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Saralasin and Sarile Are AT_2 Receptor Agonists

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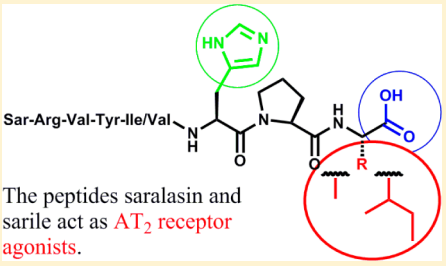
ABSTRACT: Saralasin and sarile, extensively studied over the past 40 years as angiotensin II (Ang II) receptor blockers, induce neurite outgrowth in a NG108-15 cell assay to a similar extent as the endogenous Ang II. In their undifferentiated state, these cells express mainly the AT_2 receptor. The neurite outgrowth was inhibited by preincubation with the AT_2 receptor selective antagonist PD 123,319, which suggests that the observed outgrowth was mediated by the AT_2 receptor. Neither saralasin nor sarile reduced the neurite outgrowth induced by Ang II proving that the two octapeptides do not act as antagonists at the AT_2 receptor and may be considered as AT_2 receptor agonists.



KEYWORDS: *Saralasin, sarile, AT_2 receptor agonist, peptide ligands, C21/M024*

The octapeptide angiotensin II (Ang II) is a major component of the renin−angiotensin system (RAS) and an important modulator of cardiovascular function. Ang II exerts a pronounced hypertensive effect. Forty years ago it was reported that minor modifications of the amino acid residue sequence of Ang II blocked the action of Ang II.[1] Two of the octapeptide Ang II antagonists, saralasin ([Sar[1],Val[5],Ala[8]]Ang II) and sarile ([Sar[1],Ile[8]]Ang II), were subsequently evaluated in the clinic.[2,3] However, both compounds failed to find any therapeutic use, mainly due to their peptidic character (for reviews see refs 4−6) (Chart 1). Nevertheless, the in vivo data obtained with these compounds lead to the emergence of the RAS as a suitable target for drug intervention. The ACE inhibitors were subsequently disclosed[7] followed by the angiotensin receptor blockers (ARB)[5] and more recently the renin inhibitors.[8]

Sarile is characterized by a sarcosine residue in position 1 that improves the metabolic stability and potency[9] and by an isoleucine in position 8. In saralasin, the 1-sarcosin is retained with a valine in the 5 position and an alanine residue in the 8 position. The C-terminal amino acid residue is considered a primary determinant of agonist and antagonist activity at the AT_1 receptor and a side chain with an aromatic ring with proper orientation renders agonism while an aliphatic side chain renders antagonism (or partial agonism) (for reviews on Ang II structure−activity relationships (SAR), see refs 4−6).[10,11] Both saralasin and sarile block the AT_1 receptor, but these peptides are not selective and exhibit approximately equal affinities also to the AT_2 receptor.[12]

To the best of our knowledge, it has not been known whether these prototype antagonists, studied in detail in a large variety of systems, indeed act as agonists or antagonists at the AT_2 receptor.[4] On the basis of the structural similarities in the C-terminal part of sarile and the AT_2 receptor selective agonists, e.g., C21/M024 (Chart 2), we were intrigued to examine whether the nonselective AT_1 receptor blockers sarile and saralasin indeed might act as agonists rather than antagonists at the AT_2 receptor. We herein report that saralasin and sarile are AT_2 receptor agonists equally potent to the endogenous Ang II.

The functional effects of saralasin and sarile at the AT_2 receptor were evaluated in a neurite outgrowth assay with NG108−15 cells. These cells express mainly the AT_2 receptor in their undifferentiated state[13,14] and a three-day treatment with Ang II or the selective AT_2 receptor agonist CGP-42112A[13,15] induces neurite outgrowth, which is one of the steps in neuronal differentiation and the end point of AT_2 receptor stimulation.[16,17] The results show that saralasin and sarile both induce neurite outgrowth to a similar extent as Ang II. The neurite outgrowth is inhibited by preincubation with the AT_2 receptor selective antagonist PD 123,319,[18] suggesting that the effect is mediated by the AT_2 receptor (Figure 1A). Neither saralasin nor sarile decreased the Ang II-induced neurite outgrowth, demonstrating that these two octapeptides do not act as antagonists at the AT_2 receptor (Figure 1B).

