BELGIAN SOCIETY OF
PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting
Friday October 18th 2013

PROGRAMME
&
ABSTRACT BOOK

Venue
Palace of the Academies
Hertogsstraat / Rue Ducale 1
1000 Brussels

Organisation
Prof. Dr. H. Bult
Division of Pharmacology
Faculty of Medicine and Health Sciences
University of Antwerp
B-2610 Wilrijk Antwerpen

with support of the
Royal Flemish Academy of Belgium for Science and the Arts
Main Lecture

10.00-11.00 “Producing NO out of Thin Air – Reflection of a cardiovascular pharmacologist about the biochemical foundation of our physiological tolerance to hypoxia”.

Prof. Dr. Martin FEELISCH (Department of Experimental Medicine and Integrative Biology – University of Southampton, United Kingdom).

Oral Communications

11.00-11.15 W.J. KWANTEN, F. COUTURIER, J. GOVAERTS, B.Y. DE WINTER, J.G. DE MAN, P.A. PELCKMANS, P.P. MICHELESEN, S.M. FRANCOUE (UAntwerpen). The role of nitric oxide and cyclooxygenase in vascular responsiveness in non-cirrhotic NAFLD-induced portal hypertension (PHT) compared to other models of PHT.


12.00-12.15  A. LELOUP (presented by D. SCHRYVERS UAntwerpen)
Development and validation of a new non-invasive technique to assess
arterial stiffness in mice.

12.15-13.45  Lunch – Guided Poster Session – General Assembly

Posters (height 120 cm – width 100 cm)

1.  J.T.H. VAN LANGEN, C.E. VAN HOVE, P. FRANSEN, H. BULT (UAntwerpen)
Differences in nitric oxide synthase activity and uncoupling in atherosclerosis-
prone and resistant mouse aortic regions.

2.  K. DECALUWÉ, B. PAUWELS, C. BOYDENS, J. VAN DE VOORDE (UGent)
Mechanisms involved in the vasorelaxing influence of CO-releasing molecule-2
(CORM-2) on mice aorta.

3.  E. BOCKSTEINS, D.J. SNYDERS (UAntwerpen)
Modulation of heterotetrameric Kv2.1/Kv6.4 channels by auxiliary β-subunits.

4.  J. GILISSEN, S. DILLY, N. DUPUIS, B. PIROTTE, J. HANSON (ULiège)
Identification of ligands for SUCNR1(GPR91) through a SOSA approach.

5.  N. DUPUIS, J. GILISSEN, J.C. TWIZERE, B. PIROTTE, J. HANSON (ULiège)
Development of an original β-arrestin complementation assay for orphan
GPCR GPR27(SREB1) ligand and signaling pathways determination.

6.  O. SCHAKMAN, F. SEGHERS, O. DEVUYST, P. GAILLY (UCLouvain)
Blood osmolarity control in TRPC1 mice.

J. VRIENS (KULeuven)
Functional characterization of transient receptor potential channels in human
endometrium.

TRUNG, C.A. CORNIL, V. SEUTIN (ULiège)
Mechanism of the medium-duration afterhyperpolarization in rat serotonergic
neurons.
Oral Communications

Impaired gastrointestinal motility in different animal models of sepsis.

14.00-14.15  B.I. TOTH, S. PINTO, T. VOETS (KULeuven)
Rapamycin and its analogs activate the cold sensitive ion channel
Transient Receptor Potential Melastatin 8 (TRPM8) – Role of a “menthol-
like” moiety.

14.15-14.30  K. HELD, S.J. PINTO, S. KERSELAERS, K. DE CLERCQ, P. CHALTIN,
T. VOETS, J. VRIENS (KULeuven)
TRPM3 – A promising target for analgesic treatment.

14.30-14.45  N. AOURZ, J. PORTELLI, J. COPPENS, Y. MICHOTTE, I. SMOLDERS
(VUBrussel)
Sst₂ and sst₃ – but not GHS-R1a- receptors are involved in the
anticonvulsant effects of cortistatin-14.

14.45-15.00  N. TAJEDDINE, B. BOUTIN, P. GAILLY (UCLouvain)
Phosphorylation of IP3R1 decreases ER calcium content and renders
prostate cancer cells resistant to androgen deprivation.

15.00-15.15  J. STAS, E. BOCKSTEINS, A.J. LABRO, D.J. SNYDERS (UAntwerpen)
Diversity of the pharmacological profile of heterotetrameric channels
consisting of Kv2.1 and silent voltage-gated potassium subunits.

15.15-15.30  M. ROMBOUTS, I. VAN BRUSSEL, S. GORREBEECK, N.E. RUYSSERS,
M. HEYLEN, B.Y. DE WINTER, N. COOLS, D.M. SCHRIJVERS (UAntwerpen)
Effects of immunosuppression on allogeneic T cell activation by mature
conventional dendritic cells.

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ABSTRACTS

Legend
O = Oral communication numbered
P = Poster numbered

O-01 (11.00-11.15)

THE ROLE OF NITRIC OXIDE AND CYCLOOXYGENASE IN VASCULAR RESPONSIVENESS IN NON-CIRRHOTIC NAFLD-INDUCED PORTAL HYPERTENSION (PHT) COMPARED TO OTHERS MODELS OF PHT

W.J. Kwanten¹, F. Couturier¹, J. Govaerts¹, B.Y. De Winter¹, J.G. De Man¹, P.A. Pelckmans¹,², P.P. Michielsen¹,², S.M. Francque¹,²

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Background: Steatosis without fibrosis has previously shown to induce a significant increase in portal pressure associated with features of hyperdynamic circulation and vascular hyporesponsiveness to vasoconstrictors. The underlying mechanisms of these alterations are unknown. In classical models of PHT, NO is believed to be a key player in its pathophysiology, as well as prostaglandins. Aim: To study the role of NO- and COX-mediated mechanisms of vascular responsiveness to vasoconstrictors in steatosis and other models of portal hypertension (PHT).

