

Physiological Research Pre-Press Article

The time-course of agonist-induced solubilisation of trimeric $G_q\alpha/G_{11}\alpha$ proteins resolved by two-dimensional electrophoresis

Short title: Trimeric Ga protein solubilisation

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Summary

Prolonged agonist stimulation results in specific transfer of activated Ga subunits of $G_q\alpha/G_{11}\alpha$ family from particulate membrane fraction to soluble (cytosol) cell fraction isolated as 250 000 x g supernatant. In this study, we have used 2D electrophoresis for more defined resolution of Ga subunits of $G_q\alpha/G_{11}\alpha$ family and followed the time course of solubilisation effect. The small signal of soluble G proteins was detected already in control, hormone-unexposed cells. Hormone stimulation resulted in slow but continuous increase of both intensity and number of immuno-reactive signals/spots of these G proteins (10, 30, 60, 120 and 240 min). At longer times of agonist exposure (>2 hours), marked increase of $G_q\alpha/G_{11}\alpha$ proteins was detected. The maximal level of soluble $G_q\alpha/G_{11}\alpha$ proteins was reached after 16 hours of continuous agonist exposure. At this time interval, eight individual immuno-reactive signals of $G_q\alpha/G_{11}\alpha$ proteins could have been resolved. The relative proportion among these spots was 15:42:10:11:7:7:2:5. Solubilisation of this class of Ga proteins was thus observed after prolonged agonist stimulation only, induced by ultra high concentration of hormone and in cells expressing large number of GPCRs. **Our data therefore indicate rather tight/persisting binding of $G_q\alpha/G_{11}\alpha$ proteins to the membrane.**

Keywords:

G proteins, solubilisation, G_q/G_{11} , two-dimensional electrophoresis, thyrotropin-releasing hormone, TRH.

Introduction

Number of independent studies indicated that the exposure of intact cells to hormonal stimulation results in redistribution of the cognate G-proteins from plasma membrane (PM) to the cell interior (*internalisation*). In biochemical studies, G-protein α subunits were redistributed from plasma membranes to the light-vesicular membrane fractions distinct from PM (Haraguchi and Rodbell 1990; Svoboda et al., 1992, 1996; Kvapil et al. 1994; Svoboda and Milligan 1994); when analysed by confocal immuno-fluorescence microscopy, G_α subunits were transferred from the cell surface into the intracellular sites of various origin (Drmotá et al. 1998; 1999). Transfer of G_α subunits from the particulate membrane fraction to the soluble cell fraction (*solubilisation*) was also demonstrated - for review see Svoboda and Novotný (2002). Agonist-induced subcellular redistribution of trimeric G-protein subunits is worth of further studies because it represents potential mechanism for heterologous desensitisation of hormone response (Lohse 1993).

Solubilisation of G protein α subunits had been reported for the first time in S49 lymphoma cells. Stimulation of β_2 -adrenergic receptors by isoprenaline resulted in redistribution of about half of the cellular $G_s\alpha$ from membranes to 250 000 x g supernatant (Ransnas et al., 1989). The agonist-induced solubilisation of $G_s\alpha$ was also reported in myocardial membranes when exposed to the same agonist (Ransnas et al. 1991; 1992). In mouse mastocytoma cells, solubilisation of G_α was induced by iloprost (via stimulation of IP prostanoid receptors). Thrombin induced translocation of $G_{12}\alpha$ from membranes to soluble fraction was also reported in these cells (Negishi et al. 1992; Takahashi et al. 1991). Agonists acting on VIP-receptors (vasoactive intestinal peptide) in rat pituitary GH4C1 cells induced release of activated $G_s\alpha$ from membranes to cytosol along with its down-regulation, i.e. decrease of total cellular amount (Yajima et al. 1998). Besides G-proteins of $G_s\alpha$ and $G_i\alpha/G_o\alpha$ families, evidence for solubilisation of $G_q\alpha/G_{11}\alpha$ proteins has been also provided. In stably transfected HEK-293 cells, prolonged stimulation by thyrotropin-releasing hormone receptors caused a dramatic shift of $G_q\alpha/G_{11}\alpha$ from membranes to the high-speed supernatant 250 000 x g (Svoboda et al. 1996; Drmotá et al. 1998; 1999). Agonist-induced translocation of $G_q\alpha/G_{11}\alpha$ immuno-reactivity from plasma membranes to cytosol fraction was also reported by Arthur et al. (1999) in MDCK cells and the soluble forms of $G_i\alpha$ and $G_s\alpha$ proteins were identified in brain (Ihnatovych et al. 2001) and heart-muscle (Novotný et al. 2001) and found

to be altered by physiological state of the tissue such as ontogenetic development.

