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MINIREVIEW

Subcellular Redistribution of Trimeric G-Proteins – Potential Mechanism of Desensitization of Hormone Response: Internalization, Solubilization, Down-regulation

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Summary

Agonist-induced subcellular redistribution of G-protein coupled receptors (GPCR) and of trimeric guanine-nucleotide binding regulatory proteins (G-proteins) represent mechanisms of desensitization of hormone response, which have been studied in our laboratory since 1989. This review brings a short summary of these results and also presents information about related literature data covering at least small part of research carried out in this area. We have also mentioned sodium plus potassium dependent adenosine triphosphatase (Na, K-ATPase) and ^{3}H-ouabain binding as useful reference standard of plasma membrane purity in the brain.

Key words

Brain • Subcellular fractionation • Trimeric G-proteins •· Desensitization • Na, K-ATPase

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Introduction

Hormones or neurotransmitters bind to the stereo-specific site of the receptor at the cell surface. This binding reaction represents the first step in complicated sequence of molecular events transmitting the signal into the cell interior and initiating the ultimate physiological response. In all eukaryotic organisms, a family of heterotrimeric GTP-binding and hydrolyzing proteins (G-proteins) plays an essential transducing role in linking many cell-surface receptors to effector proteins at the plasma membrane (Hepler and Gilman 1992). In G-protein-mediated pathway (Gilman 1987), the hormone/neurotransmitter binding induces conformational change of receptor molecule, which, in the next step, induces dissociation of trimeric G-protein complex (non-active) into the free Gα (active) and Gβγ (also active) subunits. Subsequently, both Gα and Gβγ activate the number of enzyme activities (effectors) or ionic channels, which then regulate the intracellular concentrations of secondary messengers.

Switching on and off the G protein cycle

The activation of receptor and dissociation of the non-active trimeric G-protein complex into individual free (active) Gα and Gβ subunits is quickly followed by switching off the signal transmission. This is caused by hormone (agonist)-induced stimulation of endogenous high-affinity GTPase located in the Gα subunit. This enzyme activity hydrolyses the terminal γ-phosphate of Gα-GTP; the non-active Gα-GDP is formed; Gα-GDP subunits then exhibit high-affinity binding capacity towards the free Gβγ subunits, the two subunits bind together and the (non-active) trimeric G-protein complex Gαβγ is thus reassembled again and, in this way, the

G-protein molecule is prepared for another round of an activation/deactivation cycle. The activation/deactivation cycle of trimeric G-proteins is rapid process, its turnover rate ranges among different G-protein families from *milliseconds* (transducin) to *seconds* range (Stryer 1991).

Desensitisation of hormone response

Besides the short-term switching on and off the signal initiated by agonist binding to GPCR, there are further types of dampening of the hormone response (Lefkowitz *et al*. 1980, Hausdorff *et al*. 1990, Dohlman *et al*. 1991, Lohse *et al.* 1993, Geshengorn 1994). These mechanisms proceed in seconds-minutes time-scale and are followed by even longer "negative feedback" regulatory loops proceeding within hours-days. The former type of desensitization is represented by receptor phosphorylation, sequestration and internalization. In this way, receptors are physically removed from the plasma (cell) membrane and transferred into the cell interior – internalized. Thus, the receptor is excluded from functional pool located at the cell surface and transferred into an inactive pool located in the cell interior. The latter type of negative feedback regulation of GPCR action proceeds within hours-days. It is represented by down-regulation of receptor molecules and represents the terminal stage of receptor "life". In this way, the total number of receptors in the cell, which has been stimulated for a long time by respective agonist, is decreased.

The short-term desensitization of GPCR action is primarily based on serine- or threonine-oriented phosphorylation at the C-terminus of receptor protein. Phosphorylation of receptor is followed by arrestin-induced functional uncoupling of receptor from G protein (Lefkowitz *et al*. 1980, Collins *et al*. 1992). Subsequently, the sequestration and internalization of receptors occurs within time-scale of minutes. Major part of the agonist-induced internalization of GPCR proceeds *via* clathrin-coated vesicles (Hausdorff *et al*. 1990).

G protein-based mechanisms of desensitization

Besides receptor-based mechanisms of desensitization of hormone response, changes in subcellular localization of trimeric G-proteins have been suggested as potential mechanism of desensitization of hormone response (Svoboda and Novotný 2002). These mechanisms were revealed by studies of subcellular localization of G proteins in cells exposed to an agonist for relatively long periods of time (>30 min). The long-term stimulation of target cells is associated with internalization, solubilization and "down-regulation" of trimeric G-proteins $G_s\alpha$ and $G_q\alpha/G_{11}\alpha$ (Ransnas *et al*. 1989, 1991, 1992, Levis and Bourne 1992, Svoboda *et al.* 1992, 1994, 1996a, Kvapil *et al*. 1994, 1995a,b, Novotný *et al.* 1995, Milligan *et al*. 1995, Novotný and Svoboda 1998, Novotny *et al.* 2001, Durchánková *et al*. 2007). The internalization of $G_q\alpha/G_{11}\alpha$ proceeds independently from internalization of their cognate membrane receptor, TRH-R and vice versa (Drmota *et al*. 1998, 1999, Yu and Hinkle 1999). The agonist-induced internalization of trimeric G proteins is not confined to $G_q\alpha/G_{11}\alpha$ but has been also demonstrated in other signaling cascades, such as β_2-adrenergic receptor and $G_s\alpha$ protein in S49 lymphoma cells (Ransnas *et al*. 1991, 1992, Yu and Rasenick 2002). It seems likely, that G-protein internalization and/or solubilization represents the general homeostatic mechanism protecting the target cell against excessive, long-term stimulation. Considering the generally accepted view that many GPCR act through the same class of G-proteins, the internalization or solubilization of given type of G-protein represents the basis for *heterologous* desensitization of hormone response. Overexpression of $G_{11}\alpha$ prevented the homologous desensitization of Ca^{2+} response to thyrotropin-releasing hormone (Novotný *et al*. 1999a). The simultaneous presence of both *homologous* and *heterologous* types of desensitization was recently noticed in cells expressing (endogenously) angiotensin II receptors and large amount of TRH-R and $G_{11}\alpha$ protein (Ostašov *et al*. 2007).