Methods: Male Wister Rats were divided in 4 groups: Sham-operated (n=10), rats (n=14) fed a methionin-choline-deficient diet (MCDD) for 4 weeks (steatosis group), CBDL (Common bile duct ligation, n=12) and PPVL (partial portal vein ligation, n=10). Vasoconstrictor response to phenylephrine (PE) was studied on abdominal aorta rings, in basal conditions, after NO-synthase inhibition by L-NAME, COX inhibition by piroxicam, or a combination of both in an organ bath set-up. Contractions were noted as a percentage of potassium induced pre-contraction. Results were analysed by Two-Way ANOVA and student-T-test. Results: Maximum contraction of aortic rings was significantly lower in models of PHT (steatosis, CBDL and PPVL) compared to controls (sham-operated): 143.89±12.86%, 133.70±7.41% and 137.50±8.73% respectively compared to 181.67±11.86 (p<0.0001). Piroxicam significantly reduced vascular response in the sham-operated and PPVL-group, whereas it had no influence in steatosis and only modestly in CBDL. L-NAME restored responses in PPVL. Also in steatosis L-NAME restored vascular contractility to values comparable to controls with L-NAME: 160.91±6.65% vs. 166.36±7.94% (p=0.1732). In CBDL L-NAME only slightly ameliorated vascular response. L-NAME/piroxicam combination did not significantly alter arterial contraction in any of the models. Conclusions: This study confirms vascular hyporeactivity in a model of non-cirrhotic NAFLD-associated PHT comparable to what is observed in classical models PHT. In normal conditions vascular reactivity is regulated by both vasodilatory NO and vasoconstrictor COX activity. Hyporesponsiveness in steatosis and CBDL (both models of sinusoidal PHT) is in part caused by absence/reduction of COX-mediated vasoconstriction and by NO overproduction. By contrast, COX-mediated vasoconstriction is maintained in PPVL (a model of prehepatic PHT), in which hyporesponsiveness is solely caused by NO overproduction.
Autophagy is a life-sustaining process for lysosomal degradation of long-lived or damaged organelles and proteins. Recent studies have shown that autophagy deficiency in macrophages exacerbates atherosclerotic plaque development by promoting macrophage apoptosis and plaque necrosis. Since autophagy also occurs in smooth muscle cells (SMCs) in the fibrous cap of atherosclerotic plaques, we focused on the role of autophagy in SMC function. We investigated the effect of autophagy inhibition on SMC survival and phenotype by genetic targeting of Atg7. Autophagy deficient SMCs (Atg7<sup>F/F</sup>SM22α-Cre<sup>+</sup> SMCs) showed a pronounced decrease in proliferation, an increase in collagen synthesis and a transition into a hypertrophic, dedifferentiated and senescent phenotype, indicating that autophagy is crucial for both SMC function and morphology. Moreover, Atg7<sup>F/F</sup>SM22α-Cre<sup>+</sup> SMCs were much more resistant to oxidative stress-induced cell death as compared to Atg7<sup>+/+</sup>SM22α-Cre<sup>+</sup> SMCs. This effect was attributed to nuclear translocation of the transcription factor Nrf2 resulting in the upregulation of several antioxidant enzymes such as glutathione S-transferase α (GSTα) and NAD(P)H:quinone oxidoreductase 1 (NQO1). In contrast to SMCs, Atg7<sup>F/F</sup>LysM-Cre<sup>+</sup> macrophages did not upregulate GSTα and were therefore more susceptible to cell death as compared to Atg7<sup>+/+</sup>LysM-Cre<sup>+</sup> macrophages. This finding suggests that the role of autophagy in survival is cell type dependent. We can conclude that autophagy plays a major role in the regulation of SMC survival and phenotype. The cell type dependent roles of autophagy must be taken into consideration when developing new strategies for targeting autophagy in the treatment of atherosclerosis.
AUTOPHAGY DEFICIENCY IN SMOOTH MUSCLE CELLS ALTERS VASCULAR FUNCTION


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Autophagy is a subcellular housekeeping mechanism, that protects the cell by degrading damaged organelles and protein aggregates. Recently, defective autophagy has been linked to several human disorders including cancer, neurodegeneration and cardiovascular diseases. In the present study, smooth muscle cell (SMC) contractility (organ baths), calcium homeostasis (FURA-2) and arterial stiffness (myograph) were investigated in mice containing a SMC-specific deletion of the essential autophagy gene Atg7 (Atg7^{F/F} SM22α-Cre^+) and in corresponding controls (Atg7^{+/+} SM22α-Cre^+). By measuring isometric force development of thoracic aorta segments in organ baths, we could demonstrate that voltage gated calcium channels of Atg7^{F/F} SM22-α Cre^+ mice were more sensitive to potassium-induced depolarization. As a result, SMC contraction developed at lower KCl concentrations (EC_{50} Atg7^{F/F} vs Atg7^{+/+} : 17.9±0.4 vs 20.7±0.4 mM KCl, p<0.001). Furthermore, segments of Atg7^{F/F} SM22α-Cre^+ mice showed significantly higher inositol triphosphate-mediated transient contractions under calcium-free conditions. Also, expression (Western blotting) of the sarco/endoplasmic reticulum calcium ATPase-2 (SERCA2) as well as the influx of calcium after emptying of the sarcoplasmic reticulum, known as the capacitative calcium influx, were higher in Atg7^{F/F} SM22α-Cre^+ SMCs. Interestingly, SMCs of Atg7^{F/F} SM22α-Cre^+ mice showed increased cytosolic calcium concentrations, which could be abolished by the non-selective cation channel blocker 2-aminoethoxydiphenyl borate. Stress-strain experiments revealed that the aortas of Atg7^{F/F} SM22α-Cre^+ mice were more flexible due to a reduction in medial collagen (positive area: 13±1 vs 32±7 %, p<0.05). Overall, our study indicates that SMC autophagy plays an important role in the vasculature and that an autophagy defect in SMCs leads to changes in vascular function.
MENTAL STRESS IN A MOUSE MODEL OF ATHEROSCLEROTIC PLAQUE RUPTURE

