Biased Agonism of the Angiotensin II Type 1 Receptor

C.M. Godin and S.S.G. Ferguson*

J. Allyn Taylor Centre for Cell Biology, Molecular Brain Research Group, Robarts Research Institute and the Department of Physiology & Pharmacology, The University of Western Ontario 100 Perth Dr. London, Ontario, N6A 5K8, Canada

Abstract: G protein-coupled receptors (GPCRs) can be activated by multiple ligands and exhibit the capacity to couple to numerous intracellular signal transduction pathways. This property allows GPCRs to be modulated by biased agonists that selectively activate specific subsets of GPCR-regulated cellular signaling proteins. The angiotensin II type 1 receptor (AT_1R) is a GPCR that endogenously binds to the peptide ligand angiotensin II. More recently it has been demonstrated that a modified peptide, [Sar^1I-le^4-Ile^8]-angiotensin II (SII) acts as a biased agonist towards the AT_1R. SII binds to the AT_1R without promoting heterotrimeric G protein-coupling, but serves to link the receptor to the beta-arrestin-dependent activation of the mitogen activated protein kinase pathway. The present mini-review summarizes current knowledge regarding the role of biased agonists in stimulating biased AT_1R signaling.

Keywords: Angiotensin II, SII, biased agonism, GPCR, G-protein.

INTRODUCTION

The angiotensin II type 1 receptor (AT_1R) is a member of the G protein-coupled receptor (GPCR) superfamily. The binding of angiotensin II (AngII) to the AT_1R causes a conformational change which allows for coupling of the receptor to a heterotrimeric G protein, G-alpha(q), but the AT_1R can also couple to G-alpha(i) and G-alpha(12/13) [1]. G-alpha(q) activates the enzyme phospholipase C-beta (PLC-beta), which hydrolyses phosphoinositol 4,5-bisphosphate (PIP_2) leading to the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). Both of these enzymatic products act as intracellular second messengers. IP_3 binds to its receptor in the endoplasmic reticulum, causing the release of Ca^{2+} from intracellular stores thereby increasing intracellular Ca^{2+} levels. This increase in intracellular Ca^{2+} along with DAG activates protein kinase C. Stimulation of the AT_1R also activates other signaling cascades, such as the mitogen-activated protein kinase (MAPK) pathway which requires extracellular regulated kinase 1/2 (ERK1/2) phosphorylation by mitogen activated kinase kinase (MEK). It has now been established that AT_1R activates ERK1/2 by multiple pathways G protein-dependent and -independent pathways (Fig. **1**). ERK1/2 activation is mediated by G-alpha(q), beta-arrestin 2, tyrosine kinases and epidermal growth factor receptor transactivation [2, 3, 4]. Although the function of beta-arrestin in regulating the desensitization of the AT_1R is well documented, beta-arrestin 2 is now recognized to play a role in AT_1R-mediated ERK1/2 activation. Beta-arrestin 2 interacts directly with both Raf1 and ERK1/2 and functions as a scaffold for the recruitment of the Raf1/MEK/ ERK1/2 to the AT_1R [2, 5, 6]. The internalized AT_1R/beta-arrestin 2 complex is also found to be colocalized with ERK1/2 in early endosomal vesicles [2]. Targeting of ERK1/2 to endosomes prevents the nuclear translocation of ERK1/2 and ERK1/2-mediated gene transcription [5, 7, 8, 9]. In the absence of beta-arrestin 2 expression, the AT_1R primarily activates ERK1/2 phosphorylation *via* the activation of PKC [3, 9].

The fact that the AT_1R couples to both G protein-dependent and -independent mechanisms for the activation of downstream signaling cascades raises the question: are each of these cascades equally activated by agonist stimulation or do some ligands preferentially activate G protein-dependent versus -independent signaling cascades? This question can be addressed by the use of biased agonists.