The mechanism of desensitization of hormone response was studied in our laboratory not only in various cell lines such as S49 lymphoma, CHO or HEK293 cells, but we have also studied this phenomenon in natural tissue – brown adipose tissue (Svartengren *et al*. 1982, 1984, Svoboda *et al.* 1984a,b, 1993, 1996b). Methodological experience collected over the years has been more recently applied for studies of brain tissue.

Subcellular fraction of the brain tissue

Original subcellular fractionations of the brain tissue were based on combination of differential and density gradient centrifugation in sucrose media (DePierre and Karnovsky 1973, Whittacker 1984). These procedures resulted in separation of myelin, nerve-

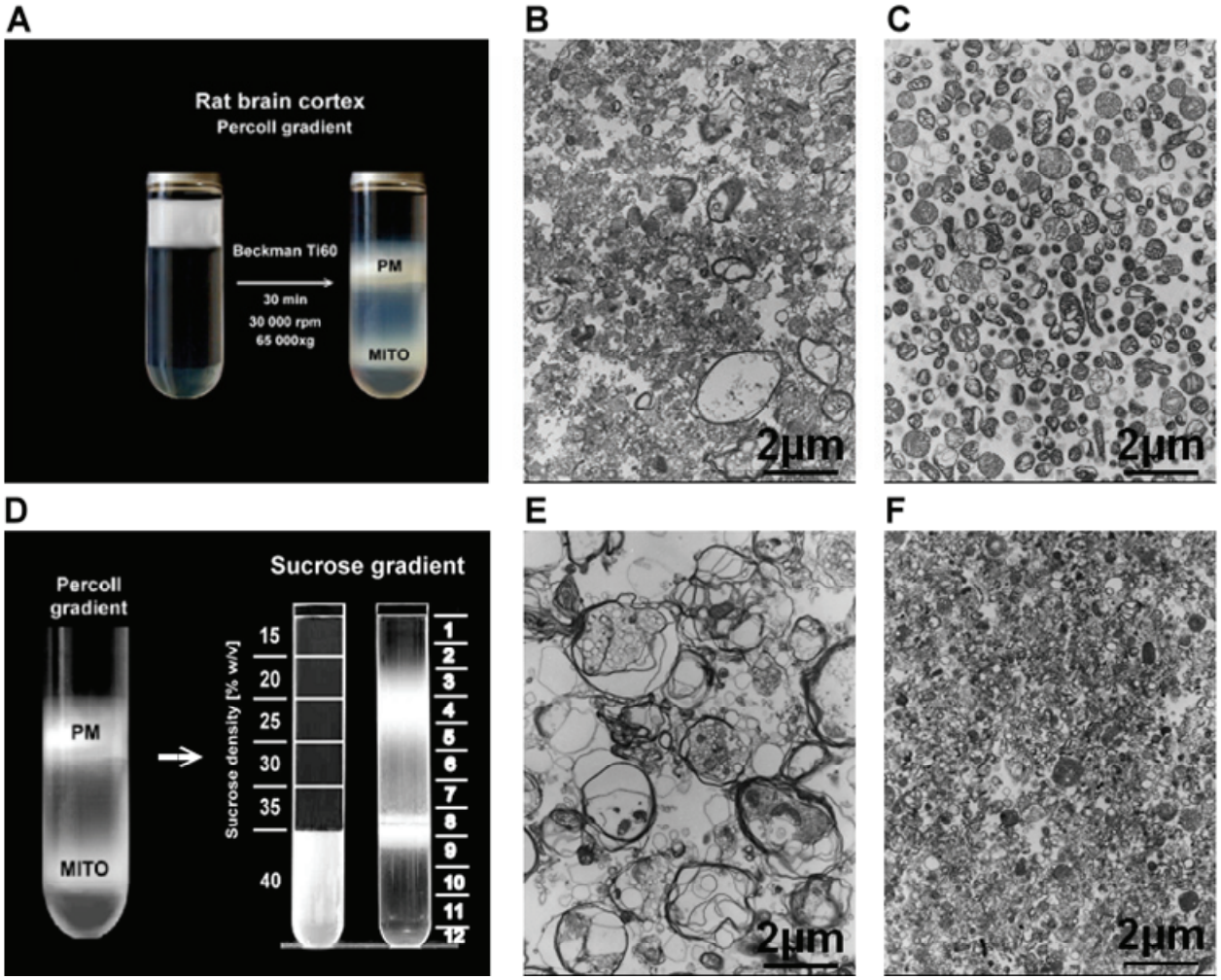

Fig. 1. Subcellular fractionation of rat brain cortex. **A**, *Separation of plasma membrane and mitochondrial fractions in Percoll^R gradient.* Post-nuclear supernatant was prepared from cerebral cortex of the rat and fractionated in Percoll^R gradient. The upper layer of plasma membranes (PM) was separated from lower layer of mitochondria (MITO). **B**, plasma membrane fraction represented mixture of large and small vesicular structures together with sheets of myelin; **C**, in mitochondrial fraction, pure mitochondria were detected. **D**, *Flotation of plasma membrane fraction in sucrose gradient.* The upper layer collected from Percoll^R gradient (PM) was fractionated by flotation in 15/20/25/30/35/40 % w/v sucrose gradient. Low-density plasma membrane (LPM), represented by hazy area in 15/20 % sucrose (fractions 3-5), were resolved from bulk of PM observed as distinct, optically dense band in 35 % sucrose or at 35/40 % sucrose interface (fractions 7-8). **E**, LPM were composed from large synaptosomal membrane particles and myelin; **F**, Bulk of plasma membrane (BPM) contained heterogeneous mixture of small vesicular structures (magnification 11700x).

endings (synaptosomes) and mitochondrial fractions mutually contaminated to larger or smaller extent. Bearing in mind that in brain and other tissues such as brown adipose tissue or heart muscle (Svoboda and Lodin 1972, Ihnatovych *et al*. 2001, 2002a,b, Hejnová *et al*. 2002, Stohr *et al.* 2004, 2005, Svoboda *et al*. 1984a,b, 1993, 1996b, Bouřová *et al*. 1999a,b, 2000, Novotný *et al.* 1999b, 2001, Hrbasová *et al.* 2003), the major part of subcellular membrane structures is represented by mitochondria, we have applied Percoll^R medium with the aim to achieve the clear separation of plasma membrane and mitochondrial fractions (for review of Percoll^R applications, see Pertoft 2000).