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Apolipoprotein E-deficient (ApoE−/−) mice with a heterozygous mutation in the fibrillin-1 gene (Fbn1C1039G+/−, Marfan phenotype) show an increase in arterial stiffness due to fragmentation of the elastin fibres. We previously showed that this results in exacerbated atherosclerosis and spontaneous plaque ruptures, accompanied by stroke, myocardial infarction and sudden death. In the present study, we focused on the influence of mental stress on atherosclerosis in the ApoE−/− Fbn1C1039G+/− mouse model. Female ApoE−/− Fbn1C1039G+/− mice were fed a Western type diet (WD) for up to 25 weeks. At week 10 of WD, the mice were divided in a control (n=17) and stress (n=18) treatment group. The stress protocol consisted of 3 triggers: water avoidance, damp bedding and restraint stress. The stress group was subjected to these triggers in a randomly assigned order during 6 hours of every weekday for 15 weeks. Mental stress resulted in a decrease in survival of the ApoE−/− Fbn1C1039G+/− mice from 71% to 50% at 25 weeks of WD. The plaque size and percentage of necrotic core in the proximal ascending aorta (Aoprox) were not influenced by mental stress. However, the plaque composition in the Aoprox was altered. The amount of macrophages was significantly increased (control: 1.5±0.3%, stress: 3.0±0.6%; p=0.021) and type I collagen was decreased (control: 4.3±0.4%, stress: 2.7±0.5%; p=0.021). The thickness of the fibrous cap was also reduced from 12.8±1.6 µm in the control group to 6.5±1.0 µm in the stress group (p=0.014). Moreover, mental stress led to an increase in the frequency of myocardial infarctions (control: 41%, stress: 67%). The degree of perivascular fibrosis of the coronary arteries was also elevated (control: 36±7%, stress: 67±7%; p=0.006). In conclusion, mental stress increased the vulnerability of atherosclerotic plaques and reduced survival in the ApoE−/− Fbn1C1039G+/− mouse model.
DEVELOPMENT AND VALIDATION OF A NEW NON-INVASIVE TECHNIQUE TO ASSESS ARTERIAL STIFFNESS IN MICE

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Introduction: The most important in vivo parameter of arterial stiffness is carotid-femoral pulse wave velocity (cfPWV). This parameter strongly correlates with cardiovascular events and all-cause mortality, but unlike its clinical value, techniques to determine cfPWV in mice are scarce. Up to now, the only way to determine this parameter non-invasively in mice is by using ultrasound echo Doppler velocimetry. Here, we developed a novel non-invasive technique to assess arterial stiffness in mice by measuring cfPWV in vivo with applanation tonometry (AT).

Methods: AT was applied to measure cfPWV in wild-type (WT, C57Bl6) mice and in two mouse models of endothelial dysfunction: endothelial NO synthase knock-out (eNOS−/−) mice and mice receiving the eNOS inhibitor LNAME in the drinking water (0.75 mg/ml). Both models have been described to display increased cfPWV. In vitro determination of aortic stiffness was performed in the myograph, whereas vascular reactivity of aortic segments was measured in organ baths. Results: cfPWV in WT mice was 3.24±0.04 m/s (n=24) and corresponded very well with published cfPWV values determined with echo Doppler velocimetry in C57Bl6 mice. In eNOS−/− mice cfPWV increased significantly to 3.79±0.02 m/s (n=20) and could be reversed by chronic ACE-inhibition with perindopril (16.7μg/ml drinking water). In vivo eNOS inhibition by LNAME increased cfPWV from 3.22±0.09 m/s in control mice (n=5) to 3.99±0.09 m/s (n=5) after 4 weeks treatment with LNAME. In vitro, we demonstrated in these hypertensive LNAME-drinking mice a calcium-dependent increase in aortic stiffness, a decrease in basal NO effectivity, and an increase in depolarisation-mediated contractions. Conclusion: These results all point to an important role of basal NO release to maintain the important pulse-smoothing properties of large elastic arteries. Endothelial dysfunction leads to an increase of cfPWV and aortic stiffness. Applanation tonometry is an easy-to-use and non-expensive technique to assess arterial stiffness non-invasively in mice and allows to perform longitudinal studies in mouse models of endothelial dysfunction.
IMPAIRED GASTROINTESTINAL MOTILITY IN ANIMAL MODELS OF SEPSIS

