



HAL
open science

Sartan- AT receptor interactions: evidence for insurmountable antagonism and inverse agonism

I. van Liefde, G. Vauquelin

► To cite this version:

I. van Liefde, G. Vauquelin. Sartan- AT receptor interactions: evidence for insurmountable antagonism and inverse agonism. *Molecular and Cellular Endocrinology*, 2009, 302 (2), pp.237. 10.1016/j.mce.2008.06.006 . hal-00532037

HAL Id: hal-00532037

<https://hal.science/hal-00532037>

Submitted on 4 Nov 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: Sartan- AT₁ receptor interactions: *In vitro* evidence for insurmountable antagonism and inverse agonism

Authors: I. Van Liefde, G. Vauquelin

PII: S0303-7207(08)00274-8
DOI: doi:10.1016/j.mce.2008.06.006
Reference: MCE 6903

To appear in: *Molecular and Cellular Endocrinology*

Received date: 26-3-2008
Revised date: 9-6-2008
Accepted date: 12-6-2008

Please cite this article as: Van Liefde, I., Vauquelin, G., Sartan- AT₁ receptor interactions: *In vitro* evidence for insurmountable antagonism and inverse agonism, *Molecular and Cellular Endocrinology* (2007), doi:10.1016/j.mce.2008.06.006

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



Sartan- AT₁ receptor interactions: *in vitro* evidence for insurmountable antagonism and inverse agonism.

by

I. Van Liefde and G. Vauquelin

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

Correspondence: Prof. Georges Vauquelin
Dept. Molecular and Biochemical Pharmacology,
Building E.5.10,
Vrije Universiteit Brussel (VUB),
Pleinlaan 2
B-1050 Brussel
Belgium.

Tel: (+32) 2-6291955,
Fax: (+32) 2-6291358,
Email: <gvauquel@.vub.ac.be>

Keywords

Sartans

AT₁ receptors

Insurmountable antagonism

Inverse agonism

G proteins

Cardiomyocytes

Summary

Sartans are non-peptide AT₁ receptor antagonists used to treat hypertension and related pathologies. Their effects on the G protein-dependent responses of angiotensin II (Ang II) were the same in vascular tissues and in isolated cell systems. All are competitive but, when pre-incubated, they act surmountably (only rightward shift of the Ang II concentration-response curve) or insurmountably (also decreasing the maximal response). Insurmountable behaviour reflects the formation of tight sartan-receptor complexes; it is often partial due to the co-existence of tight and loose complexes. Their ratio positively correlates with the dissociation half-life of the tight complexes and depends on the sartan: i.e. candesartan > olmesartan > telmisartan \approx EXP3174 > valsartan > irbesartan >> losartan. When AT₁ receptors display sufficient basal activity (in case of receptor over-expression, mutation and, especially, tissue stretching) sartans may also act as inverse agonists. This rather affects long-term, G protein-independent hypertrophic responses leading to cardiovascular remodelling.

Introduction.

The octapeptide angiotensin II (Ang II) is the main active peptide hormone of the renin–angiotensin–aldosterone system. It mediates most of its physiological (and pathological) functions by activating the AT₁ receptor, a member of the superfamily of G protein-coupled receptors (GPCR) (de Gasparo et al., 2000). By stimulating these receptors Ang II will, among others, produce constriction of vascular smooth muscle and aldosterone secretion from adrenal glomerulosa cells and lead to trophic changes in the heart and in blood vessels. Much research has been focused on the molecular aspects of AT₁ receptor signalling and it has now become clear that, besides the traditional short-term G protein dependent effects on blood pressure (via inositol triphosphate production, cytosolic calcium transients and vascular smooth muscle contraction), there are also long-term G protein-independent signal transduction mechanisms leading to pathological remodelling of the heart and blood vessels (via activation of MAP kinases and other proteins involved in hypertrophic responses) (Oro et al., 2007). By acting in concert, these distinct signal transduction mechanisms will contribute to the pathophysiological consequences of excessive AT₁ receptor activation.

AT₁ receptor-selective, orally active non-peptide antagonists have been found to be highly successful to treat hypertension and related pathologies (Timmermans, 1999; Oparil, 2000; Norris and Vaughn, 2003). These molecules are designated as “sartans” and, while their substituted imidazole core is highly variable (Table 1), they rather consistently contain a biphenylmethyl moiety with an acidic group (either a tetrazole or carboxylic acid) at the 2'-position (Berellini et al., 2005). The sartans have in common that they all competitively block the access of the AT₁ receptor to Ang II and related agonists (Vanderheyden et al., 2000a; Le et al., 2007). This implies that their binding sites do at least partially overlap with the one of Ang II. As a number of sartans are currently used in clinical therapy, it is of interest to find out how they can be differentiated from each other. To this end, a number of studies have explored, and sometimes compared, additional pharmacological properties of the sartans with special focus on their ‘insurmountable antagonism’ and ‘inverse agonism’. Here, we will briefly

review the recent advances in our understanding about the molecular basis of these somewhat exotic properties.

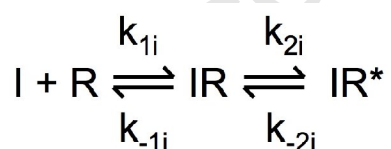
Insurmountable antagonism.

Contraction studies with rabbit aortic strips or other isolated vascular tissues have also shed light on some marked differences regarding some of their other pharmacodynamic properties. In this traditional paradigm, the tissues are invariably pre-incubated with antagonist before their challenge with agonist (Leff and Martin, 1986) and losartan (the prototype of the sartans) and a few others were shown only to produce parallel rightward shifts of the Ang II dose-response curve (Wienen et al., 1992; Dickinson et al., 1994; Vanderheyden et al., 1999). This phenomenon is designated as “surmountable” antagonism (i.e. Ang II can surmount the receptor blockade by such antagonists) and it is typical for fast-dissociating antagonists. Yet, most sartans including irbesartan, valsartan, EXP3174 (the active metabolite of losartan) and telmisartan also produced a partial depression of the maximal response and some like olmesartan and candesartan even an almost complete depression (Vauquelin et al., 2006; Le et al., 2007). This latter behaviour is qualified as “insurmountable” and, because of the competitive nature of the sartans (with respect to Ang II), it is related to their ability to slowly dissociate from the AT₁ receptor (Vanderheyden et al., 2000a; Vauquelin et al., 2001a, 2002). In line with this interpretation, *in vitro* studies with intact Chinese Hamster Ovary Cells expressing the transfected human AT₁ receptor (CHO-hAT₁ cells) clearly established that a pre-incubation step with the antagonist is essential for observing its insurmountable effect (Fierens et al., 1999a).