In the beginning of our experimental efforts, clear separation of upper (plasma membranes, PM) and lower (mitochondria, MITO) bands was not achieved. The total amount of protein applied per Percoll^R gradient as well as homogenization intensity had to be decreased. The tightly-fitting homogenizers (Brown, clearance 0.002-0.004 mm) used in our previous fractionations of HEK293 cells (Bouřová *et al*. 2003) had to be changed for loosely-fitting ones and homogenization had to be performed mildly with the aim to prevent degradation of mitochondria. The starting brain homogenate was filtered through a nylon nets of decreasing pore size (330, 110 and 75 µm) and centrifuged at low speed to sediment nuclear fraction and cell debris (5 min at 3000xg). Optimum results were found when applying filtered post-

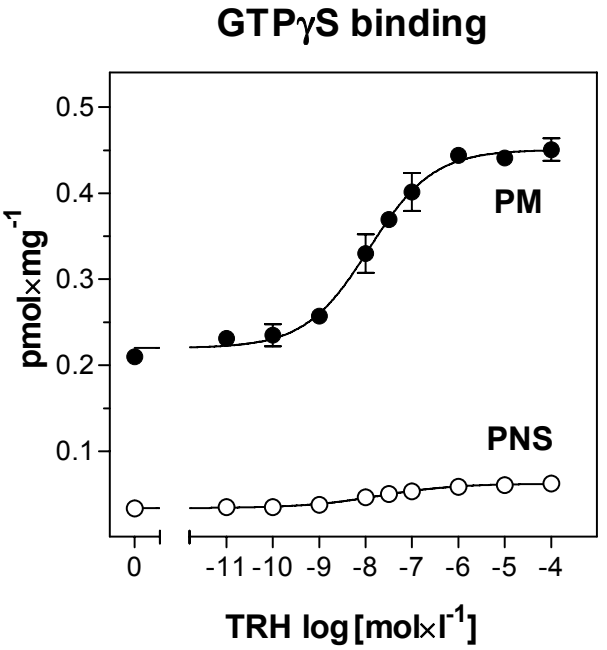

Fig. 2. Detection of TRH-stimulated G-protein functional activity in purified PM fraction; comparison with post-nuclear supernatant. HEK293 cells stably expressing TRH-R and $G_{11}\alpha$ protein were collected from 15x80 cm^2 flasks, homogenized, centrifuged at a low-speed and the post-nuclear supernatant (PNS) was applied on the top of 30 % Percoll. Centrifugation was performed for 30 min at 65000xg and resulted in separation of upper (plasma membrane, PM) and mitochondrial fractions. Agonist (TRH)-stimulated G-protein activity was measured by [^{35}S]GTPγS binding assay and compared in PNS and PM fractions.

nuclear supernatant containing 33-50 mg of protein on top of 30 ml of 30% Percoll^R in STE medium. After centrifugation in self-forming Percoll^R gradient, the upper layer of plasma membranes was separated from lower layer of mitochondria. Electron microscopy revealed very pure mitochondrial preparation. The upper layer of PM consisted of mixed population of large and small vesicular structures, synaptosomes and myelin (Fig. 1, A, B and C).

Subsequent sub-fractionation of the plasma membrane fraction was performed by flotation in step-wise 15/20/25/30/35/40% sucrose gradient designed in similar way as in our previous studies dealing with membrane domains/caveolae (Moravcová *et al*. 2004). Low-density plasma membrane fragments in 20/25% sucrose (Fig. 1D, LPM) were separated from the bulk of plasma membranes (Fig. 1E, BPM). LPM were enriched in large vesicular structures of synaptosomal origin. Major part of plasma membrane markers Na, K-ATPase, β-AR, DOR and $GABA_B$-R, caveolin 1,2, flotillin and $G_i1/G_i2\alpha$ proteins were co-localized in BPM. Majority of PM protein was also recovered in BPM.

Subcellular fractionation of transfected cell lines stably expressing defined signaling molecules

The best methodological approach how to isolate plasma (cell) membrane fragments is represented by density gradient centrifugation. Our earlier studies used the "sedimentation" types of continuous and later discontinuous sucrose gradients. When using this protocol, cell homogenate or post-nuclear supernatant was applied on the top of sucrose layer of the lowest density and subcellular particles like microsomes, plasma (cell) membrane derived vesicles, mitochondria, lysosomes and Golgi fragments migrated down through sucrose layers of increasing density. In this case, centrifugation was performed for relatively short period of time: 30-60 min in Beckman SW28 rotor. This type of centrifugation is denominated as *rate-zonal* mode of centrifugation (Svoboda *et al.* 1992, 1993, 1994, 1996a,b).

