New complexes formed by Mas and Angiotensin receptors. Mas/AT_1R and Mas/AT_2R altered heteromers expression in a rat model of Parkinson disease

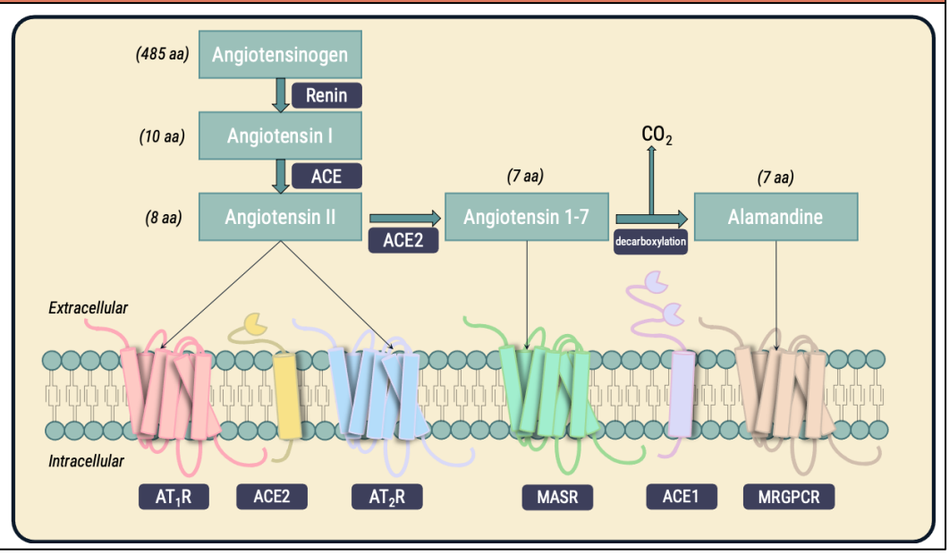
IECBS 2021

Rafael Rivas-Santisteban[a,b], Jaume Lillo[a,b], Ana Muñoz[b,c], Ana I. Rodriguez-Perez[b,c], José Luis Labandeira-García[b,c], Nerea Domínguez-Madrid [a], Gemma Navarro[b,d], Rafael Franco[a,b]

a) Molecular Neurobiology Laboratory, Department of Biochemistry and Molecular Biomedicine, Universitat de Barcelona, Barcelona, Spain. , b) Centro de Investigación en Red, Enfermedades Neurodegenerativas (CIBERNED), Instituto de Salud Carlos III, Madrid, Spain. , c) Laboratory of Cellular and Molecular Neurobiology of Parkinson's disease, Research Center for Molecular Medicine and Chronic Diseases (CIMUS), Dept. of Morphological Sciences, IDIS, University of Santiago de Compostela, Santiago de Compostela; Spain. , d) Dept. Biochemistry and Physiology. School of Pharmacy and Food Sciences. Universitat de Barcelona. Barcelona. Spain.