Biased agonism has been defined as the property of a ligand to stabilize a conformation of a GPCR that preferentially activates a specific set of downstream effector proteins [10]. GPCRs exist in multiple tertiary conformations in assembly with other proteins. Some of these conformations can be termed 'active states', allowing the receptor to activate downstream signaling molecules [11]. Upon the binding of a biased agonist, the receptor adopts a conformation most highly stabilized by that particular agonist at the expense of other conformations, and results in the coupling of the receptor to a specific subset of effectors [11]. In the absence of a ligand, GPCRs reside in a predominantly inactive conformation that is uncoupled from the activation of heterotrimeric G proteins. A full agonist, such as angiotensin II (AngII), elicits a change in receptor conformation to an active state that couples the receptor to heterotrimeric G proteins, which results in the recruitment of beta-arrestin and activates multiple signaling pathways. One or more receptor conformational states exists in between these two states that facilitates GPCR coupling to specific effector proteins, but do not necessarily require heterotrimeric G protein-coupling. It has been proposed that

*Address correspondence to this author at the J. Allyn Taylor Centre for Cell Biology, Molecular Brain Research Group, Robarts Research Institute, 100 Perth Dr. London, Ontario, N6A 5K8, Canada; Tel: (519) 931-5706; Fax: (519) 931-5252; E-mail: Ferguson@robarts.ca

