BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting

Saturday, October 24 2009

A B S T R A C T B O O K

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Organisation

Prof. Dr. I. Smolders
Prof. Dr. A. Dupont
Onderzoeksgroep Experimentele Farmacologie
Vrije Universiteit Brussel
Laarbeeklaan 103
1090 Brussel-Jette

BELGIAN SOCIETY OF FUNDAMENTAL AND CLINICAL PHYSIOLOGY AND PHARMACOLOGY

Autumn Meeting
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VRIJE UNIVERSITEIT BRUSSEL
Research Group Experimental Pharmacology
Vrije Universiteit Brussel
Campus Jette – gebouw A
Laarbeeklaan 103
1090 BRUSSEL JETTE

Main Lecture

Auditorium Piet Brouwer

10.00-10.50 Prof. Dr. Rob VOSKUYL
(Stichting Epilepsie Instellingen Nederland (SEIN) – Heemstede/Zwolle en Leiden/Amsterdam Center for Drug Research (Division of Pharmacology, Leiden)

Solving the problem of therapy resistance in epilepsy: a challenge for basic and clinical research.

Oral Communications

Fundamental Physiology and Pharmacology
Auditorium Piet Brouwer

11.00-11.15 E. CARRETTE[1], X. DE TIÈGE[2], K. VONCK[1], A. MEURS[1], L. GOOSSENS[1], V. DE HERDT[1], A. VAN DYCKE[1], R. EL TAHRY[1], R. RAEDT[1], E. THIERY[1], K. DEBLAERE[1], M. OP DE BEECK[2], M. BOURGUIGNON[2], S. GOLDMAN[2], D. VAN ROOST[1], P. VAN BOGAERT[2], P. BOON[1] ([1]UGent, [2]ULBruxelles).
Clinical added value of MEG in patients with refractory partial epilepsy and non-localizing conventional presurgical evaluation.

11.15-11.30 B. MERTENS, P. VANDERHEYDEN, Y. MICHOTTE, S. SARRE (VUBrussel).
Angiotensin II type 2 receptor stimulation decreases tyrosine hydroxylase activity in the rat striatum.

11.30-11.45 B. KOENER, S. GOURSAUD, M. VAN DE STADT, A.G. CALAS, A.P. JEANJEAN, J-M MALOTEAUX, E. HERMANS (UCLouvain).
The pharmacodynamic profile of aripiprazole is not altered in a model of striatal dopaminergic hypersensitivity.

11.45-12.00 I. DE MEYER, W. MARTINET, G.R.Y. DE MEYER (UAntwerpen).
Lithium chloride induces macrophage apoptosis in rabbit atherosclerotic plaques.

12.00-12.15 N. MAENHAUT, C. BOYDENS, J. VAN DE VOORDE (UGent).
Hypoxia enhances the relaxing influence of perivascular adiopose tissue.

12.15-12.45 **Bullet Session**
Oral presentations of posters 1,2,3,4,5,6

Clinical Pharmacology
Auditorium Vanden Driessche

11.00-11.15 L. DECOSTER[1], I. VANDE BROEK[1], E. ANCKAERT[1], D. DECLERQ[1], J. DE MEY[1], F. MAJOIS[2], JF BAURAIN[2], H. DENYS[3], B. NEYNS[1], J. DE GRÈVE[1] ([1]VUBrussel, [2]UCLouvain, [3]UGent).
Activity of sunitinib in advanced malignant melanoma and its correlation with potential predictive biomarkers.

11.15-11.30 T. DILLES[1], R. VANDER STICHELE[2], M. LOPEZ HARTMANN[1], L. VAN BORTEL[2], M.M. ELSEVIERS[1] ([1]UAntwerpen, [2] UGent).
Nursing students' pharmacological knowledge and calculation skills.

11.30-11.45 B.J. VAN DER SCHUEREN, R. VERBESSELT, A. VAN HECKEN, M. DEPRÉ, F.H. VERBRUGGE, J.N. DE HOON (KULeuven).
No evidence for increased activity of the L-arginine/NO pathway in migraine patients.