Introduction

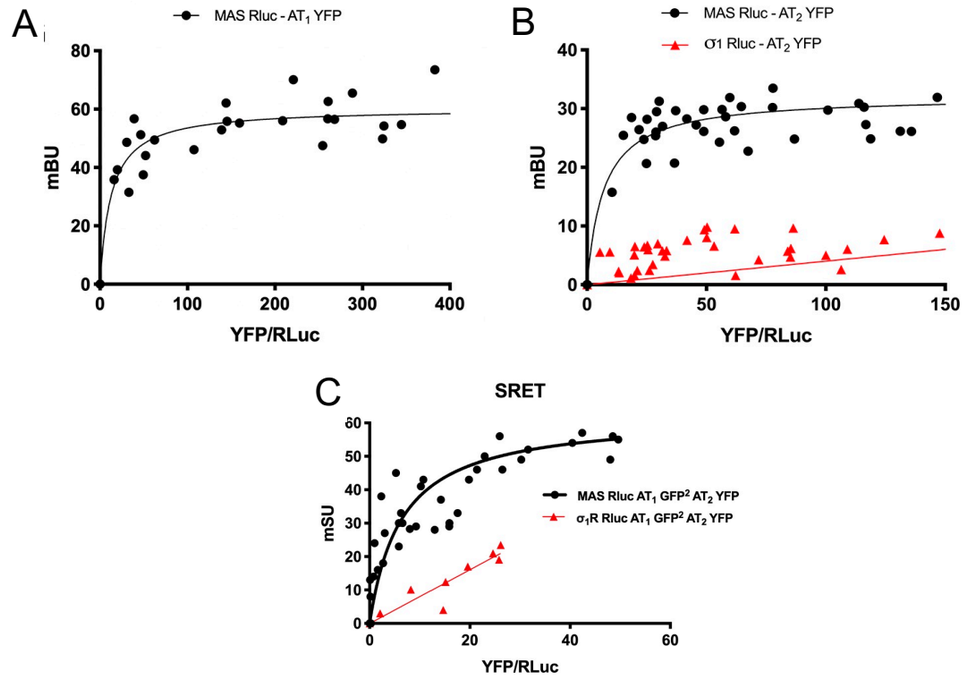
The **renin/angiotensin system (RAS)** is composed of angiotensin-converting enzymes, like ACE or ACE2, that produce different angiotensin (Ang) peptides and of cell surface receptors that convey cytocrin signals to achieve specific cell responses. Angiotensin (AT_1 and AT_2) and Mas receptors belong to the superfamily of G-protein-coupled receptors. RAS has been abundantly studied in the periphery, mainly in relation with the control of arterial tension. However, RAS also participates in almost every process to maintain homeostasis in mammals. RAS components in activated microglia warrant attention in drug-development approaches to address neurodegeneration.



Results

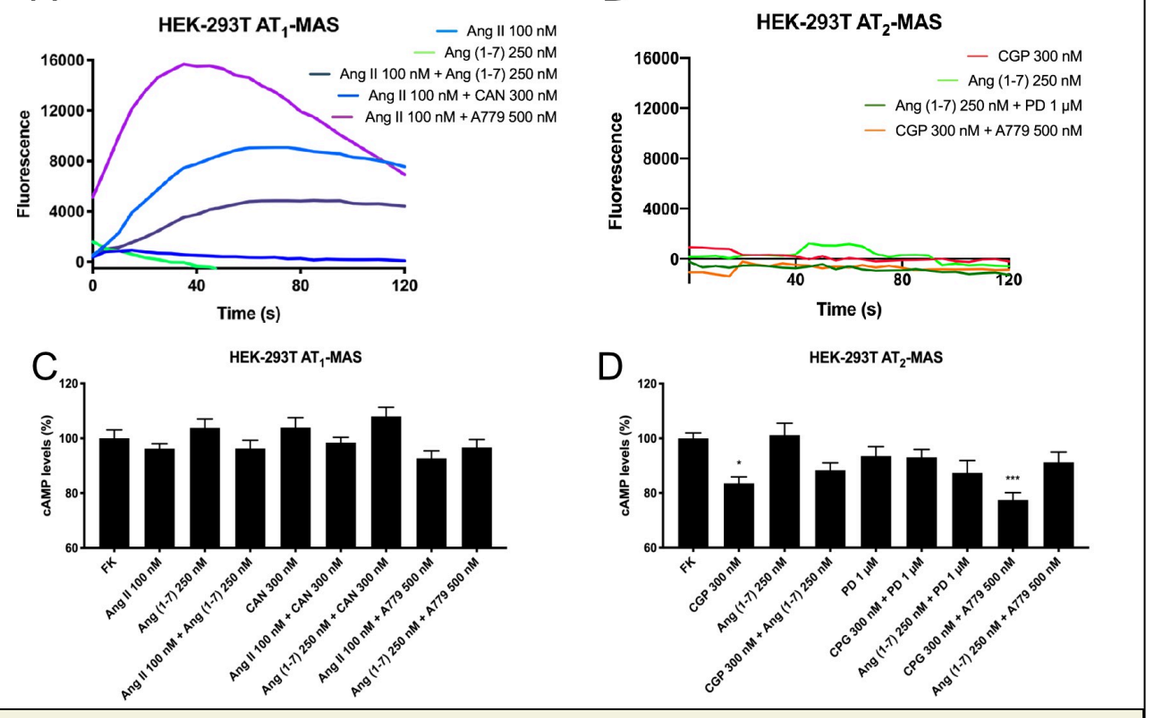
Interaction between angiotensin (AT_1 and AT_2) and Mas receptors in a heterologous expression system