The second version of centrifugation, which was used in our more recent studies of *membrane domains* and had originally been successfully used for preparation of Golgi compartments, is much more time-consuming. It is represented by "flotation" of membrane fragments or vesicles *up* into equilibrium density zones, density of which is equal to buoyant density of a given membrane structure/vesicle (Pešanová *et al*. 1999, Bouřová *et al*. 2003, Moravcová *et al*. 2004, Matoušek *et al.* 2003a,b, 2005a,b, Rudajev *et al.* 2005). All these "flotation gradients" were performed with cell homogenate or crude membrane preparation applied to the bottom of the tube and subsequently, overlayed with sucrose layers of decreasing density. In the future, we would like to use the same procedure, however, with purified PM as starting material. Therefore we applied Percoll^R-medium for resolution of plasma membrane and mitochondrial fractions from HEK293 cells stably expressing δ-opioid receptor, DOR (Moon *et al*. 2001), TRH-R (Kim *et al.* 1994), TRH-R plus large amount of $G_{11}\alpha$ (Svoboda *et al*. 1996a) or VSV-TRH-R-GFP hybrid molecule (Drmota *et al*. 1999). In this case, an optimum separation of PM and MITO bands was achieved when applying post-nuclear supernatant prepared from 15 x 80 cm^2 flasks on the top of Percoll^R gradient in one Beckman Ti60 tube. Surprisingly, the efforts to use swing-out rotors for separation of PM and MITO bands were not successful. Angle rotors have to be used for proper separation of PM and MITO fractions when using Percoll^R medium for

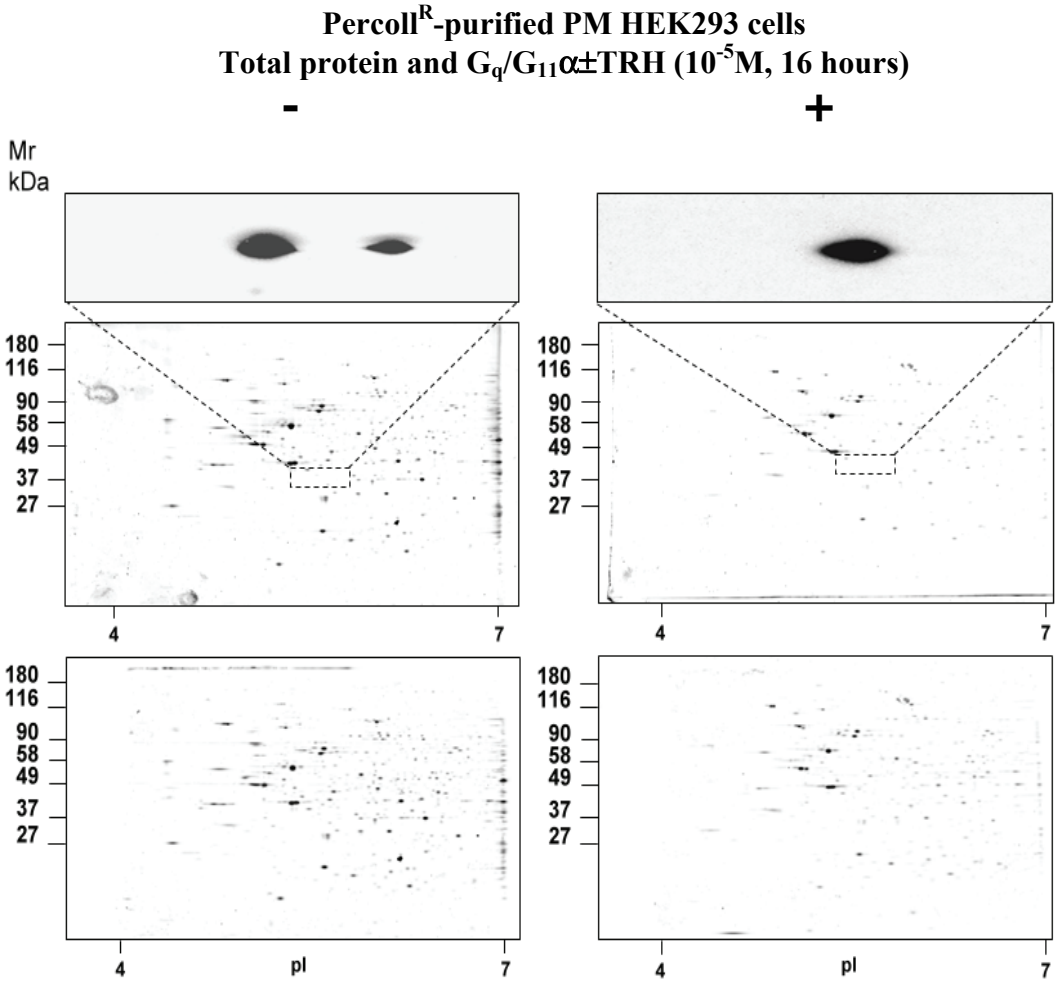

Fig. 3. Resolution of plasma membrane proteins by 2-D electrophoresis. PM fraction was isolated from HEK293 cells stably expressing TRH-R and $G_{11}\alpha$ protein as described in legend to Fig. 2. PM proteins were resolved by 2-D electrophoresis and visualized by Sypro Ruby stain according to manufacturers protocol (Amersham). In parallel sample, the membrane proteins were transferred to nitrocellulose membrane and Gq/G11α proteins detected by immubloting with specific antibodies.

subcellular fractionation.

As shown in Figures 2 and 3, purification of PM is not just methodological perfectionism of a biochemist obsessed with purity. Separation of PM-enriched fraction allows detection of physiologically significant functions such as agonist-stimulated trimeric G-protein activity with high efficiency (Fig. 2); it also allows detailed analysis of membrane proteins by 2-D electrophoresis (Fig. 3). It increases specificity of immunoblot assays when detecting very small amounts of membrane-bound signaling molecules such as trimeric G proteins.

Detection of Na, K-ATPase as internal standard of plasma membrane purity

Receptor number (B_{max}) or an intensity of an immunoblot signal of G-protein in a given PM preparation is related to the amount of protein present in a given sample. Thus, in studies of plasma membrane-bound signaling molecules such as receptors, G-proteins or adenylyl cyclase molecules, the contaminating proteins decrease the specific binding capacity or the specific content of signaling molecule. This does not mean a great problem if: i) the extent of contamination is the same, ii) protein is determined by the same method, iii) the same protein standard is used over the whole set of experiments, and iiii) data are collected from the linear range of calibration curve.

