



University of Zurich
Zurich Open Repository and Archive

Winterthurerstr. 190
CH-8057 Zurich
<http://www.zora.uzh.ch>

Year: 2009

Calreticulin enhances B2 bradykinin receptor maturation and heterodimerization

Abd Alla, J; Reeck, K; Langer, A; Streichert, T; Qwitterer, U

Abd Alla, J; Reeck, K; Langer, A; Streichert, T; Qwitterer, U (2009). Calreticulin enhances B2 bradykinin receptor maturation and heterodimerization. *Biochemical and Biophysical Research Communications*, 387(1):186-190.

Postprint available at:
<http://www.zora.uzh.ch>

Posted at the Zurich Open Repository and Archive, University of Zurich.
<http://www.zora.uzh.ch>

Originally published at:
Biochemical and Biophysical Research Communications 2009, 387(1):186-190.

Calreticulin enhances B2 bradykinin receptor maturation and heterodimerization

Joshua Abd Alla^a, Kristin Reeck^b, Andreas Langer^a, Thomas Streichert^b, Ursula Quitterer^{a,*}

^a*Department of Molecular Pharmacology, Swiss Federal Institute of Technology and University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland*

^b*Department of Clinical Chemistry/Central Laboratories, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany*

*Corresponding author. Address: Department of Molecular Pharmacology, Swiss Federal Institute of Technology and University of Zurich, Y17M70, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland. Fax: +41 44 635 6881.

E-mail address: ursula.quitterer@pharma.ethz.ch (U. Quitterer).

Abstract

In different native tissues and cells the receptor for the vasodepressor bradykinin, B₂, forms dimers with the receptor for the vasopressor angiotensin II, AT₁. Because AT₁/B₂ heterodimers may contribute to enhanced angiotensin II-stimulated signalling under pathophysiological conditions, we analyzed mechanisms of AT₁/B₂ heterodimerization. We found that efficient B₂ receptor maturation was a prerequisite for heterodimerization because only the fully mature B₂ receptor was capable to interact with AT₁. To identify chaperones involved in B₂ receptor maturation and heterodimerization we performed microarray gene expression profiling of human embryonic kidney (HEK293) cells. The expression of the chaperone calreticulin was up-regulated in cells with efficient B₂ receptor maturation. Vice versa, upon down regulation of calreticulin expression by RNA interference, B₂ receptor maturation and AT₁/B₂ receptor heterodimerization were significantly impaired. Concomitantly, the B₂ receptor-mediated enhancement of AT₁-stimulated signalling was reduced. Thus, calreticulin enhances B₂ receptor maturation and heterodimerization with AT₁.

Keywords:

bradykinin

angiotensin II

B2 bradykinin receptor

Type-1 angiotensin II receptor

G-protein-coupled receptor

protein maturation

chaperone

calreticulin

receptor dimerization

Introduction

Angiotensin II is an important peptide hormone regulating vascular tone and blood pressure. The vasopressor actions of angiotensin II are mediated mainly by the AT₁ receptor. In contrast, bradykinin is a vasodepressor peptide, which exerts major effects via the B₂ bradykinin receptor. The angiotensin II and bradykinin systems are intertwined at multiple levels. The angiotensin-converting enzyme (ACE) regulates the availability of both peptides in vivo by releasing angiotensin II and inactivating bradykinin. With more than 30% identity, the AT₁ receptor is one of the closest relatives of the B₂ receptor. In agreement with the high homology, a direct interaction between the angiotensin II AT₁ receptor and the bradykinin B₂ receptor in vivo in rat brain is known since 1993 [1]. We followed those original studies, and identified AT₁/B₂ receptor heterodimers in different other native systems and in patients with preeclampsia [2,3]. In addition to the in vivo data, AT₁/B₂ interactions were also reconstituted in a transfected in vitro system [2,3].

Heterodimerization of AT₁ with B₂ receptors leads to signal enhancement of AT₁ [2,3]. Signal enhancement as a consequence of heterodimerization with the B₂ receptor is also effective for the closely related angiotensin II AT₂ receptor [4]. Both studies were performed with native cells [3,4]. Thus, a native, correctly folded B₂ receptor protein seems to be required for receptor heterodimerization and signal enhancement.

