2737-Pos Board B344
Binding Modes of Full and Partial Agonists in the Orthosteric Binding Site of the Glycine Receptor
Marc A. Dämgen[1], Timo Greiner[2], Remigijus Lape[2], Lucia G. Sivilotti[2], Philip C. Biggin[1].
[1]Structural Bioinformatics and Computational Biochemistry, Department of Biochemistry, University of Oxford, Oxford, United Kingdom, [2]Department of Neuroscience, Physiology and Pharmacology, University College London, London, United Kingdom.
The glycine receptor is a well-characterized member of the Cys-loop superfamily of receptor proteins. Upon the binding of agonist the transmembrane pore opens to allow the passage of chloride ions into the cell. Previous research has identified agonists that bind with comparable affinity to the receptor's orthosteric binding site, but differ in their ability to open the ion channel pore. This phenomenon of partial agonism is still poorly understood at atomistic resolution. Knowledge of how different agonists modulate the receptor's response is not only of fundamental importance, but also has direct implications for drug design. Previously, on the basis of homology models, we suggested that water-mediated interactions may play a significant role in determining the binding modes of agonists. We have extended this approach using the recent cryo-EM and crystal structures. Our molecular dynamics simulations support our original hypotheses with respect to the role of water in the binding pocket. In addition, via the use of Markov State Modelling, we are able to provide a detailed picture of the complex nature of ligand-protein interactions for the glycine receptor that may have implications for the interpretation of efficacy of different agonists.

2738-Pos Board B345
Structure-Based Discovery of Novel Glycinergic Modulators
Marta M. Wells, Nathan Reinert, Pei Tang, Yan Xu.
University of Pittsburgh, Pittsburgh, PA, USA.
Marijuana's analgesic effects can be attributed to the allosteric modulation of glycine receptors (GlyRs) by Δ9-tetrahydrocannabinol (THC). GlyRs are pentameric ligand-gated chloride channels that control inhibitory neurotransmission in the brainstem and spinal cord. The glycinergic mechanism of cannabis-induced analgesia is independent of the other psychoactive effects of THC. Compounds specifically targeting the well-defined THC-binding site in GlyRs are likely to provide pain relief with fewer unwanted side effects. Here, we screened over 2 million drug-like molecules from the ZINC database on an ensemble of α3GlyR structures obtained from molecular dynamics simulations of the closed-state α3GlyR crystal structure (PDB ID: 5CFB) and a homology model derived from the open-state α1GlyR NMR structure (PDB ID: 2M6I). Computational dockings were specifically targeted to the known THC-binding site in the α3GlyR transmembrane domain. Each screened compound was ranked based on its total predicted binding affinity across the ensemble of α3GlyR structures. Top ranked compounds were selected for functional measurements in *Xenopus laevis* oocytes expressing human α3GlyR. Several lead candidates have been identified as strong modulators of α3GlyR, exhibiting positive and/or negative allosteric effects at micromolar concentrations. Further molecular dynamics simulations of α3GlyR in the presence of modulators revealed that in the closed-state α3GlyR, potentiating compounds showed an increased probability of contacting with residue S296 compared to inhibitors occupying the same THC-binding site. S296 on the third transmembrane helix has been previously shown to be critical for THC potentiation of GlyRs. In contrast, inhibitors showed a higher probability of close contact with residue S241 in the first transmembrane helix. Interestingly, the adjacent residue I240 has been previously reported as an inhibitory site. This study suggests that different interaction residue partners, even within the same binding site, may lead to distinctly different allosteric modulations in α3GlyR. Research supported by NIH grants.

