_1 ICL_2 and D_1 ICL_3, and to 26 °C for D_1-CTSF and D_1-CTLF. The cells were harvested after 6 h (3000×g, 10 min, Sorvall SS-1 centrifuge), resuspended in the solution containing (in mmol/L): NaCl 140, KCl 2.7, Na_2HPO_4 10, KH_2PO_4 18, pH 7.30 (PBS; 50 mL buffer per mL culture) and incubated (15 min, 25 °C) with the solution consisting of lysosyme (final conc. 0.2 mg/mL), Triton X-100 (final conc. 0.1%) and phenylmethylsulfonylfluoride (PMSF; final conc. 17 mg/L). After that, Na-deoxycholate (final conc. 1.6 mg/mL), $MgCl_2$ (final conc. 10 mmol/L) and DNA-se (final conc. 20 mg/mL) were introduced and the incubation continued (15 min, 25 °C). The lysates were centrifuged (13000×g, 20 min, Sorvall SS-1) and supernatants loaded onto GSH-Sepharose (1.0 mL gel *per* 1000 mL culture) equilibrated with 10 vol. of ice-cold PBS containing 1.0 mmol/L EDTA. Proteins were eluted with 10 mmol/L GSH, 50 mmol/L Tris, pH 8.0. The fractions containing fusion proteins were pooled and dialyzed overnight against 1.0 mmol/L EDTA, 10 mmol/L Tris, pH 8.0, at 4 °C. Purified proteins were concentrated by PEG-20 000 and stored at 20 °C in 40% (v/v) glycerol until used.

Expression and purification of His-Gα proteins

This was done exactly as described by Lee et al. (20).

1-Chloro-2,4-dinitrobenzene (CDNB) assay for GST-fusion protein activity determination

This was performed as suggested by Pabst et al. (21) and Simonović et al. (22).

Assay for quantitative estimation of His-Gα subunit interactions with fusion proteins

His-Bind Resin was equilibrated with the solution of 0.1% ovalbumin and 10 mmol/L Tris, pH 7.4, by repeated centrifugations (2000×g, 1 min, Fisher Sci. microfuge). Varying concentrations of His-Gαs, His-Gαo, or His-Gαi_1 (0.019 14.286 mmol/L, 0.013 13.636 nmol/L and 0.012 14.634 nmol/L, respectively) prepared in the same solution and preincubated with 1.0 mmol/L GDP and 5 mmol/L $MgCl_2$, were mixed with 40 mL of His-Bind Resin. The mixtures (final vol. 90 mL) were incubated (24 °C, 60 min, constant shaking) and after that, unbound His-proteins were removed by double washing with the above solution. Fusion proteins (D_1-ICL_2-GST-14 nmol; D_1-ICL_3-GST 10 nmol; D_1-CTSF-GST-21 nmol; D_1-CTLF-GST 16 nmol) were added and incubated (24 °C, 60 min, constant shaking). Unbound fusion proteins were removed by double washing with ice-cold 10 mmol/L Tris-HCl, pH 7.4. The samples were subjected to CDNB assay. GST reaction was terminated after 40 min with 90 μL of 2 mol/L HCl. After that, the mixtures were centrifuged (2000xg, 2 min, Fisher Sci. microfuge) and absorbancy (340 nm) in the resulting supernatants was recorded.

Assay for qualitative estimation of His-Gα subunits interaction with fusion proteins

Glutathione-Sepharose was equilibrated with of binding buffer (0.3% BSA, 10 mmol/L Tris pH 7.4, 0.1% Triton X-100, 360 mmol/L NaCl). Fusion proteins (12 μg) were mixed with 40 μL of Glutathione-Sepharose and incubated for 45 min (25 °C, constant shaking). His-Gα subunits (20 mg) were added and the incubation continued for 60 min. Unbound proteins were removed by 3 × 1.0 mL washing with ice cold binding buffer and centrifugation. Electrophoretic sample buffer (40 μL) was added and the samples were prepared for SDS-PAGE electrophoresis (23).

Data analysis

Saturation binding data were analyzed and graphically displayed by nonlinear curve fitting using the Microcal Origin 6.0 software. Kd values were calculated using the same program.