For various G-protein-coupled receptors, conditions of efficient protein folding were investigated. Different chaperones are known to be involved in the correct folding of AT₁ receptors such as BiP, calnexin, and D₁RiP78 [5]. By contrast, little is known about chaperones, which assist the folding of B₂ receptors and AT₁/B₂ receptor heterodimerization. We therefore analyzed requirements for efficient B₂ receptor maturation and the potential interrelationship between B₂ receptor maturation and heterodimerization with the angiotensin II AT₁ receptor.

Materials and methods

Cell culture and cell transfection. HEK293 cells were routinely grown in DMEM (100 mg/dl glucose) supplemented with 10 % (v/v) FCS unless otherwise indicated. Cells were transfected with Lipofectamine Plus (Invitrogen). Transfection efficiency of HEK293 cells was ≥ 90 %. Plasmids encoding the human B₂ and AT₁ receptor under control of the CMV promoter were used. Cellular inositol phosphate levels were determined similarly as described [2]. For down-regulation of calreticulin expression, HEK293 cells were transfected with stealth RNAi (20 pmol/ml) targeting the coding sequence of the human calreticulin cDNA (nucleotides 59-83, RNAi-Calreticulin1 and nucleotides 367-391, RNAi-Calreticulin2). Quantification of B₂ receptors was performed on HEK cells with 50 nM of

[¹²⁵I]-labeled F(ab)₂ fragments of affinity-purified B₂ receptor-specific antibodies (~1 μCi) similarly as described [6].

Protein detection in immunoblot and co-enrichment of receptors. Protein detection in immunoblot was performed with membranes prepared by sucrose density gradient centrifugation followed by partial enrichment as described [2]. For co-enrichment of AT₁ and B₂ receptors, membranes of HEK cells were solubilized with RIPA buffer (including protease inhibitor cocktail) and subjected to immuno-affinity chromatography by anti-AT₁ receptor antibodies using 0.1 ml immunoaffinity matrix (Affigel 10; 15 mg affinity-purified antibodies per ml gel). After extensive washing, proteins were eluted with 0.2 M glycine, pH 2.5, neutralized, desalted, delipidated and precipitated [2]. Eluted proteins were dissolved and separated by urea-containing SDS-PAGE under reducing conditions. Enriched AT₁ and co-enriched B₂ receptors were identified in immunoblot with F(ab)₂ fragments of the respective anti-receptor antibodies. Enrichment of the B₂ receptor was performed analogously with anti-B₂ receptor antibodies. Chemical deglycosylation of the enriched B₂ receptor was performed with trifluoromethanesulfonic acid (TFMS) at 0 °C as described [7].

Cross-linking of bradykinin to the B₂ receptor. Cross-linking of bradykinin (5 nM) to the B₂ receptor was performed with 0.2 mM *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) in the absence or presence of a 400-fold molar excess of the B₂-specific antagonist HOE140 at 4 °C using membranes prepared from B₂ receptor-expressing HEK293 cells as described previously [2,8]. Solubilized proteins were separated by SDS-PAGE under reducing conditions including 6 M urea followed by immunoblotting with anti-bradykinin antibodies [2].

Antibodies used for immunoblotting and immunofluorescence. All antibodies used for immunoblotting, receptor immunoaffinity enrichment and immunofluorescence were characterized in previous studies [2,3,6,8-10]. Immunofluorescence was performed with paraformaldehyde-fixed (4 % w/v) cells [6].

Microarray gene expression profiling. For microarray gene expression analysis, HEK293 cells were cultivated for 7 days in DMEM supplemented with 10 % FCS and 100 mg/dl (low) or 450 mg/dl (high) glucose. Total RNA was isolated with the RNeasy Mini kit (Qiagen). Procedures for cDNA synthesis, labeling and hybridization were carried out according to the protocol of the manufacturer (Affymetrix GeneChip Expression Analysis Technical Manual; Rev. 5). For hybridization, 15 μg of fragmented cRNA were incubated with the chip (Affymetrix GeneChip Human genome U133 Plus 2.0 Array) in 200 μl of hybridization solution in a Hybridization Oven 640 (Affymetrix) at 45 °C for 16 h. GeneChips were then washed and stained using the Affymetrix Fluidics Station 450. Microarrays were scanned with the Affymetrix GeneChip Scanner 7G, and the signals were processed using GCOS (v. 1.4, Affymetrix). Gene expression data are available at GEO accession no GSE15575.

Statistics. Unless otherwise stated, data are expressed as mean \pm S.E. To determine significance between two groups, we made comparisons using the unpaired two-tailed Student's t-test. *P* values of < 0.05 were considered significant.