These criteria, though appearing trivial, are not easy to be met in reality of physiological research and bear strong impact on interpretation of receptor or G-protein studies. Studies of ontogenetic development, effects of nutrition, long-term hormonal or drug exposure, long-term mechanisms of desensitization of hormone response or drug addiction, environmental effects such as cold-acclimation and many others, require comparison of

PM preparations of constant protein composition. In reality this means the work with PM preparation contaminated by a non-plasma membrane proteins to different extent. So, what sort of internal standard, inherently and exclusively bound/attached to and located in PM under all *imaginable* conditions may be used?

The sodium plus potassium activated, magnesium dependent adenosine-triphosphatase (EC 3.6.1.3) (Jorgensen 1975, 1988) is localized exclusively in the plasma membrane, represents an integral membrane protein strongly bound into PM (because of multiple transmembrane segments) and is expressed in central nervous system in high quantity. Therefore, detection of this enzyme activity might serve as a satisfactory tool for characterization of purity of PM. However, there is a better, more quantitative and simple way how to estimate the purity of PM preparation with the help of Na, K-ATPase: the usage of radioactively labeled inhibitor – ^{3}H-ouabain.

Na, K-ATPase activity is selectively inhibited by cardioactive glycosides and its brain isoform was studied in our laboratory for a prolonged period of time (Svoboda and Mosinger 1981a,b, Svoboda *et al.* 1984c,d, 1986, Vyskočil *et al*. 1983, 1987, Amler *et al*. 1987, 1988a,b, Teisinger *et al*. 1992). Among the wide range of cardiac glycosides, ouabain (strophantin G), as the water-soluble derivative, was often used when detecting this enzyme in PM preparations. We have also used it for characterization of membrane polarity of Na, K-ATPase molecule in brain microsomes (Svoboda *et al.* 1988). Surprising feature of this radioligand is very low non-specific binding, at least in brain.

It could be mentioned in this respect that [^{3}H]ouabain is by far the most specific ligand used by us in the course of 20 years of laboratory practice. The non-specific binding of radioactively labeled antagonists or agonists of GPCR [^{3}H-dihydroalprenolol (β-AR antagonist), ^{3}H-CGP12157 (β-AR antagonist), ^{125}I-azido-benylcarazolol (β-AR antagonist), ^{125}I-cyanopindol (β-AR antagonist), ^{3}H-TRH (TRH-R agonist), ^{3}H-CGP54566 ($GABA_B$-R antagonist), ^{3}H-baclofen ($GABA_B$-R agonist), ^{3}H-naltrindol (DOR agonist), ^{3}H-DADLE (DOR agonist), ^{3}H-DAMGO (MOR agonist), etc.] was significantly higher.

We have used ^{3}H-ouabain for comparison of PM isolated from brown adipose tissue of control and cold-acclimated hamsters (Svoboda *et al.* 1993, 1996b). Cold-acclimation induces dramatic increase of respiratory chain enzyme activities per cell; consequently, PM isolated from brown adipose tissue of cold-acclimated hamsters were contaminated by mitochondrial fragments to higher degree than PM isolated from control animals. Introduction of ^{3}H-ouabain as reference standard of PM purity, helped to overcome this problem. It is therefore logical to use this radioligand as "internal standard" of various PM compartments isolated from brain tissue in the future research as one should not forget that brain is the most oxidative tissue in the body of man and mammals and contains numerous (too many) mitochondria.

Conflict of Interest

There is no conflict of interest.

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References

AMLER E, TEISINGER J, SVOBODA P: Mg^{2+}-induced changes of lipid order and conformation of Na,K-ATPase. *Biochim Biophys Acta* **905:** 376-382, 1987.

AMLER E, SVOBODA P, TEISINGER J, ZBOROWSKI J: The role of carboxyl groups of Na^{+}/K^{+}-ATPase in the interaction with divalent cations. *Biochim Biophys Acta* **945:** 367-370, 1988a.

AMLER E, TEISINGER J, SVOBODA P, VYSKOČIL F: The changes in conformation of Na, K-ATPase from rat brain membranes are accompanied by changes of protein segment movements in the nanosecond range. *Physiol Bohemoslov* **37:** 145-148, 1988b.

BOUŘOVÁ L, NOVOTNÝ J, SVOBODA P: The decrease in the short variant of $G_s\alpha$ protein is associated with an increase in [^{3}H]CGP12177 binding, [^{3}H]ouabain binding and Na, K-ATPase activity in brown adipose tissue plasma membranes of cold-acclimated hamsters. *J Mol Endocrinol* **22:** 55-64, 1999a.

BOUŘOVÁ L, NOVOTNÝ J, SVOBODA P: Resolution and identification of $G_q/G_{11}\alpha$ and $G_i\alpha/G_o\alpha$ proteins in brown adipose tissue: effect of cold acclimation. *J Mol Endocrinol* **23:** 223-229, 1999b.

BOUŘOVÁ L, PEŠANOVÁ Z, NOVOTNÝ J, SVOBODA P: Differentiation of cultured brown adipocytes is associated with selective increase of the short variant of G_s alpha protein. Evidence for higher functional activity of $G_s\alpha$S. *Mol Cell Endocrinol* **167:** 23-31, 2000.

BOUŘOVÁ L, KOŠTRNOVÁ A, HEJNOVÁ L, MORAVCOVÁ Z, MOON HE, NOVOTNÝ J, MILLIGAN G, SVOBODA P: δ-Opioid receptors exhibit high efficiency when activating trimeric G proteins in membrane domains. *J Neurochem* **85:** 34-49, 2003.

COLLINS S, CARON MG, LEFKOWITZ RJ: From ligand binding to gene expression: new insights into the regulation of G-protein-coupled receptors. *Trends Biochem Sci* **17:** 37-39, 1992.

DEPIERRE JW, KARNOVSKY M: Plasma membrane of mammalian cells. *J Cell Biol* **56**: 273-303, 1973.

DOHLMAN HG, THORNER J, CARON MG, LEFKOWITZ RJ: Model systems for the study of seven-transmembrane-segment receptors. *Annu Rev Biochem* **60:** 653-688, 1991.