Miscellaneous

Proteins were determined by micro method of Lowry et al. (24), using BSA as a reference. Degree of protein purity was checked by SDS-PAGE electrophoresis (23).

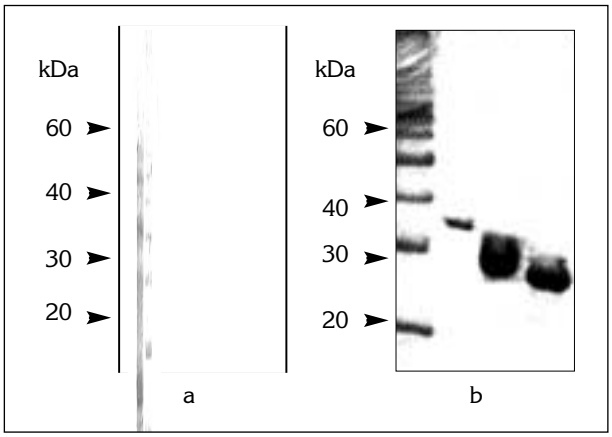

Figure 1 SDS-PAGE of purified ICL2-GST and ICL3-GST C-terminal parts of GST-fragments of human D_1dopamine receptor.
a. Lanes: 1-ICL2-GST eluate; 2-GibcoBRL marker; 3-GibcoBRL marker; 4-ICL3-GST eluate, b. Lanes: 1-GibcoBRL marker; 2-GST-CTLF-D_1 eluate; 3-GST-CTSF-D_1 eluate; 4-glutathione-S-transferase.

Results

Expression and purification of fusion proteins

D_1-ICL_2, D_1-ICL_3, D_1-CTSF and D_1-CTLF were cloned into BamHI-EcoRI sites of the prokaryotic expression plasmid pGEX-2T. *E. coli* BL21 strain was used as a host for pGEX-2T-D_1-ICL_2 and pGEX2T-D_1-ICL_3 and *E. coli* BL21 DH_3 strain for pGEX2T-D_1-CTLF. To avoid extensive proteolysis of fusion proteins, the expression proceeded at 25 °C for D_1-ICL2-GST and D_1-ICL_3-GST and at 26 °C for D_1-CTSF-GST and D_1-CTLF-GST. Under these conditions the fusion proteins were successfully expressed and purified (*Figure 1*) in soluble form yielding 1.04 mg D_1-ICL_2-GST per liter bacterial culture (final conc. 0.23 mg/mL in 4.5 mL), 0.36 mg D_1-ICL3-GST per mL bacterial culture (final conc. 0.8 mg/mL), 2.34 mg D_1-CTSF-GST per mL bacterial culture (final conc. 0.52 mg/mL) and 0.68 mg D_1-CTLF-GST per mL bacterial culture (final conc. 0.17 mg/mL in 4.0 mL).

Expression of Gα subunits

Gα subunits were expressed as His-tagged proteins all being tagged at C-terminus and purified in soluble form to provide 2.04 mg Gαs/L bacterial culture (0.51 mg/mL in 4.0 mL); 2.15 mg Gαo/L bacterial culture (0.53 mg/mL in 4.0 mL) and 1.88 mg Gαi_1/L bacterial culture (0.47 mg/mL in 4.0 mL).

Quantification of His-Gα subunit interactions with fusion proteins

Interactions of soluble form of fusion proteins with Gα subunits were measured using His-Bind Resin. GST activity was determined by CDNB assay (21,