Results

Transfected HEK293 cells synthesize a monomeric B₂ receptor that resembles the native B₂ receptor protein

Protein maturation was assessed with HEK293 cells as an expression system because the B₂ receptor protein of transfected HEK293 cells appeared as a pure monomer (Fig. 1A). Even at high receptor expression levels (>5 pmol/mg protein), the B₂ receptor expressed in HEK293 cells did not show significant protein aggregation under reducing conditions of SDS-PAGE supplemented with urea (Fig. 1A). The B₂ receptor protein of transfected HEK293 cells showed a similar apparent molecular mass in SDS-PAGE as the native B₂ receptor protein of various native tissues and cells (Fig. 1B). Detection of the B₂ receptor by immunofluorescence revealed the predominant membrane localization of the transfected B₂ receptor on HEK293 cells (Fig. 1C). Altogether, these findings indicate that HEK293 cells express all the proteins necessary for efficient B₂ receptor folding.

High glucose induces an immature B₂ receptor protein in HEK293 cells

Expression of mature and correctly folded receptors on the plasma membrane depends on the general chaperone system of the endoplasmic reticulum (ER) controlling protein synthesis, folding and assembly [11]. To assess the impact of the general chaperone system on B₂ receptor folding and AT₁/B₂ receptor heterodimerization, we down-regulated the general chaperone system of HEK293 cells by high glucose [12]. Cultivation of HEK293 cells in a standard medium with high glucose (450 mg/dl) led to the appearance of an "immature" B₂ receptor-reactive band of $\sim 53 \pm 4$ kDa in addition to the (mature) B₂ receptor of 67 ± 5 kDa (Fig. 2A).

The B₂ receptor-reactive band of 53 kDa did not show a high-affinity interaction with the agonist bradykinin as assessed by affinity cross-linking (Fig. 2B). Chemical deglycosylation of the "mature" and "immature" B₂ receptor proteins produced the deglycosylated B₂ receptor of ~ 43 kDa (Fig. 2C). These experiments strongly suggest that the 53 kDa protein is indeed an immature B₂ receptor which is not fully glycosylated. Interestingly, the immature B₂ receptor of 53 kDa did not interact with the AT₁ receptor as assessed by co-immunoenrichment (Fig. 2D). Thus, efficient B₂ receptor maturation seems to be required for heterodimerization with the AT₁ receptor.

Microarray gene expression profiling of the general chaperone system of HEK293 cells

To determine chaperone(s) involved in B₂ receptor maturation and AT₁/B₂ receptor heterodimerization, we performed microarray gene expression profiling of HEK293 cells cultivated in low and high glucose medium, respectively. Differentially expressed proteins of the ER protein quality-control system were identified by selection of probe sets with (i) ≥ 2.5 -fold higher expression in low versus high glucose, (ii) ER localization according to gene ontology (GO) analysis, and (iii) involvement in the general chaperone system according to the literature (Fig. 3A). The approach identified 14 ER-localized members of the protein quality-control system that showed a more than 2.5-fold higher expression signal in cells cultivated in low glucose relative to high glucose (Fig. 3A). The microarray gene expression data were verified by immunoblotting as exemplified for GRP78 (Fig. 3B).

The chaperone calreticulin co-localizes with the B₂ receptor

Several of the identified proteins of the general chaperone system are reported to be involved in folding of membrane proteins [5]. Searching for chaperones that are specifically required for the maturation of B₂ receptors, we focused on calreticulin, because gene inactivation studies revealed an interrelationship between calreticulin and functional B₂ receptor protein levels [13]. Immunoblotting confirmed the microarray-based evidence of a significantly higher calreticulin protein expression in HEK293 cells cultivated in low glucose relative to high glucose (Fig. 3B, middle panel). As a control, actin protein levels of HEK293 cells were not significantly different (Fig. 3B, lower panel). In agreement with a potential role of calreticulin in B₂ receptor folding and maturation, immunofluorescence revealed the cellular co-localization of the B₂ receptor with calreticulin (Fig. 3C).

Down-regulation of calreticulin expression by RNA interference impairs B₂ receptor maturation and AT₁/B₂ receptor heterodimerization

To determine the potential involvement of calreticulin in B₂ receptor maturation, we down-regulated the expression of calreticulin by RNA interference. Upon down-regulation of calreticulin, the maturation of the B₂ receptor protein was significantly affected as evidenced by the appearance of the immature B₂ receptor protein band of 53 kDa even when cells were cultivated in a medium with a physiological (low) glucose concentration (Fig. 4A).

