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## **Abstracts**

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## P-381

### Combinatorial treatment with antihypertensive drugs prevents HELLP syndrome exacerbated by endothelial dysfunction in pregnant hypertensive mice

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HELLP (Hemolysis, Elevated Liver enzymes, and Low Platelets) syndrome is a severe variation of preeclampsia, a common pregnancy disorder including hypertension, proteinuria, and intrauterine growth retardation (IUGR) in late pregnancy, and life threatening to mother and neonate. Current hypotheses propose that preeclampsia and HELLP syndrome is due to the abnormal dynamic state of vasoactive factors. These have gained wide acceptance, but scientific evidence remains less well-established. As the model of preeclampsia, we previously generated a transgenic mouse model that developed pregnancy-associated hypertension (PAH) by mating females expressing human angiotensinogen with males expressing human renin. PAH mice exhibit severe hypertension by the overproduction of angiotensin II, a potent vasopressor, on late pregnancy, which is accompanied by the maternal and fetal disorders including proteinuria, intrauterine growth retardation, and neonatal death. Here, we found the diagnostic features of HELLP syndrome in PAH mice, and revealed the impaired vasodilation with endothelial dysfunction. Although these maternal and fetal abnormalities are partially ameliorated by hydralazine, an antihypertensive authorized drug for preeclampsia and HELLP syndrome, angiotensin II type 1 receptor blocker (ARB) substantially prevents the development of these defects including the neonatal death. We also showed that a simultaneous lower-dose drug treatment of ARB and calcium channel blocker dramatically ameliorated the maternal and neonatal abnormalities in PAH mice. Our findings highlight the combined effect of antihypertensive drugs as a therapeutic target in HELLP syndrome.

#### KEYWORDS:

HELLP syndrome, preeclampsia, renin-angiotensin system, genetically engineered mouse model, vascular endothelial dysfunction

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## P-382

### Rho-kinase activation contributes to hypertension in rat model

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Our aim was to characterize spontaneously hypertensive stroke prone (SHRSP), and normotensive wistar kyoto (WKY) rat strains, to find possible differences in their blood pressure and cardiac system.

In our experiments, we detected the diameter changes of cannulated muscle arterioles, and the contractile force of the femoral artery. We measured the body, the heartweight, and the blood pressure. The body weight of the SHRSP rats ( $257 \pm 2$ g) was smaller than the WKY rats ( $361 \pm 10$ g,  $p < 0.01$ ). The heart/body ratio (0.0047), the systolic ( $210 \pm 12$  Hgmm) and diastolic ( $161 \pm 8$  Hgmm) blood pressure of the SHRSP was significantly higher than in WKY rats (0.0027,  $147 \pm 9$  Hgmm,  $115 \pm 7$  Hgmm). The myogenic tone of the muscle arterioles was similar in the two groups. After pretreatment with Rho-kinase inhibitor fasudil (10  $\mu$ M) the myogenic tone decreased more in the SHRSP group ( $82.5 \pm 6.2\%$ ) than in the WKY group ( $34.4 \pm 9.8\%$ ). In the case of the femoral artery, treatment with 10-60mM KCL resulted in higher contractile force in SHRSP ( $8.96 \pm 1.44$  mN) than in WKY ( $6.39 \pm 1.09$  mN). Pretreatment with fasudil resulted in the normalisation of the higher contractile force in the SHRSP group (decreased to  $4.08 \pm 1.49$  mN), but had no effect on the contractile force of the WKY group ( $6.79 \pm 1.65$  mN). By using serotonin we detected similar reaction (contraction for SHRSP:  $14.33 \pm 1.44$  mN, which decreased to  $9.25 \pm 1.51$  mN in the presence of fasudil, contraction for WKY:  $8.17 \pm 1.81$  mN, which was  $8.95 \pm 1.84$  mN after using fasudil).

According to our results, the Rho-kinase system probably plays an important role in the pathogenesis of high blood pressure. Better understanding of the pathogenesis may lead to a more effective drug development.

#### KEYWORDS:

Hypertension, Rho-kinase, SHRSP

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## P-407

### Inhibition of Egr1 expression: A novel cell-type specific mechanism underlying cAMP anti-mitogenesis in vascular smooth muscle cells

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Drug eluting stents limit angioplasty restenosis but the drugs used are non-specific, inhibiting both endothelial and vascular smooth muscle cell (VSMC) proliferation, thereby contributing to the clinical complications associated with angioplasty. To identify new targets for VSMC-selective anti-proliferative agents we studied cAMP signalling, which inhibits VSMC but not EC proliferation. Anti-mitogenic properties of cAMP in VSMC are dependent on activation of protein kinase A (PKA) and exchange protein activated by cAMP (EPAC), but the mechanisms are unclear.

