Angiotensin II AT2 receptors are functionally coupled to protein tyrosine dephosphorylation in N1E-115 neuroblastoma cells

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Murine N1E-115 neuroblastoma cells are shown to express a single class of angiotensin II (Ang II) receptors that display all the pharmacological properties defining the Ang II receptor subtype 2 (AT2): high affinity for ¹²⁵I-labelled AT2-selective agonist CGP 42112 (K_d 91 ± 19 pM); expected rank order of potency (CGP 42112 = (Sar¹,Ile⁸)Ang II ≥ Ang II > PD 123319 > DUP 753) for several Ang II analogues; increased binding in the presence of the reducing reagent dithiothreitol (DTT); and insensitivity to analogues of GTP. Molecular cloning of cDNA encoding AT2 receptors from N1E-115 cells reveals nucleotide sequence identity with the AT2 subtype expressed in fetal tissue. Murine AT2 receptors transiently expressed in COS cells display the same pharmacological profile as endogenous Ang II receptors of N1E-115 cells. Taken together, these data

reveal the exclusive presence of the AT2 receptor subtype in N1E-115 cells. Incubation of N1E-115 cells with Ang II leads to a marked decrease in the level of tyrosine phosphorylation of several proteins with apparent molecular masses of 80, 97, 120, 150 and 180 kDa respectively. Tyrosine dephosphorylation of the same set of proteins is observed after treatment with the AT2-specific agonist CGP 42112. The response to both effectors is rapid and transient, showing a maximum between 5 and 10 min, and returning to basal levels after 20–30 min. In both cases, tyrosine dephosphorylation can be prevented by co-incubation with an excess of the antagonist Sarile. These data thus establish that AT2 receptor activation leads to protein tyrosine dephosphorylation in N1E-115 cells, and support a possible role for AT2 receptors in the negative regulation of cell proliferation.

INTRODUCTION

The octapeptide angiotensin II (Ang II) exerts various effects on its target tissues through binding to membrane receptors, which have been classified into two pharmacologically distinct subtypes, AT1 and AT2. The AT1 receptor is defined by its high sensitivity to losartan (DUP 753), whereas the AT2 subtype is insensitive to losartan but displays high affinity for peptidic and non-peptidic synthetic compounds such as CGP 42112 and PD 123319 respectively. AT1 and AT2 receptor subtypes are also distinguished by their sensitivity to reducing reagents; dithiothreitol (DTT) strongly reduces the binding of Ang II analogues to AT1 receptors but increases the affinity of these ligands for AT2 receptors [1,2].

AT1 receptors belong to the superfamily of G-protein-coupled receptors with seven hydrophobic domains [3,4], and are functionally coupled to multiple classic second-messenger pathways such as hydrolysis of phosphatidyl inositols, inhibition of adenylate cyclase and opening of Ca²⁺ channels [5]. In addition, AT1 receptors have recently been shown to stimulate protein tyrosine phosphorylation [6–9], and to activate a complex cascade of protein kinases including protein kinase C, Raf-1, mitogen-activated protein kinases, and a still undefined intracellular tyrosine kinase [10]. It is commonly accepted that activation of the AT1 receptor subtype would account for most of the known physiological effects of Ang II, including regulation of blood pressure, hormone release, cell growth and proliferation.

The AT2 subtype of receptors is much less characterized, and

physiological functions associated with this receptor still remain to be elucidated. Recent studies have suggested that this subtype may be involved in the formation of neointima after injury in rat carotid artery [11], and in angiogenesis in the chorioallantoic membrane model [12]. Transient expression of high levels of AT2 receptors in late stages of embryonic development [13,14] and in immature brain [15] strongly suggests a developmental role for this subtype.

Recently, cDNA encoding AT2 receptors have been isolated from rat PC12W cells [16] and from fetal tissues of rat and mouse [17,18]. These receptors present the seven hydrophobic stretches characteristic of the G-protein-coupled receptor superfamily and exhibit moderate (32%) amino acid sequence identity with AT1 receptors. AT2 receptors, however, do not display the usual features of G-protein-coupled receptors, e.g. sensitivity to analogues of GTP [17,19–21], and do not affect inositol triphosphate production or adenylate cyclase activity [17,20–22]. AT2 receptors have been shown to mediate prostaglandin synthesis [23], to modulate T-type Ca²⁺ channels [24], and to inhibit cyclic GMP production [25–27]. In addition, two groups have reported that AT2 receptors modulate the activity of protein tyrosine phosphatases (PTPases), but whether the enzymatic activity is stimulated [26,28] or inhibited [16,29] by Ang II is still controversial.