In this work, we decided to resolve members of G_q/G_{11} family by 2D electrophoresis and analyse the time-course of agonist effect on solubilisation of these G proteins.

Materials and Methods

Materials. All materials for tissue cultures were supplied by Sigma-Aldrich (Poole, U.K.) and Invitrogen (Paisley, U.K.). Complete protease inhibitor cocktail was from Roche Diagnostics Ltd. (Lewes, U.K.). Immobiline DryStrips, IPG buffer and secondary anti-rabbit antibody marked with horseradish peroxidase were purchased from Amersham Biosciences (Chalfont St. Giles, U.K.). Duracryl (30% acrylamide, 0,8% bisacrylamide) was from Genomic Solutions (Huntingdon, U.K.). All other chemicals and materials were from Sigma-Aldrich and Merck (Darmstadt, Germany) and were of the best grade available. The rabbit anti- G_q/G_{11} antiserum, CQ was oriented against C-terminus decapeptide of G_q and G_{11} and was prepared in Prague laboratory.

Stable transfection of HEK-293 cells. Clone E2M11 of HEK-293 cells stably expressing high levels of the rat TRH receptor and murine G_{11} , was prepared as described previously by [Svoboda et al. \(1996\)](#). Briefly, a full-length rat (long isoform) TRH receptor cDNA (2,2 kb) ([Sellar et al. 1993](#)) was subcloned into the eukaryotic expression vector pcDNA1 (Invitrogen), which is driven by the cytomegalovirus (CMV) promoter. HEK-293 cells were co-transfected with linearized pcDNA1/TRH receptor (800 ng) and pSPneo (200 ng, Invitrogen) using Lipofectin reagent (30 mg, Life Technologies, Inc., Paisley, Strathclyde, UK) in serum free Dulbecco's modified Eagle's medium (DMEM). Resultant geneticin-resistant clones were picked, and TRH receptor containing clones were identified as those in which TRH produced a rise in total inositol phosphate production. Expression of the TRH receptor in membranes from these clones was assessed by the specific binding of [3 H]TRH. Clone E2, which expresses some 14 pmol of the receptor/mg of membrane protein ([Kim et al. 1994](#)), was selected for further transfection with plasmid pCMV, into which a cDNA encoding murine G_{11} was inserted, and with the plasmid pBABE hygro, which allows expression of resistance to the antibiotic hygromycin B. Clones were selected on the basis of resistance to hygromycin B, and the continued expression of the TRH receptor and novel expression of murine G_{11} were examined ([Kim et al. 1994](#); [Svoboda et al. 1996](#)).

Cell culture. HEK-293 cells (clone E2M11) were maintained in Dulbecco's modified Eagle's medium supplemented with 0,55 mg/ml L-

glutamine and 10% (v/v) heat-inactivated newborn calf serum and with selection markers geneticin (0,8 mg/ml) and hygromycin B (0,2 mg/ml) at 37°C in a 5% CO₂ humidified atmosphere. E2M11 cells were cultivated in cell culture dishes according to the protocol of Svoboda et al. (1996).