Using microarray analysis, we identified Egr1 as a PKA and EPAC target gene that was required for proliferation and the anti-mitogenic effects of cAMP in VSMC. Numerous cAMP elevating stimuli (Forskolin, adenosine, A2B receptor agonist BAY60-6583 and Cicaprost) significantly inhibited Egr1 expression in VSMC but not endothelial cells. Inhibition of Egr1 was independent of effects of cAMP on ERK1/2 or cAMP response element binding protein (CREB) activity. However, it was dependent on inhibition of serum response element (SRE) activity that occurred in response to inhibition of Rac1 activity resulting in cell-type specific loss F-actin stress fibres and elevation of monomeric actin.

Taken together, this data highlights the importance of cAMP-dependent remodelling of the actin-cytoskeleton and rapid inhibition of Egr1 expression as key mechanisms underlying the cell-type specific anti-mitogenic effects of cAMP in VSMC. This data suggests that targeting these cAMP-dependent mechanisms that control actin-cytoskeleton remodeling and Egr1 expression may aid development of novel cell-type specific anti-proliferative agents that will minimise the complications associated with the current generation of drug eluting stents.

#### KEYWORDS:

smooth muscle cell, proliferation, 3'-5'-cyclic adenosine monophosphate, Egr1, cytoskeleton

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## P-408

### Transient Receptor Potential Melastatin-4 (TRPM4) mediates vasoconstriction in rat model

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The transient receptor potential melastatin-4 (TRPM4) is a calcium-activated monovalent cation channel. TRPM4 has a major role in pressure-induced depolarization and vasoconstriction in intracranial arteries. Our aim was to study the regulatory function of TRPM4 in basilar (isometric measurements, precontraction by about 5mN) and mesenteric (isobaric conditions, at 80mmHg) arteries. Arteries were isolated from Wistar-Kyoto (WKY) rats and experiments were performed in oxygenated Krebs buffer.

Initial diameter of the mesenteric arteries was  $325 \pm 18\mu\text{m}$  ( $n=9$ ). Extracellular  $\text{K}^+$  (120mM) evoked a vasoconstriction (decrease in diameter from  $316 \pm 30\mu\text{m}$  to  $155 \pm 40\mu\text{m}$ ,  $n=4$ ,  $p=0.03$ ), similarly to norepinephrine (10 $\mu\text{M}$ , decrease from  $387 \pm 17\mu\text{m}$  to  $127 \pm 18\mu\text{m}$ ,  $n=5$ ,  $p<0.01$ ). Arteries were pre-constricted with norepinephrine and treated with the TRPM4 channel inhibitor 9-phenanthrol (1  $\mu\text{M}$ -300mM), which evoked a dilation (increase of diameter from  $206 \pm 22\mu\text{m}$  to  $390 \pm 10\mu\text{m}$ ,  $n=5$ ,  $p<0.01$ ). Extracellular  $\text{K}^+$  mediated constriction was also inhibited by 9-phenanthrol. Extracellular  $\text{K}^+$  (60mM) evoked an increase in contractile force in the basilar arteries of the rat (contractile force from  $2.6 \pm 0.9\text{mN}$  to  $9.3 \pm 1.3\text{mN}$ ,  $n=4$ ,  $p<0.01$ ). This extracellular  $\text{K}^+$  mediated constriction was also inhibited by 9-phenanthrol (contractile force from  $8.3 \pm 1.4\text{mN}$  to  $2.9 \pm 0.9\text{mN}$ ,  $n=4$ ,  $p=0.02$ ). The presumed TRPM4 activator, A23187 caused vasoconstriction accompanied by the rise of intracellular  $\text{Ca}^{2+}$  concentration. The evoked vasoconstriction was antagonized by the inhibitor.

Our data suggest that TRPM4 has a major role in the modulation of contraction followed by various agonist stimuli in different arteriolar beds of the rat. TRPM4 may participate in pathological conditions characterized by increased arteriolar tone, such as hypertension.

#### KEYWORDS:

TRP, TRPM4, hypertension, arteriole, vascular smooth muscle