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Recent studies acknowledge that ileus, the impaired propulsion of gastrointestinal (GI) contents following surgery or severe illness, is not only the result but also contributes to sepsis by impairing the mucosal barrier function. Different animal models of sepsis are currently in use worldwide. Our goal was to quantify impaired GI motility in two animal models, in order to study the effect of different pharmacological compounds on both GI motility and severity of sepsis. Sepsis was induced in two different animal models. In the LPS-model, OF-1 mice received an i.p. injection of lipopolysaccharide (E. coli 055B5, 20 mg/kg) or equivalent dose of saline. Mice received an oral gavage with 25 green glass beads (diameter 0.4-0.5 mm) 17 hours following the injection. In the ‘caecal ligation and puncture’ (CLP)-model, mice were subjected to a surgical procedure during which the caecum was ligated for 50%, and punctured once with a 25G needle. Sham mice were subjected to a laparotomy. Mice received a gavage either 2 or 7 days following the procedure. Two hours following the gavage, the entire GI tract was removed and divided into 10 segments. Percentage of gastric emptying (%GE) and geometric centre (GC) were calculated. The unpaired Student’s t-test or One-way ANOVA with post-hoc Bonferroni were applied where necessary. Injection of LPS resulted in a significantly lower %GE (37.07 ± 11.17 vs 81.20 ± 10.05% in controls, p = 0.009), and lower GC (2.11 ± 0.31 vs 4.37 ± 0.59, p = 0.003). Mice subjected to CLP also displayed a significantly lower %GE at day 2 (42.50 ± 13.48 vs 88.96 ± 8.13 in sham mice, p = 0.020) and day 7 (32.89 ± 14.45, p = 0.023). Furthermore did we measure a lower GC at day 2 (2.25 ± 0.50 vs 4.87 ± 0.45, p = 0.002) and day 7 (1.93 ± 0.33, p = 0.004). Both the LPS- and CLP-model thus resulted in a significant delay of GI transit, and will be used for future research and testing of pharmacological compounds in septic ileus in our lab.
RAPAMYCIN AND ITS ANALOGS ACTIVATE THE COLD SENSITIVE ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MELASTATIN 8 (TRPM8) – ROLE OF A “MENTHOL-LIKE” MOIETY

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Rapamycin (Sirolimus), a potent mammalian target of rapamycin (mTOR) inhibitor macrolid immunosuppressant was found to activate human TRPM8, a cold sensitive member of TRP family of ion channels. Rapamycin evoked transient increase of the intracellular Ca$^{2+}$ concentration and induced an outwardly rectifying current on HEK-293T cells overexpressing TRPM8 channels. The rapamycin was more potent than the typical TRPM8 agonist menthol and its effect was completely blocked by the TRPM8 antagonist N-(3-aminopropyl)-2-[[3-methylphenyl] methyl]oxy]-N-(2-thienylmethyl) benzamide hydrochloride (AMTB). Moreover, rapamycin failed to activate TRPM3, another thermosensitive close relative of TRPM8 or Transient Receptor Potential Ankyrin 1 (TRPA1), another cold sensitive member of TRP family. Kinetic analysis of the current traces and in silico modelling suggested that rapamycin, like menthol but even more effectively, stabilizes the open state of TRPM8 channels. Another macrolid immunosuppressant, the calcineurin inhibitor FK-506 (Tacrolimus) possessing the same “menthol-like” moiety as rapamycin was also found to activate TRPM8. The substitution of a hydroxyl group in this moiety of rapamycin and FK-506 (Everolimus and Pimecrolimus, respectively) resulted in a dramatically decreased activation of TRPM8. On TRPM8+/+ mice, rapamycin applied subcutaneously also evoked wet-dog-shake, a TRPM8 specific behavioral response. Our results introduce a new class of TRPM8 activators, as well as a new target of rapamycin-like macrolids calling the attention for potential novel beneficial therapeutic effects.
TRPM3 – A PROMISING TARGET FOR ANALGESIC TREATMENT

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The superfamily of Transient Receptor Potential (TRP) ion channels consists of 28 different members in mammals. The sensitivity of TRP channels to a broad array of stimuli allows them to function as biological sensors involved in processes ranging from vision to taste, and tactile sensation. The temperature sensitive TRP channels (so called thermoTRPs) are typically expressed in sensory neurons, where they act as sensors for the detection of innocuous and noxious temperatures. Lately, our research group characterized TRPM3 as a heat sensitive nociceptor expressed in a subset of sensory neurons, where it plays a decisive role in the nocifensive response to pregnenolone sulphate (PS) and heat and in the development of heat hyperalgesia during inflammation. This project aims to validate TRPM3 as a potential target for the development of new analgesics. Therefore we purpose to identify new potent and selective TRPM3 blockers, and show their ability to different pain conditions in vivo. In close collaboration with the Centre of Drug Design and Development (CD3) of the KU Leuven, we were able to identify different groups of potent and highly selective TRPM3 blockers. Within newly identified TRPM3 blockers, CIM056741 and CIM057941 displayed favorable properties with respect to their inhibitory potency (IC$_{50}$ < 50 nM). Both compounds exhibited a marked specificity for TRPM3 compared with other sensory TRP channels, and blocked PS-induced [Ca$^{2+}$]$_i$ signals in freshly isolated DRG neurons. Furthermore, both compounds significantly reduced the sensitivity of mice and rats to noxious heat and PS-induced chemical pain. Interestingly, injection of CIM056741 strongly reduced the inflammatory induced mechanical hyperalgesia and was without effect on body core temperature, heart rate and locomotor activity. In conclusion, our data validate TRPM3 as a possible target for the development of novel analgesics in humans.