In the present study, we have investigated the modulation of intracellular tyrosine phosphorylation by AT2 receptors in murine N1E-115 neuroblastoma cells. We provide pharmacological and molecular evidence that N1E-115 cells express

Abbreviations used: Ang II, angiotensin II; AT Ang II receptor subtype; DTT, dithiothreitol; GTPγS, guanosine 5'-O-(thiotriphosphate); PTPase, protein tyrosine phosphatase; Sarile, (Sar¹,Ile⁸)Ang II.

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exclusively Ang II receptors of the AT2 subtype, and we show that stimulation of these receptors leads to a rapid decrease in the phosphorylation of several cellular proteins on tyrosine residues.

MATERIALS AND METHODS

Cell culture and transfections

The N1E-115 neuroblastoma cells, kindly provided by Drs. P. Hordijk and W. H. Moolenaar (N.C.I., Amsterdam, The Netherlands), were cultured as previously described [30] in Dulbecco's Modified Eagle's Medium supplemented with 10% (v/v) fetal calf serum, and were used at passages 30–41.

COS cells were transiently transfected (48–72 h) with plasmid DNA using the DEAE-dextran/chloroquine method as described [31].

Cell membrane preparation

After washing with PBS, preconfluent N1E-115 cells or confluent transfected COS cells were harvested by EDTA treatment and resuspended at a concentration of 2.5×10^7 cells/ml in ice-cold lysis buffer (25 mM Tris/HCl, pH 7.4, containing 0.5 mM phenylmethanesulphonyl fluoride). All subsequent operations were performed at 4 °C. The cells were incubated for 15 min in the lysis buffer and broken using a Potter homogenizer. Following centrifugation (1500 g, 8 min), the supernatant was collected and the debris were resuspended in the same volume of lysis buffer and submitted once more to the same treatment. The two supernatants were combined and centrifuged (1500 g, 8 min) to remove remaining cell debris. The supernatant was then centrifuged (50 000 g, 60 min), and the resulting membrane pellet was resuspended in a minimal volume of PBS containing 10% (v/v) glycerol, 5 µg/ml leupeptin and 5 µg/ml pepstatin and stored at –80 °C until further use. Protein content was assayed (BCA Kit, Pierce) using BSA as a standard.

Radioligand binding assays

Membrane preparations (15–40 µg of proteins) were assayed for binding to 125 I-labelled (Sar¹,Ile⁸)Ang II (Sarile) or 125 I-labelled CGP 42112 for 90 min at room temperature in 200 µl of binding buffer as described [32]. Saturation experiments were performed with varying concentrations (5–2000 pM) of radioligand, and non-specific binding was estimated in the presence of 1 µM corresponding unlabelled compound. Competition experiments were carried out at 250 pM 125 I-Sarile with variable concentrations of unlabelled ligands. Non-specific binding, defined as that occurring in the presence of 10 µM Sarile, was 5–10% of total binding. Incubations were terminated by filtration through GF/B filters on a Brandel apparatus, and the retained radioactivity was measured using a LKB 1282 Compugamma counter. 125 I-Sarile (2000 Ci/mmol) was from Amersham, 125 I-CGP 42112 (2000 Ci/mmol) was from Anawa (Switzerland), and unlabelled ligands Ang II and Sarile were purchased from Sigma.

Saturation and competition binding experiments were analysed using the EBDA program (Biosoft-Elsevier, Cambridge, U.K.) [33]. The resulting data are presented as means $\pm$ S.E.M. of 2–4 independent experiments performed in duplicate.

cDNA cloning

A cDNA library of 3×10^6 independent clones was constructed from N1E-115 poly(A⁺) RNA (10 µg) by oligo(dT) priming, size fractionation (>1.5 kb) of the cDNA, and ligation into the

*Bsr*XI site of the mammalian expression vector pcDNA1 (Invitrogen) as described [31]. Clones (1×10^5) were screened by hybridization to a random primed, 32 P-labelled, 559 bp DNA fragment encompassing the 5' half of the murine AT2 coding region. This DNA fragment used as a probe was obtained from N1E-115 mRNA by reverse transcription followed by PCR using *Thermophilus aquaticus* polymerase (Promega) as described by the manufacturer. Sense (5'-CACCAGCAGAAACATCACC-3') and antisense (5'-ACAAGCATTACACCTAAGT-3') primers were derived from the rat AT2 cDNA sequence at positions 27–45 and 566–585 respectively [17]. Approximately 200 positive clones were obtained and tested for functionality by transient expression in COS cells and subsequent binding of the transfected cells with the 125 I-CGP 42112 ligand (250 pM). Nucleotide sequencing was performed on both strands by the dideoxy chain termination method using Sequenase T7 DNA polymerase (U.S. Biochemical Corp.)