Cell fractionation. E2M11 cells were grown to 70-80% confluence and the day before the experiment the medium was replaced by fresh medium. Cells were incubated with 10 µM TRH in growth medium at the following time points - 0 min (control, naive, hormone unexposed cells), 10 min, 30 min, 1 h, 2h, 4 h and 16 h at 37°C in a 5% CO₂ humidified atmosphere prior to the harvest. Cells were harvested from cell culture dishes (152 cm² each, 1,96 x 10⁶ cells per dish); 7 dishes were used per each time point. Agonist stimulation was stopped by placing dishes on ice and by aspirating of medium. Cells were scraped in ice-cold phosphate-buffered saline solution, PBS (138 mM NaCl, 2.8 mM KCl, 1.5 mM KH₂PO₄, 10 mM Na₂HPO₄, pH 7,4) and washed twice in this solution by spinning at 3 000 rpm for 5 min, 4°C. The final cell sediment was frozen at -80°C for at least 1 h.

The resulting sediment was re-suspended by repeated pipetting in 3 500 µl (500 µl per dish) of 250 mM sucrose, 50 mM Tris-HCl, pH 7.4, 3 mM MgCl₂, 1 mM EDTA (STE buffer) containing freshly added 1 mM PMSF and protease inhibitors. The cell suspension was homogenised by hand in glass-glass homogeniser for 7 min on ice. Each sample was then spun at 1 000 rpm for 5 min at 4°C and the resulting supernatant (*post-nuclear supernatant*) was spun again at 250 000 x g for 2 h at 4°C. The resulting supernatant represented *soluble (cytosol) fraction* and was stored at -80°C until use.

TCA precipitation and extraction in diethylether / ethanol. Post-nuclear supernatant and soluble (cytosol) fractions were prepared as described in previous paragraph and precipitated with ice-cold 7.5% (w/v) TCA on ice for 1 h. The mixture was centrifuged at 16 000 x g for 10 min at 4°C. The supernatant was discarded and the pellet was treated with 1 ml of ice-cold 5% (w/v) TCA on ice for 10 min. The sample was centrifuged at 16 000 x g for 5 min at 4°C. The supernatant was aspirated and the pellet was washed twice with ice-cold diethylether/ethanol (1:1, v/v) by mixing end-over-end for 30 min at room temperature. The pellet was regained in each step by centrifugation at 16 000 x g for 10 min at 4°C. For 2D electrophoresis the pellet was air-dried for a couple of minutes and dissolved in denaturing *2D sample buffer* containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 1% (v/v) IPG buffer, pH 4-7, 1% (w/v) DTT by shaking for 1 hr at room temperature. The sample was cleared by centrifugation at 16 000 x g for 10 min at room temperature. The supernatant arising from this centrifugation was referred to as *fraction 1*. The remaining pellet was vortexed with freshly added *2D sample buffer* for 1 hr at room temperature, spun at 16

000 x g for 10 min at room temperature. The supernatant was added to the *fraction 1*.

Isoelectric focusing (IEF). The 18 cm linear gradient pH 4-7 ready-to-use Immobiline DryStrips were loaded with 400 µg of protein in 360 µl of 2D sample buffer with trace of bromophenol blue per strip by passive re-hydration overnight at room temperature. Strips were transferred into coffins and covered with thin layer of mineral oil to prevent evaporation. Isoelectric focusing was performed on an Amersham IPGphor device, using a step-and-hold protocol of increasing voltage as follows: 30 V for 15 Vhr, 150 V for 1000 Vhr, 500 V for 500 Vhr, 1500 V for 1500 Vhr, 3000 V for 3000 Vhr, 8000 V for 8500 Vhr, and 2000 V for 24-48 h. The electrofocused strips were stored at -20°C before the second dimension.