This intact cell approach offers several advantages compared to previous investigations on intact tissues and cell membrane preparations. Among others, it allows both radioligand binding and functional assays to be carried out under similar experimental conditions so that both can be directly compared to each-other. Compared to the classical “organ bath” experiments, agonist/antagonist co-incubation experiments are easy to perform and, compared to experiments with membrane preparations, it is much easier to remove free ligands and, hence, to investigate the AT₁ receptor dissociation

characteristics of the sartans. Last but not least, dissociation of candesartan and other sartans also appears to proceed appreciably slower from intact cells than from leaky ones and membrane preparations (Fierens et al., 2002; Verheyen et al., 2004). Despite the commonness of radioligand binding studies on plasma membranes or broken cell preparations, it is clear that GPCRs and membrane-associated proteins in general lose part of their natural environment (e.g. differences between the ionic composition and redox potential at both sides of the plasma membrane) when cells have been disrupted. This could explain why certain other receptors also display different ligand binding characteristics when experiments were done on intact cells instead of membrane preparations thereof (Hara et al., 1998; Packeu et al., 2008). It is clear that, because of their higher physiological relevance, intact cell experiments are to be preferred over those with broken cell preparations.

Functional and radioligand binding experiments with intact CHO-hAT₁ cells have allowed us to advance a plausible model to describe the partial nature of insurmountable AT₁ receptor antagonism by the majority of the sartants (Fierens et al, 1999a, Vauquelin et al., 2001b,c). Indeed, the competitive and partially insurmountable properties of the sartans can most easily be explained by a two-step model in where the initial sartan (I) - receptor (R) interaction yields a fast reversible/ surmountable complex (I.R). For insurmountable antagonism, IR must be further converted into a tight binding (I.R*) state. (Fierens et al, 1999a; Vauquelin et al., 2001b,c).



Computer simulations according to this model suggest that the biphenyltetrazole-based sartans display similar potency for the initial binding step (i.e. formation of IR) (Vauquelin et al., 2001b). Based thereon, it was proposed that the biphenyl moiety of these molecules plays an essential role in the initial binding process. The amino acids of the receptor that take part in this process have not been unambiguously been identified

but amino acids such as Arg¹⁶⁷ or Tyr¹¹³ are likely to play a predominant role (Vauquelin et al., 2001d; Baleanu-Gogonea et al., 2006). More recently, telmisartan was calculated to display 4.5-times lower potency for this initial interaction (Table 1) (Le et al., 2007). This behaviour of telmisartan is still compatible with the important role of the biphenyl moiety in the initial binding process if one assumes that the replacement of the tetrazole substituent by a carboxyl group decreases the potency (i.e. k_{1i}/k_{-1i} ratio) of the equilibrium.

The $IR \rightleftharpoons IR^*$ equilibrium represents the second step in this model. Inherent to this model is that only part of the sartan- AT_1 receptor complexes reside in the IR^* state while the remaining complexes are in the IR state. IR^* is apparently too unstable to be observed in the case of losartan but many other sartans stabilise IR^* sufficiently well for an equilibrium between IR and IR^* to be observed under the form of partial insurmountable antagonism in functional studies. The $[IR^*]/([IR]+[IR^*])$ ratio (i.e. the degree of insurmountability) is specific for each sartan and largely dictated by the dissociation rate of each sartan (Table 1). In this respect, closely the same dissociation rates were recorded for each sartan when investigated in direct as well as indirect radioligand binding studies as well as in functional wash-out experiments (Fierens et al., 1999b, Vanderheyden et al., 2000a,b; Verheijen et al., 2000; Le et al., 2007). As shown in Fig. 1, there is a hyperbolic relationship between the degree of insurmountability of each of the sartans we investigated and their dissociation half-life (i.e. $t_{1/2diss} = 0.69/k_{-2i}$). This is to be expected from the above equation if the conversion of IR into IR^* (given by k_{2i}) occurs with the same rate for all sartans (since $[IR^*]/([IR]+[IR^*]) = 1/(1+0.69/(t_{1/2diss}*k_{2i}))$). Of special interest is the very similar behaviour of telmisartan and the EXP 3174 (a biphenyltetrazole- based sartan): both display $\pm 70\%$ insurmountability and this effect disappears with closely the same rate ($t_{1/2}$ of ± 25 min for telmisartan and 30 min for EXP 3174 (Vanderheyden et al., 2000; Le et al., 2007). This similarity is in line with the proposed link between the degree of insurmountability and the dissociation rate of the sartans. Moreover, as only EXP 3174 is a biphenyltetrazole- based sartan (Table 1), this similarity is also in line with structure-activity relationship studies stressing the essential role of the imidazole core of these molecules in stabilising the IR^* complex. In

this respect, the insurmountable behaviour of sartans like candesartan and EXP 3174 can clearly be linked to the presence of a carboxyl group at their imidazole-derived moiety (Noda et al., 1993; Mochizuki et al., 1995). Indeed, this group is either absent or esterified in their prodrugs candesartan cilexetil, and losartan and these are unable to display insurmountable antagonism (Noda et al., 1993; Mochizuki et al., 1995). In the case of candesartan analogues, it was also clearly established that their degree of insurmountability was closely associated to the sterical position of this carboxyl group (Noda et al., 1993). Yet, telmisartan, valsartan and some other sartans (Wienen et al., 1993; Criscione et al., 1993; Schambye et al., 1994; De Arriba et al., 1996) show insurmountable behaviour as well despite the absence of such carboxylic group at their imidazole-derived moiety (Table 1). Moreover, it has also been reported that the nature of its alkyl substituents may also determine whether sartans like UR-7280 are surmountable or insurmountable (De Arriba et al., 1996). This clearly illustrates that sartans may also utilise other pharmacophores to stabilise IR*.

Site- directed mutagenesis studies involving the systematic substitution of basic amino acids (Arg, Lys and His in its protonated state) of the AT₁ receptor into neutral ones suggest that the carboxyl end of angiotensin II interacts with Lys199 (Noda et al., 1995,1996). The observation that mutating Lys¹⁹⁹ in the human AT₁ receptor into Gly decreases its affinity for sartans in a way that closely matches their degree of insurmountability suggests that this amino acid may play an important role in the stabilisation of IR* by such antagonists (Fierens et al., 2000; Vauquelin et al., 2001d). It also suggests that the binding pockets for Ang II and sartans are partially overlapping.

The above equation also implies that the degree of insurmountability and the dissociation rate of the different sartans should be positively correlated to their “macroscopic” receptor affinity (Table 1) provided that the initial binding process is the same (Vauquelin et al., 2001b). Competition binding studies on CHO-hAT₁ cells with radioligands as diverse as [³H]candesartan, [³H]irbesartan, [³H]angiotensin II, [³H]valsartan, [³H]olmesartan and [³H]telmisartan all revealed the same order of potencies for the biphenyltetrazole-based sartans (i.e. candesartan ~ olmesartan ~

EXP3174 > valsartan ~ irbesartan >> losartan) (Vauquelin et al., 2006; Le et al., 2007). EXP3174 was about 2 times less potent as candesartan and, despite the same dissociation rate and extent of insurmountability as EXP3174, telmisartan was found to be about 10 times less potent than candesartan. The about 5-fold lower potency of telmisartan versus EXP3174 cannot be explained by differences between the kinetic parameters describing the $IR \Leftrightarrow IR^*$ equilibrium but fit perfectly with its (above mentioned) lower potency at the level of the initial $I + R \Leftrightarrow IR$ interaction.