DRMOTA T, NOVOTNÝ J, KIM GD, EIDNE KA, MILLIGAN G, SVOBODA P: Agonist-induced internalisation of the G protein $G_{11}\alpha$ and thyrotropin-releasing hormone receptors proceed on different time scales. *J Biol Chem* **273:** 21699-21707, 1998.

DRMOTA T, NOVOTNÝ J, GOULD GW, SVOBODA P, MILLIGAN G: Visualization of distinct patterns of subcellular redistribution of the thyrotropin-releasing hormone receptor-1 and $G_q\alpha/G_{11}\alpha$ induced by agonist stimulation. *Biochem J* **340:** 529-538, 1999.

DURCHÁNKOVÁ D, NOVOTNÝ J, SVOBODA P: The time-course of agonist-induced solubilisation of trimeric $G_q\alpha/G_{11}\alpha$ proteins resolved by two-dimensional electrophoresis. *Physiol Res* **57:** 195-203, 2008.

GERSHENGORN MC: Excessive stimulation is bad, so desensitization is ubiquitous. *Endocrinology* **134:** 5-6, 1994.

GILMAN AG: G proteins: transducers of receptor-generated signals. *Annu Rev Biochem* **56:** 615-649, 1987.

HAUSDORFF WP, CARON MG, LEFKOWITZ RJ: Turning off the signal: desensitisation of β-adrenergic receptor function. *FASEB J* **4**: 2881-2889, 1990.

HEJNOVÁ L, IHNATOVYCH I, NOVOTNÝ J, KUBOVÁ H, MAREŠ P, SVOBODA P: Modulation of adenylyl cyclase activity by baclofen in the developing rat brain: difference between cortex, thalamus and hippocampus. *Neurosci Lett* **330:** 9-12, 2002.

HEPPLER JR, GILMAN AG: G proteins. *Trends Biochem Sci* **17:** 383-387, 1992.

HRBASOVÁ M, NOVOTNÝ J, HEJNOVÁ L, KOLÁŘ F, NECKÁŘ J, SVOBODA P: Altered myocardial G_s protein and adenylyl cyclase signalling in rats exposed to chronic hypoxia and normoxic recovery. *J Appl Physiol* **94:** 2423-2432, 2003.

IHNATOVYCH I, HEJNOVÁ L, KOŠTRNOVÁ A, MAREŠ P, SVOBODA P, NOVOTNÝ J: Maturation of rat brain is accompanied by differential expression of the long and short splice variants of $G_s\alpha$ protein: identification of cytosolic forms of $G_s\alpha$. *J Neurochem* **79:** 88-97, 2001.

IHNATOVYCH I, NOVOTNÝ J, HAUGVICOVÁ R, BOUŘOVÁ L, MAREŠ P, SVOBODA P: Opposing changes of trimeric G protein levels during ontogenetic development of rat brain. *Brain Res Dev Brain Res* **133:** 57-67, 2002a.

IHNATOVYCH I, NOVOTNÝ J, HAUGVICOVÁ R, BOUŘOVÁ L, MAREŠ P, SVOBODA P: Ontogenetic development of the G protein-mediated adenylyl cyclase signalling in rat brain. *Brain Res Dev Brain Res* **133:** 69-75, 2002b.

JORGENSEN PL: Isolation and characterization of the components of the sodium pump. *Q Rev Biophys* **7:** 239-274, 1975.

JORGENSEN PL: Purification of Na, K-ATPase: enzyme sources, preparative problems and preparation from mammalian kidney. *Methods Enzymol* **156:** 29-43, 1988.

KIM GD, CARR IC, ANDERSON LA, ZABAVNIK J, EIDNE KA, MILLIGAN G: The long isoform of the rat thyrotropin-releasing hormone receptor down-regulates G_q proteins. *J Biol Chem* **269:** 19933-19940, 1994.

KVAPIL P, NOVOTNÝ J, SVOBODA P, RANSNAS LA: The short and long forms of the α subunit of the stimulatory guanine-nucleotide-binding protein are unequally redistributed during (–)-isoproterenol-mediated desensitization of intact S49 lymphoma cells. *Eur J Biochem* **226:** 193-199, 1994.

KVAPIL P, NOVOTNÝ J, RANSNAS LA: Splicing variants of the α subunit of the stimulatory G-protein ($G_s\alpha$) differ in their ability to interact with adenylyl cyclase in S49 lymphoma cells. *FASEB J* **9:** A1411, 1995a.

KVAPIL P, NOVOTNÝ J, RANSNAS LA: Prolonged exposure of hamsters to cold changes the levels of G proteins in brown adipose tissue plasma membranes. *Life Sci* **57:** 311-318, 1995b.

LEFKOWITZ RJ, WESSELS MR, STADEL JM: Hormones, receptors, and cyclic AMP: their role in target cell refractoriness. *Curr Top Cell Regul* **17:** 205-230, 1980.

LEVIS ML, BOURNE H: Activation of the alpha subunit of G_s in intact cells alters its abundance, rate of degradation and membrane avidity. *J Biol Chem* **119:** 1297-1307, 1992.

LOHSE MJ: Molecular mechanisms of membrane receptor desensitisation. *Biochim Biophys Acta* **1179:** 171-188, 1993.

MATOUŠEK P, HODNÝ Z, ŠVANDOVÁ I, SVOBODA P: Different methods of membrane domains isolation result in similar 2-D distribution patterns of membrane domain proteins. *Biochem Cell Biol* **81:** 365-372, 2003a.