O-09 (14.30-14.45)

**SST2 AND SST3 –BUT NOT GHS-R1A- RECEPTORS ARE INVOLVED IN THE ANTICONVULSANT EFFECTS OF CORTISTATIN-14**

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Unlike somatostatin(SST)-14, anticonvulsant actions of cortistatin(CST)-14 have not been extensively studied. Both peptides are structurally related and have high affinities for the five SST receptor subtypes (sst₁-sst₅). Despite these homologies, CST-14 seems to act also on other receptors. It has been suggested that the growth hormone secretagogue receptor (GHS-R1a) may fulfill this role. We here aim to unveil which receptors are involved in the anticonvulsant effects of CST-14. We use *in vivo* microdialysis and telemetry-based electrocorticography(ECoG) in rats and mice. In rats, CST-14 (0.1µM-1µM-10µM) was administered in the presence and absence of sst₂ and sst₃ receptor antagonists via intrahippocampal administration. Seizures were evoked by intrahippocampal pilocarpine perfusion (12mM, 40min) and seizure severity was assessed using a behavioural scoring system and ECoG. In mice, CST-14(0.1µM-1µM-10µM) was administered intrahippocampally to confirm the anticonvulsant actions observed in rats. The involvement of GHS-R1a was tested by administering an anticonvulsant dose (1µM) of CST-14 in both GHS-R1a knock-out (KO) and wild-type (WT) mice. Seizures were evoked by intrahippocampal pilocarpine perfusion (12mM, 40min). Seizure severity was assessed by ECoG, by means of seizure duration. Intrahippocampal administration of 1µM and 10µM CST-14 in rats was anticonvulsant against pilocarpine-induced seizures. Furthermore, we showed that CST-14(1µM)-mediated anticonvulsant actions were reversed in the presence of 0.1µM Cyanamid, a selective sst₂ antagonist or 0.1µM SST3-ODN8, a selective sst₃ antagonist. Intrahippocampal perfusion of these antagonists alone did not affect the pilocarpine-induced seizure severity per se. In mice, 1µM CST-14 -but not 0.1µM and 10µM- was able to protect the animals against pilocarpine-induced seizures. Anticonvulsant effects of CST-14(1µM) were confirmed in GHS-R1a WT animals since the seizure duration in these treated animals was significantly lower when compared to the pilocarpine control group. Perfusion of CST-14(1µM) in KO mice showed no difference in seizure duration when compared to the CST-14 treated WT animals. Our results show that CST-14 prevents seizures in a focal pilocarpine model and that selective sst₂ or sst₃ receptor antagonism abolishes these anticonvulsant actions in rats. Our findings also demonstrate that the anticonvulsant actions of CST-14 in mice are not mediated via the GHS-R1a receptor.
PHOSPHORYLATION OF IP3R1 DECREASES ER CALCIUM CONTENT AND RENDERS PROSTATE CANCER CELLS RESISTANT TO ANDROGEN DEPRIVATION.

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Reference treatment of advanced prostate cancer (PCa) relies on pharmacological or surgical androgen deprivation therapy. However, it is only temporarily efficient, tumour cells inevitably adapting to low testosterone environment and becoming hormone-refractory (HRPCa). We observed that androgen removal in HRPCa-derived LNCaP cells cause different alterations of their Ca^{2+} homeostasis among which a reduction of ER Ca^{2+} content. We show that the [Ca^{2+}]_{ER} decrease is due to a mild overexpression of type 1 IP3R made leakier by an increased phosphorylation on the PKA consensus site Ser-1716. Accordingly, Ca^{2+} store content was restored by siRNA-mediated depletion of IP3R1 and by inhibition of its phosphorylation by competition with a permeant TAT-peptide containing the Ser-1716 consensus phosphorylation sequence. Moreover, inhibition of the IP3R1 phosphorylation by PKA inhibitor H89 or TAT-peptide sensitized LNCaP cells to androgen deprivation. This suggests that the modulation of the channel function of IP3R1 is a protective mechanism by which HRPCa-derived cells manage to overcome cell death despite the absence of androgenic stimulation.
DIVERSITY IN PHARMACOLOGICAL PROFILE OF HETEROTETRAMERIC CHANNELS CONSISTING OF KV2.1 AND SILENT VOLTAGE-GATED POTASSIUM SUBUNITS

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Voltage-gated K⁺ (Kv) are tetramers of α-subunits each consisting of 6 transmembrane segments (S1-S6) and a cytoplasmic N- and C-terminus. The S5-S6 segments of each subunit assemble to generate the central pore while the S1-S4 segments form the voltage-sensing domains. The PXP motif in the middle of S6 provides a degree of flexibility to the bottom half of the S6 segment which is necessary for channel gating. This region is also critical for the interaction with channel blockers. Based on sequence homology, eight Shaker-related Kv subfamilies have been identified: Kv1-Kv6, Kv8-Kv9. The silent (KvS) subunits (Kv5-Kv9) cannot form homotetramers but assemble with Kv2 subunits into Kv2/KvS heterotetramers that display unique biophysical properties. KvS subunits lack the 2nd proline residue of the PXP motif which may impact on the pharmacological profile of channel blockers. We tested this hypothesis by using the Kv1.5(P511G) mutant in which the 2nd proline of the PXP motif was replaced by a glycine. Homotetrameric Kv1.5(P511G) channels were insensitive to 4-AP while heterotetrameric Kv1.5-Kv1.5(P511G) channels (stoichiometry controlled by using dimers), still displayed current inhibition. However, Kv1.5-Kv1.5(P511G) channels were significantly less sensitive displaying an IC₅₀ values of 16 mM instead of 270 µM for wild type (WT) Kv1.5. Similarly, heterotetrameric Kv2/KvS channels displayed an altered affinity for 4-AP compared to WT Kv2.1; 18 mM (IC₅₀ for Kv2.1) inhibited 17%, 60%, 82% and 13% of Kv5.1, Kv6.3, Kv8.1 and Kv9.3-containing currents, respectively. Furthermore, the heterotetrameric Kv2/KvS channels displayed also a subtle change in the affinity for the open channel blockers quinidine and flecainide. These results suggest that the absence of a complete PXP motif in one or two out of four subunits alters the pharmacological profile.
EFFECTS OF IMMUNOSUPPRESSION ON ALLOGENEIC T CELL ACTIVATION BY MATURE CONVENTIONAL DENDRITIC CELLS