Two-dimensional SDS-polyacrylamide gel electrophoresis. The IPG strips were rinsed thoroughly with distilled water, dried quickly on filter paper and equilibrated prior to the second dimension electrophoresis in 4 ml of *equilibration buffer* [30% (v/v) glycerol, 2% (w/v) SDS, 6 M urea, 50 mM Tris-HCl, pH 6,8] in Immobiline Dry Strip Re-swelling Tray. Subsequently, disulphide bridges present in protein molecules and other oxidised groups were reduced in equilibration buffer containing 2% (w/v) DTT and alkylated in equilibration buffer containing 4.5% (w/v) IAA, for 20 min each. After this, the IPG strips were rinsed thoroughly with distilled water, dried quickly on filter paper and treated with *Laemmli solubilisation buffer* [25 mM Tris, 192 mM glycine, 0,1% (w/v) SDS] for 10 min. The 14 cm x 16 cm x 1 mm gels were cast with 10% (v/v) polyacrylamide mixture with double amount of SDS [0,2% (w/v) SDS final concentration], using Hoefer SE 600 unit (2 x 16 cm x 18 cm). Equilibrated strips were cut 3 cm from left side and 2 cm from right side and loaded horizontally on the top of the gel. Molecular weight markers were loaded onto a piece of filter paper and placed close to the acidic side of the strip. The strip and molecular weight markers were sealed in place with 0,5% (w/v) agarose in Laemmli buffer (*SLB*) containing bromophenol blue. Gels were run vertically in SLB at a constant voltage of 30 V for 30 min and then at constant voltage of 50 V overnight till the bromophenol blue dye reached the end of the gel (the apparatus was cooled to 20°C throughout electrophoresis).

Production of antisera G_{qa}/G_{11a} protein oriented antibody was rabbit polyclonal antipeptide serum raised in rabbits obtained from VELAZ (Prague). Immunisation was performed with a glutaraldehyde conjugate of keyhole limpet hemocyanin and synthetic peptide QLNLKEYNLV representing the C-terminal decapeptide, which is conserved between G_{qa} and G_{11a}. This antiserum identifies both polypeptides equally (Mitchell et al. 1991).

Immunoblotting After the second dimension, proteins were transferred to nitrocellulose membrane by semi-dry blotting. Transfer was performed at 21 V (constant voltage) for 90 min. Membranes were blocked with 5% fat-free milk in PBS with 0.05% (v/v) Tween 20 for 1 hr at room temperature. Subsequently, the blots were incubated in 1% fat-free milk, 0.05% (v/v) Tween 20 in PBS with appropriate primary antibody for 2 hr at room temperature. Rabbit antiserum against $G_{q\alpha}/G_{11\alpha}$ (84/1) was used at a 1:5 000 dilution. After extensive wash with 0.05% (v/v) Tween 20 in PBS (3x10 min), the membranes were incubated for 1 hr at room temperature with horseradish peroxidase-conjugated with secondary anti-rabbit antibody which was diluted 20 000 x in 1% fat-free milk in PBS with 0.05% (v/v) Tween 20. After 3x 10 min washes with 0.05% (v/v) Tween in PBS, the blots were developed by ECL Plus Kit (Amersham).

Image Analysis The immunoblot signals were visualised on films, subjected to densitometric scanning and analysed by PDQuest.

Results

HEK293 cells expressing large amount of TRH-receptors and exogenous, mouse isoform of $G_{11\alpha}$ (clone E2M11) were incubated with 10 μ M TRH for increasing periods of time – 0 min (control), 10 min, 30 min, 60 min, 120 min, 4 h and 16h. Harvested control and hormone-treated cells were subjected to homogenisation and low-speed centrifugation in order to isolate post-nuclear supernatant from nuclear fraction. Subsequently, the cytosol fraction was prepared by high-speed centrifugation of post-nuclear supernatant (2h at 250 000 x g, 4°C) as described in Methods. The cytosol fraction was concentrated by TCA precipitation, extracted in diethylether/ethanol and resolved in the first dimension according to isoelectric points (1st D). The standard SDS-PAGE in 10% polyacrylamide gel was used afterwards for resolution in the second dimension according to relative molecular weights (2nd D). $G_{q\alpha}/G_{11\alpha}$ proteins were then detected by Western blotting and ECL with specific anti- $G_{q\alpha}/G_{11\alpha}$ antibodies. The immunoblots were quantified by densitometric scanning and analysed by PDQuest.