We analyzed the interaction between angiotensin and Mas receptors at the plasma membrane **Bioluminiscence Resonance Energy Transfer Assays (BRET),** confirmed the specific **interaction between AT_1 or AT_2 with Mas receptors (A, B),** and **Sequential BRET-FRET (SRET) (C).**



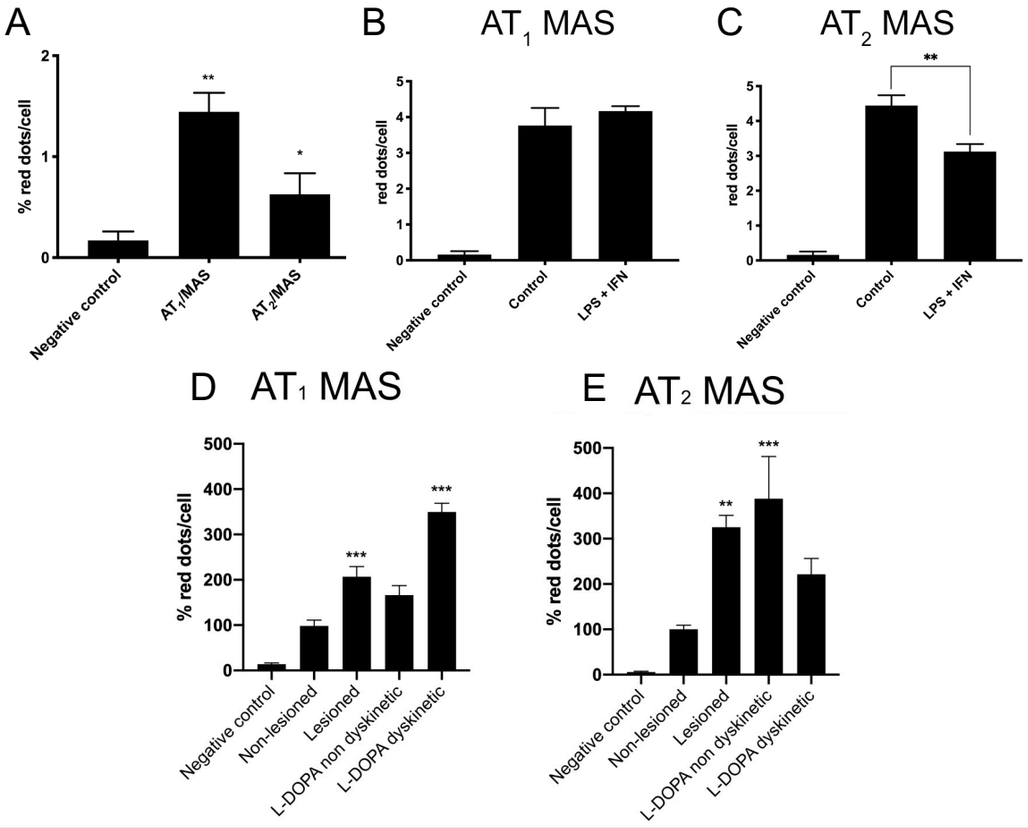
Functionality of AT_1Mas and AT_2Mas Hets in a heterologous expression system

To characterize the heteromers functionality, signaling assays were performed in cotransfected AT_1Mas or AT_2Mas hets expressing HEK-293T cells. **Cytosolic calcium levels** (A,B) **and cAMP determinations** (C,D) were performed.