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Regulation of immune processes by conventional dendritic cells (cDC) is a key event in a cascade of processes occurring during the development and progression of atherosclerotic plaques. Therefore, strategies aimed at immunosuppression in plaques using DC-based vaccination are very promising to prevent atherothrombotic disease. cDC can be converted \textit{in vitro} into tolerogenic DC which elicit a regulatory anti-inflammatory response against disease-related antigens (e.g. plaque-antigens) via the formation of regulatory T cells. Previous studies suggest that both IL-10 and helminth proteins are able to induce tolerance. The aim of this study was to evaluate whether IL-10 or helminth proteins are suitable immunosuppressive agents for the induction of tolerance in cDC that are reactive to atherosclerosis related antigens. Stimulation of cDC with LPS and IFN\gamma resulted in mature cDC characterized by a significant increase in the expression of costimulatory molecules CD40 (95.05\pm2.13 \% positive cells) and CD86 (71.35\pm5.44 \% positive cells) compared to immature cDC (p<0.0001), together with a high production of IL-12p70 (901.4\pm239.7 pg/ml). When cDC were stimulated with a total lysate from mouse atherosclerotic plaques, morphologically mature cDC characterized by the expression of CD40 (28.2\pm5.7 \% positive cells, p=0.03) could also be observed. Allogeneic activation of splenic naïve T cells by mature cDC resulted in the secretion of proinflammatory cytokines IFN\gamma, TNF\alpha, IL-6 and IL-2. This secretion can be reduced by the addition of IL-10 but not by \textit{Schistosoma mansoni} worm proteins. In fact, the latter induced an even higher secretion of inflammatory cytokines. In conclusion, these pilot data indicate that IL-10 has proven its role as immunosuppressant by suppressing the polarization of naïve T cells into proinflammatory effector T cells by mature cDC. Helminth proteins however do not exert such immunosuppressive effects and should not be considered for the induction of cDC tolerance in the treatment of atherosclerotic plaques.
DIFFERENCES IN NITRIC OXIDE SYNTHASE ACTIVITY AND UNCOUPLING IN ATHEROSCLEROSIS-PRONE AND RESISTANT MOUSE AORTIC REGIONS

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Atherosclerotic plaques develop preferably at sites subjected to low shear stress or turbulent flow. Here we studied differences in basal and acetylcholine (ACh)-stimulated endothelial nitric oxide (NO) synthase (eNOS) activity and eNOS uncoupling in the atherosclerosis-prone ascending aorta and aortic arch compared to the atherosclerosis-resistant descending aorta of C57/Bl6 mice. Aortic rings were mounted in organ baths, and basal NO activity was assessed by the suppression of phenylephrine (PE, 1 µM)-induced isometric force at 1h after isolation. After stabilisation of the PE responses, ACh (0.003-10 µM) was given. Catalase (2400 U/ml) was used to determine the contribution of hydrogen peroxide, while nitrosylation, eNOS monomerisation and Akt and eNOS phosphorylation were studied using immunoblotting. The ascending aorta displayed less basal NO release, but increased sensitivity to ACh compared to the atherosclerosis-resistant descending aorta. In contrast, the distal aortic arch displayed severely attenuated ACh-induced relaxation that was largely inhibited by catalase. eNOS was found primarily as dimers and there were no differences in eNOS dimerisation between regions. Nitrosylation was enhanced in ascending aorta and aortic arch. The ascending aorta demonstrated increased eNOSThr495 phosphorylation, while in the aortic arch eNOSSer615 phosphorylation was decreased. In conclusion, basal NO release was diminished in the ascending aorta despite increased responsiveness to ACh. The augmented Thr495 phosphorylation in this region suggests a role for this site in regulating basal eNOS activity. Despite high basal NO production, the distal aortic arch demonstrated decreased responses to ACh and extensive ‘physiological’ eNOS uncoupling, which was associated with decreased Ser615 phosphorylation. These results suggest that differences between basal and stimulated eNOS activity and eNOS uncoupling are of (patho)physiological relevance.
MECHANISMS INVOLVED IN THE VASORELAXING INFLUENCE OF CO-RELEASING MOLECULE-2 (CORM-2) ON MICE AORTA

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The currently available CO-releasing molecules (CORMs) have shown encouraging pharmacological actions, particularly in the vasculature, suggesting pleiotropic properties of these compounds in the resolution of vascular disorders. However, the precise cellular and molecular targets involved in CORM-mediated responses remain ambiguous. The present study aimed to better understand the mechanisms involved in CORM-2-induced vasorelaxations. Mice thoracic aorta ring segments were mounted in small-vessel myographs for isometric tension recordings. Vasodilatation elicited by CORM-2 was significantly attenuated in the presence of i) an inhibitor of NADPH reductases, 7-ER (10 µM), ii) a high K⁺ (30 mM) solution and iii) an inhibitor of Na⁺/K⁺-ATPase pumps, ouabain (100 µM). Moreover, CORM-2-induced vasorelaxations were strongly reduced in vessels pre-contracted with the thromboxane A₂ mimetic U46619 (5 nM) or PGF₂α (15 µM) compared to vessels pre-contracted with norepinephrine (5µM), phenylephrine (10 µM), serotonin (1 µM), ET-1 (1 µM) or PGE₂ (5 µM). Surprisingly it was found that the CO-carrying moiety of CORM-2, RuCl₃, also exerts profound vasodilatory effects similar to CORM-2. Comparable to CORM-2, the relaxing effects of RuCl₃ were significantly attenuated in vessels pre-constricted with U46619 compared to vessels pre-contracted with norepinephrine. It is concluded that the vascular responses of CORM-2 in mice aorta are largely mimicked by the CO-carrier ruthenium moiety of the CO-donor. In addition, CORM-2 induces relaxations in part by activation of NADPH-dependent reductases and Na⁺/K⁺-ATPases. Moreover, the vasodilatory responses of CORM-2 are remarkably less pronounced in U46619 or PGF₂α pre-contracted preparations compared to norepinephrine, phenylephrine, serotonin, ET-1 and PGE₂ pre-constricted vessels, although an explanation for this observation remains to be found.
MODULATION OF HETEROTETRAMERIC KV2.1/KV6.4 CHANNELS BY AUXILIARY 
B-SUBUNITS