Small signal of the two soluble $G_{q\alpha}/G_{11\alpha}$ proteins was detected already in control, hormone-unexposed cells (spot 7 and 8, **Fig. 1, time zero**). Hormone stimulation for 10, 30, 60 and 120 min resulted in an increase of both intensity and number of immuno-reactive signals/spots of these G proteins (**Fig. 1, 0.12-2 hours**); at long time intervals (>2 hours), significant increase of $G_{q\alpha}/G_{11\alpha}$ -related immuno-reactivity was detected (**Fig. 1, 2-16 hours**). Comparison of the data collected from various time intervals indicated that the maximal increase of $G_{q\alpha}/G_{11\alpha}$ in soluble fraction was reached after 16 h of incubation with TRH. At

this time period, eight distinct immuno-reactive proteins were clearly resolved and the total intensity of these spots was 15x higher than in control cells ([Table 1](#), see the next paragraph for further details).

Various numbers of immunoblot signals of $G_{q/a}/G_{11a}$ proteins were observed in the course of the hormone stimulation. The two signals were detected already in control cells (spot 7 and 8) and exposure to TRH for 10 and 30 min resulted in increase in intensity of these spots and appearance of the third one (spot 3). Two major and three minor spots were identified after 2 hours of stimulation and, as already mentioned in the previous paragraph, eight immuno-reactive signals were identified after 16h of hormone stimulation ([Fig. 1](#)). The relative proportion among these spots was 15:42:10:11:7:7:2:5 ([Table 2](#)). It is reasonable to assume that the two major signals correspond to exogenously (over) expressed mouse isoform of G_{11a} and endogenous, human $G_{q/a}$. Our previous studies indicated that the presence of human G_{11a} was very low (Kim et al., 1994; Matousek et al., 2004, 2005). It may be assumed that the minor signals, which were also clearly distinguished, represent covalently modified forms of the two major G proteins exhibiting the same relative molecular weight but different isoelectric points.

The isoelectric points of the two major forms of $G_{q/a}/G_{11a}$ were estimated from the isoelectric focusing data to be at pH 5.7 and 5.9 ([Fig. 2](#)). Previously we studied resolution of $G_{q/a}/G_{11a}$ proteins in E2M11-HEK-293 cells by two-dimensional electrophoresis, but in membrane preparations ([Matousek et al. 2004, 2005](#)). Five different signals were detected indicating either resolution of multiple members of $G_{q/a}/G_{11a}$ family or some post-translational modification of the dominant proteins of $G_{q/a}/G_{11a}$ family. In membranes, the two major spots corresponding to exogenously (over) expressed G_{11a} and endogenous $G_{q/a}$ were reduced; the minor spots were diminished to zero level (after 16 h of hormonal stimulation). ***Both lines of experimental evidence thus converge to each other and indicate that prolonged agonist stimulation of TRH-R in cells expressing large amount of these receptors results in dramatic but slow decrease of membrane bound complement of $G_{q/a}/G_{11a}$. This decrease is accompanied by an increase of these proteins in the soluble, cytosol fraction, which is also very slow.***

Discussion

Post-translational modification of $G\beta\gamma$ subunits is regarded as an important cause of membrane localisation of trimeric G-protein complex in hormonally non-stimulated cells. $G\gamma$ subunits are prenylated ([Mumby et al. 1990a](#)) and α subunits of G_i family are myristoylated ([Mumby et al. 1990b](#)). In addition, nearly all $G\alpha$ subunits are acylated on cysteine residues near amino termini by

formation of thio-esters, usually with palmitate (Parenti et al. 1993; Linder et al. 1993). Interaction with Gβγ is required for membrane targeting and palmitoylation of G_sα and G_qα (Evanko et al. 2000) and Gβγ isoforms selectively rescue plasma membrane localisation and palmitoylation of mutant G_sα and G_qα (Evanko et al. 2001). Although there is general agreement that activation of G-proteins causes an increased turnover of palmitate on Ga, the topological fate of these proteins is not settled. Wedegaertner and Bourne proposed that activation and de-palmitoylation of Ga promote its release from the membrane (Wedegaertner and Bourne 1994; Wedegaertner et al. 1995). It has been also reported that functional interaction between the α_{1b}-adrenoreceptor and G₁₁α is reduced by de-palmitoylation of the G protein (Novotny et al. 2006). On the other hand, data of Huang et al. (1997; 1999) supported a different conclusion: membrane association of Ga persists whether the protein is activated and/or de-palmitoylated.