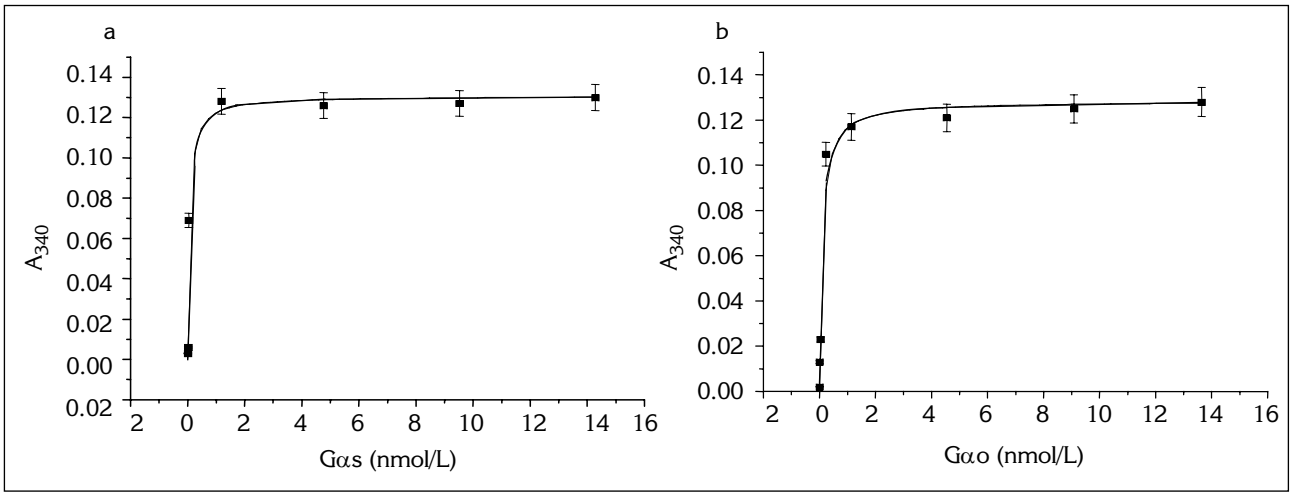

Figure 2 Binding curves of Gαs and Gαo to intracellular D1 receptor fragments.
Graph a. Saturation binding curve of Gαs-His-GDP binding to GST-CTSF-D1. Concs. of His-Gαs ranged from 0.019 14.2 nmol/L and that of GST-CTSF-D_1 was 0.21 μmol/L. Various concs. of His-Gαs, pre-incubated with 1 μmol/L GDP were prepared to final vol. of 85 μL and incubated with 30 μL of 50 % His-Bind Resin suspension. Graph b. Saturation binding curve of Gαo-His-GDP binding to GST-CTSF-D_1. Concs. of Gαo-His-GDP ranged from 0.013 13.6 nmol/L, and that of GST-CTSF-D_1 was 0.16 μmol/L. Final GDP conc. of 1.0 mmol/L was used. Gαs-His-GDP was employed within the range of concentrations from 0.019 14.286 nmol/L. The results are the means ±S.E.M. from at least 3 experiments done in triplicate.

Table I Kd values of GST-CTSF-D_1 interaction with His-Gαs and GST-CTLF-D_1 interaction with His-Gαo

Interaction	Kd (nmol/L)
His-Gαs/GST-CTSF	0.07 ± 0.02
His-Gαo/GST CTLF	0.10 ± 0.03

22). Various concentrations of His-Gα proteins were immobilized on His-Bind Resin and titrated with fusion proteins. The results presented as saturation binding curves are shown in *Figure 2, graphs a e*. Kd values for the interaction of D_1-CTSF-GST with His-Gαs and D_1-CTLF-GST with His-Gαo, calculated from saturation binding curves are listed in *Table I*. No other interactions were recorded (*Figure 2, graphs c e*).

It can be seen (*Figure 2, graphs a* and *b*; *Table I*) that His-Gαs and His-Gαo have the highest binding affinity at D_1-CTSF-GST and D_1-CTLF-GST, while expressing no affinity to bind at $Gαi_1$. Also, D_1-ICL_2-GST and D_1-ICL3-GST did not interact with either of Gα-subunits.