Immunoblotting

N1E-115 cells (1×10^6) were incubated for various periods of time at 37 °C in the culture medium in the presence of various concentrations of effectors. The reaction was stopped by aspiration of the medium, immediate washing with ice-cold PBS containing 0.1 mM Na₃VO₄, and subsequent addition to the cells of 100 µl of SDS (2%) lysis buffer containing 0.1 mM Na₃VO₄. Cell lysates were boiled for 10 min before samples (15 µl) were subjected to 8%-SDS/PAGE and analysed by immunoblotting with anti-phosphotyrosine antibodies as previously described [34]. For internal control of the amount of proteins in each lane, bound antibodies were stripped from the membranes by incubation with 0.1 M glycine, pH 2.5, for 10 min before subsequent incubation with anti-ezrin antibodies. Mouse monoclonal anti-phosphotyrosine antibody was purchased from UBI (Lake Placid, NY, U.S.A.). Rabbit polyclonal anti-ezrin antibodies were kindly provided by Dr. P. Mangeat (U.S.T.L., Montpellier, France). Peroxidase-coupled sheep anti-(mouse IgG) antibodies, peroxidase-coupled donkey anti-rabbit antibodies and ECL reagents were from Amersham (Les Ulis, France).

RESULTS

N1E-115 neuroblastoma cells express only the AT2 subtype

Membranes prepared from N1E-115 neuroblastoma cells were assayed for binding to the non-discriminative Ang II antagonist 125 I-Sarile as well as to the AT2-specific 125 I-CGP 42112. Scatchard representations of the saturation curves (Figure 1a) reveal that each radioligand binds to a single class of high-affinity binding sites, with K_d values of 233 ± 33 pM for 125 I-Sarile and 91 ± 19 pM for 125 I-CGP 42112. The same total number of sites is labelled by each of the two radioligands (estimated B_{max} values of 298 ± 53 and 321 ± 42 fmol/mg of protein for 125 I-Sarile and 125 I-CGP 42112 respectively), suggesting that, in N1E-115 cells, all the 125 I-Sarile binding sites are of the AT2 subtype.

The binding of 125 I-Sarile (250 pM) to N1E-115 membranes is totally displaced by Ang II and by the AT2-specific compounds CGP 42112 and PD 123319 with monophasic curves (Figure 1b), whereas the AT1-selective ligand DUP 753 is virtually ineffective at concentrations up to 1 µM. The obtained IC₅₀ values (Table 1) indicate a rank order of potency Sarile = CGP 42112 > Ang II > PD 123319 > DUP 753, characteristic of AT2 receptors. Taken together, these binding studies indicate that N1E-115 cells express significant levels of AT2 and no detectable AT1 binding site.

Figure 1 Binding characteristics of ^{125}I -Sarile and ^{125}I -CGP 42112 to membranes prepared from N1E-115 cells

(a) Scatchard representation of the specific binding of ^{125}I -Sarile (●) and ^{125}I -CGP 42112 (○) to membranes from N1E-115 cells. The data shown yield respective K_d and B_{max} values of 205 pM and 372 fmol/mg of protein for ^{125}I -Sarile, and 101 pM and 367 fmol/mg of protein for ^{125}I -CGP 42112. Each plot is representative of 3–4 independent experiments performed on different membrane preparations. (b) Displacement of specific ^{125}I -Sarile binding by increasing concentrations of Sarile (■), CGP 42112 (□), Ang II (●), PD 123319 (○) and DUP 753 (▲). The data shown derive from one representative example of 3–4 experiments performed in duplicate. (c) Displacement of total ^{125}I -Sarile binding by increasing concentrations of Ang II alone (□) or in the presence of GTP γ S (○), DTT (■), or DTT + GTP γ S (●). The curves shown are representative of three experiments performed in duplicate.