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Based on sequence homology, eight Shaker-related subfamilies of voltage-gated K⁺ (Kv) channel α-subunits have been identified: Kv1-Kv6 and Kv8-Kv9. The Kv1-Kv4 subfamilies generate functional channels in both homo- and heterotetrameric configuration while the silent (KvS) channel subunits (Kv5-9) do not form functional homotetramers due to retention in the endoplasmic reticulum (ER). This ER retention is relieved by assembly with Kv2 subunits generating functional Kv2/KvS heterotetramers with unique biophysical properties. The diversity within the Kv2 subfamily is further increased by interactions with auxiliary β-subunits; Kv2 subunits form complexes with both cytoplasmic (such as AMIGO) and transmembrane (such as KCNE1) β-subunits resulting in changed biophysical Kv2.1 properties compared to Kv2.1 alone. Despite that Kv2.1 subunits interact with both the modulatory KvS α-subunits and auxiliary β-subunits, it has not been investigated yet whether the auxiliary β-subunits also interact with Kv2/KvS heterotetramers. We demonstrate here that the transmembrane β-subunit KCNE1 also interact with Kv2.1/Kv6.4 heterotetramers. Co-expression of KCNE1 with Kv2.1 and Kv6.4 shifts the voltage-dependence of activation to hyperpolarizing potentials compared to that of Kv2.1/Kv6.4 alone without influencing the voltage-dependence of the Kv2.1/Kv6.4 inactivation. This potential Kv2.1/Kv6.4/KCNE1 interaction has been confirmed by Fluorescence Resonance Energy Transfer (FRET) experiments. Co-expression of N-terminal CFP-tagged Kv6.4 with C-terminal YFP-tagged KCNE1 in HEK293 cells did not result in FRET while co-expression of CFP-Kv6.4 with YFP-KCNE1 and unlabeled Kv2.1 yielded a FRET efficiency of 6 % which is similar to the FRET efficiency of the positive CFP-Kv2.1 + YFP-KCNE1 combination. Furthermore, RT-PCR experiments revealed that Kv6.4 and KCNE1 subunits posses an overlapping expression pattern. These results suggest that a triple complex consisting of Kv2.1, Kv6.4 and KCNE1 subunits can be formed which might also exist and play a function in vivo.
IDENTIFICATION OF LIGANDS FOR SUCNR1(GPR91) THROUGH A SOSA APPROACH

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Succinic acid is a metabolic component, which takes part in the Krebs cycle, also termed citric acid cycle. It has been recently described as the cognate agonist for the orphan receptor SUCNR1 (GPR91). This receptor belongs to the G Protein-Coupled Receptor family (GPCR), the largest class of membrane receptors characterized by 7 transmembrane domains. GPCRs are involved in many physiological functions and represent 30% of targets for currently marketed drugs. Several studies have identified a role for succinic acid as a marker of cellular ischemic stress to adjacent tissues through its receptor. The activation of SUCNR1 can induce angiogenesis, release of renin, hematopoiesis and enhancement of immunity. Besides, succinic acid has been shown to induce platelet aggregation. Nevertheless, the proper identification of SUCNR1 roles is limited by the lack of small molecule as pharmacological tools. The aim of this project is to identify active molecules modulators for SUCNR1 and use these tools to validate the potential therapeutic interest of this protein and understand its physiological functions. Therefore, we performed a SOSA (selective optimization of side activities) library screening based on a luciferase assay able to measure cAMP levels in a kinetic way. We selected ebastin, the most potent molecule (EC₅₀ = 10 µM), a histamine receptor (H₁) antagonist. To obtain more active and selective ligands for SUCNR1, we synthesized ebastin derivatives and developed a new bioluminescent pharmacological assay based on the real-time measure of [Ca²⁺] by a biosensor for the routine evaluation of these small molecules on both H₁ and SUCNR1.
DEVELOPMENT OF AN ORIGINAL β-ARRESTIN COMPLEMENTATION ASSAY FOR ORPHAN GPCR GPR27(SREB1) LIGAND AND SIGNALING PATHWAYS DETERMINATION

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The largest family of membrane receptors is represented by G protein-coupled receptors (GPCRs), which are characterized by 7 transmembrane domains. Even if marketed drugs currently target only 10% of all GPCRs, they represent more than 30% of all small molecules based therapies. The physiological and pathophysiological role of a GPCR is defined by its expression pattern, signaling pathway and specific ligand. GPCRs which have not yet been associated to a physiological ligand are called orphan GPCRs and represent ~100 of the ~370 human non-odorant GPCRs. This project aims at identifying and developing pharmacological tools for GPR27 (SREB1), one of these orphan receptors. GPR27 has recently been shown to have a role in the regulation of insulin promoter activity and insulin secretion. Nevertheless, the pharmacology of GPR27 remains elusive and the lack of appropriate pharmacological tools dramatically restricts the understanding of its function and its validation as a drug target. Thus, we plan to study its signaling pathways by developing original complementation assay, first based on β-arrestin recruitment. In addition, the assay will allow us to identify small molecules able to interact with GPR27 (screening of a DOS library). These are important steps toward understanding its function and evaluating GPR27 as a potential drug target, for instance in insulin-related metabolic disorders such as type II diabetes or in other pathologies where it might be involved.
BLOOD OSMOLARITY CONTROL IN TRPC1−/− MICE