Qualitative estimation of His-Gα subunit interaction with fusion proteins

Interaction of fusion proteins D_1-CTSF-GST and D_1-CTLF-GST with Gα subunits were checked using GSH-Sepharose. Known concentrations of fusion proteins were immobilized on GSH-Sepharose and treated with known concentrations of Gα subunits. The results obtained by SDS-PAGE are shown in *Figure 3*. As demonstrated by the above assay, D_1-CTSF-GST

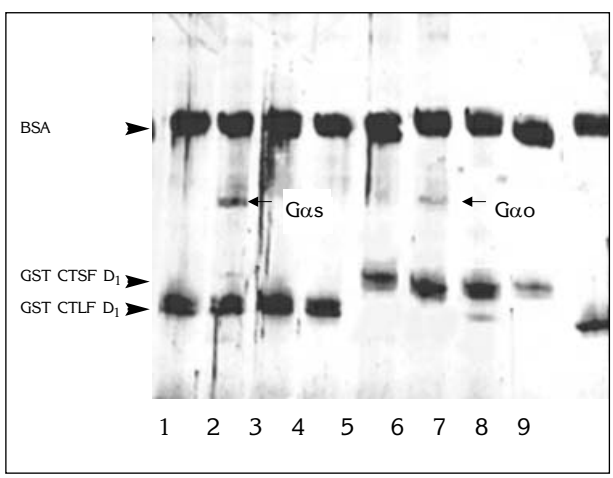

Figure 3 SDS-PAGE showing interactions of Gα subunits with intracellular fragments of C-terminal of human D_1 dopamine receptor. Lines: 1 - GST-CTSF-D_1; 2 - GST-CTSF-D_1 + Gαs-His; 3 - GST-CTSF-D_1 + Gαo-His; 4 - GST-CTSF-D_1 + $Gαi_1$-His; 5 - GST-CTLF-D_1; 6 - GST- CTLF-D_1 + Gαs-His; 7 - GST- CTLF-D_1 + Gαo-His; 8 - GST- CTLF-D_1 + $Gαi_1$-His; 9 - Glutathione-S-transferase. GST-fusion proteins (12 mg) were mixed with 40 mL of Glutathione--Sepharose equilibrated in binding buffer (0.3% BSA, 10 mmol/L Tris pH 7.4, 0.1% Triton X-100, 360 mmol/L NaCl) and incubated for 45 min at 25 °C with constant agitation. His-Gα subunits (20 mg) were added and the incubation continued for 60 min. Unbound proteins were removed by 3 × 1 mL washing by centrifugation with ice-cold binding buffer. Glutatione-Sepharose pellets were treated with 40 μL Laemmli sample buffer (23) and the supernatants were run on 12% SDS-PAGE.

and D_1-CTLF-GST expressed the highest affinity for the binding at Gαs and Gαo subunits.

Discussion

Sidhu et al. (25) and Kimura et al. (9) showed that D_1 dopamine receptor couples to Gs and Go proteins. Several studies were concentrated on the examinations of which parts of the D_1 receptor interact with G proteins (12 14). All these authors pointed out the importance of the second and third intracellular loops, as well as of C-terminal part of this receptor in coupling to G proteins. However, procedures employed were rather expensive and time consuming.

In this work the method of Simonović et al. (22) was applied, previously shown to be both rapid and simple for qualitative and quantitative analyses of the interactions of the third intracellular domain of the D_{2s} receptor with Gi proteins. Using this method and bearing in mind the results of the above authors, we attempted to show which part(s) of the human D_1 dopamine receptor plays a role in coupling to G proteins.

All fragments of the D_1 receptor were cloned in pGEX-2T plasmid and in this way fusion proteins with glutathione-S-transferase (GST) were created. Such constructs facilitated purification of the D_1 receptor fragments and enabled to estimate the interactions by measuring the activity of GST, as an active enzyme. All Gα subunits were expressed as His-tagged proteins and purified on His-Bind Resin which was used for immobilization of these proteins in quantitative assay.

Our results show that just C-terminal part of the human D_1 dopamine receptor, interacts specifically with Gαs and Gαo proteins as suggested earlier by Konig and Gratzel (14) and quite recently by Jackson et al. (26). Konig and Gratzel (14) demonstrated that synthetic peptides, with amino acid sequence of the second and third intracellular loop and C-terminal part of rat D_1 dopamine receptor interact with Gαs protein. The data of Jackson et al. (26) who pointed to the role cytoplasmic tails of human D_1 and D_5 dopamine receptors play in coupling to G proteins are in the accordance with our results reported in the present study.

