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ORAL ABSTRACTS
CARDIOVASCULAR PHARMACOLOGY

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5-HT_2 RECEPTOR BLOCKADE ENHANCES SYMPATHO-INHIBITORY SEROTONERGIC ACTIONS AT CARDIOVASCULAR LEVEL IN RAT

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Introduction/Objectives: 5-HT evokes a variety of responses influencing vascular tone. 5-HT_2 receptors are associated with platelet aggregation, vasoconstriction, adrenaline release and tachycardia[1,2,3]. The aim of this study was to evaluate whether 5-HT_2 receptor blockade changes serotonergic modulation of sympathetic neurotransmission in pithed rats.
Material/Methods: Wistar rats were orally treated with sarpogrelate (a 5-HT_2 receptor antagonist) during 14 days (30 mg/kg/day). After CNS destruction[4], electrical stimulation of sympathetic outflow (monophasic pulses, 1 ms duration, 15 ± 3 V at increasing frequencies 0.1, 0.5, 1 and 5 Hz) or administration of exogenous noradrenaline (0.01, 0.05, 0.1 and 0.5 μg/kg) were performed. Western blotting for 5-$HT_{1A/1B/1D/7}$ receptors was also carried out.
Results: Electrical stimulation or administration of noradrenaline resulted in frequency- or dose-dependent increases in mean blood pressure. Continuous infusion of 5-carboxamidotryptamine (5-CT, 5-$HT_{1/7}$ receptor agonist; 0.005, 0.1 and 5 μg/kg/min) exerted a dose-dependent inhibition of sympathetic outflow, significantly greater in sarpogrelate-treated rats than in non-treated rats[4]. This effect was mimicked by L-694,247 and AS-19, 5-HT_{1D} and 5-HT_7 receptor agonists respectively (0.1, 5 and 10 μg/kg/min each). However, 1-Phenylbiguanide, CGS-12066B and 8-OH-DPAT, (5-HT_3, 5-HT_{1B} and 5-HT_{1A} receptor agonists, respectively; 5 μg/kg/min each) failed to reproduce 5-CT inhibitory action. A mixture of LY310762 and SB258719 (5-HT_{1D} and 5-HT_7 receptor antagonists, respectively; 1 mg/kg each) completely abolished 5-CT inhibitory effect. None of the agonists modified the pressor responses induced by exogenous noradrenaline. Western blot showed increased expression of 5-HT_{1D} receptors in sarpogrelate-treated animals.
Conclusions: In conclusion, 5-HT_2 receptor blockade potentiates sympatho-inhibitory 5-carboxamidotryptamine actions at cardiovascular level, mainly mediated by prejunctional 5-HT_{1D} and 5-HT_7 receptors.
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RICE BRAN ENZYMATIC EXTRACT REDUCES ATHEROSCLEROTIC LESIONS IN APO E (−/−) MICE

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Background and aims: rice bran is a byproduct of rice milling rich in antioxidants, sterols and γ-oryzanol that is underused mainly due to its low water solubility. We aimed to evaluate the effect of a novel water soluble rice bran enzymatic extract (RBEE) supplemented diet on atherosclerosis.
Methods: $ApoE^{-/-}$ mice were fed standard (ST) or high fat (HF) diet supplemented or not with 1% or 5% RBEE for 23 weeks. Serum lipids, oxLDL and nitrites were measured using spectrophotometry, ELISA and Griess reaction, respectively. Atherosclerotic lesions were assessed in the aortic sinus and brachiocephalic artery by Mac-3 inmunostaining and Oil Red O staining. ICAM-1 and VCAM-1 expression were measured by WB in aorta homogenates.
Results: 1% and 5% RBEE supplementation reduced the elevation of nitrites observed in HF fed animals ($P < 0.05$ and $P < 0.01$, respectively) and augmented HDL cholesterol ($P < 0.05$) while total cholesterol and triglycerides decreased only in HF 5% ($P < 0.05$). $ApoE^{-/-}$ showed increased serum and aorta oxLDL that was reduced by 1% and 5% RBEE supplementation in serum in ST diet ($P < 0.05$) and in aorta in HF fed mice ($P < 0.001$). Macrophage infiltration and lipid deposition in the aortic sinus was reduced by 1% and 5% RBEE supplementation in HF fed mice. However, in the brachiocephalic artery, only ST 5% showed reduced lipid deposition ($P < 0.01$) while macrophage infiltration was also reduced in HF 1% and HF 5% ($P < 0.01$). VCAM-1 and ICAM-1 expression was reduced in HF 5% ($P < 0.01$ and $P < 0.05$, respectively).
Conclusions: RBEE supplemented diet improved lipid profile and prevented atherosclerotic lesions development showing its interest as functional food in atherosclerosis disease.

