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Ligand binding and functional properties of human angiotensin AT_1 receptors in transiently and stably expressed CHO-K1 cells

Minh Tam Le[a,*], Jean-Paul De Backer[a], László Hunyady[b], Patrick M.L. Vanderheyden[a], Georges Vauquelin[a]

[a]*Department of Molecular and Biochemical Pharmacology, Institute for Molecular Biology and Biotechnology, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussel, Belgium*

[b]*Department of Physiology, Semmelweis University, Faculty of Medicine, H-1444 Budapest, P.O. Box 259, Hungary*

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Abstract

Chinese Hamster Ovary Cells (CHO-K1) were transiently and stably transfected to express the human angiotensin AT_1 receptor. Cell surface receptor expression was maximal 2 days after transient transfection. Their pharmacological and signalling properties differed from stably expressed receptors. Receptor reserve was significant in the transient cells but not in stable cells, explaining the higher potency of angiotensin II and the lower degree of insurmountable inhibition by candesartan in the transient cells. [Sar^1Ile^8]angiotensin II (sarile) is a potent angiotensin AT_1 receptor antagonist for the stable cells but is a partial agonist, producing 19% of the maximal response by angiotensin II, in transient cells. Internalization of [^{3}H]angiotensin II and [^{125}I]sarile (i.e., acid-resistant binding) was more pronounced in stable cells. CHO-K1 cells were also transiently transfected with the enhanced green fluorescence-AT_1 receptor gene. Confocal microscopy revealed rapid internalization induced by angiotensin II and sarile but not by candesartan. The above disparities may result from differences in receptor maturation and/or cellular surrounding.

Keywords: CHO-K1; Angiotensin II; Angiotensin AT_1 receptor; Internalization; Receptor reserve

1. Introduction

The octapeptide hormone angiotensin II exerts most of its physiological effects by stimulating angiotensin AT_1 receptors. Starting with the activation of Gq, the angiotensin AT_1 receptors elicit an increased production of inositol phosphate and a transient rise in cytoplasmic calcium concentration. By its combined effects on cardiovascular, endocrine and neuronal systems, angiotensin II is one of the most potent regulators of blood pressure and it is an important etiological factor in hypertension and cardiovascular disease (De Gasparo et al., 2000). Two strategies have been highly successful in counteracting these pathologies. Angiotensin converting enzyme inhibitors have been introduced to decrease the plasma level of angiotensin II and angiotensin AT_1 receptor antagonists have been developed to selectively block the access of angiotensin II to this receptor (Timmermans, 1999; Unger, 1999).

Initial attempts to block the actions of angiotensin II relied on the development of synthetic analogues of this peptide. These were obtained by substituting one or more of the original amino acids of angiotensin II by natural or synthetic amino acids. However, such peptide analogues often display partial agonistic activity and this constituted one of the reasons for the failure of the therapeutic use of saralasin ([Sar^1,Val^5,Ala^8]angiotensin II) (Pals et al., 1979). [Sar^1,Ile^8]angiotensin II (sarile) is another typical example and, in its radioiodinated form, it is widely used for the labelling of angiotensin AT_1 as well as angiotensin AT_2 receptors in radioligand binding studies. Like angiotensin II,

* Corresponding author. Tel.: +32 2 6291993; fax: +32 2 6291358.
E-mail address: tmleminh@vub.ac.be (M.T. Le).

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it displays high affinity (i.e., K_d in the nanomolar range) for both receptor subtypes. Based on its ability to inhibit angiotensin II-mediated angiotensin AT_1 receptor stimulation, sarile appears to behave as a potent angiotensin AT_1 receptor antagonist. In the same line 'in vitro' contraction studies of rabbit aorta and rat portal vein revealed that sarile produced a significant, concentration-dependent depression of the maximum contractile force mediated by angiotensin II (Pendleton et al., 1989; Wienen et al., 1992; Zhang et al., 1994). This depression, referred to as "insurmountable inhibition," occurred when the vascular tissue was exposed to sarile before its challenge with angiotensin II. Therefore, it attributed to the long-lasting action of this antagonist (Pendleton et al., 1989; Wienen et al., 1992). In agreement with its insurmountable inhibition, the binding of [^{125}I]sarile to angiotensin AT_1 receptor containing membranes (Wienen et al., 1992) and cells (Le et al., 2003) has been shown to be slowly reversible.