Moreover, down-regulation of calreticulin strongly reduced the AT₁/B₂ receptor interaction because only the fully mature B₂ receptor of 67 kDa was capable to interact with AT₁ whereas the immature B₂ receptor of 53 kDa was not significantly co-enriched with AT₁

(Fig. 4B). Concomitantly, the B₂ receptor-mediated enhancement of the AT₁-stimulated signal - an additional indicator of AT₁/B₂ receptor heterodimerization - was strongly decreased upon down-regulation of calreticulin (Fig. 4C). Together these data suggest that endogenous calreticulin expression levels of HEK293 cells are sufficient to enhance B₂ receptor maturation and AT₁/B₂ receptor heterodimerization.

Discussion

In various in vivo systems the receptor for the vasodepressor bradykinin, B₂, undergoes a functional interaction with the receptor for the vasopressor angiotensin II, AT₁ [1-3,14]. The covalently bonded AT₁/B₂ receptor heterodimers are distinguished from individual, dissociable receptors by a kinetically favored interaction with G-proteins leading to enhanced AT₁-stimulated signaling [2-3]. Because increased formation of AT₁/B₂ receptor heterodimers could constitute a pathological feature of hypertensive disorders [3,14], the current study investigated mechanisms required for functional AT₁/B₂ receptor heterodimerization.

Several lines of evidence are presented supporting that efficient protein maturation of the B₂ receptor is a prerequisite for functional interaction with AT₁. First, only the mature B₂ receptor interacted with co-expressed AT₁ as determined by co-enrichment. Second, microarray gene expression profiling revealed a correlation between efficient AT₁/B₂ receptor heterodimerization and the expression of the general chaperone system. Finally, RNA interference studies determined the involvement of the chaperone calreticulin in B₂ receptor maturation and AT₁/B₂ receptor heterodimerization. Future studies will have to assess whether the herein identified requirement of specific chaperone(s) for AT₁/B₂ receptor heterodimerization is a factor involved in the induction of AT₁/B₂ receptor heterodimers under pathophysiological conditions.

Acknowledgments

The work was supported in part by the Swiss National Science Foundation.

References

- [1] D.R. Fior, P.B. Hedlund, K. Fuxe, Autoradiographic evidence for a bradykinin/angiotensin II receptor-receptor interaction in the rat brain, *Neurosci. Lett.* 163 (1993) 58-62.
- [2] S. AbdAlla, H. Lothar, U. Quitterer, AT₁-receptor heterodimers show enhanced G-protein activation and altered receptor sequestration, *Nature* 407 (2000) 94-98.