Inverse agonism.

The simplest mechanism of GPCR stimulation assumes the existence of only two receptor conformations, an inactive and an active one, and that the presence of a bound agonist is absolutely necessary for the receptor activation process. Yet, this model is inadequate to explain the fact that many GPCRs exhibit some basal activity in the absence of agonist molecules (which is defined as “constitutive” receptor activity). Ligands which inhibit the basal receptor activity in a dose-dependent fashion are termed “inverse agonists” (Kenakin, 1996; Milligan, 2003). The wild-type AT_1 receptor does not display significant constitutive activity but some receptor mutants do (Parnot et al., 2000). Among these, one of the most studied mutations deals with the replacement of Asn^{111} on TM3 by glycine (no side chain) or by residues bearing a smaller side chain such as alanine or serine (Balmforth et al., 1997; Groblowski et al., 1997; Le et al., 2003). The ability of Ang II to further stimulate the constitutively active $Asn^{111}Gly$ AT_1 receptor mutant suggests that it adopts a “pre-activated” conformation that is intermediate between the inactive and fully active states of the receptor (Le et al., 2002; Hunyady et al., 2003). This is in line with the actual view that receptor activation proceeds according to a multi-step process starting with the release of conformational constraints within the receptor (Hulme et al., 1999; Gether et al., 2000; Vauquelin and Van Liefde, 2005).

The potential inverse agonistic properties of different sartans has only been addressed in a relatively small number of studies and most of them were performed on Asn^{111} -mutated AT_1 receptors (Table 2). This is presumably because of the clearly discernible levels of constitutive activity in those systems. In this context, it is of interest to note that various

sartans display lower affinity for the Asn¹¹¹-mutated AT₁ receptors than for the wild-type receptor (Le et al., 2003). A plausible explanation for this behaviour is that such ligands preferentially bind to the inactive conformation of the receptor than to its pre-activated conformation and, consequently, that they have the potential to act as inverse agonists. Yet, as different Asn¹¹¹ substitutions decreased the receptor's affinity for losartan to the same extent as for the insurmountable sartans irbesartan, EXP3174 and candesartan (Le et al., 2003), such preference should be unrelated to the insurmountable nature of the sartans and, more particularly, to the presence of a carboxyl group at their substituted imidazole core.

Different sartans were also effectively found to exhibit inverse agonist-like behaviour but clear-cut structure-activity relationship considerations are still hampered by the lack of consistency among the results from different teams. Indeed, while Groblewski et al. (1997) noticed that losartan (i.e. Dup 753) reduced the basal activity the Asn¹¹¹Ala AT₁ receptor mutant in COS-7 cells after one hour incubation, no such effect of losartan was seen after 'short-term' (ranging from 15 min to 2 h) incubations for the Asn¹¹¹Gly mutant in CHO-K1 cells by us (Le et al., 2003). Under such short incubation conditions, insurmountable sartans such as candesartan, EXP3174 and irbesartan did not show inverse agonism either. Yet, losartan as well as the above-mentioned insurmountable sartans produced a clear-cut decrease of the constitutive activity when recombinant CHO-K1 cells expressing the Asn¹¹¹Gly AT₁ receptor mutant were pre-incubated with these antagonists for 18 h (Le et al., 2003). Based on the rationale that the inter-conversion between the pre-activated and inactive states might be extremely slow in the case of Asn¹¹¹-mutated AT₁ receptors, the same long incubation times have also been adopted by others (Noda et al., 1996; Miura et al., 2006). Contrary to our observations (Le et al., 2003), no inverse agonism by losartan could be observed by Miura et al. (2003) for the Asn¹¹¹Gly receptor mutant in COS-1 cells. However, these investigators documented the ability of EXP3174, valsartan and olmesartan to decrease the constitutive activity of this receptor mutant and gathered evidence that the carboxyl and hydroxyl groups at the substituted imidazole core of olmesartan play an important role in this process (Miura et al., 2003, 2006, 2008).

However, even with the disparate results for losartan being set aside, this “receptor mutant” approach incites two critical considerations. First, because of the long incubation times needed, it cannot be excluded that the ability of the sartans to diminish basal activities reflect phenomena like post-translational modifications of the mutant receptors, their internalisation or even their down-regulation instead of inverse agonism. Although such phenomena were previously attributed to agonists only, there is now a widespread opinion that receptors can adopt a broad range of conformations and that there is only a partial overlap between the conformations that mediate distinct receptor functions (Kenakin, 1996). Secondly, one should keep in mind that the amino acid sequence of mutated receptors is distinct from that of the wild type receptor and, hence, that they are structurally different proteins (Giraldo, 2004). This also implies that agonist-, antagonist- as well as inverse agonist interactions with wild-type and constitutively active receptor mutants could produce completely different outcomes or, in other words, that results obtained with such mutant receptors do not provide pertinent information with respect to the wild-type receptor.

Over-expression of wild-type receptors constitutes an alternative approach to generate constitutive receptor activity (Kenakin, 1996) and, because of the unaltered receptor structure, such systems are likely to provide more reliable information about inverse antagonists. For the study of sartans, this approach is severely hindered by the only marginal basal activity of the wild-type AT₁ receptors in endogenously expressing as well as in potentially over-expressing recombinant cell systems (Le et al., 2003; Miura et al., 2006, 2008). Yet, using such approach, candesartan was shown to significantly decrease the basal extracellular signal-regulated protein kinase (ERK) activity in HEK293- and COS7 cells transiently expressing wild-type AT₁ receptors (Zou et al., 2004). In the same line, olmesartan was shown to decrease the basal inositol triphosphate production in COS1 cells transiently expressing wild-type AT₁ receptors (Miura et al., 2006) and, more recently, valsartan was also reported to produce a small decrease of the basal activity in this recombinant system as well as in endogenous AT₁ receptor- expressing vascular smooth muscle cells (Miura et al., 2008). A major note of concern with respect to the

physiological relevance of such studies could be that basal AT₁ receptor activities are very low when compared the fully stimulated ones. While this may be the case when considering the inositol triphosphate production- related short-term AT₁ receptor responses like blood pressure control, the situation could be quite different when considering hypertrophic responses in tissues that are subject to mechanical stress.