MATOUŠEK P, NOVOTNÝ J, SVOBODA P: Resolution of $G_s\alpha$ and $G_q\alpha$ /$G_{11}\alpha$ proteins in membrane domains by 2D electrophoresis. The effect of long term agonist stimulation. *Physiol Res* **53:** 295-303, 2003b

MATOUŠEK P, NOVOTNÝ J, RUDAJEV V, SVOBODA P: Prolonged agonist-stimulation does not alter the protein composition of membrane domains in spite of dramatic change in specific signalling cascade. *Cell Biochem Biophys* **42:** 21-40, 2005a

MATOUŠEK P, DURCHÁNKOVÁ D, ŠVANDOVÁ I, NOVOTNÝ J, MILLIGAN G, SVOBODA P: Agonist-induced tyrosine-phosphorylation of $G_q/G_{11}\alpha$ requires intact structure of membrane domains. *Biochem Biophys Res Commun* **328:** 526-532, 2005b.

MILLIGAN G, WISE A, MACEWAN DJ, GRASSIE MA, KENNEDY FR, LEE TW, ADIE EJ, KIM GD, MCCALLUM JF, BURT A, CARR IC, SVOBODA P, SHAH BH: Mechanisms of agonist-induced G-protein elimination. *Biochem Soc Trans* **23:** 166-170, 1995.

MOON HE, BAHIA DS, CAVALLI A, HOFFMAN M, MILLIGAN G: Control and efficiency of agonist-induced information transfer and stability of the ternary complex containing the δ-opioid receptor and the alpha subunit of Gi1 by mutation of a receptor/G protein contact interphase. *Neuropharmacology* **41:** 321-330, 2001.

MORAVCOVÁ Z, RUDAJEV V, NOVOTNÝ J, ČERNÝ J, MATOUŠEK P, PARENTI M, MILLIGAN G, SVOBODA P: Long-term agonist stimulation of IP prostanoid receptor depletes the cognate $G_s\alpha$ protein from membrane domains but does not affect the receptor level. *Biochim Biophys Acta* **1691:** 51-65, 2004.

NOVOTNÝ J, KVAPIL P, BOKOCH GM, RANSNAS LA: Isoproterenol-induced subcellular redistribution of G-protein β subunits in S49 lymphoma cells demonstrated by a novel competitive ELISA. *Arch Physiol Biochem* **103:** 202-210, 1995.

NOVOTNÝ J, SVOBODA P: The long (G_sL) and short (G_sS) variants of the stimulatory guanine-nucleotide-binding protein. Do they behave in an identical way? *Mol Endocrinol* **20:** 163-173, 1998.

NOVOTNÝ J, KRŮŠEK J, DRMOTA T, SVOBODA P: Over-expression of the G protein $G_{11}\alpha$ prevents desensitization of Ca^{2+} response to thyrotropin-releasing hormone. *Life Sci* **65:** 889-900, 1999a.

NOVOTNÝ J, BOUŘOVÁ L, MÁLKOVÁ O, SVOBODA P, KOLÁŘ F: G proteins, β-adrenoreceptors and β-adrenergic responsiveness in immature and adult rat ventricular myocardium: influence of neonatal hypo- and hyperthyroidism. *J Mol Cell Cardiol* **31:** 761-772, 1999b.

NOVOTNÝ J, BOUŘOVÁ L, KOLÁŘ F, SVOBODA P: Membrane-bound and cytosolic forms of heterotrimeric G proteins in young and adult rat myocardium: influence of neonatal hypo- and hyperthyroidism. *J Cell Biochem* **82:** 215-224, 2001.

OSTAŠOV P, KRŮŠEK J, DURCHÁNKOVÁ D, SVOBODA P, NOVOTNÝ J: Ca^{2+}-responses to thyrotropine-relasing hormone and angiotensin II. The role of plasma membrane integrity and effect of G_{11}alpha protein overexpression on homologous and heterologous desensitization. *Cell Biochem Function* **26**: 264-274, 2007.

PEŠANOVÁ Z, NOVOTNÝ J, CERNY J, MILLIGAN G, SVOBODA P: Thyrotropin-releasing hormone-induced depletion of $G_q\alpha/G_{11}\alpha$ proteins from detergent-insensitive membrane domains. *FEBS Lett* **464:** 35-40, 1999.

PERTOFT H: Fractionation of cells and subcellular particles with Percoll. *J Biochem Biophys Methods* **44:** 1-30, 2000.

RANSNAS LA, SVOBODA P, JASPER JR, INSEL PA: Stimulation of β-adrenergic receptors of S49 lymphoma cells redistributes the α subunit of the stimulatory G protein between cytosol and membranes. *Proc Natl Acad Sci USA* **86:** 7900-7903, 1989.

RANSNAS LA, LEIBER D, INSEL PA: Inhibition of subunit dissociation and release of the stimulatory G-protein, G_s, by βγ-subunits and somatostatin in S49 lymphoma cell membranes. *Biochem J* **280:** 303-307, 1991.

RANSNAS LA, JASPER JR, LEIBER D, INSEL PA: β-adrenergic-receptor-mediated dissociation and membrane release of the Gs protein in S49 lymphoma-cell membranes. Dependence on Mg^{2+} and GTP. *Biochem J* **283:** 519-524, 1992.

RUDAJEV V, NOVOTNÝ J, HEJNOVÁ L, MILLIGAN G, SVOBODA P: Thyrotropin-releasing hormone receptor is excluded from lipid domains. Detergent-resistant and detergent-sensitive pools of TRH receptor and $G_q\alpha/G_{11}\alpha$ protein. *J Biochem (Tokyo)* **138:** 111-125, 2005.

STÖHR J, BOUŘOVÁ L, HEJNOVÁ L, IHNATOVYCH I, NOVOTNÝ J, SVOBODA P: Increased baclofen-stimulated G protein coupling and deactivation in rat brain cortex during development. *Dev Brain Res* **151:** 67-73, 2004.

STÖHR J, NOVOTNÝ J, SVOBODA P: Characterisation of [^{3}H]-forskolin binding sites in young and adult rat brain cortex: identification of suramin as a competitive inhibitor of [^{3}H]-forskolin binding. *Can J Physiol Pharmacol* **83:** 1-9, 2005.

STRYER L: Visual excitation and recovery. *J Biol Chem* **266:** 10711-10714, 1991.