1875-5607/12 $58.00+.00

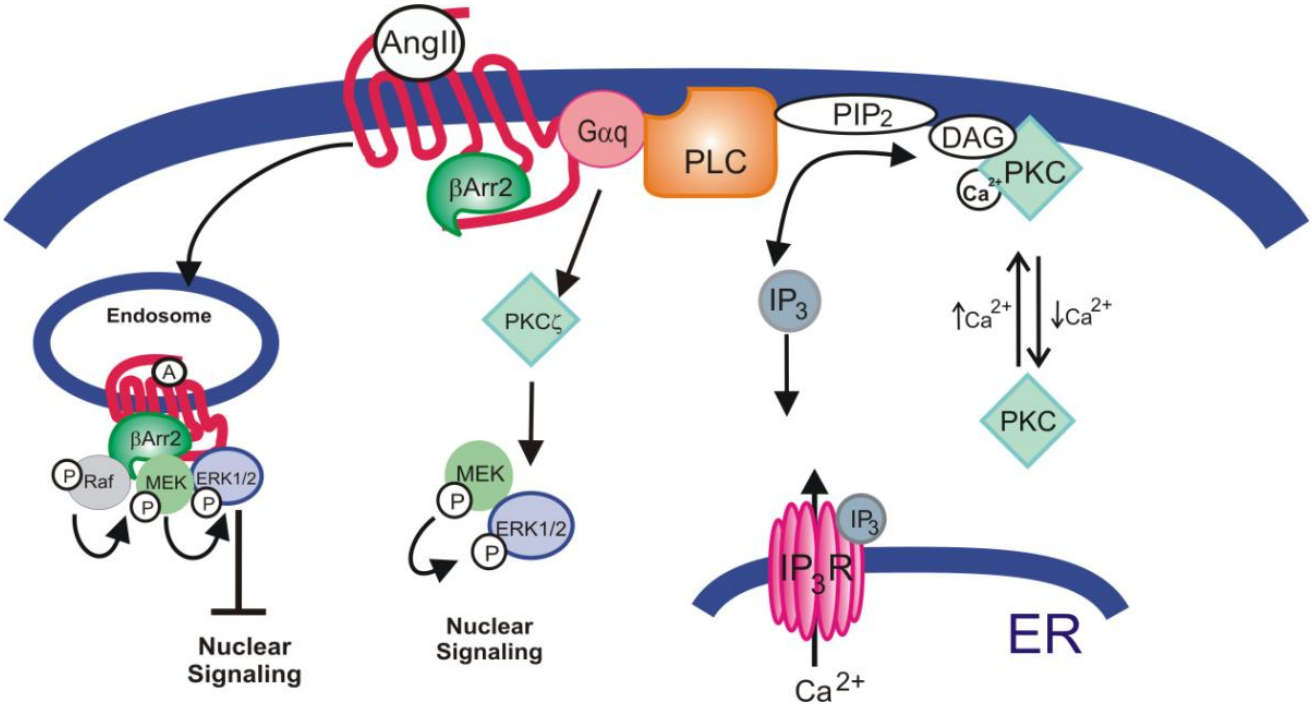


Fig. (1). Angiotensin II induced signalling of the AT_1R. AngII binds to the AT_1R which induces a conformational change allowing for the interaction of receptor with G-alpha(q). G-alpha(q) activates phospholipids C-beta (PLC-beta) which hydrolyses the membrane phospholipids, phosphoinositol-4,5 bisphosphate (PIP_2) to produce inositol-1,4,5 trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 binds to the IP_3 receptor (IP_3R) in the endoplasmic reticulum (ER) to cause the release of Ca^{2+}. The production of DAG along with increases in intracellular Ca^{2+} activates protein kinase C (PKC). PKC translocates from the cytosol to the plasma membrane where it phosphorylates AT_1R and other targets. AT_1R activation of G-alpha(q) proteins leads to activation of protein kinase C-zeta (PKC-zeta) which in turn leads to phosphorylation of ERK1/2 by mitogen activated kinase kinase (MEK). This phospho-ERK1/2 translocates to the nucleus to activate transcription. Beta-arrestin 2 is recruited to activated AT_1R, internalizing with the receptor and acting as a scaffold for ERK1/2. Beta-arrestin 2 causes a robust phosphorylation of ERK1/2, which is then retained in the endosome and antagonizes gene transcription.

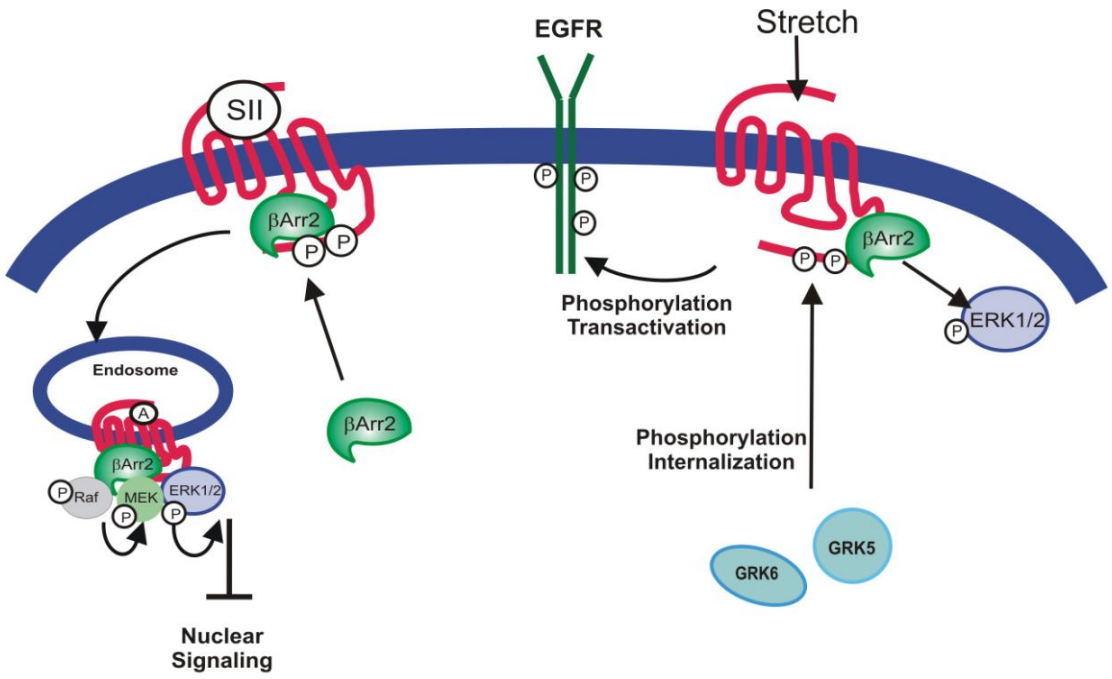
signaling molecules interact with different regions of the receptor, and that distinct receptor conformations will not be uniformly coupled to downstream effectors, allowing for biased agonism [11]. This property has been demonstrated for AT_1R, where the AngII analogue [Sar^1I-le^4-Ile^8]-AngII (SII) antagonizes AT_1R-mediated G protein-coupling, but promotes the beta-arrestin 2-dependent activation of ERK1/2 [12, 13].