However, although several authors (12, 13) suggested that the second and third intracellular loop of the D_1 dopamine receptor are important for the coupling to G proteins, our results did not support such an opinion. It is very interesting to note that two different fragments of C-terminal part of the human D_1 dopamine receptor (D_1-CTSF and D_1-CTLF) take part in coupling to two different Gα proteins (Gαs and Gαo). This leads to a hypothesis that different Gα proteins are coupled to different sequences within the same receptor, but to prove this assumption further studies are necessary.

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INTERAKCIJA INTRACELULARNIH PETLJI DOPAMINSKOG D_1 RECEPTORA SA PODJEDINICAMA G PROTEINA

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Kratak sadržaj: Razvijena je jednostavna i brza metoda za kvalitativano i kvantitativno određivanje interakcija podjedinica Gα proteina sa drugom i trećom intracelularnom petljom, kao i sa terminalnim krajem D_1 dopaminskog receptora čoveka. U tu svrhu su D_1-ICL_2 i D_1-ICL_3 klonirani u vektor pGEX-2T i eksprimirani u *E. coli* BL21 kao fuzioni proteini sa glutation-S-transferazom (D_1-ICL_2-GST i D_1-ICL_3-GST). C-terminalni deo je rastavljen u dva fragmenta koji su klonirani u pGEX-2T i eksprimirani in *E. coli* BL21 DE3 kao fuzioni proteini sa glutation-S-transferazom (D_1-CTSF-GST i D_1-CTLF-GST). Dobijeni solubilni konstrukti su prečišćavani afinitetnom hromatografijom na glutation-sefarozi. Podjedinice Gα su eksprimirane i prečišćene kao His-obeleženi proteini (Gαo i $Gαi_1$ u *E. coli* BL21 DE3, a Gαs u *E. coli* JM 109). Za kvantitativno određivanje su različite koncentracije prečišćenih His-obeleženih podjedinica Gα imobilisane na smoli His-Bind i titrovane fuzionim proteinima, a interakcije su određivane kolorimetrijskim postupkom za određivanje aktivnosti GST. Za kvalitativno dokazivanje interakcija je primenjen sličan pristup. U tu svrhu su poznate koncentracije fuzionih proteina imobilisane na glutation-sefarozi i tretirane poznatim koncentracijama prečišćenih His-obeleženih podjedinica Gα. Tako tretirani kompleksi su eluirani sa glutation-sefaroze i analizirani SDS elektroforezom na poliakrilamidnim gelovima (SDS-PAGE). Pokazano je da D_1-CTSF specifično interaguje sa podjedinicom Gαs, a D_1-CTLF sa podjedinicom Gαo. Druge interakcije nisu zapažene. Kd vrednosti izračunate na osnovu eksperimenata vezivanja liganda do zasićenja bile su u nanomolarnom opsegu koncentracija, što ukazuje na najviši afinitet vezivanja His-Gαs za D_1-CTSF-GST i His-Gαo za D_1-CTLF-GST.

Ključne reči: D_1 dopaminski receptor čoveka, intracelularne petlje, G proteini, podjedinice Gα, interakcije.