SVARTENGREN J, SVOBODA P, CANNON B: Desensitisation of β-adrenergic responsiveness in vivo. Decreased coupling between receptors and adenylate cyclase in isolated brown-fat cells. *Eur J Biochem* **128:** 481-488, 1982.

SVARTENGREN J, SVOBODA P, DRAHOTA Z, CANNON B: The molecular basis for adrenergic desensitisation in hamster brown adipose tissue: uncoupling of adenylate cyclase activation. *Comp Biochem Physiol C* **78:** 159-170, 1984.

SVOBODA P, LODIN Z: Postnatal development of some mitochondrial enzyme activities of cortical neurons and glial cells. *Physiol Bohemoslov* **21:** 457-465, 1972.

SVOBODA P, MOSINGER B: Catecholamines and the brain microsomal Na, K-adenosinetriphosphatase. I. Protection against lipoperoxidative damage. *Biochem Pharmacol* **30:** 427-432, 1981a.

SVOBODA P, MOSINGER B: Catecholamines and the brain microsomal Na, K-adenosinetriphosphatase. II. The mechanism of action. *Biochem Pharmacol* **30:** 433-439, 1981b.

SVOBODA P, SVARTENGREN J, NÁPRSTEK J, JIRMANOVÁ I: The functional and structural reorganisation of the plasma membranes of brown adipose tissue induced by cold acclimation of the hamster. I. Changes in catecholamine-sensitive adenylate cyclase activity. *Mol Physiol* **5:** 197-210, 1984a.

SVOBODA P, SVARTENGREN J, DRAHOTA Z: The functional and structural reorganisation of the plasma membranes of brown adipose tissue induced by cold acclimation of the hamster. II. The β-adrenergic receptor. *Mol Physiol* **5:** 211-220, 1984b.

SVOBODA P, TEISINGER J, PILAŘ J, VYSKOČIL F: Vanadyl (VO_2^+) and vanadate (VO_3^-) ions inhibit the brain microsomal Na,K-ATPase with similar affinities. Protection by transferrin and noradrenaline. *Biochem Pharmacol* **33:** 2485-2491, 1984c

SVOBODA P, TEISINGER J, VYSKOČIL F: Vanadyl (VO_2^+)induced lipoperoxidation in the brain microsomal fraction is not related to VO_2^+ inhibition of Na,K-ATPase. *Biochem Pharmacol* **33:** 2493-2497, 1984d.

SVOBODA P, TEISINGER J, VYSKOČIL F: Effect of catecholamines and metal chelating agents on the brain and brown adipose tissue Na,K-ATPase. *Comp Biochem Physiol C* **84:** 283-290, 1986.

SVOBODA P, AMLER E, TEISINGER J: Different sensitivity of ATP + Mg + Na (I) and Pi + Mg (II) dependent types of ouabain binding to phospholipase A_2. *J Membr Biol* **104:** 211-221, 1988.

SVOBODA P, KVAPIL P, INSEL PA, RANSNAS LA: Plasma-membrane-independent pool of the α subunit of the stimulatory guanine-nucleotide-binding regulatory protein in a low-density-membrane fraction of S49 lymphoma cells. *Eur J Biochem* **208:** 693-698, 1992.

SVOBODA P, UNELIUS L, CANNON B, NEDERGAARD J: Attenuation of $G_s\alpha$ coupling efficiency in brown-adipose-tissue plasma membranes from cold-acclimated hamsters. *Biochem J* **295:** 655-661, 1993.

SVOBODA P, MILLIGAN G: Agonist-induced transfer of the α subunits of the guanine-nucleotide-binding regulatory proteins G_q and G_{11} and of muscarinic M1 acetylcholine receptors from plasma membranes to a light-vesicular membrane fraction. *Eur J Biochem* **224:** 455-462, 1994.

SVOBODA P, KIM GD, GRASSIE MA, EIDNE KA, MILLIGAN G: Thyrotropin-releasing hormone-induced subcellular redistribution and down-regulation of $G_{11}\alpha$: analysis of agonist regulation of co-expressed $G_{11}\alpha$ species variants. *Mol Pharmacol* **49:** 646-655, 1996a.

SVOBODA P, UNNELIUS L, DICKER A, CANNON B, NEDERGAARD J: Cold-induced reduction in Gi proteins in brown adipose tissue. Effects of cellular hypersensitization to noradrenaline caused by pertussis-toxin treatment. *Biochem J* **313:** 761-768, 1996b

SVOBODA P, NOVOTNÝ J: Hormone-induced subcellular redistribution of trimeric G proteins. *Cell Mol Life Sci* **59:** 501-512, 2002.

TEISINGER J, ZEMKOVÁ H, SVOBODA P, AMLER E, VYSKOČIL F: Ouabain binding, ATP hydrolysis, and Na^+, K^+-pump activity during chemical modification of brain and muscle Na^+, K^+-ATPase. *J Neurochem* **58:** 1066-1072, 1992.

WHITTACKER VP: The structure and function of cholinergic synaptic vesicles. *Biochem J* **12**: 561-576, 1984.

VYSKOČIL F, PILAŘ J, ZEMKOVÁ H, SVOBODA P, VÍTEK V, TEISINGER J: Bleomycin stimulates both membrane (Na^+-K^+) ATPase and electrogenic (Na^+-K^+) pump and partially removes the inhibition by vanadium ions. *Biochem Biophys Res Commun* **116**: 783-790, 1983.

VYSKOČIL F, ZEMKOVÁ H, TEISINGER J, SVOBODA P: Arachidonate activates muscle electrogenic sodium pump and brain microsome Na^+,K^+-ATPase under suboptimal conditions. *Brain Res* **436:** 85-91, 1987.

YU R, HINKLE PM: Signal transduction and hormone-dependent internalisation of the thyrotropin-releasing hormone receptor in cells lacking G_q and G_{11}. *J Biol Chem* **274:** 15745-15750, 1999.

YU JZ, RASENICK MM: Real-time visualization of a fluorescent G(α)(s): dissociation of the activated G protein from plasma membrane. *Mol Pharmacol* **61:** 352-359, 2002.