Hormonal or neurotransmitter stimulation results in an uncoupling of Ga from Gβγ subunits. Though evidenced mainly from functional point of view (Gilman 1987), it is reasonable to assume that in hormonally stimulated cells, membrane attachment of Ga is weakened when compared with un-stimulated cells. Aliphatic chains of fatty acids such as palmitic and myristic acid represent the only sites, which in the presence of hormone are linking Ga subunits to the membrane and protecting Ga from detachment from the membrane, i.e. solubilisation (Iiri et al. 1996; Evanko et al. 2000; 2001). Potential for detachment from the membrane and solubilisation of Ga subunits is further increased in conditions of sustained agonist stimulation and/or in cells expressing high amount of corresponding receptors. It is reasonable to assume that under such conditions, equilibrium between Ga-GTP and Ga-GDP is shifted to „free” Ga-GTP, which is more susceptible for detachment from the membrane.

With the aim to bring more detailed picture of agonist-induced release of G proteins from the membrane, the time-course of TRH (thyrotropin-releasing hormone) solubilisation of G_qα/G₁₁α was analysed in HEK-293 cells stably expressing large amount of TRH-R. The soluble (cytosolic) forms of G_qα/G₁₁α were resolved by 2D electrophoresis. In spite of large number of receptors activated by continuous agonist presence, G_qα/G₁₁α proteins were almost undetectable in soluble fraction till the 10th minute of continuous agonist exposure. The significant increase was observed after 0.5-2 h of stimulation and prolongation of hormone stimulation up to 16 h was reflected by the further increase of G_qα/G₁₁α immuno-reactivity in soluble fraction. Membrane association of various species of G_qα/G₁₁α thus persists under drastic conditions of hormonal stimulation and the release of these G α subunits does not seem to

follow rather fast palmitoylation/de-palmitoylation cycles and/or interaction(s) with Gβ γ subunits.

Acknowledgements

This work was supported by project LC 554 of Ministry of Education of the Czech Republic (Centrum of Neurosciences), Grant Agency of Czech Republic (305/06/0121) and Academy of Sciences of the Czech Republic (AVOZ 5011922).

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Figure legends

Fig. 1

The time-course of TRH-induced solubilisation of G_qa/G₁₁a proteins

E2M11-HEK-293 cells were either untreated (**0**) or exposed to 10 μ M TRH for 10 min (**0.12 h**), 30 min (**0.5 h**), 60 min (**1 h**), 2 (**2 h**), 4 (**4h**) or 16 hours (**16 h**). Cell harvest and homogenisation was performed as described in Methods. The soluble (cytosol) cell fraction was prepared by centrifugation of post-nuclear supernatant at 250 000 x g for 2h. Cytosolic (soluble) proteins were concentrated by TCA precipitation, resolved by 2D electrophoresis and G_qa/G₁₁a proteins identified by immuno-blotting with CQ antiserum oriented against C-terminal decapeptide of G_qa and G₁₁a. The results show the average intensity of immunoblot signals (expressed in arbitrary units) collected from 4 independent experiments at each time interval of agonist exposure.

Spot 1, open squares; Spot 2; open circles; spot 3, stars, spot 4, crosses, spot 5, full triangles; spot 6, full circles; spot 7, full squares, spot 8, full rhombuses.

Table 1

Quantitative analysis of the time-course of TRH-induced solubilisation of G_qa/G₁₁a proteins

The same legend as in Fig. 1. The results show the average intensity of immunoblot signals collected from 4 independent experiments at each time interval of agonist exposure. The total signal in control, hormone-unexposed cells (100%) was compared with the total signal of all spots resolved at a given time interval of 0.12, 0.5, 1, 2, 4 and 16 hours.