The results presented herein demonstrate that the two important research tools saralasin and sarile both exert agonistic effects at the AT_2 receptor. Agonist and antagonist SAR both regarding peptide ligands and nonpeptide ligands acting at the AT_1 receptor has been thoroughly investigated and reviewed.[4−6] Much less are known about SAR and agonism and antagonism exerted by peptidic AT_2 receptor ligands. In fact, only two AT_2 receptor selective peptide agonists, i.e., CGP 42112A[13,15] and (4-NH_2−Phe[6])Ang II[19] that are structurally very different, have been studied in detail and utilized as

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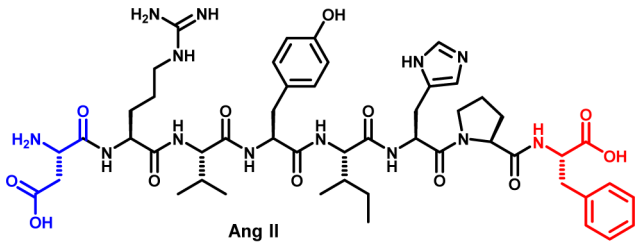
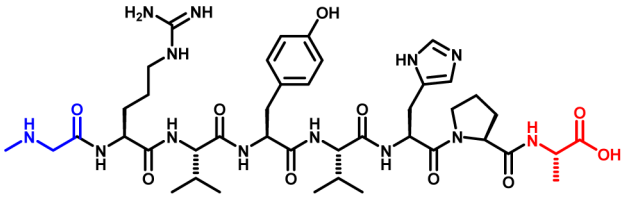
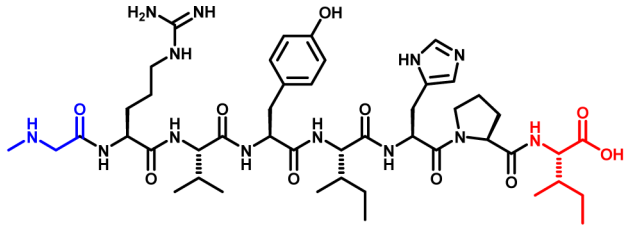
Chart 1. Structures and Biological Activities of Ang II, Saralasin, and Sarile



Ang II
K_i (nM)[20]
AT_1: 0.95
AT_2: 0.2
Agonist (AT_1/AT_2)

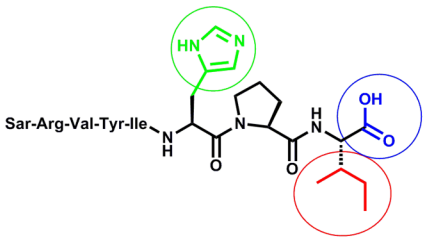


Saralasin ([Sar^1,Val^5,Ala^8]Ang II)
K_i (nM)[12]
AT_1: 0.22
AT_2: 0.38
Antagonist (AT_1)
Agonist (AT_2)

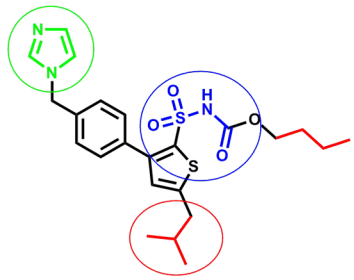


Sarile ([Sar^1,Ile^8]Ang II)
K_i (nM)[12]
AT_1: 0.65
AT_2: 0.14
Antagonist (AT_1)
Agonist (AT_2)

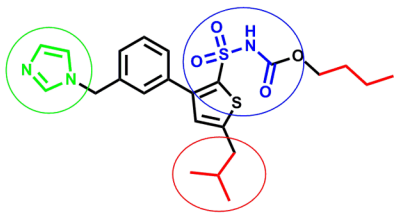
Chart 2. Structures of Sarile, the Nonpeptide AT_2 Receptor Agonist C21/M024, and the Structurally Related Antagonist C38/M132 with Possible Important Pharmacophore Elements Highlighted