Cardiac Muscle Mechanics and Structure II

2739-Pos Board B346
Atrial Remodeling in Hypertrophic Cardiomyopathy
Cecilia Ferrantini[1], Josè Manuel Pioner[2], Francesca Gentile[1], Raffaele Coppini[1], Cristina Morelli[1], Nicoletta Piroddi[1], Beatrice Scellini[1], Elisabetta Cerbai[1], Jil Tardiff[2], Chiara Tesi[1], Iacopo Olivotto[1], Corrado Poggesi[1].
[1]Physiology, University of Florence, Florence, Italy, [2]Physiology, University of Arizona, Tucson, AZ, USA.
Changes in myofilament function related to HCM-associated mutations contribute to the diastolic dysfunction observed in the in vivo patient heart and in intact ventricular preparations from patient samples. HCM mutations that are ubiquitously expressed in the heart (e.g. cMyBP-C or cTnT) could also affect atrial function. Here we investigate whether HCM-associated atrial myopathy is a consequence of mutation-driven sarcomere dysfunction or results from atrial remodeling due to the diastolic dysfunction and increased LV filling pressures. In one HCM patient carrying the Lys814del cMyB-C mutation, changes in sarcomere function (increased myofilament Ca^{2+} sensitivity and increased cross bridges detachment rate under isometric conditions) were similar in atrial and ventricular myofibrils compared to donor preparations. However, isometric twitch mechanics and kinetics of intact trabeculae from the right atrium of 4 cMyB-C-mutant patients were unaffected as compared to trabeculae from non-HCM patients (N=8), or mutation negative HCM patients (N=3), or HCM patients carrying mutations in beta-myosin (N=2). We extended the study to HCM mouse models carrying mutations in cTnT. In the E163R mouse, atrial and ventricular sarcomere kinetics and energetics were similarly altered compared to WT mice. Isometric ATPase, both at rest and at maximal Ca^{2+}-activation and the energy cost of tension generation were increased in both atrial and ventricular preparations of E163R vs WT. However, isometric twitch kinetics were prolonged in intact ventricular trabeculae of E163R mice vs WT while they were unaffected in atrial trabeculae. In the R92Q mouse model, that is associated with a much more severe degree of LV diastolic dysfunction and left atrial dilatation compared to the E163R, left atrial trabeculae showed prolonged twitch contractions, increased spontaneous activity and a number of E-C coupling alterations that resemble those observed in ventricular preparations. HCM-mutations in cMyBP-C and cTnT induce similar alterations in both atrial and ventricular sarcomeres. However, likely due to the different working conditions of the two chambers, sarcomere dysfunction can significantly alter the mechanics and kinetics of the intact myocardium only in the ventricles. Atrial muscle dysfunction in HCM is induced by remodeling processes that depend on the increased filling pressures.

2740-Pos Board B347
Myosin Activator Omecamtiv Mecarbil Differentially Impacts the Contractile Properties of Skinned Myocardium from Failing and Donor Human Hearts
Ranganath Mamidi[1], Kenneth S. Gresham[2], Jiayang Li[1], Julian E. Stelzer[1].
[1]Case Western Reserve University, Cleveland, OH, USA, [2]Temple University, Philadelphia, PA, USA.
Omecamtiv mecarbil (OM) is a compound that selectively targets the cardiac sarcomere and enhances myosin crossbridge (XB) activation and force generation. Because of these properties OM has been proposed as a treatment for systolic heart failure in humans, however, there are no studies that have directly examined the functional effects of OM, at XB level, in remodeled human failing myocardium. Thus, we tested whether OM differentially impacts contractile properties in failing and donor human myocardium. Isometric force, myofilament Ca^{2+}-sensitivity (pCa_{50}), rates of XB detachment (k_{rel}), recruitment (k_{df}), and magnitude of XB recruitment (P_{df}) were measured both before and after OM incubation in chemically-skinned myocardial preparations isolated from failing and donor hearts. Because the failing myocardium exhibited an enhanced pCa_{50}, dynamic XB parameters were measured at pCa's 6.6 and 6.3, respectively for failing and donor groups in order to achieve equivalent levels of activation (~22% and ~35% of maximal force, respectively for pre- and post-OM incubations). OM incubation did not alter the pCa_{50} but significantly enhanced the sub-maximal force production at forces lower than 50%, an affect that was less pronounced as Ca^{2+} levels increased. Furthermore, OM differentially impacted the contractile properties of failing and donor myocardium. Specifically, OM-induced force increases were more pronounced in the failing myocardium (~79% increase vs. ~28% in donor myocardium). Additionally, the OM-induced slowing of k_{rel} and k_{df}, and increase in P_{df} were also more pronounced in the failing myocardium. Our results suggest that OM is effective in increasing force generation in myocardium isolated from failing hearts by increasing XB on-time and enhancing cooperative XB recruitment.

2741-Pos Board B348
Biochemical and Mechanical Properties of the S532P and R712L Cardiomyopathy Myosin Mutants
Ailian Xiong[1], Yingying Liu[1], Bipasha Barua[2], Betty Belknap[1], Howard White[1], Donald Winklemann[2], Eva Forgacs[1].
[1]Physiological Sciences, Eastern Virginia Medical School, Norfolk, VA, USA, [2]Pathology, Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ, USA.
Human cardiomyopathiesincludes hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM), which affect 1 in every 500 and 1 in every 250 people respectivelyin the U.S. The highest percentage of disease-causing