BIASED AGONISTS OF THE AT_1R

[Sar^1-Ile^4-Ile^8]-Angiotensin II

[Sar^1I-le^4-Ile^8]-AngII (SII) is an angiotensin receptor-specific peptide ligand that will activate G protein-independent signaling in the absence of AT_1R coupling to G-alpha(q) [13]. The substitutions of Asp^1 with scarcosine, Tyr^4 and Phe^8 with isoleucines results in an AngII analogue that retains binding affinity for AT_1R, does not stimulate IP_3 formation, does not effectively promote AT_1R phosphorylation, but activates ERK1/2 phosphorylation [12,14]. The SII ligand allows for the distinction between G protein-dependent and -independent signaling *via* the AT_1R and has been integral in determining numerous G protein-independent signal mechanisms [7]. SII competes for binding with AngII and has a binding affinity 187 times lower than AngII [12]. Stimulation of the AT_1R with SII causes recruitment of beta-arrestin 2, but this is not as robust as that observed for AngII stimulation. SII also stimulates the beta-arrestin-dependent phosphorylation of multiple proteins and regulates gene transcription, as well as promoting the internalization of AT_1R [7] (Fig. **2**).

SII stimulation of the AT_1R expressed in HEK 293 cells results in the plasma membrane recruitment of beta-arrestin 2 and promotes internalization of AT_1R, as a complex with beta-arrestin 2 [12,13]. The robust activation of ERK1/2 mediated by SII is abolished following siRNA down-regulation of beta-arrestin 2, but not beta-arrestin 1 protein expression [13]. A recent study using stable isotope labeling of amino acid residues in cell culture, suggested that SII and AngII stimulation of the AT_1R results in the phosphorylation of differential subsets of downstream protein substrates [15]. Specifically, Christensen *et al.* [15] have demonstrated using phosphopeptide enrichment and high performance mass spectrometry that SII stimulation of the AT_1R only contributed to 36% of the phosphopeptides that are phosphorylated in response to AT_1R with AngII [15]. There is also a striking difference in the protein kinases activated in response to the stimulation of the AT_1R by either AngII or SII. However, there does appear to be some overlap between the kinases activated by AngII and SII, with protein kinase D (PKD) being activated by both G-alpha(q)-dependent and -independent pathways [15].



		K_D
AngII	NH2-Asp-Arg-Val-Tyr-Val-His-Pro-Phe-COOH	1.6 nM
SII	Sar-Arg-Val-Ile-Val-His-Pro-Ile-COOH	300 nM

Fig. (2). [Sar[1]-Ile[4]-Ile[8]]-Angiotensin II induced signaling of the AT_1R. Stimulation of the receptor with SII causes recruitment of beta-arrestins and internalization of the receptor, however the receptor does not couple to G-alpha(q) proteins. Beta-arrestin 2 acts as a scaffold for ERK1/2 signaling leading to an SII-induced beta-arrestin-dependent ERK1/2 signal. SII differs from AngII by substitution of Asp[1] with scarcosine, Tyr[4] and Phe[8] with isoleucines, which results in a higher K_D value[12]. Stimulation of AT_1R by mechanical stretch causes phosphorylation of AT_1R by GRK5 and GRK6 and receptor internalization. Stretch activates ERK1/2 and causes phosphorylation and transactivation of the epidermal growth factor receptor (EGFR).