More recently, a fair number of nonpeptide angiotensin AT_1 receptor-selective antagonists have been developed and some of those belonging to the biphenyltetrazole group have been highly successful in clinical practice. In vascular smooth muscle contraction studies, some of these antagonists also act in an insurmountable, long-lasting fashion while others only produce parallel rightward shifts of the angiotensin II concentration–response curve (i.e., "surmountable inhibition"). "In vitro" studies on recombinant systems such as CHO cells stably expressing the human angiotensin AT_1 receptor (CHO-hAT_1 cells) as well as on cell lines which express endogenous angiotensin AT_1 receptors (Verheijen et al., 2003) improved our understanding about the molecular action mechanism of these antagonists. In these cells, all investigated biphenyltetrazole antagonists were competitive with angiotensin II (Vanderheyden et al., 2000) but they displayed marked differences with respect to their ability to adopt fast reversible/surmountable and tight binding/insurmountable complexes with the angiotensin AT_1 receptor (Vauquelin et al., 2001a). The behaviour of these antagonists in CHO-hAT_1 cells and other cell lines with endogenous angiotensin AT_1 receptors is remarkably similar to that in more complex experimental systems like vascular smooth muscle preparations. Whereas losartan, the prototype of the nonpeptide antagonists, is only able to form fast reversible complexes, other antagonists like candesartan almost exclusively form tight binding complexes. In accordance with the formation of tight-binding complexes, [^{3}H]candesartan was found to bind with high affinity to intact CHO-hAT_1 cells and to dissociate slowly from these cells (Fierens et al., 1999a,b; Vauquelin et al., 2001a).

When comparing different studies, it emerges that the interaction of angiotensin AT_1 receptors with peptide and nonpeptide ligands could be affected by its mode of expression. Whereas candesartan pre-incubation produced a more than 90% inhibition of the maximal angiotensin II-stimulated inositol phosphate accumulation in CHO-hAT_1 cells and human cell lines with endogenous angiotensin AT_1 receptors (Fierens et al., 1999b; Verheijen et al., 2002), the inhibition did not exceed 80% in CHO-K1 cells that were transient expressing human angiotensin AT_1 receptors (Le et al., 2003). In the same vein, while sarile completely inhibited the angiotensin II receptor-mediated calcium influx in bovine adrenal glomerulosa cells (Ambroz and Catt, 1992), this peptide behaved as a partial agonist for recombinant cell lines transiently expressing angiotensin AT_1 receptors (Noda et al., 1995; Hines et al., 2001; Miserey-Lenkei et al., 2001; Le et al., 2003). These discrepancies prompted us to perform a comparative characterisation of the binding and functional properties of sarile and candesartan in CHO cells stably and transiently expressing human angiotensin AT_1 receptors. Differences in the inhibitory characteristics of candesartan in functional studies appeared to be dictated by the extent of "receptor reserve" in each cell system. On top of this, differences in the functional and binding characteristics of sarile between both cell systems were also observed.

2. Materials and methods

2.1. Materials

2-Ethoxy-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]-1H-benzimidazoline-7-carboxylic acid (candesartan) and [^{3}H]candesartan (17 Ci/mmol) were obtained from AstraZeneca (Mölndal, Sweden). [^{3}H]angiotensin II (68 Ci/mmol) was obtained from Amersham, Biosciences (The Netherlands). Angiotensin II and [Sar^{1}Ile^8]angiotensin II (sarile) were obtained from Neosystem (France). LipofectAMINE was from Invitrogen (Belgium). [^{125}I]-[Sar^{1}Ile^8]angiotensin II (2200 Ci/mmol) was from Perkin Elmer Life Science (USA). Monoclonal anti-HA mouse IgG antibodies and FITC-labeled sheep antimouse IgG antibodies were from Sigma. All other chemicals were of the highest grade commercially available.

2.2. Cell culture and transient transfection

CHO cells stably expressing the human angiotensin AT_1 receptor were obtained and cultured as described (Vanderheyden et al., 1999). The cells were cultured in 12- or 24-well plates in DMEM (Dulbecco's modified essential medium) supplemented with 10% foetal bovine serum, 2 mM L-glutamine, 100 I.U./ml penicillin, 100 μg/ml streptomycin, 1% solution of non-essential amino acids, and 1 mM sodium pyruvate. Cells were grown in 5% CO_2 at 37 °C. To obtain transient expression, the human angiotensin AT_1 receptor gene was transfected into CHO-K1 cells at 80% confluence. Transfection was performed in Opti-MEM I by replacing the culture medium with the mixture of LipofectAMINE (8 μl/ml) and plasmid DNA (1.5 μg/ml). The cells were then incubated for 5 h and the supplemented DMEM