Heterogeneity of AT2 receptors in the rat brain, according to their sensitivity to analogues of GTP [35] and to DTT [36], prompted us to examine the binding properties of the AT2

Table 1 Parameters for displacement by various ligands of ^{125}I -Sarile binding to membranes of N1E-115 neuroblastoma cells and COS-AT2 cells

Data represent means $\pm$ S.E.M. (n given in parentheses).

Ligand	N1E-115 IC_{50} (nM)	COS-AT2 IC_{50} (nM)
Sarile	0.45 ± 0.09 ($n = 4$)	0.39 ± 0.07 ($n = 2$)
CGP 42112	0.53 ± 0.15 ($n = 3$)	0.45 ± 0.08 ($n = 2$)
Ang II	1.22 ± 0.12 ($n = 3$)	0.84 ± 0.28 ($n = 2$)
PD 123319	4.60 ± 0.21 ($n = 3$)	4.74 ± 0.55 ($n = 3$)
DUP 753	> 10000	> 10000

Figure 2 Characteristics of ^{125}I -Sarile and ^{125}I -CGP 42112 binding to membranes prepared from COS cells transfected with the AT2 cDNA

(a) Scatchard plot of the specific binding of ^{125}I -CGP 42112 to membranes from COS-AT2. The data shown are representative of two experiments performed in duplicate on different membrane preparations, and yield a K_d value of 70 pM and a B_{max} of 140 fmol/mg of protein. (b) Displacement of specific ^{125}I -Sarile binding to COS-AT2 membranes by increasing concentrations of Sarile (■), CGP 42112 (□), Ang II (●), PD 123319 (○) and DUP 753 (▲). The data shown derive from one representative experiment out of 2–3 experiments performed in duplicate.

receptors of N1E-115 cells in the presence of these two reagents. Displacement curves of ^{125}I -Sarile binding by the agonists Ang II (Figure 1c) and CGP 42112 (results not shown) reveal that the binding properties of AT2 receptors from N1E-115 cells remain unaffected in the presence of 20 μM guanosine 5'-O-(thiotriphosphate) (GTP γ S), whereas total binding to the radioligand is augmented in the presence of 5 mM DTT (Figure 1c).

Figure 3 Time-course of tyrosine dephosphorylation induced by Ang II in N1E-115 cells

(a) N1E-115 cells were incubated with Ang II (10 nM) at 37 °C for the period of time indicated at the top of the lanes, before lysis and subsequent immunoblotting with anti-phosphotyrosine antibodies as described in the Materials and methods section. The data shown derive from a representative example of three experiments performed in duplicate on independent cultures of N1E-115 cells, each cell lysate being subsequently loaded on to 2–3 different gels for immunoblotting. The positions of standard molecular-mass markers are indicated on the left-hand side of the Figure. The arrow on the right-hand side indicates the position of the 80 kDa phosphorylated protein. (b) After total removal of bound antibodies, the same blot was re-incubated with anti-ezrin antibodies for internal control of the amount of proteins in each lane.

Cloning and expression of AT2 receptors from N1E-115 cells

To characterize the molecular subtype of AT2 receptors expressed in N1E-115 cells, we have undertaken the cloning of the corresponding cDNA. A cDNA library was constructed from N1E-115 poly(A⁺) RNA, and probed using a radiolabelled, PCR-amplified DNA fragment corresponding to the murine AT2 receptor (see the Materials and methods section). Several full-length cDNA clones were isolated, and found by nucleotide sequencing (Genbank accession no. L32840) to be identical to AT2 receptors isolated from mouse fetal tissues [18].

COS cells transiently transfected with the murine AT2 receptor cDNA (thereafter referred to as COS-AT2 cells) display saturable and specific binding to ¹²⁵I-CGP 42112 (Figure 2a). Scatchard representation of the saturation curves yields an estimated $B_{\max}$ of 125 ± 11 fmol/mg of protein, and a K_d value (75 ± 4 pM) consistent with that obtained with membranes from N1E-115 cells (91 ± 19 pM). Displacement of ¹²⁵I-Sarile (250 pM) binding to membranes from COS-AT2 cells is achieved by Ang II and by AT2-selective ligands with monophasic curves (Figure 2b), showing the expected rank order of potency: Sarile = CGP 42112 > Ang II > PD 123319 > DUP 753. The IC_{50} values obtained for these ligands (shown in Table 1) are very close to those found with membranes from N1E-115 cells, therefore corroborating our initial results (Figure 1) which show that N1E-115 cells express exclusively the AT2 receptor subtype.