Mechanical stress has indeed been shown to produce hypertrophic responses in diverse cell cultures including cardiomyocytes, bladder smooth muscle cells and chondrocytes (Sadoshima et al., 1993; Kudoh et al., 1998; Nguyen et al., 2000; Fukunaga et al., 2003; Zou et al., 2004). In cultured rat neonatal cardiomyocytes, these responses include (among others) the activation of ERKs and other mitogen-activated protein (MAP) kinases (Kudoh et al., 1998). This seems to take place via AT₁ receptor -dependent and -independent mechanisms (van Kesteren et al., 1999; Lal et al., 2007). Indeed, on the one hand, stress-mediated ERK activation and induction of the c-fos gene still took place in cardiomyocytes from AT₁ receptor knockout mice (Kudoh et al., 1998; Harada et al., 1998). On the other hand, sartans like losartan, valsartan or candesartan have been shown to inhibit MAP kinase activation, electrical remodelling and other hypertrophic responses in wild-type cardiomyocytes at various occasions (Sadoshima et al., 1993; Yamazaki et al., 1995 a,b; Kudoh et al., 1998; Zou et al., 2004; Iekushi et al., 2007; Saygili et al., 2007).

In this respect, two mechanisms have been proposed to explain the link between of cardiomyocyte stretching and AT₁ receptor activation. On the one hand, mechanical stress has been found to stimulate the secretion of Ang II from secretory granules in cardiac myocytes, suggesting that AT₁ receptor activation by its natural messenger contributes to the hypertrophic response (Sadoshima et al., 1993; Yamazaki et al., 1995a; Kudoh et al., 1998). In this respect, Ang II has also been suggested to act as an important autocrine mediator of stretch-induced phenomena in other cell types (Becker et al., 1998; Park et al., 1998). Yet, Zou et al. (2004) recently reported that, upon mechanical stretching of cultured rat neonatal myocytes, the AT₁ receptors could also be activated without the intervention of Ang II (i.e. without noticeable autocrine secretion of Ang II) and that this activation was blocked by candesartan. These authors also demonstrated the

ability of candesartan to block mechanically stress- induced hypertrophic responses as well as inositol triphosphate production in HEK293- and COS7 cells transiently expressing wild-type AT₁ receptors. It has therefore been suggested that AT₁ receptors may also function as Ang II-independent mechanoreceptors and/or that they are physically complexed with integrins and other mechanosensors (Zou et al., 2004; Oro et al., 2007). Accordingly, it has been proposed that sartans could block stretch-generated hypertrophic responses by acting as inverse AT₁ receptor agonists (Zou et al., 2004; Miura et al., 2006, 2008). Mechanical stretching of cultured rat neonatal myocytes also failed to trigger noticeable autocrine secretion of Ang II in an earlier study (van Kesteren et al., 1999) but here, losartan failed to inhibit the generated hypertrophic response. Because of the limited information presently available, it is still too early to proclaim whether the difference in the reported protecting behaviours of candesartan and losartan has a genuine mechanistic foundation or whether it is merely due to subtle differences in the experimental conditions. A rigorous comparison (and preferably in a single study) of the propensity of different sartans to block stretch-generated non-autocrine hypertrophic responses is absolutely needed to clarify this issue. Based on so-obtained information, a link could be made between the end-organ protecting behaviour of sartans and certain particularities of their chemical structure and/or other pharmacological properties of interest such as their degree of insurmountability.

Concluding remarks.

It is becoming increasingly clear that the pathological consequences of an over-active renin–angiotensin–aldosterone system result from the ability of Ang II to produce fast, G protein dependent rises in blood pressure as well as longer-term, G protein- independent hypertrophic cellular responses leading to remodelling of heart and blood vessels (Oro et al., 2007). The recent finding that valsartan significantly inhibited periostin expression (a putative soluble extracellular matrix protein) and improved cardiac dysfunction without change in blood pressure and heart rate in a rat myocardial infarction model (Iekushi et al., 2007) illustrates that there is no necessary causal link between the short-and long-term effects of Ang II. Yet, sartans have been found to be highly successful to treat both hypertension and the related pathologies (Timmermans, 1999; Oparil, 2000; Norris and

Vaughn, 2003). This could be achieved by two distinct mechanisms. The most thoroughly investigated one simply deals with the ability of the sartans to prevent the access of Ang II to the AT₁ receptor by virtue of competitive inhibition. This plays an essential role for blood pressure reduction and, in this respect, marked differences have been found in the ability of different sartan- AT₁ receptor complexes to adopt a slow-dissociating state and in the dissociation rate thereof. These kinetic properties provide a sufficient explanation for the insurmountable effect of sartan in the classical rabbit aortic strip contraction experiments and for the often partial nature thereof. Yet, seen the often much longer plasma half-life of these sartans, it is to be questioned whether their receptor dissociation is sufficiently slow to produce a tangible contribution to their long-lasting anti-hypertensive effect *in vivo* without the intervention of additional processes such as rebinding (Oparil., 2000; Schmidt and Schieffer, 2003; Vauquelin and Van Liefde, 2006; Lindström et al., 2007, Vauquelin and Szczuka., 2007). Experiments with constitutively active AT₁ receptor mutants suggest that at least some of the sartans display inverse agonism. Yet, inverse agonism is unlikely to have a major impact on G protein-dependent *in vivo* responses because of very low basal activity of the wild-type AT₁ receptor and the continuous presence of circulating and/or locally produced Ang II. In this respect, it is noteworthy that, at least in mice, the levels of Ang II in heart and kidney are about 15- and 25-fold higher than in plasma (Campbell et al., 2004). Yet, inverse agonism may play an important role when considering the prevention by sartans of AT₁ receptor mediated hypertrophic and other pathophysiological effects in cardiovascular tissues. Indeed, it appears that such effects are G protein- independent and that they can occur without the intervention of Ang II or an increase in blood pressure (Zou et al., 2004; Iekushi et al., 2007). Unfortunately a rigorous comparison of the propensity of different sartans to block stretch-generated hypertrophic responses intact cell systems has not been performed yet. However, such experiments should provide us a more complete and maybe more subtle picture of the *in vivo* actions of these therapeutically highly successful molecules.

Legends to Tables.**Table 1.**

Sartan structure and interaction with the human AT₁ receptor stably expressed in recombinant CHO-hAT₁ cells.

The percentage of fast- and slow-dissociating complexes refer to surmountable and insurmountable effects of sartan pre-incubation on Ang II- mediated inositol triphosphate production during 5 min. Sartan dissociation half-lives are calculated from radioligand binding and/or functional experiments. Their potencies relative to candesartan are from [³H]valsartan competition binding experiments (data from Fierens et al., 1999a,b; Vanderheyden et al., 2000a,b; Verheijen et al., 2000; Le et al., 2007).

Table 2.

Effect of sartans on G protein -dependent (production of inositol triphosphate) and -independent (ERK activation and/or other hypertrophic responses) responses in intact cell systems.