References

1. Gingrich JA, Caron MG. Recent advances in the molecular biology of dopamine receptors. Ann Rev Neurosci 1993; 16: 299 321.
2. Civelli O, Bunzow JR, Grandy DK. 1993. Molecular diversity of the dopamine receptors. Ann Rev Pharmacol Toxicol 1993; 33: 281 307.
3. Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998; 78: 189- 225.
4. Undie AS, Weinstock J, Sarau HM, Friedman E. Evidence for a distinct D_1-like dopamine receptor that couples to activation of phosphoinositide metabolism in brain. J Neurochem 1994; 62, 2045 2048.
5. McMillan MK, He XP, Hong JS, Pennypacker KR. Dopamine stimulates [3H]phorbol 12,13-dibutyrate binding in cultured striatal cells. J Neurochem 1992; 58: 1308 12.
6. Piomelli D, Pilon C, Giros B, Sokoloff P, Martres MP, Schwartz JC. Dopamine activation of the arachidonic acid cascade as a basis for D_1/D_2 receptor synergism. Nature, London 1991; 353: 164 7.
7. Bertorello A, Aperia A. Regulation of Na^+-K^+-ATPase activity in kidney proximal tubules: involvement of GTP binding proteins. Am J Physiol 1989; 256: Pt 2, F57 62.
8. Laitinen JT. Dopamine stimulates K^+ efflux in the chick retina via D_1 receptors independently of adenylyl cyclase activation. J Neurochem 1993; 61: 1461 9.
9. Kimura K, White BH, Sidhu A. Coupling of human D-1 dopamine receptors to different guanine nucleotide binding proteins. Evidence that D-1 dopamine receptors can couple to both Gs and G(o). J Biol Chem 1995; 270: 14672 8.
10. Cai G, Gurdal H, Smoth C, Wang H-Y, Friedman, E. Inverse agonist properties of dopaminergic antagonists at the D_{1A} dopamine receptor: Uncoupling of the D_{1A} dopamine receptor from Gs protein. Mol Pharmacol 2001; 56: 989 96.
11. Jin L-Q, Wang H-Y, Friedman E. Stimulated D1 dopamine receptors couple to multiple Gα proteins in different brain regions. J Neurochem 2001; 78: 981 90
12. O'Dowd BF, Hnatowich M, Regan JW, Leader WM, Caron MG, Lefkowitz RJ. Site-directed mutagenesis of the cytoplasmic domains of the human β_2-adrenergic receptor. Localization of regions involved in G protein-receptor coupling. J Biol Chem 1988; 263: 15985 92.
13. Ostrowski J, Kjelsberg MA, Caron MG, Lefkowitz RJ. Mutagenesis of the β_2-adrenergic receptor: How structure elucidates function. Ann Rev Pharmacol Toxicol 1992; 32: 167 83.
14. Konig B, Gratzel M. Site of dopamine D1 receptor binding to Gs protein mapped with synthetic peptides. Biochim Biophys Acta 1994; 1223: 261 6.
15. Sugamori KS, Demchyshyn LL, Chung M, Niznik HB. D_{1A}, D_{1B} and D_{1C} dopamine receptors from *Xenopus laevis*. Proc Natl Acad Sci U S A 1994; 91: 10536 40.
16. Okuma Y, Reisine T. Immunoprecipitation of α2a-adrenergic receptor-GTP-binding protein complexes using GTP-binding protein selective antisera. Changes in receptor/GTP-binding protein interaction following agonist binding. J Biol Chem 1992; 267: 14826 31.
17. Law SF, Yasuda K, Bell GI, Reisine T. Giα3 and G(o)α selectively associate with the cloned somatostatin receptor subtype SSTR2. J Biol Chem 1993; 268: 10721 7.
18. Allgeier A, Offermanns S, Van Sande J, Spicher K, Schultz G, Dumont JE. The human thyrotropin receptor activates G-proteins Gs and Gq/11. J Biol Chem 1994; 269: 13733 5.
19. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbour Lab. Cold Spring Harbour, NY, 1989; 1 1626
20. Lee E, Linder ME, Gilman AG. Expression of G-Protein α Subunits in *Escherichia coli*, Meth Enzymol 1994; 237: 146 63.
21. Pabst MJ, Habig WH, Jakoby WB. Glutathione-S-transferase A. A novel kinetic mechanism in which the major reaction pathway depends on substrate concentration. J Biol Chem 1974; 249: 7140 7.
22. Simonović M, Šoškić V, Joksimović J. Quantification of human dopamine D2s receptor interactions with $Gα_{(i,1,2)}$- and $Gα_{(o)}$-proteins. Neurochem Int 1998; 33: 271 5.
23. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacterio-phage T4. Nature, London 1970; 227: 680 5.
24. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 265 75.
25. Sidhu A, Sullivan M, Kohout T, Balen P, Fishman PH. D_1 dopamine receptors can interact with both stimulatory and inhibitory guanine nucleotide binding proteins. J Neurochem 1991; 57: 1445 51.
26. Jackson A, Iwasiow RM, Tiberi M. Distinct function of the cytoplasmic tail in human D_1-like receptor ligand binding and coupling. FEBS Lett 2000; 470: 183 8.

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