AT2 receptors mediate protein tyrosine dephosphorylation

The effect of Ang II on tyrosine phosphorylation in N1E-115 cells was investigated by immunoblotting of cell lysates with anti-phosphotyrosine antibodies. As shown in Figure 3(a), Ang II (10 nM) mediates a significant reduction in tyrosine phosphorylation of several proteins with apparent molecular masses of 80, 97, 120, 150 and 180 kDa respectively. Incubation of the same blot with anti-ezrin antibodies (Figure 3b) confirms that similar amounts of proteins are present in each lane. Strong dephosphorylation of a major protein at 80 kDa ('p80') (Figure 3a) is

Figure 4 Inhibition of Ang II-induced tyrosine dephosphorylation in the presence of an excess of Sarile

N1E-115 cells were incubated for 10 min at 37 °C in medium alone (0) or with 10 nM Ang II (10) in the presence (+) or absence (–) of an excess (1 μ M) of the antagonist Sarile. Cell lysates were submitted to immunoblotting analysis with anti-phosphotyrosine antibodies as indicated in the legend of Figure 3(a). The arrow on the right-hand side indicates the position of the 80 kDa phosphorylated protein.

consistently observed following Ang II treatment. Densitometry scanning of the autoradiographs indicates that the intensity of the signal on the p80 phosphoprotein is reduced by $43.3 \pm 6.5\%$ ($n = 9$) relative to the basal level. In the following experiments, the conditions for electrophoresis and immunoblotting were therefore optimized for the detection of p80.

The reduction of tyrosine phosphorylation induced by Ang II (Figure 3a) is detectable at 2 min and is maximal between 5 and 10 min. Basal levels are recovered by 20–30 min. This rapid and transient time course of dephosphorylation is indicative of a direct, ligand-dependent effect that does not involve protein synthesis. The specificity of the effect of Ang II on tyrosine phosphorylation was shown by co-incubating the cells with an excess (1 μ M) of the antagonist Sarile (Figure 4); under these conditions, the intensity of the signal of the p80 phosphoprotein remained unchanged ($98.4 \pm 2.3\%$, $n = 2$) relative to the basal level).

The N1E-115 cells used in this study express no detectable AT1 binding site, therefore implying that the effect of Ang II on tyrosine phosphorylation is mediated by the AT2 receptor subtype. This conclusion is further confirmed by experiments using the AT2-selective agonist CGP 42112 (Figure 5). Incubation of N1E-115 cells with CGP 42112 (10 nM) also leads to protein tyrosine dephosphorylation of several intracellular proteins, among which the protein migrating at 80 kDa is the most strongly regulated. Densitometry scanning of autoradiographs obtained from independent experiments indicates that the intensity of the signal detected on the p80 phosphoprotein is reduced by $41.6 \pm 6.4\%$ ($n = 4$) following treatment with CGP 42112. The response to CGP 42112 is rapid and transient (detectable at 5 min, maximal at 10 min, and returning to basal levels after 30 min), and follows essentially the same time-course as that observed with Ang II. Moreover, the effect of CGP 42112 is also inhibited when co-incubated with an excess (1 μ M) of the antagonist Sarile (Figure 5); under these conditions, the intensity of the p80 phosphoprotein signal corresponds to $87 \pm 1\%$ ($n = 2$) of the basal level.

DISCUSSION

This paper reports the pharmacological, molecular and functional characterization of AT2 receptors in the murine N1E-115

Figure 5 Time-course of tyrosine dephosphorylation induced by CGP 42112 (10 nM) in N1E-115 cells

N1E-115 cells were incubated with CGP 42112 (10 nM) at 37 °C for the period of time indicated at the top of the lanes, in the presence (+) or absence (–) of the antagonist Sarile (1 μM). The same experiment was performed twice in duplicate on independent cultures of N1E-115 cells, and each cell lysate was loaded on two different gels for immunoblotting with anti-phosphotyrosine antibodies as described in the Materials and methods section. Longer exposures of the autoradiographs allowed the detection of additional phosphoproteins of 97, 120 and 150 kDa. On the left-hand side to the Figure are indicated the positions of standard molecular-mass markers. The arrow on the right-hand side indicates the position of the 80 kDa phosphorylated protein.

neuroblastoma cells. Several lines of pharmacological evidence (Figure 1) demonstrate that these cells express exclusively Ang II receptors of the AT2 subtype and no detectable AT1 binding site. The similarity of binding parameters between endogenous Ang II receptors of N1E-115 cells and cloned AT2 receptors expressed in COS cells further reinforces this conclusion. AT2 receptors expressed in N1E-115 cells exhibit insensitivity to analogues of GTP and show increased binding in the presence of DTT. These receptors belong to the same molecular subtype as AT2 receptors recently isolated from rat [16,17] and mouse [18] fetal tissues.