- [3] S. AbdAlla, H. Lothar, A. el Massiery, U. Quitterer, Increased AT(1) receptor heterodimers in preeclampsia mediate enhanced angiotensin II responsiveness, *Nat. Med.* 7 (2001) 1003-1009.
- [4] P.M. Abadir, A. Periasamy, R.M. Carey, H.M. Siragy, Angiotensin II type 2 receptor-bradykinin B2 receptor functional heterodimerization, *Hypertension* 48 (2006) 316-322.
- [5] C. Dong, C.M. Filipeanu, M.T. Duvernay, G. Wu, Regulation of G protein-coupled receptor export trafficking, *Biochim. Biophys. Acta* 1768 (2006) 853-870.
- [6] S. AbdAlla, H. Lothar, A. el Missiry, A. Langer, P. Sergeev, Y. el Faramawy, U. Quitterer, Angiotensin II AT2 receptor oligomers mediate G-protein dysfunction in an animal model of Alzheimer disease, *J. Biol. Chem.* 284 (2009) 6554-6565.
- [7] A.S. Edge, C.R. Faltynek, L. Hof, L.E. Reichert, P. Weber, Deglycosylation of glycoproteins by trifluoromethanesulfonic acid, *Anal. Biochem.* 118 (1981) 131-137.
- [8] S. AbdAlla, E. Zaki, H. Lothar, U. Quitterer, Involvement of the amino terminus of the B(2) receptor in agonist-induced receptor dimerization, *J. Biol. Chem.* 274 (1999) 26079-26084.
- [9] S. AbdAlla, H. Lothar, A. Langer, Y. el Faramawy, U. Quitterer, Factor XIIIa transglutaminase crosslinks AT1 receptor dimers of monocytes at the onset of atherosclerosis, *Cell* 119 (2004) 343-354.
- [10] S. AbdAlla, H. Lothar, A. el Missiry, P. Sergeev, A. Langer, Y. el Faramawy, U. Quitterer, Dominant-negative AT2 receptor oligomers induce G-protein arrest and symptoms of neurodegeneration, *J. Biol. Chem.* 284 (2009) 6566-6574.
- [11] L. Achour, C. Labbé-Jullie, M.G. Scott, S. Marullo, An escort for GPCRs: implications for regulation of receptor density at the cell surface, *Trends Pharmacol. Sci.* 29 (2008) 528-535.
- [12] S.P. Ramachandra Rao, R. Wassell, M.A. Shaw, K. Sharma, Profiling of human mesangial subproteomes reveals a role for calmodulin in glucose uptake, *Am. J. Physiol. Renal Physiol.* 292 (2007) F1182-F1189.
- [13] K. Nakamura, A. Zuppini, S. Arnaudeau, J. Lynch, I. Ahsan, R. Krause, S. Papp, H. De Smedt, J.B. Parys, W. Muller-Esterl, D.P. Lew, K.H. Krause, N. Demaurex, M. Opas, M. Michalak, Functional specialization of calreticulin domains, *J. Cell. Biol.* 154 (2001) 961-972.
- [14] S. AbdAlla, A. Abdel-Baset, H. Lothar, A. el Massiery, U. Quitterer, Mesangial AT1/B2 receptor heterodimers contribute to angiotensin II hyperresponsiveness in experimental hypertension, *J. Mol. Neurosci.* 26 (2005) 185-192.