Transcriptional regulation has traditionally been thought to be mediated *via* a G protein-dependent mechanism. However, using the peptide SII, Christensen *et al*. [16] have also demonstrated that G protein-independent signaling represses gene transcription. The study indicates that AT_1R-mediated gene transcription is predominantly G protein-dependent, whereas G protein-independent signaling modified the expression of only a few genes which involves the modification of the gene expression mediated by other GPCRs. However, there is some transcriptional regulation that is G protein-independent and SII is found to activate transcriptional regulators such as LARP, MIER and TRIM33, all of which are known to be transcriptional repressors. The cell death protein and translation suppressor, PDCD4 is also phosphorylated in response to SII. In response to phosphorylation, PDCD4 translocates to the nucleus and functions to repress AP-1-mediated gene transcription by reducing levels of phosphorylated c-Jun [17]. Transcriptional repression may also be due impart to the phosphorylation of CAMKII2δ, which is phosphorylated in response to SII near the region of nuclear translocation, potentially leading to its exclusion from the nucleus [15]. When gene regulation between SII and AngII treatment are compared by microarray analysis, it is found that only 25 genes are regulated by SII out of the 212 genes that are regulated by AngII [16]. The temporal dynamics of both SII and AngII stimulation of the AT_1R also differ, as SII-mediated AT_1R activation results in a delayed, but sustained regulation of gene transcription, when compared to AngII treatment.

SII stimulation of the AT_1R results in the phosphorylation of three proteins that are linked to the ubiquitin-protein degradation pathway, these are, calpastatin, TRIM33 and PRAJA1 [15]. Thus, there is the potential that these proteins play a role in the down-regulation of AT_1R expression. In addition, there are several proteins that are phosphorylated in response to SII activation that may contribute to AT_1R-mediated cytoskeletal regulation and endocytosis including GIT1 and REPS1 and PACS1. GIT1 is implicated in the regulation of $Beta_2AR$ endocytosis and REPS1 is involved in the internalization and trafficking of epidermal growth factor receptors [18, 19].

Mechanical Stretch

Mechanical stretch leads to the activation of the AT_1R [20]. However, mechanical stretch selectively activates the ERK pathway, but not G protein-dependent signaling, thus displaying the properties of a biased agonist. Mechanical stretch induces ERK1/2 phosphorylation in an AT_1R-dependent mechanism in the mouse heart. In the absence of ligand, mechanical stretch also induces AT_1R phosphorylation in a GRK5- and GRK6-dependent manner and promotes the internalization of the receptor. AT_1R phosphorylation as the consequence of stretch promotes the recruitment of beta-arrestin and results in both the G protein-independent phosphorylation of ERK1/2 and epidermal growth factor receptor transactivation (Fig. **2**). The effects of stretch can be blocked by pretreatment with the AT_1R antagonist Losartan, indicating that stretch is mediating these effects through the activation of the AT_1R. These effects were similar to those induced by the biased agonist SII [20].

Cardiovascular Disease

Biased signaling may also provide a novel means for the treatment of cardiovascular diseases. TVR120027 (Sar-Arg-Val-Ile-His-Pro-D-Ala-OH) competitively antagonizes AT_1R-mediated G protein signaling, but promotes beta-arrestin recruitment leading to the activation of ERK1/2 [21]. In addition, the compound is found to increase cardiomyocyte contractility, reduce mean arterial pressure and increase cardiac performance.

CONCLUSIONS AND IMPLICATIONS

Biased agonism of the AT_1R is achieved by use of either the peptide ligand [Sar^1-Ile^4-Ile^8]-AngII or by mechanical stretch. Both theses stimuli lead to AT_1R phosphorylation, recruitment of beta-arrestin, internalization of AT_1R and the beta-arrestin-dependent activation of ERK1/2 [12, 13, 20]. The study of SII has allowed for a better understanding of G protein-independent signaling of AT_1R, and has expanded our view of how GPCRs function. The simplified view of receptor activity states was that GPCRs existed in either an inactive or an active conformation. We now know that multiple conformations are possible and that these are stabilized by ligands that have traditionally been thought to function as either antagonists or inverse agonists to allow for differential coupling to various down stream effector molecules, selectively activating one or more pathways [11]. This property of the GPCR allows for the potential to either activate or inhibit one signaling pathway, without affect to the activity of alternative signaling pathways providing the possibility for the development of more effective pharmacological treatments for disease.