* Stands for hypertrophic responses without evidence for autocrine Ang II release.

Legends to Figures.**Figure 1.**

Correlation between the degree of insurmountability (i.e. the $[IR^*]/([IR]+[IR^*])$ ratio) and the corresponding experimental half-lives ($t_{1/2}$ in min) of sartan dissociation from the human AT_1 receptor stably expressed in recombinant CHO-h AT_1 cells. Data are from Table 1; $t_{1/2}$ was arbitrarily set to 1 min for losartan. The curve was drawn according to a hyperbolic function with GraphPad Prism software (GraphPad Software Inc., San Diego, CA).

Table 1.

References:

Baleanu-Gogonea, C., Karnik, S., 2006. Model of the whole rat AT₁ receptor and the ligand-binding site. *J. Mol. Model.* 12, 325–337.

Balmforth, A.J., Lee, A.J., Warburton, P., Donnelly, D., Ball S.G., 1997. The conformational change responsible for AT₁ receptor activation is dependent upon two juxtaposed asparagine residues on transmembrane helices III and VII. *J. Biol. Chem.* 272, 4245-4251.

Becker, B.N., Yasuda, T., Kondo, S., Vaikunth, S., Homma, T., Harris, R.C., 1998. Mechanical stretch/relaxation stimulates a cellular renin-angiotensin system in cultured rat mesangial cells. *Exp. Nephrol.* 6, 57-66.

Berellini, G., Cruciani, G., Mannhold, R., 2005. Pharmacophore, drug metabolism, and pharmacokinetics models on non-peptide AT₁, AT₂, and AT₁/AT₂ angiotensin II receptor antagonists. *J Med. Chem.* 48, 4389-4399.

Campbell, D.J., Alexiou, T., Xiao, H.D., Fuchs, S., McKinley, M.J., Corvol, P., Bernstein, K.E., 2004. Effect of reduced angiotensin- converting enzyme gene expression and angiotensin- converting enzyme inhibition on angiotensin and bradykinin peptide levels in mice. *Hypertension* 43, 854–859.

Criscione, L., de Gasparo, M., Bühlmayer, P., Whitebread, S., Ramjoué, H.P., Wood, J., 1993. Pharmacological profile of valsartan: a potent, orally active, nonpeptide antagonist of the angiotensin II AT₁ receptor subtype. *Brit. J. Pharmacol.* 110, 761-771.

De Arriba, A.F., Gomez-Casajus, L.A., Cavalcanti, F., Almansa, C., Garcia-Rafanel, J., Forn, J., 1996. In vitro pharmacological characterization of a new selective angiotensin AT₁ receptor antagonist, UR-7280. *Eur. J. Pharmacol.* 318, 341-347.

de Gasparo, M., Catt, K.J., Inagami, T., Wright, J.W., Unger, T., 2000. International Union of Pharmacology. XXIII. The Angiotensin II Receptors. *Pharmacol. Rev.* 52, 415-472.

Dickinson, K.E., Cohen, R.B., Skwish, S., Delaney, C.L., Serafino, R.P., Poss, M.A. Gu, Z., Ryono, D.E., Moreland, S., Powell, J.R., 1994. BMS-180560, an insurmountable inhibitor of angiotensin II-stimulated responses: comparison with losartan and EXP3174. *Br it. J. Pharmacol.* 113, 179-189.

Fierens, F.L.P., Vanderheyden, P.M.L., De Backer, J.-P., Vauquelin, G., 1999a. Insurmountable angiotensin II AT₁ receptor antagonists: the role of tight antagonist binding. *Eur. J. Pharmacol.* 372, 199-206.

Fierens, F.L.P., Vanderheyden, P.M.L., De Backer, J.-P., Vauquelin, G., 1999b. Binding of the antagonist [³H]candesartan to angiotensin II AT₁ receptor- transfected Chinese hamster ovary cells. *Eur. J. Pharmacol.* 367, 413-422.

Fierens, F., Vanderheyden, P.M.L., Roggeman, C., Vande Gucht, P., De Backer, J.-P., Vauquelin, G., 2002. Distinct binding properties of the AT₁ receptor antagonist [³H]candesartan to intact cells and membrane preparations. *Biochem. Pharmacol.* 63, 1273-1279.

Fukunaga, T., Yamashiro, T., Oya, S., Takeshita, N., Takigawa, M., Takano-Yamamoto, T., 2003. Connective tissue growth factor mRNA expression pattern in cartilages is associated with their type I collagen expression. *Bone.* 33, 911-918.

Gether, U., 2000. Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr. Rev.* 21, 90-113.

Giraldo, J., 2004. Agonist induction, conformational selection, and mutant receptors. *FEBS Lett.* 556, 13-18.

Groblewski, T., Maigret, B., Larguier, R., Lombard, C., Bonnafous, J.C., Marie, J., 1997. Mutation of Asn¹¹¹ in the third transmembrane domain of the AT_{1A} angiotensin II receptor induces its constitutive activation. *J. Biol. Chem.* 272, 1822–1826.

Hara, M., Tozawa, F., Itazaki, K., Mihara, S., Fujimoto, M., 1998. Endothelin ET(B) receptors show different binding profiles in intact cells and cell membrane preparations. *Eur. J. Pharmacol.* 345, 339-342.

Harada, K., Komuro, I., Shiojima, I., Hayashi, D., Kudoh, S., Mizuno, T., Kijima, K., Matsubara, H., Sugaya, T., Murakami, K., Yazaki, Y., 1998. Pressure overload induces cardiac hypertrophy in angiotensin II type 1A receptor knockout mice. *Circulation* 97, 1952-1959.

Hulme, E.C., Lu, Z.L., Ward, S.D., Allman, K., Curtis, C.A., 1999. The conformational switch in 7-transmembrane receptors: the muscarinic receptor paradigm. *Eur. J. Pharmacol.* 375, 247-260.

Hunyady, L., Vauquelin, G., Vanderheyden, P., 2003. Agonist induction and conformational selection during activation of a G-protein-coupled receptor. *Trends Pharmacol. Sci.* 24, 81-86.

Iekushi, K., Taniyama, Y., Azuma, J., Katsuragi, N., Dosaka, N., Sanada, F., Koibuchi, N., Nagao, K., Ogihara, T., Morishita, R., 2007. Novel mechanisms of valsartan on the treatment of acute myocardial infarction through inhibition of the antiadhesion molecule periostin. *Hypertension.* 49, 1409-1414.

Kenakin, T., 1996. The classification of seven transmembrane receptors in recombinant expression systems. *Pharmacol. Rev.* 48, 413-463.

Kudoh, S., Komuro I., Hiroi, Y., Zou, Y., Harada, K., Sugaya, T., Takekoshi, N., Murakami, K., Kadowaki, T, and Yazaki, Y., 1998. Mechanical stretch induces hypertrophic responses in cardiac myocytes of angiotensin II type 1a receptor knockout mice. *J Biol. Chem.* 273, 24037-24043.