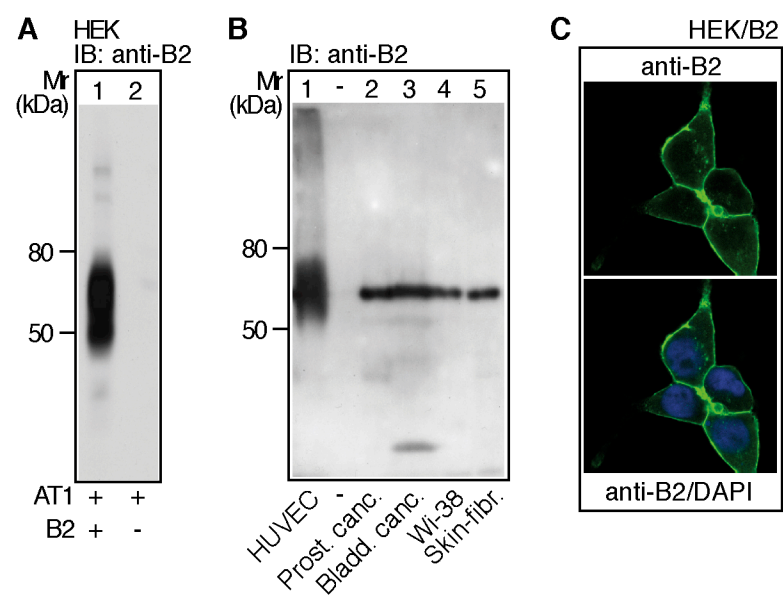
Figure legends

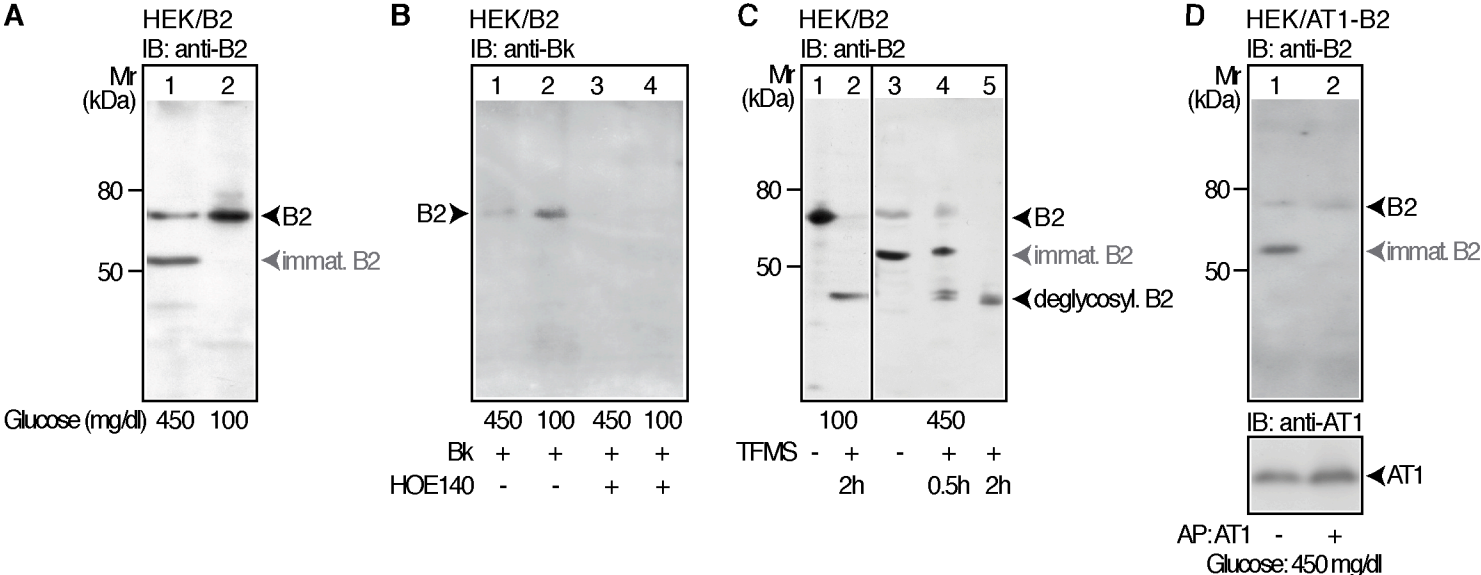
Fig. 1. (A) Immunoblot detection of the B₂ receptor (IB: anti-B₂) with F(ab)₂ fragments of affinity-purified B₂-specific antibodies on enriched membranes of HEK293 cells (HEK) expressing ~ 5 pmol B₂ receptor/mg protein (lane 1). As a control, AT₁ receptor-expressing HEK cells were used (lane 2). (B) Immunoblot detection of B₂ receptors on the enriched and acetone-precipitated, solubilized membrane fraction prepared from different native tissues, i.e. human umbilical vein endothelial cells (HUVEC; lane 1), prostate cancer tissue (Prost. canc., lane 2), bladder cancer tissue (Bladd. canc., lane 3), WI-38 fibroblasts (lane 4), and human skin fibroblasts (Skin-fibr., lane 5). (C) Immunofluorescence detection of the B₂ receptor on B₂ receptor-transfected HEK293 cells (HEK/B₂). The lower panel shows merged pictures of B₂ receptor localization and DAPI-stained cell nuclei (anti-B₂/DAPI). (Original magnification, 630 x).

Fig. 2. (A) Immunoblot detection of the B₂ receptor (IB: anti-B₂) on enriched membranes of HEK293 cells (HEK/B₂; expression of ~ 240 fmol B₂/mg protein) cultivated in a medium with high (450 mg/dl) or low (100 mg/dl) glucose. (B) Affinity-crosslinking of bradykinin (Bk) to the B₂ receptor expressed in HEK293 cells (HEK/B₂) cultivated in medium with 450 or 100 mg/dl glucose as indicated (450; 100), and detection of cross-linked bradykinin in immunoblot with affinity-purified anti-bradykinin antibodies (IB: anti-Bk). B₂-specificity was assessed with the B₂-specific antagonist HOE140 (2 μM; lanes 3,4). (C) Chemical deglycosylation (deglycosyl.) by TFMS (± TFMS) of enriched B₂ receptors from HEK293 cells (HEK/B₂) cultivated in medium containing 100 or 450 mg/dl glucose followed by immunoblot detection of B₂ (IB: anti-B₂). (D) Immunoaffinity enrichment of the AT₁ receptor (± AP: AT₁) from AT₁ and B₂ receptor co-expressing HEK293 cells (HEK/AT₁-B₂) cultivated in medium containing high glucose (450 mg/dl), and detection of co-enriched B₂ receptor or enriched AT₁ receptor in immunoblot with F(ab)₂ fragments of affinity-purified B₂- or AT₁-specific antibodies, respectively (lane 2, upper and lower panel). As a control, the solubilisate was applied for immunoblot detection of the B₂ or AT₁ receptor (lane 1, upper and lower panel).