Sarile ([Sar^1,Ile^8]Ang II)
Antagonist (AT_1)
Agonist (AT_2)



C21/M024
K_i (nM)[24]
AT_1: >10,000
AT_2: 0.4
Agonist (AT_2)



C38/M132
K_i (nM)[27]
AT_1: >10,000
AT_2: 17.8
Antagonist (AT_2)

reference AT_2 agonists. More recently, constrained and linear peptide agonists at the AT_2 receptor have been disclosed.[20,21]

While nonpeptide AT_2 receptor antagonists have been known for a long time, e.g., PD 123,319[18] and the more recently disclosed but structurally related EMA401, now in clinical trials as an analgesic,[22,23] the first selective nonpeptide agonists to the AT_2 receptor were not reported until 2004. This agonist, C21/M024, was developed from a nonselective AT_1/AT_2 receptor agonist.[24] Subsequently, a large number of drug-like selective AT_2 receptor agonists were reported comprising a nitrogen containing substituent, a lipophilic chain, and a sulfonylcarbamate function, attached to a biaryl scaffold, as characteristic features.[25,26] The acidic sulfonylcarbamate group is anticipated to serve as a bioisoster of a carboxy group and occupy the same binding site as the C-terminal carboxy group of Ang II and related analogues.

The fact that sarile and saralasin were found to be as potent as Ang II in activating the AT_2 receptor suggests that (a) the lipophilic carbon chain, (b) the acidic carboxy function or the corresponding acidic sulfonylcarbamate bioisostere in C21/M024, and (c) a properly located imidazole moiety might constitute important pharmacophore elements in at least these two series of AT_2 receptor agonists. It should be emphasized that the position of the five-membered imidazole structure at the phenyl ring of the druglike molecules is critical since a meta rather than para substitution pattern creates antagonism, cf., C38/M132 (Chart 2).[27,28]

At high doses saralasin and sarile can display partial agonist activity at the AT_1 receptor.[4] Furthermore, it has been reported that the AT_1 receptor exerts functional selectivity, thus a biased agonist does not necessarily mimic the physiological agonist

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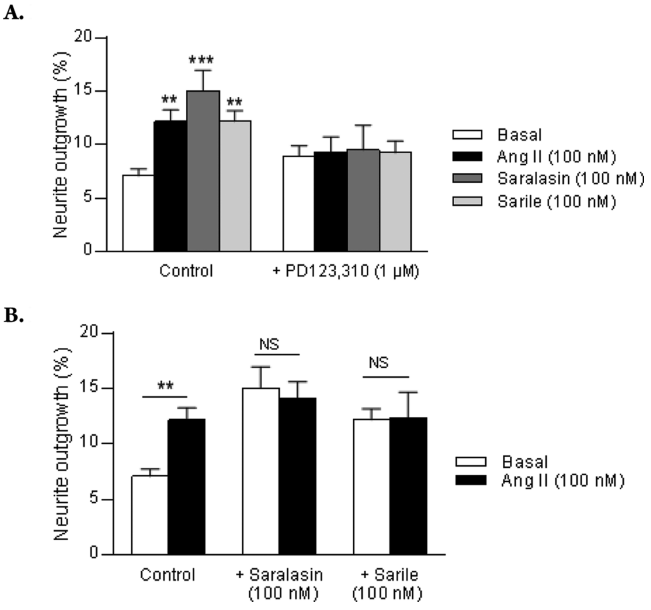

Figure 1. Effects of saralasin and sarile on neurite outgrowth in NG108-15 cells. The cells were plated at a cell density of 3.6×10^4 cells/Petri dish (35 mm) and subjects for a three-day treatment. Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth. The number of cells with neurites was expressed as the percentage of the total number of cells. (A) Cells cultured in the absence or presence of 100 nM Ang II, 100 nM saralasin, or 100 nM sarile either alone or in combination with 1 μM PD 123,319. (B) Cells cultured in the absence or presence of 100 nM Ang II either alone or in combination with 100 nM saralasin or 100 nM sarile. The results are significant according to two-way ANOVA: ***, $p < 0.001$; **, $p < 0.01$; NS = not significant.

and can thereby act with a unique signaling output.[29,30] Whether the AT_2 receptor possesses functional selectivity or if saralasin or sarile are true agonists remains to be elucidated until more specific assays on the function of the AT_2 receptor are available. The neurite outgrowth assay used in this study is a qualitative assay unable to reveal any biases in the signaling pathways.