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In recent years, we investigated the role of TRPC1 ion channel in muscle function. We showed its implication in cell migration and proliferation and in the resistance to muscle fatigue. In order to measure the consequences on mice behaviour, we measured their general activity in physiocages. Their activity was normal, but unexpectedly, we observed that TRPC1−/− mice drank more than control mice. As TRPC1 has been reported to be mechanosensitive, we hypothesized it could be activated by osmolarity changes. Besides, other isoforms (in the TRPV subfamily) have been shown to play such a role. Indeed, we and others showed that TRPV4−/− mice present troubles of osmolarity regulation, TRPV4 being the osmotic sensor in the hypothalamus. Interestingly, it has recently been shown that TRPV4 and TRPC1 could form heterotetramers and play a key role in flow-induced endothelial Ca2+ influx. In spite of this abnormally elevated water intake, TRPC1−/− mice had a 10 mM increase in serum osmolarity. When a bolus of hyper-osmotic solution was injected IP, TRPC1−/− mice increased water intake with the same time course as control mice, suggesting a normal hypothalamic response. After iso-osmotic overload, TRPC1−/− had a smaller urine excretion than control mice, pointing out to an involvement of the kidney. Moreover, water deprivation test showed that TRPC1−/− mice were unable to concentrate urines as if they were unable to diminish their renin secretion. And interestingly, at rest, renin expression measured by quantitative PCR showed a 50 % increase in TRPC1−/− mice kidneys. We plan to further characterize extracellular osmolarity control in TRPC1−/− mice by measuring volume, osmolarity and specific ion concentrations at rest and after various challenges. We also plan to further study TRPC1 role at a cellular level in juxtaglomerular cells and in glomerular mesangial cells, both expressing TRPC1.
FUNCTIONAL CHARACTERIZATION OF TRANSIENT RECEPTOR POTENTIAL CHANNELS IN HUMAN ENDOMETRIUM

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The human endometrium undergoes extensive remodeling during the menstrual cycle and plays a pivotal role in embryo implantation. Despite tremendous developments in in vitro fertilization (IVF) techniques, implantation failure remains one of the main causes of low pregnancy rates. However, recent findings suggest that local endometrial injury prior to the embryo transfer cycle improves the embryo implantation rate in women undergoing assisted reproductive techniques (ART). Additionally, it has been shown that mechanical stimuli, such as scratching the endometrium, can induce decidualization in rodents in the absence of the embryo. In our study, we aim to identify and characterize mechanosensors expressed in the human endometrium. Possible candidates are members of the Transient Receptor Potential (TRP) superfamily, since they are activated and regulated through strikingly diverse mechanisms, making them suitable candidates for cellular sensors. At the moment, little is known about the functional expression profile of TRP channels in human endometrium. Quantitative real-time PCR on RNA of endometrial biopsies from different phases of the reproductive cycle (follicular, luteal and menstrual phase) showed high RNA levels of TRPC6, TRPV2, TRPV4, and TRPM7 during the luteal phase. Evidence for the functional expression was provided by Fura 2-mediated Ca²⁺-microfluorimetry and patch-clamp experiments using specific pharmacology of the related TRP channels on primary endometrial cells. In conclusion, we showed for the first time the functional expression of TRPC6, TRPV2, TRPV4 and TRPM7 in human endometrial cells. Further experiments will be performed to identify the physiological role of these channels in the complex process of embryo implantation.
MECHANISM OF THE MEDIUM-DURATION AFTERHYPERPOLARIZATION IN RAT SEROTONERGIC NEURONS


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Most serotonergic neurons display a prominent medium-duration afterhyperpolarization (mAHP), which is mediated by small conductance Ca\(^{2+}\)-activated K\(^{+}\) (SK) channels. Recent ex vivo and in vivo experiments have suggested that SK channel blockade increases the firing rate and/or bursting in these neurons. The purpose of this study was therefore to characterize the source of Ca\(^{2+}\) which activates their mAHP channels. In voltage clamp experiments, an outward current was recorded at -60 mV after a depolarizing pulse to + 100 mV. A supra-maximal concentration of the SK channel blockers apamin or (--)bicuculline methiodide blocked this outward current. This current was also sensitive to the broad Ca\(^{2+}\) channel blocker Co\(^{2+}\) and was partially blocked by both \(\omega\)-conotoxin and mibefradil, which are blockers of N-type and T-type Ca\(^{2+}\) channels, respectively. Neither blockers of other voltage-gated Ca\(^{2+}\) channels nor DBHQ, an inhibitor of Ca\(^{2+}\)-induced Ca\(^{2+}\) release, had any effect on the SK current. In current-clamp experiments, mAHPs following action potentials were only blocked by \(\omega\)-conotoxin and were unaffected by mibefradil. This was observed both in slices from juvenile and adult rats. Finally, when these neurons were induced to fire in an in vivo-like pacemaker rate, only \(\omega\)-conotoxin was able to increase their firing rate by \(~30\%\), an effect identical to the one previously reported for apamin. Our results demonstrate that N-type Ca\(^{2+}\) channels are the only source of Ca\(^{2+}\) which activates the SK channels underlying the mAHP. T-type Ca\(^{2+}\) channels may also activate SK channels under different circumstances. Our data also emphasize that results from voltage-clamp experiments should be interpreted with caution.