Expression of AT_1Mas and AT_2Mas Hets in the striatum of parkinsonian and dyskinetic rats

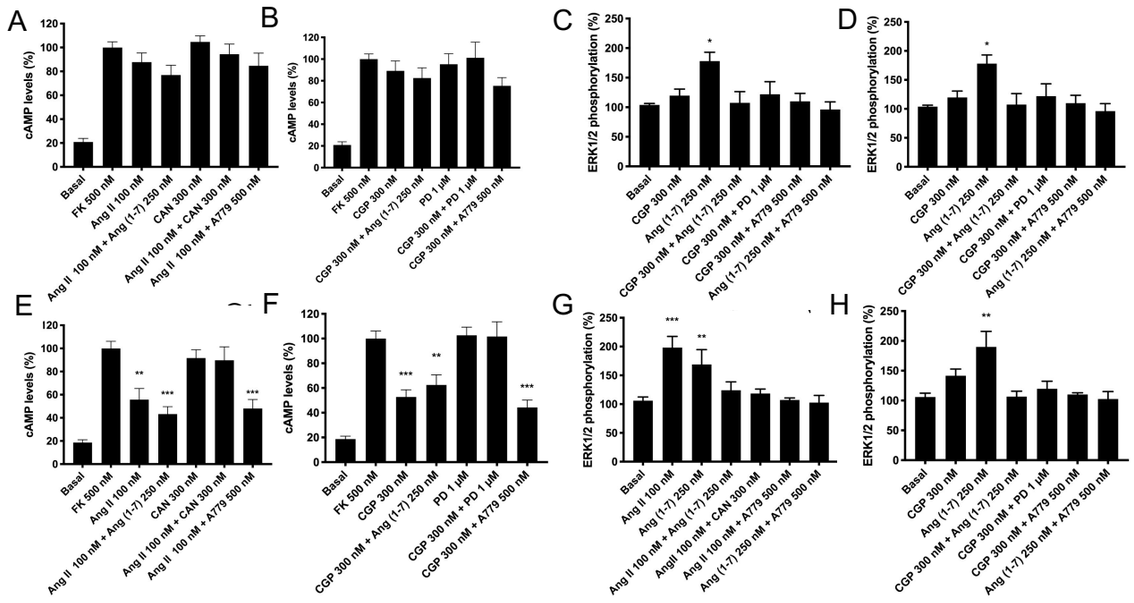
Expression of ***AT_1Mas*** **and** ***AT_2Mas*** **heteromers** in primary striatal neuron (A) and microglial (B,C) cultures were determined by **Proximity Ligation Assays (PLA)**. In order to get as close as possible to the pathological conditions of Parkinson's disease (PD), expression of these Hets in brain striatal slices of PD rat model were determined by PLA (D,E).



Functionality of AT_1Mas and AT_2Mas Hets in activated microglia

To characterize the *AT_1Mas* and *AT_2Mas* heteromers functionality in microglia signaling assays were performed:

- **cAMP determination** in the absence (A,B) or presence of LPS + IFN-γ (E,F) **and**
- **$ERK_{1/2}$ phosphorylation assays** in the absence (C,D) or presence of LPS + IFN-γ (G,H).



REAGENTS:

Agonists: Ang II (AT_1R), CGP (AT_2R), Ang (1-7) (MasR)

Antagonists: CAN (AT_1R), PD (AT_2R), A779 (MasR)

Conclusions

- Specific interaction between AT_1/Mas, AT_2/Mas receptors and trimeric interaction between AT_1/AT_2/Mas receptors has been demonstrated.
- Expression of AT_1/Mas and AT_2/Mas hets is higher in microglia than in striatum neurons.
- Both AT_1/Mas and AT_2/Mas hets have higher expression in rats PD model compared to control rats. Increased expression of AT_1/Mas het even further in rats PD model treated with L-DOPA and exhibiting dyskinesia.
- Negative crosstalk between Angiotensin (AT_1 and AT_2) and Mas receptors has been detected.