CONFLICT OF INTEREST

None declared.

ACKNOWLEDGEMENT

None declared.

ABBREVIATIONS

AngII = angiotensin II

AT_1R = angiotensin II type 1 receptor

DAG = diacylglycerol

ER = endoplasmic reticulum

ERK = extracellular regulated kinase

HEK 293 = human embryonic kidney cells 293

GRK = G-protein-coupled receptor kinase

IP_3 = inositol 1,4,5-trisphosphate

MAPK = mitogen activated protein kinase

MEK = mitogen activated kinase kinase

PKC = protein kinase C

PKD = protein kinase D

PLC = phospholipase C

SII = [Sar^1I-le^4-Ile^8]-angiotensin II

REFERENCES

[1] Touyz, R. M; Schiffrin, E. L. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Pharmacol Rev*. **2000**, *52*, 639-672.

[2] Luttrell, L. M; Roudabush, F. L; Choy, E. W; Miller, W. E; Field, M. E; Pierce, K. L; Lefkowitz, R. J. Activation and targeting of extracellular signal-regulated kinases by beta-arrestin scaffolds. *Proc Natl Acad Sci USA.* **2001**, *98*(5), 2449-2454.

[3] Godeny, M. D; Sayeski, P. P. ANG II-induced cell proliferation is dually mediated by c-Src/Yes/Fyn-regulated ERK1/2 activation in the cytoplasm and PKCzeta-controlled ERK1/2 activity within the nucleus. *Am J Physiol Cell Physiol.* **2006**, *291*(6),C1297-1307.

[4] Murasawa, S; Mori, Y; Nozawa, Y; Gotoh, N; Shibuya, M; Masaki, H; Maruyama, K; Tsutsumi, Y; Moriguchi, Y; Shibazaki, Y; Tanaka, Y; Iwasaka, T; Inada, M; Matsubara, H. Angiotensin II type 1 receptor-induced extracellular signal-regulated protein kinase activation is mediated by Ca2+/calmodulin-dependent transactivation of epidermal growth factor. *Circ Res.* **1998**, *82*, 1338-1348.

[5] Tohgo, A; Peirce, K. L; Choy, E. W; Lefkowitz, R. J; Luttrell, L. M. Beta-Arrestin scaffolding of the ERK cascade enhances cytosolic ERK activity but inhibits ERK-mediated transcription following angiotensin AT1a receptor stimulation. *J Bio Chem.***2002**, *277*(11), 9429-9436.

[6] Tohgo, A; Choy, E. W; Gesty-Palmer, D; Pierce, K. L; Laporte, S; Oakley, R. H; Caron, M. G; Lefkowitz, R. J; Luttrell, L. M.The stability of the G protein-coupled receptor-beta-arrestin interaction determines the mechanism and functional consequence of ERK activation. *J Bio Chem.* **2003**, *278*(8), 6258-6267.

[7] Wei, H; Ahn, S; Barnes, W. G; Lefkowitz, R. J. Stable interaction between beta-arrestin 2 and angiotensin type 1A receptor is required for beta-arrestin 2-mediated activation of extracellular signal-regulated kinases 1 and 2. *J Bio Chem*. **2004**, *279*(46), 48255-48261.

[8] Lee, M. H; El-Shewy, H. M; Luttrell, D. K; Luttrell, L. M. Role of β-arrestin-mediated desensitization and signalling in the control of Angiotensin AT1a receptor-stimulated transcription. *J Bio Chem.* **2008**, *283*(4), 2088-2097.