The beneficial physiological effects that were observed after administration of sarile and saralasin in early investigations were attributed to their antagonistic properties at the angiotensin II receptor(s).[5,12] Considering the new data reported herein it is tempting to suggest that at least a part of the outcomes encountered in previous experiments with saralasin and sarile could well have been mediated through AT_2 receptor stimulation. Activation of the AT_1 and AT_2 receptors frequently results in opposing outcomes. Thus, high or sustained AT_1 receptor stimulation leads to vasoconstriction, inflammation, and fibrosis, while AT_2 receptor stimulation induces vasodilatation and counteracts inflammation and fibrosis.[31]

In summary, saralasin and sarile induce neurite outgrowth of NG108-15 cells and act as AT_2 receptor agonists. We believe that at least some of the effects reported with saralasin and sarile, previously administrated as Ang II receptor antagonists, might not be entirely attributed to blockade (or partial agonism) at the AT_1 receptor but rather to stimulation of the AT_2 receptor.

EXPERIMENTAL PROCEDURES

General Considerations for In Vitro Morphological Effects. The chemicals used in the present study were obtained from the following sources: Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), HAT supplement (Hypoxanthine, Aminopterin, Thymidine), gentamycin from Gibco BRL (Burlington, Ont, Canada), and [Val^5]-angiotensin II from Bachem (Marina Delphen, CA, USA). PD 123,319 was obtained from RBI (Natick, MA, USA). All other chemicals were of grade A purity.

Cell Culture. NG108-15 cells (initially provided by Drs. M. Emerit and M. Hamon; INSERM, U. 238, Paris, France) were used to study the in vitro morphological effects. In their undifferented state, neuroblastoma x glioma hybrid NG108-15 cells have a rounded shape and divide actively. The cells were cultured form passage 18 to 25 in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Burlington, Ont., Canada) with 10% fetal bovine serum (FBS, Gibco), HAT supplement, and 50 mg/L gentamycin at 37 °C in 75 cm^2 Nunclon Delta flasks in a humidified atmosphere of 93% air and 7% CO_2, as previously described. Subcultures were performed at subconfluency. Under these conditions, cells express mainly the AT_2 receptor subtype.[13,14,17] Cells were treated during 3 days, once a day (first treatment 24 h after plating), and micrographs were taken the fourth day. For all experiments, cells were plated at the same initial density of 3.6×10^4 cells/35 mm Petri dish. Cells were treated without (control cells) or with [Val^5]-angiotensin II (100 nM) or sarile (100 nM) or saralasin (100 nM) in the absence or in the presence of PD 123,319 (1 μM), an AT_2 receptor selective antagonist. The antagonist was introduced daily 30 min prior to Ang II, sarile, or saralasin. Sarile and saralasin were also tested in the presence of Ang II (100 nM) where the compounds were introduced daily 30 min prior to Ang II, to evaluate antagonistic properties.

Determination of Cells with Neurites. Cells were examined under a phase contrast microscope, and pictures were taken at the end of the experimental period (on the fourth day). Cells with at least one neurite longer than a cell body were counted as positive for neurite outgrowth. The number of cells with neurites was reported as the percentage of the total amount of cells in the micrographs, and at least 400 cells were counted in three independent experiments and each condition was performed in duplicate, as previously described.[32]

Data Analysis. The data are presented as mean ± SEM of the average number of cells on a micrograph. Statistical analyses of the data were performed using the two-way ANOVA test. Homogeneity of variance was assessed by Bartlett's test, and p values were obtained from Dunnett's tables.

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Author Contributions

The manuscript was written through contributions of all authors.

Notes

The authors declare no competing financial interest.

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