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PHARMACOPHORE OF DRUGS THAT INCREASE $I_{KIR2.1}$

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Introduction: Drug-induced increase of the inward rectifier current (I_{K1}) generated by Kir2.1 homotetramers ($I_{Kir2.1}$) is a mechanism of drug-induced proarrhythmic effects that we recently identified. Here we analyzed whether propafenone, similarly to flecainide, also increases $I_{Kir2.1}$. Furthermore, by testing the effects of timolol, atenolol, and dronedarone we identified the chemical determinants of drug affinity to the receptor within Kir2.1 channels.
Material and Methods: Currents were recorded with the patch-clamp technique using whole-cell, inside-out, and cell-attached configurations in transfected Chinese hamster ovary CHO cells and cardiac myocytes.
Results: Propafenone (0.1 nM–1 μM) did not modify either I_{K1} recorded in human right atrial myocytes or the current generated by homo- or heterotetramers of Kir2.2 and Kir2.3 channels recorded in CHO cells. Conversely, propafenone increased $I_{Kir2.1}$ (EC_{50} = 12.0 ± 3.0 nM) as a consequence of its interaction with Cys311, an effect which decreased inward rectification of the current. Propafenone significantly increased mean open time and opening frequency at all the voltages tested which resulted in a significant increase of the mean open probability of the channel. Timolol, which interacted with Cys311, was also able to increase $I_{Kir2.1}$. On the contrary, neither atenolol nor dronedarone modified $I_{Kir2.1}$. Molecular modeling of the Kir2.1-drugs interaction allowed to the proposal of the pharmacophore of drugs that increase $I_{Kir2.1}$.
Conclusions: The results demonstrated that those drugs that are able to increase $I_{Kir2.1}$ exhibit an L-like structure with electronegative and

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PITX2 DECREASES L-TYPE CA^{2+} CURRENT AND INCREASES THE SLOW DELAYED RECTIFIER K^+ CURRENT IN CARDIAC CELLS

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Introduction: An increase in Pitx2 expression has been associated with an increased risk of atrial fibrillation (AF). On the other hand, AF produces profound changes in the expression of voltage-gated Ca^{2+} and K^+ channels. Here we analyzed the effects of Pitx2 on L-type Ca^{2+} and K^+ channels.
Methods: Currents were recorded in mouse atrial cultured HL-1 cells transfected or not with the cardiac Pitx2 isoform (Pitx2c) by using whole-cell patch-clamp. L-type Ca^{2+} current ($I_{Ca,L}$) was recorded by using Ba^{2+} as charge carrier (I_{Ba}).
Results: Pitx2c significantly reduced peak I_{Ba} density by 40.2% without modifying time- and voltage-dependent properties of the current. Regarding voltage-gated K^+ channels, under control conditions 2 groups of cells were identified based on the predominant voltage-gated K^+ current exhibited. In most of the cells (≈80%), a rapid delayed rectifier current (I_{Kr}) sensitive to dofetilide could be recorded. In the rest of the cells (≈20%), I_{Kr} was absent and the predominant current was an outward current sensitive to 4-aminopyridine (2 mM), similar to the ultrarapid delayed rectifier K^+ current (I_{Kur}) recorded in human atrial myocytes. In the presence of Pitx2c, only 10% of the cells exhibited I_{Kur} and the rest exhibited a voltage-gated, dofetilide-resistant, K^+ current with very slow activation kinetics that was completely abolished by the selective I_{Ks} blocker HMR-1556 (1 μM).
Conclusions: Pitx2c decreased $I_{Ca,L}$ and increased I_{Ks} suggesting that this transcription factor could contribute to the reduction of $I_{Ca,L}$ and the increase of I_{Ks} that characterize the AF-induced electrical remodeling.