Fig. 3. (A) Microarray gene expression profiling of HEK293 cells (HEK). Probe sets of low glucose-cultivated HEK293 cells with a ≥ 2.5-fold increased signal relative to that of high glucose-cultivated HEK293 cells (Fold increase; low/high gluc.; t-test *p* value ≤ 0.01), ER localization and involvement in the general chaperone system are listed. (B) Immunoblot analysis controlling the microarray data. (C) Co-localization of the B₂ receptor with calreticulin in HEK293 cells (HEK/B₂-calreticulin) cultivated in low glucose medium. (Original magnification: 630 x).

Fig. 4. (A) Immunoblot detection of the B₂ receptor (IB: anti-B₂) expressed in HEK293 cells (HEK/B₂) cultivated in low glucose (100 mg/dl). As indicated, membranes of control HEK/B₂ cells (-; lane 1), of cells transfected with an unrelated control RNAi duplex (Cont-RNAi; lane 2), or of cells transfected with two different RNAi duplexes targeting calreticulin by RNA interference (RNAi-Calret1, RNAi-Calret2; lanes 3,4) were applied. The lower panel shows an immunoblot of calreticulin (IB: anti-calreticulin). (B) Immuno-affinity enrichment of the AT₁ receptor (\pm AP: AT₁) from AT₁ and B₂ receptor co-expressing HEK293 cells (HEK/AT₁-B₂) transfected with an unrelated control RNAi duplex (Cont-RNAi; left panels) or an RNAi duplex targeting calreticulin by RNA interference (RNAi-Calret2; right panels), and detection of the co-enriched B₂ receptor or enriched AT₁ receptor in immunoblot with F(ab)₂ fragments of affinity-purified B₂- or AT₁-specific antibodies, respectively (lanes 2/4, upper and lower panels). Lanes 1 and 3 show detection of the B₂ or AT₁ receptor in the solubilisate. (C) Strongly reduced B₂ receptor-mediated enhancement of the angiotensin II-stimulated (AngII, 100 nM) inositol phosphate signal upon down-regulation of calreticulin (columns 1,2). As a control, transfection of an unrelated control RNAi duplex (Cont-RNAi) did not impair the B₂ receptor-mediated enhancement of the angiotensin II-stimulated inositol phosphate generation relative to control cells (Control) without RNAi duplex transfection (columns 3,4 versus 5,6). HEK293 cells expressed comparable levels of cell-surface receptors as determined with [¹²⁵I]-labeled F(ab)₂ fragments of affinity-purified B₂- and AT₁-specific antibodies (268 ± 9 fmol/mg protein of B₂ receptor and 139 ± 8 fmol/mg protein of AT₁ receptor). Data represent mean \pm S.E., n=3 (**p* < 0.04; ***p* < 0.002).





A Low glucose increases the general chaperone system of HEK cells

Gene name	Accession no.	Fold increase (low/high gluc.)	Description	No. of increased probe sets (≥ 2.5 -fold)
DNAJB9	gb:NM_012328	6.36	DNAJ (HSP40) homolog, subfamilyB, member 9	1
HYOU1 (ORP150)	gb:NM_006389	5.68	Hypoxia up-regulated 1	1
HSPA5 (GRP78/BIP)	gb:NM_005347	4.97	Heat shock 70kDa protein (glucose-regulated protein, 78kDa)	2
PPP1R15A (GADD34)	gb:NM_014330	4.96	Protein phosphatase 1, regulatory (inhibitor) subunit 15A	2
PDIA4 (ERP70)	gb:NM_004911	3.85	Protein disulfide isomerase family A, member 4	2
DNAJB5	gb:NM_001135004	3.47	DNAJ (HSP40) homolog, subfamilyB, member 5	1
ARMET	gb:NM_006010	3.34	Arginine rich, mutated in early stage tumors	1
SEL1L	gb:NM_005065	3.16	Sel-1 suppressor of lin-12-like (C. elegans)	2
FKBP2	gb:NM_004470	2.79	FK506-binding protein 2 (13 kDa)	1
OS-9	gb:NM_001017956	2.74	Amplified in osteosarcoma	1
SDF2L1	gb:NM_022044	2.68	Stromal cell-derived factor 2-like 1	1
HSEC61	gb:NM_013336	2.63	Homo sapiens sec61 homolog	1
CALR	gb: NM_004343	2.50	Calreticulin	1
CANX	gb: NM_001746	2.50	Calnexin	1