Table 2

The relative proportion among signals corresponding to G_qa/G₁₁a proteins resolved by 2D electrophoresis and detected by immuno-blotting in supernatant 250 000 x g prepared from e2m11-HEK293 cells stimulated by TRH for 16 hours

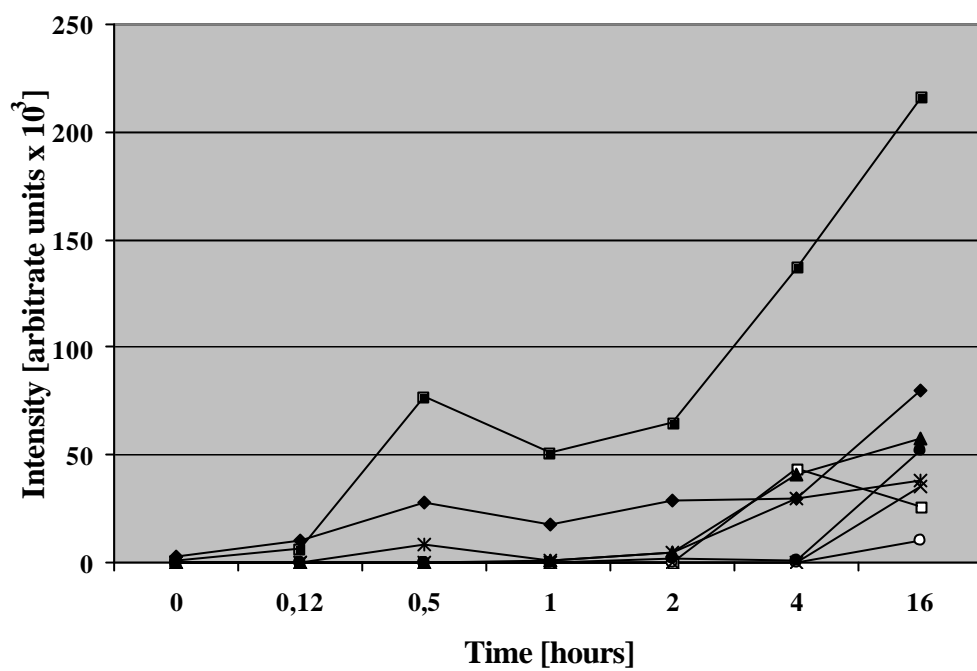
Spot	Average signal	%
1	25902	5
2	10009	2
3	37508	7
4	35440	7
5	57749	11
6	51661	10
7	215707	42
8	79901	16
Total	513876	100

Fig. 2
PDQuest images of the soluble G_qa/G₁₁a proteins resolved at various time intervals of TRH-exposure

Samples collected from hormone unexposed, naive cells (0 min) and TRH-treated cells (10 min, 30 min, 60 min, 2, 4 and 16 hours) were resolved by 2D electrophoresis and analysed by PDQuest program.

Fig. 1

**Time-course of TRH-induced solubilisation
of G_qa/G₁₁a proteins**



Spot 1 (open square), Spot 2 (open circle), Spot 3 (star), Spot 4 (cross), Spot 5 (full triangle),
Spot 6 (full circle), Spot 7 (full square), Spot 8 (full rhombus)

Tab. 1

**Quantitative analysis of TRH-induced solubilisation
of G_qa/G₁₁a proteins**

Time [hours]	0	0,12	0,5	1	2	4	16
Spot 1	ND	ND	ND	ND	ND	43349	25902
Spot 2	ND	ND	ND	ND	ND	ND	10009
Spot 3	ND	ND	8004	1284	4693	30034	37508
Spot 4	ND	ND	ND	ND	ND	ND	35439
Spot 5	ND	ND	ND	813	4920	40712	57748
Spot 6	ND	ND	ND	ND	1797	951	51661
Spot 7	1096	6489	76636	50915	64409	136745	215707
Spot 8	2368	9884	27987	17678	28849	29987	79901
Total	3463	16373	112626	70689	104668	281778	513876
%	100	473	3252	2041	3022	8136	14837

Table 2

The relative proportion among signals corresponding to G_qa/G₁₁a proteins resolved by 2D electrophoresis and detected by immuno-blotting in supernatant 250 000 x g prepared from e2m11-HEK293 cells stimulated by TRH for 16 hours

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Fig. 2

**PDQuest images of soluble $G_q\alpha/G_{11}\alpha$ proteins
resolved by 2D electrophoresis**

