as crucial players in neuroinflammation. We have shown before a crosstalk between histamine H1 receptor (H1R) and glucocorticoid receptor (GR) signaling that could have specific impact on (neuro) inflammatory conditions. The objective of the present work was to evaluate this signaling interaction in a neuroinflammatory context. In a first stage, we used the BV2 murine glial cell line. Using this system, we aimed to set an in vitro neuroinflammatory model by treating the cells with 1μg/μl of lipopolysaccharide (LPS) and evaluating the induction of the inducible nitric oxide synthase (iNOS) gene expression. Pretreatment with 1nM of the synthetic glucocorticoid dexamethasone (DEX) reduced LPS-induced iNOS response to 50%, while co-incubation with the antihistamines chlorpheniramine and diphenhydramine enhanced DEX-induced iNOS reduction to 65 and 90%. To extend these results to a human glial cell model, we used hiPSC-derived astrocytes. In this system we modeled neuroinflammation using synthetic beta-amyloid oligomers (Aβ). Treatment with 1μM of Aβ resulted in an induction of TNF-α gene expression. Pretreatment with 10nM DEX showed a significant reduction of TNF-α response to Aβ. Co-incubation with 10μM of the antihistamines mepyramine and triprolidine resulted in a 2 and 3-fold enhancement of DEX effect respectively. This enhancement was not induced by a different antihistamine, chlorpheniramine. We conclude there is a ligand specific interaction between H1R signaling and GR transcriptional activity that can have pharmacological impact on neuroinflammatory contexts where glial cells play a central role. This work was partially supported by a Boehringer Ingelheim Fonds travel grant awarded to CDZ.

281. (507) **MODULATION OF GLUCOCORTICOID RECEPTOR ACTIVITY BY HISTAMINE H2 RECEPTOR SIGNALING. INVOLVEMENT OF PI3K AND MTOR.**

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There are reports describing the interaction between membrane G-protein coupled receptors (GPCRs) signaling and glucocorticoid receptor (GR) transcriptional activity. The aim of the present work was to study the modulation of GR activity by histamine H2 receptor (H2r) signaling. HEK293T cells were co-transfected with plasmids coding for H2r, GR and a GR-driven reporter gene TAT3-Luc. In this system, 10 minutes pretreatment with 10μM H2r agonist (amthamine) significantly increased GR activity, duplicating dexamethasone-induced signal ($p<0.05$). To study the mechanism of such interaction, we co-incubated the cells with different signaling inhibitors. When cells were exposed to G-protein βγ inhibitor gallein, PI3K inhibitor wortmannin or mTOR inhibitor rapamycin, dexamethasone response was increased to levels achieved in presence of amthamine. Interestingly, co-treatment of amthamine with dexamethasone and any of the inhibitors mentioned above did not enhanced dexamethasone-induced GR activity. Consistently, as previously described in HEK293T cells, H2r agonist reduced the levels of phosphorylation of the PI3K substrate AKT, phospho-mTOR and its target phospho-S6K. The whole of these results shows that PI3K/AKT/mTOR pathway has an inhibitory effect on dexamethasone-induced GR activity, and that H2r agonist amthamine potentiates GR transcriptional activity inhibiting this signaling path. Considering the co-expression of H2r and GR in several physiological systems and the widespread use of their ligands, the interaction described herein could have an impact in glucocorticoid based therapy and grants further research.

282. (541) **BIASED AGONISM AT HISTAMINE H1 RECEPTORS**

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GPCRs (G-protein coupled receptors) exist as conformational collections in which different conformations lead to differential downstream behaviours such as G-protein activation, receptor phosphorylation or internalization. In this context, a ligand may cause differential activation of some, but not all, of the signaling events associated to a particular receptor and would lead to biased agonism. On the other hand, antihistamines used clinically as antiallergics rank among the most widely prescribed and over-the-counter drugs in the world. The aim of the present study was to investigate whether widely used histamine H1 receptor (H1R) ligands that exert therapeutic actions by blocking the effects of histamine, due to null or negative efficacy towards Gαq-phospholipase C (PLC)-inositol triphosphates (IP3) and Nuclear Factor-kB cascades, could display positive efficacy concerning receptor desensitization or internalization. We used A549 cells, derived from human lung epithelium, endogenously expressing the H1R. Pretreatment of A549 cells during 10 minutes with 1, 3, 10 and 33 μM of chlorpheniramine and triprolidine prevented the increase of cytosolic Ca2+ levels evoked by 100 μM of histamine suggesting that both ligands may promote H1R desensitization. On the contrary, pretreatment with diphenhydramine did not modify the H1R response to the agonist. To examine the mechanisms involved in these desensitizations we transfected A549 cells with GRK2 and dynamin dominant-negative mutants. Our results indicate that although these mutants potentiate calcium response to histamine and partially impaired histamine induced H1R desensitization they did not revert chlorpheniramine nor triprolidine induced desensitization. Finally, preliminary results of saturation-binding assays suggest that some of these ligands may also lead to receptor internalization. In conclusion, H1R desensitization and/or internalization promoted by these ligands demonstrate their biased nature and could explain their undesired effects. Accordingly, this study contributes to a correct classification, providing evidence for a more rational and safe use of antihistamines.

283. (546) **PRECLINICAL PHARMACOLOGY STUDY OF RATIONALLY DESIGNED GRK2 INHIBITORS**

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GPCR kinase 2 (GRK2) plays a major role in GPCRs desensitization and has been extensively validated as an effective target for heart failure (HF) treatment. Its overexpression is associated to disease progression due to the lack of cardiac BAR responsiveness. Accordingly, we have previously obtained four compounds (C2, C3, C4, C5) that exert significant in vitro GRK2 inhibitory activity, postulating them as suitable candidates for in vivo testing. However, there are several risks inherent in preclinical drug discovery that might lead to drugs attrition in late stages. Therefore, the objective of this work was to identify potential early failures of our hits before reaching in vivo phases.

To achieve this, we evaluated their ADMET (Absorption-Distribution-Metabolism-Excretion-Toxicity) properties. As a lipophilicity descriptor, experimental logP was obtained by RP-HPLC. Hits cytotoxicity was assessed in U937 and HepG2 cells by trypan blue exclusion test after 48hs treatment. Even though all compounds exhibited an appropriate lipophilicity, with logP values ranging from 1 to 3, compounds C3 and C5 stood out as they did not affect cellular viability, while C2 presented an EC50=10,5μM and C4 an EC50=17,2μM. Moreover, GRK2 desensitizes GPCRs that couple to different G-proteins. Since compounds that specifically potentiate cAMP could be of interest for HF treatment, we compared their ability to increase responsiveness of GPCRs that couple to different G-proteins. Initial cell-based screening assays proved that the hits increased cAMP response of H2R (histamine type 2 receptor). Nonetheless we observed that compound C5 also increased histamine-stimulated intracellular calcium release in A549 cell line endogenously expressing H1R (histamine type 1 receptor), revealing an undesirable promiscuous behavior.

In conclusion, we applied strategies to mitigate the risks of drug attrition in late phases of clinical trials, increasing the confidence in our candidate compound C3 for proceeding to HF animal models research.