Lal, H., Verma, S.K., Smith, M., Guleria, R.S., Lu, G., Foster, D.M., Dostal, D.E., 2007. Stretch-induced MAP kinase activation in cardiac myocytes: Differential regulation through beta1-integrin and focal adhesion kinase. *J. Mol. Cell. Cardiol.* 43, 137-147.

Le, M.T., Vanderheyden, P.M.L., Szaszák, M., Hunyady, L., Vauquelin, G., 2002. Angiotensin IV is a potent agonist for constitutive active human AT₁ receptors. Distinct roles of the N- and C-terminal amino acid residues of angiotensin II in human AT₁ receptor activation. *J. Biol. Chem.* 277, 23107-23110.

Le, M.T., Vanderheyden, P.M.L, Szaszák, M., Hunyady, L., De Backer, J.-P., Fierens, F.L.P., Vauquelin, G., 2003. Peptide and non-peptide antagonist interaction with constitutively active human AT₁ receptors. *Biochem. Pharmacol.* 65, 1329-1335.

Le, M.T., Pugsley, M., Vauquelin, G., Van Liefde, I., 2007. Angiotensin II AT₁ antagonism and insurmountability: comparison of olmesartan and telmisartan. *Brit. J. Pharmacol.* 151, 952-962.

Leff, P., Martin, G.R., 1986. Peripheral 5-HT₂-like receptors. Can they be classified with the available antagonists? *Brit. J. Pharmacol.* 88, 585-593.

Lindström, E., von Mentzer, B., Pählman, I., Ahlstedt, I., Uvebrant, A., Kristensson, E., Eriksson, R., Novén, A., de Verdier, J., Vauquelin, G., 2007. Neurokinin (NK)1 receptor antagonists: correlation between in vitro receptor interaction and in vivo efficacy. *J. Pharmacol. Exp. Ther.* 322, 1286-1293.

Milligan, G., 2003. Constitutive activity and inverse agonists of G protein-coupled receptors: a current perspective. *Mol. Pharmacol.* 64, 1271-1276.

Miura, S., Saku, K., Karnik, S.S., 2003. Molecular analysis of the structure and function of the angiotensin II type 1 receptor. *Hypertens Res.* 26, 937-943.

Miura, S., Fujino, M., Hanzawa, H., Kiya, Y., Imaizumi, S., Matsuo, Y., Tomita, S., Uehara, Y., Karnik, S.S., Yanagisawa, H., Koike, H., Komuro, I., Saku K., 2006. Molecular mechanism underlying inverse agonist of angiotensin II type 1 receptor. *J Biol. Chem.* 281, 19288-19295.

Miura, S., Kiya, Y., Kanazawa, T., Imaizumi, S., Fujino, M., Matsuo, Y., Karnik, S.S., Saku, K., 2008. Differential Bonding Interactions of Inverse Agonists of Angiotensin II Type 1 Receptor in Stabilizing the Inactive State. *Mol. Endocrinol.* 22, 139-146.

Mochizuki, S., Sato, T., Furata, K., Hase, K., Ohkura, Y., Fukai, C., Kosakai, K., Wakabayashi, A., Tomiyama, A., 1995. Pharmacological properties of KT3-671, a novel nonpeptide angiotensin II receptor antagonist. *J. Cardiovasc. Pharmacol.* 25, 22-29.

Nguyen, H.T., Adam, R.M., Bride, S.H., Park, J.M., Peters, C.A., Freeman, M.R., 2000. Cyclic stretch activates p38 SAPK2-, ErbB2-, and AT₁-dependent signalling in bladder smooth muscle cells. *Am. J. Physiol. Cell. Physiol.* 279, C1155-C1167.

Noda, M., Shibouta, Y., Inada, Y., Ojima, M., Wada, T., Sanada, T., Kubo, K., Kohara, Y., Naka, T., Nishikawa, K., 1993. Inhibition of rabbit aortic angiotensin II (AII) receptor by CV-11974, a new neuropeptide AII antagonist. *Biochem. Pharmacol.* 46, 311-318.

Noda, K., Saad, Y., Karnik, S.S., 1995. Interaction of Phe⁸ of angiotensin II with Lys¹⁹⁹ and His²⁵⁶ of AT₁ receptor in agonist activation. *J. Biol. Chem.* 270, 28511-28514

Noda, K., Feng, Y.H., Liu, X.P., Saad, Y., Husain, A., Karnik, S.S., 1996. The active state of the AT₁ angiotensin receptor is generated by angiotensin II induction. *Biochemistry* 35, 16435-16442.

Norris, K., Vaughn, C., 2003. The role of renin-angiotensin-aldosterone system inhibition in chronic kidney disease. *Expert Rev. Cardiovasc. Ther.* 1, 51-63.

Oparil, S., 2000. Newly emerging pharmacologic differences in angiotensin II receptor blockers. *Am. J. Hypertens.* 13, 18S-24S.

Oro, C., Qian, H., Thomas, W.G., 2007. Type 1 angiotensin receptor pharmacology: signalling beyond G proteins. *Pharmacol. Ther.* 113, 210-226.

Packeu, A., De Backer, J-P., Van Liefde, I., Vanderheyden, P., Vauquelin, G., 2008. Antagonist- dopamine D_{2L}- receptor interactions in intact cells. *Biochem. Pharmacol.* (in press).

Park, J.M., Borer, J.G., Freeman, M.R., Peters, C.A., 1998. Stretch activates heparin-binding EGF-like growth factor expression in bladder smooth muscle cells. *Am. J. Physiol.* 275, C1247-C1254.

Parnot, C., Bardin, S., Miserey-Lenkei, S., Guedin, D., Corvol, P., Clauser, E., 2000. Systematic identification of mutations that constitutively activate the angiotensin II type 1A receptor by screening a randomly mutated cDNA library with an original pharmacological bioassay. *Proc. Natl. Acad. Sci. USA.* 97, 7615-7620.

Sadoshima, J., Xu, Y., Slayter, H.S., Izumo, S., 1993. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes in vitro. *Cell* 75, 977-984

Saygili, E., Rana, O.R., Saygili, E., Reuter, H., Frank, K., Schwinger, R.H., Müller-Ehmsen, J., Zobel, C., 2007. Losartan prevents stretch-induced electrical remodeling in

cultured atrial neonatal myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 292, H2898-H2905.

Schambye, H.T., von Wijk, B., Hjorth, S.A., Wiene, W., Entzeroth, M., Bergsma, D.J., Schwartz, T.W., 1994. Mutations in transmembrane segment VII of the AT₁ receptor differentiate between closely related insurmountable and competitive angiotensin antagonists. *Brit. J. Pharmacol.*, 113, 331-333.