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NAV1.5 N-TERMINUS EXHIBITS A PDZ BINDING DOMAIN AND INCREASES THE KIR2.1 CHANNEL DENSITY

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Introduction: The N-terminal domain of Nav1.5 channels (Nter), a 132 aminoacids peptide increases the Nav1.5 current ($I_{Nav1.5}$) density. Additionally, there is a reciprocal modulation of the expression of Nav1.5 and Kir2.1 and Kir2.2 channels. Thus, we tested whether Nter is able to increase the expression of Kir2.x channels.
Material and Methods: Currents were recorded using the patch-clamp technique in Chinese hamster ovary cells transiently transfected with wild type or site directed mutated Nter, Kir2.1, Kir2.2, and Nav1.5 channels.
Results: Cotransfection of Nter with Kir2.1 and Kir2.2 channels significantly increased Kir2.1 current ($I_{Kir2.1}$) and $I_{Kir2.2}$. Conversely, cotransfection of Nter with Kir2.3 channels, did not significantly modify $I_{Kir2.3}$. Nter did not increase the current density generated by Nav1.5 channels lacking their C-terminal PDZ domain (Nav1.5ΔPDZ). Noteworthy, Nav1.5ΔPDZ channels still co-immunoprecipitated with syntrophin. We identified in the Nter peptide a sequence which could act as a "PDZ-like" binding domain (18-RESLA). Site directed mutagenesis demonstrated that mutants of all residues, except p.S20A Nter, increased $I_{Kir2.x}$. Finally, adult rat ventricular myocytes were enzimatically dissociated, cultured, and infected with either a control adenoviral construction (Ad-GFP) or an Nter codifying adenoviral construction (Ad-Nter). Results demonstrated that myocyte infection with Ad-Nter significantly increased both the inward sodium (I_{Na}) and inward rectifier (I_{K1}) currents.
Conclusions: The N-terminal domain of Nav1.5 channels exerts "chaperon-like" effect increasing $I_{Nav1.5}$ and $I_{Kir2.x}$ densities, this effect depends on the residue Ser20 which probably determines the binding to syntrophin via an "internal" PDZ binding domain.

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GRAPHENE DERIVATIVES AS SCAFFOLD FOR EX VIVO SURVIVAL AND MATURATION OF DOPAMINERGIC SN4741 CELLS

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Carbon nanomaterial Graphene (G) can form a three-dimensional porous structure with efficient bioconjugation and cell differentiation properties, providing a promising scaffold for neural regeneration.
Aims: To study this putative new application of G, we cultured a clonal substantia nigra dopaminergic neuronal progenitor cell line (SN4741) in presence of G as scaffold.
Methods: Cells were cultured in DMEM/10% FCS to about 80% confluence and incubated with different concentrations (0.001–1 mg/ml) of three chemically different G derivatives (G oxide (GO); partially reduced GO (PRGO) and fully reduced GO (FRGO)) and two different presentation matrixes as powder and films. Cell viability was measured by the MTT assay. To study cellular characterization, morphology and assessment of cell engraftment into G films, we analyzed the immunostaining of the neuronal marker NeuN, the anti-rat Beta-3-tubulin antibody, and the anti-rabbit DCX as immature neuronal marker. Reactive oxidative species (ROS) and the mitochondrial membrane potential after JC-1 incubation were measured by flow cytometry. Lactate dehydrogenase was measured in the culture supernatant.
Results: We found similar increase of survival and metabolism (30-40%) at low concentrations of PRGO and FRGO (0.05-0.01 mg/ml) compared with the higher concentration (1 mg/ml), no changes were seen in the GO group. PRGO or FRGO films showed an increased in the effective anchorage capacity to nest into the G matrix and in the maturation of the dopaminergic SN 4741 cells.
Conclusions: G scaffolds could offer a powerful platform for neural stem cells, direct cell conversion techniques and neural tissue engineering.

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THE EFFECTS OF TREATMENT ON DISEASE SYMPTOMS AND PROGRESSION OF STRUCTURAL CHANGES IN KNEE OSTEOARTHRITIS PARTICIPANTS FROM THE OSTEOARTHRITIS INITIATIVE PROGRESSION COHORT

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Introduction: To explore the effects of commonly used medications for treatment of knee osteoarthritis (OA) on structural progression.