[9] Kim, J; Ahn, S; Rajagopal, K; Lefkowitz, R. J. Independent beta-arrestin2 and Gq/protein kinase Czeta pathways for ERK stimulated by angiotensin type 1A receptors in vascular smooth muscle cells converge on transactivation of the epidermal growth factor receptor. *J Bio Chem.***2009**, *284*(18), 11953-11962

[10] Kenakin, T.Collateral efficacy in drug discovery: taking advantage of the good (allosteric) nature of 7TM receptors. *Trends Pharmacol Sci.* **2007**, *28*(8), 407-415.

[11] Kenakin, T. Biased agonism. *F1000 Biol Rep*. **2009**, *26*(1), pii: 87.

[12] Holloway, A. C; Qian, H; Pipolo, L; Ziogas, J; Miura, S; Karnik, S; Southwell, B. R; Lew, M. J; Thomas, W. G. Side-chain substitutions within angiotensin II reveal different requirements for

signaling, internalization, and phosphorylation of type 1A angiotensin receptors. *Mol Pharmacol.* **2002**, *61*(4), 768-777.

[13] Wei, H; Ahn, S; Shenoy, S. K; Karnik, S. S; Hunyady, L; Luttrell, L. M; Lefkowitz, R. J. Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci USA.* **2003**, *100*(19), 10782-10787.

[14] Miura, S; Feng, Y. H; Husain, A; Karnik, S. S. Role of aromaticity of agonist switches of angiotensin II in the activation of the AT1 receptor. *J Biol Chem*. **1999**, *274*(11), 7103-7110.

[15] Christensen, G. L; Kelstup, C. D; Lynso, C; Sarwar, U; Bogebo, R; Sheikh, S. P; Gammeltoft, S. Olsen, J. V; Hansen, J. L. Quantitative Phosphoproteomics Dissection of Seven-transmembrane Receptor Signaling Using Full and Biased Agonists. *Mol Cell Proteomics*. **2010**, *9*(7), 1540-1553.

[16] Christensen, G. L; Knudsen, S; Schneider, M; Aplin, M; Gammeltoft, S; Sheikh, S. P; Hansen, J. L. AT_1 receptor Gαq protein-independent signalling transcriptionally activates only a few genes directly, but robustly potentiates gene regulation from the β2-adrenergic receptor. *Mol. Cell Endocrinol.* **2011**, *331*(1), 49-56.

[17] Lankat-Buttgereit, B; Göke, R. The tumour suppressor Pdcd4: recent advances in the elucidation of function and regulation. *Biol Cell*. **2009**, *101*(6), 309-317.

[18] Claing, A; Perry, S. J, Achiriloaie, M; Walker, J. K; Albanesi, J. P; Lefkowitz, R. J; Premont, R. T.Multiple endocytic pathways of G protein-coupled receptors delineated by GIT1 sensitivity. *Proc. Natl. Acad. Sci. U. S. A.* **2000**, *97*(3), 1119-1124.

[19] Cullis, D.N.; Philip, B; Baleja, J. D; Feig, L. A.Rab11-FIP2, an adaptor protein connecting cellular components involved in internalization and recycling of epidermal growth factor receptors. *J. Biol. Chem.* **2002**, *277*(51), 49158-49166.

[20] Rakesh, K; Yoo, B; Kim, I. M; Salazar, N; Kim, K. S; Rockman, H. A. beta-Arrestin-biased agonism of the angiotensin receptor induced by mechanical stress. *Sci Signal*. **2010**, *8*(125), ra46.

[21] Violin, J. D; DeWire, S. M; Yamashita, D; Rominger, D. H; Nguyen, L; Schiller, K; Whalen, E. J; Gowen, M; Lark, M. W. Selectively engaging β-arrestins at the angiotensin II type 1 receptor reduces blood pressure and increases cardiac performance. *J. Pharmacol. Exp. Ther*. **2010**, *335*(3), 572-579.

Received: February 04, 2011 Revised: September 06, 2011 Accepted: September 08 2011