Schmidt, B., Schieffer, B., 2003. Angiotensin II AT₁ Receptor Antagonists. Clinical Implications of Active Metabolites. *J. Med. Chem.* 46, 2261-2270.

Timmermans, P.B., 1999. Pharmacological properties of angiotensin II receptor antagonists. *Can. J. Cardiol.* 15, 26F-8F.

Vanderheyden, P.M.L., Fierens, F.L.P., De Backer, J-P., Vauquelin, G., 2000a. Reversible and syntopic interaction between angiotensin II AT₁ receptor antagonists and human AT₁ receptors expressed in CHO-K1 cells. *Biochem. Pharmacol.* 59, 927-935.

Vanderheyden, P.M.L., Fierens, F.L.P., Verheijen, I., De Backer, J.-P., Vauquelin, G., 2000b. Binding characteristics of [³H]irbesartan to human recombinant angiotensin II type 1 receptors. *J. Renin- Angiotensin- Aldosterone System.*1, 159-165.

van Kesteren, C.A., Saris, J.J., Dekkers, D.H., Lamers, J.M., Saxena, P.R., Schalekamp, M.A., Danser, A.H., 1999. Cultured neonatal rat cardiac myocytes and fibroblasts do not synthesize renin or angiotensinogen: evidence for stretch-induced cardiomyocyte hypertrophy independent of angiotensin II. *Cardiovasc. Res.* 43, 148-156.

Vauquelin, G., Fierens, F.L.P., Verheijen, I., Vanderheyden, P.M.L., 2001a. Distinctions between non-peptide angiotensin II AT₁ receptor antagonists. *J. Renin- Angiotensin- Aldosterone System*, 2, S24-S31.

Vauquelin, G., Morsing, P., Fierens, F.L., De Backer, J.P., Vanderheyden, P.M., 2001b. A two-state receptor model for the interaction between angiotensin II type 1 receptors and non-peptide antagonists. *Biochem. Pharmacol.* 61, 277-284.

Vauquelin, G., Fierens, F., Verheijen, I., Vanderheyden, P., 2001c. Insurmountable AT₁ receptor antagonism: the need for different antagonist binding states of the receptor. *Trends Pharmacol. Sci.* 22, 343-344.

Vauquelin, G., Fierens, F.L.P., Gáborik, Z., Le Minh, T., De Backer, J.-P., Hunyady, L., Vanderheyden, P.M.L., 2001d. Role of basic amino acids of the human angiotensin type 1 receptor in the binding of the non-peptide antagonist candesartan. *J. Renin- Angiotensin- Aldosterone System* 2, S32-S36.

Vauquelin, G., Van Liefde, I., Vanderheyden, P.M.L., 2002. Models and methods for studying insurmountable antagonism. *Trends Pharmacol. Sci.* 23, 514-518.

Vauquelin, G., Van Liefde, I., 2005. G protein- coupled receptors: a count of 1001 conformations. *Fund. Clin. Pharmacol.* 19, 45-56.

Vauquelin, G., Fierens, F., Van Liefde, I., 2006. Long-lasting AT₁ receptor binding and protection by Candesartan: comparison to other biphenyl-tetrazole sartans. *J. Hypertens.* 24, S23-S30.

Vauquelin, G., Van Liefde, I., 2006. From slow antagonist dissociation to long- lasting receptor protection. *Trends Pharmacol. Sci.* 27, 355-359.

Vauquelin, G., Szczuka, A., 2007. Kinetic versus allosteric mechanisms to explain insurmountable antagonism and delayed ligand dissociation. *Neurochem. Int.* 51, 254-260.

Verheijen, I., Fierens, F.L.P., De Backer, J.-P., Vauquelin, G., Vanderheyden, P.M.L., 2000. Interaction between the partially insurmountable antagonist valsartan and human recombinant angiotensin II type 1 receptors. *Fund. Clin. Pharmacol.* 14, 577-585.

Verheijen, I., Turlousse, D., Vanderheyden, P.M.L., De Backer, J.P., Vauquelin, G., 2004. Effect of saponin and filipin on antagonist binding to AT₁ receptors in intact cells. *Biochem. Pharmacol.* 67, 601-606.

Wienen, W., Maunz, A.B., Van Meel, J.C., Entzeroth, M., 1992. Different types of receptor interaction of peptide and nonpeptide angiotensin II antagonists revealed by receptor binding and functional studies. *Mol. Pharmacol.* 41, 1081-1088.

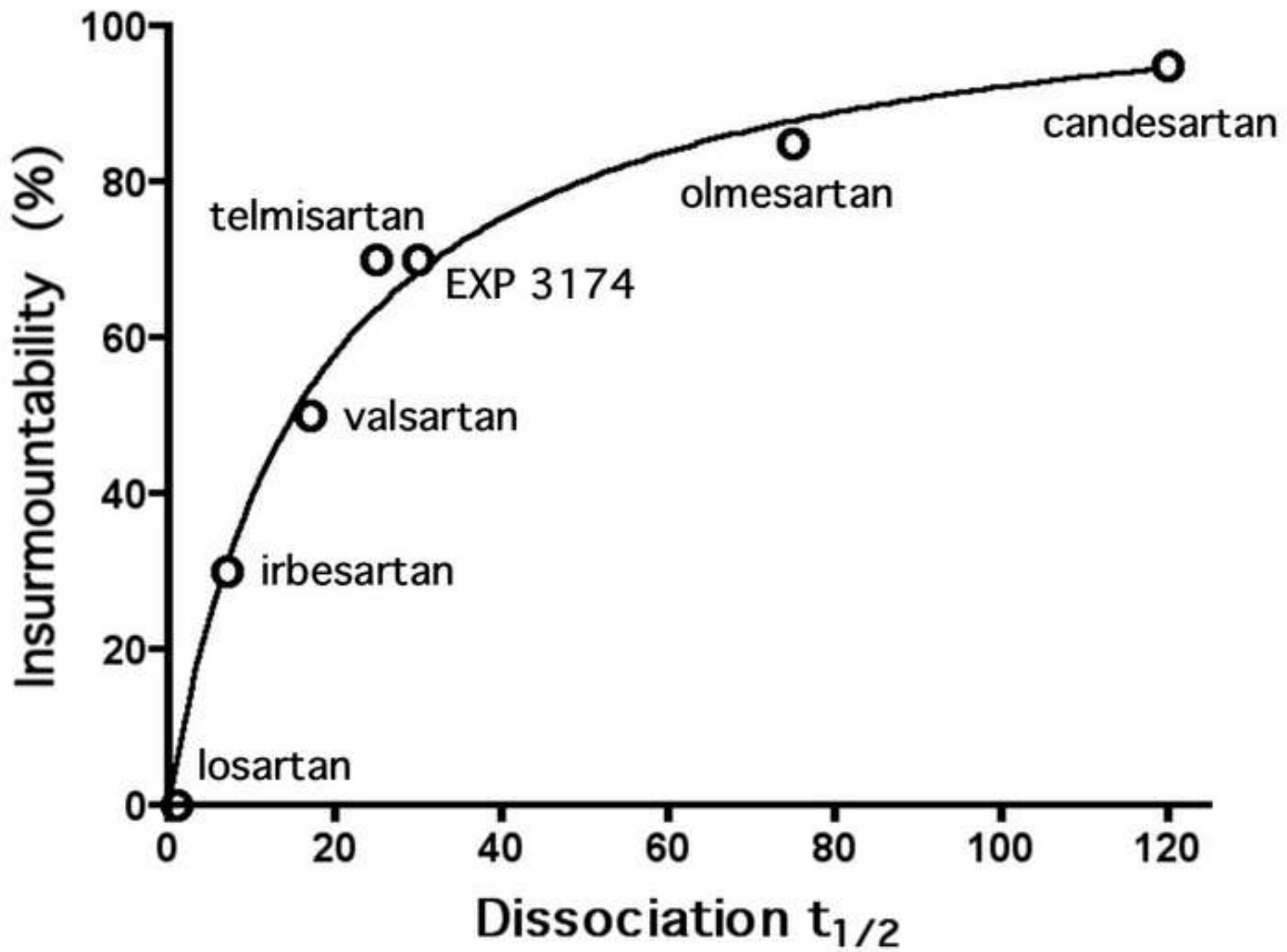
Wienen, W., Huel, N., Van Meel, J.C., Narr, B., Ries, U., Entzeroth, M., 1993. Pharmacological characterization of the novel nonpeptide angiotensin II receptor antagonist, BIBR 277. *Brit. J. Pharmacol.* 110, 245-252.