Other groups [37,38] have reported that N1E-115 cells express both AT1 and AT2 binding sites. This apparent contradiction can be explained by the divergence occurring between cell lines of common origin that are cultured separately for a long period of time.

We show here that activation of AT2 receptors leads to a marked decrease in intracellular protein tyrosine phosphorylation in N1E-115 cells. This effect is rapid and transient, and is reproducibly obtained using either the natural ligand Ang II or the synthetic AT2-selective agonist CGP 42112. The response to both ligands is prevented by co-incubation with an excess of the antagonist Sarile. Tyrosine dephosphorylation following AT2 receptor activation affects a set of intracellular proteins with apparent molecular masses of 80, 97, 120, 150 and 180 kDa. The identification of these phosphoproteins will be of great help in understanding the molecular pathway of signal transduction by AT2 receptors.

This is the first demonstration of AT2 receptor-mediated tyrosine dephosphorylation in N1E-115 cells. Decreased phosphorylation of several endogenous substrates strongly argues in favour of an increased PTPase activity. Other hormones, such as dopamine [39] or somatostatin [40], also acting through G-protein-coupled receptors, have been reported to rapidly stimulate a PTPase activity. In the case of AT2 receptors, however, studies conducted in the PC12W cell line have led to controversial results, as AT2 receptors were found either to stimulate [26,28] or to inhibit [16,29] the activity of a PTPase, measured using exogenous synthetic substrates. Given the complex regulation exerted on protein kinases and phosphatases inside the cell, it is indeed possible that stimulation of AT2 receptors leads to a cascade of events, involving both activation

and inhibition of specific sets of PTPases acting on different substrates. Our study, measuring the phosphorylation of endogenous substrates in intact cells, differs from previous analyses of PTPase activity *in vitro*, and is more representative of the overall effect of AT2 receptors on the regulation of intracellular protein tyrosine phosphorylation.

In cell types known to express only AT1 receptors, Ang II induces a rapid elevation of intracellular protein tyrosine phosphorylation [6–10]. These observations raise the interesting possibility that a single hormone peptide may have opposite intracellular effects through binding to different receptor subtypes. As Ang II exhibits similar affinity towards AT1 and AT2 receptors, one may speculate that activation of AT2 receptors would lead to negative regulation of intracellular effects mediated by AT1 receptors. It will be interesting to determine whether regulation of tyrosine phosphorylation by AT1 and AT2 receptors affects the same set of proteins; transfection of N1E-115 cells with the AT1 receptor cDNA should provide clues about possible cross-talk between AT1 and AT2 receptor subtypes.

Tyrosine phosphorylation is most often associated with cell proliferation and differentiation. So far, a number of ligands, either peptides [6–10,34] or phospholipids [41], have been shown to promote a rapid elevation of intracellular tyrosine phosphorylation through binding to G-protein-coupled receptors. In every case, these ligands have also been reported to have mitogenic or growth factor activity. To our knowledge, AT2 receptors constitute the only demonstrated example of a neuropeptide receptor mediating a reduction rather than an elevation of intracellular tyrosine phosphorylation. Our results thus suggest that AT2 receptors may participate in the negative regulation of cell proliferation. Anti-proliferative effects of AT2 receptors *in vivo* have been suggested by experiments performed by Janiak et al. [11], showing that administration of the AT2-selective agonist CGP 42112 to rats leads to a reduction of the formation of neointima after carotid injury.

In conclusion, we report here the pharmacological and molecular characterization of AT2 receptors in the N1E-115 cell line, and we present evidence that these receptors are functionally coupled to tyrosine dephosphorylation. This cell line thus offers a useful model in which to dissect the molecular determinants recruited in signal transduction by AT2 receptors, and to investigate the possible role of these receptors in the control of cell proliferation.

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