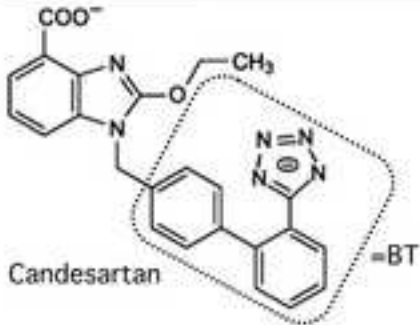
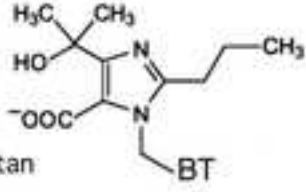
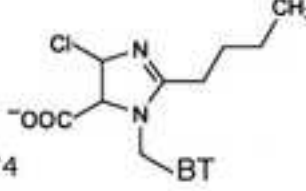
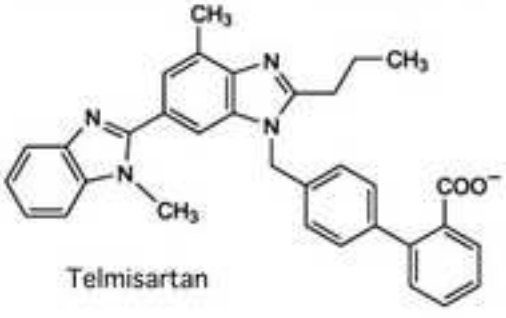
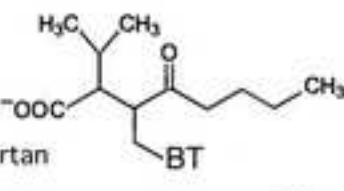
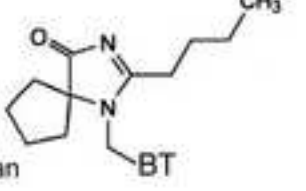
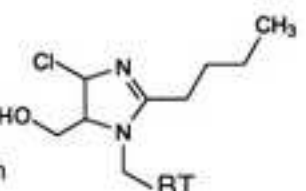
Yamazaki, T., Komuro, I., Kudoh, S., Zou, Y., Shiojima, I., Mizuno, T., Takano, H., Hiroi, Y., Ueki, K., Tobe, K., Kadowaki, T., Nagai, R., Yazaki, Y., 1995a. Angiotensin II partly mediates mechanical stress-induced cardiac hypertrophy. *Circ. Res.* 77, 258-265.

Yamazaki, T., Komuro, I., Shiojima, I., Mizuno, T., Kudoh, S., Zou, Y., Takano, H., Hiroi, Y., Ueki, K., Tobe, K., Kadowaki, T., Nagai, R., Yazaki, Y., 1995b. Mechanical stress activates protein kinase cascade of phosphorylation in neonatal rat cardiac myocytes. *J. Clin. Invest.* 96, 438-446.

Zou, Y., Akazawa, H., Qin, Y., Sano, M., Takano, H., Minamino, T., Makita, N., Iwanaga, K., Zhu, W., Kudoh, S., Toko, H., Tamura, K., Kihara, M., Nagai, T., Fukamizu, A., Umemura, S., Iiri, T., Fujita, T., Komuro, I., 2004. Mechanical stress activates angiotensin II type 1 receptor without the involvement of angiotensin II. *Nat. Cell Biol.* 6, 499-506.

Figure 1



Structure	Receptor interaction $I + R \rightleftharpoons IR \rightleftharpoons IR^*$		Dissociation $t_{1/2}$ (min)	Binding potency (candesartan = 1)
 Candesartan	5%	95%	120	1
 Olmesartan	15%	85%	75	0.73
 EXP3174	30%	70%	30	0.45
 Telmisartan	30%	70%	25	0.083
 Valsartan	50%	50%	17	0.17
 Irbesartan	70%	30%	7	0.15
 Losartan	100%	0%	fast	0.014

AT₁ receptor activity	sartan	effect	reference	
Wild-type receptor: basal inositol phosphate production	valsartan	decrease	Miura et al., 2008	
	olmesartan	decrease	Miura et al., 2006	
Wild-type receptor: basal ERK activity	candesartan	decrease	Zou et al., 2004	
Wild-type receptor: hypertrophic response to mechanical stress	candesartan	decrease	Yamazaki et al., 1995a,b	
	candesartan	decrease	Kudoh et al., 1998	
	candesartan	decrease*	Zou et al., 2004	
	valsartan	decrease	Iekushi et al., 2007	
	losartan	decrease	Sadoshima et al., 1993	
	losartan	none*	van Kesteren et al., 1999	
	losartan	decrease	Saygili et al., 2007	
	Asn ¹¹¹ -mutated receptor: basal inositol phosphate production	losartan	decrease	Groblewski et al., 1997
		losartan	decrease	Le et al., 2003
		losartan	none	Miura et al., 2003
irbesartan		decrease	Le et al., 2003	
valsartan		decrease	Miura et al., 2008	
EXP 3174		decrease	Le et al., 2003	
olmesartan		decrease	Miura et al., 2006	
candesartan		decrease	Le et al., 2003	