11.45-12.00 B.J. VAN DER SCHUEREN, K. VAN LAERE, N. GERARD, G. BORMANS, J.N. DE HOON (KULeuven)
Increased cerebral type 1 cannabinoid receptor availability in migraine patients.

12.00-12.15 K. VAN CALSTEREN[1], R. VERBESSELT[1], N. OTTEVANGER[2], M. HALAKSA[3], L. HEYNS[1], E. DE BRUYN[1], J. DE HOON[1], F. AMANT[1] ([1]KULeuven, [2]Univ. Nijmegen, [3]Charles Univ. Prague, Czech Republic).
Pharmacokinetic study of chemotherapeutic agents in pregnant women.

12.15-12.45 **Bullet Session**
Oral presentations of posters 23, 24, 25, 26, 27, 28

Lunch (Atrium – studentenrestaurant)
Poster Session (Atrium – studentenrestaurant)
General Assembly of the Society (Auditorium Piet Brouwer)

Oral Communications

Fundamental Physiology and Pharmacology
Auditorium Piet Brouwer

14.15-14.30 M. GEES, W. EVERAERTS, Y. KARASHIMA, A. APETREI, B. NILIUS, T. VOETS, K. TALAVERA (KULeuven).
TRPV1 activation by allyl isothiocyanate.

14.30-14.45 S. DILLY, C. LAMY, J.-F. LIEGEOIS, V. SEUTIN (ULiège).
Combined experimental and computational approaches to study the action of blockers of small conductance calcium-activated potassium (SK) channels.

14.45-15.00 M. DE BOCK[1], M. CULOT[2], E. DE VUYST[1], N. WANG[1], E. DECROCK[1], M. VAN MOORHEM[1], M. BOL[1], R. CECCHELLI[2], L. LEYBAERT[1] ([1]UGent, [2]Univ. Lille Nord de France, France).
Connexin channels contribute to endothelial Ca^{2+} dynamics and alter blood-brain barrier function.

15.00-15.15 A-G CEULEMANS, T. ZGAVE, R. KOOIJMAN, S. HACHIMI-IDRISSI, S. SARRE, Y. MICHOTTE (VUBrussel).
Mild hypothermia after a transient focal cerebral ischemia in rats: influence on cytokines.

15.15-15.30 B. COLSOUL[1], A. SCHRAENEN[1], K. LEMAIRE[1], R. QUINTENS[1], L. VAN LOMMEL[1], A. SEGAL[1], R. MARGOLSKEE[2], Z. KOKRASHVILI[2], G. OWSIANICK[1], K. TALAVERA[1], T. VOETS[1], P. GILON[3], B. NILIUS[1], F. SCHUIT[1], R. VENNEKENS[1] ([1]KULeuven, [2]Mount Sinai School of Medicine, New York NY USA, [3]UCLouvain).
A reduction of glucose-induced bursting frequency in pancreatic islets correlates with decreased insulin release an impaired glucose tolerance in $Trpm5^{/-}$ mice.

15.30-15.45 L. JIN, X. DEDEKEN, C. MASSART, J. VAN SANDE, J.E. DUMONT, R. BEAUWENS (ULBruxelles).
Expression and localization of Na^{+}-HCO_3^{-} cotransporter (NBCe1) in thyroid and its regulation by TSH.

15.45-16.30 **Poster Session** (Atrium – studentenrestaurant)
Coffee - Tea

Posters

(dimensions: height 120 cm – width 90 cm)

1. A. AL HAIDAR, C. SANDERSEN, E. VAN ERCK, S. DELEUZE, F. FARNIR, H. AMORY (ULiège).
Body size affects morphological echocardiographic parameters in the equine species.

2. E. LOYENS[1], A. SCHALLIER[1], D. DE BUNDEL[1], H. DEMAEGDT[1], S.Y. CHAI[2], A.L. ALBISTON[2], G. VAUQUELIN[1], P. VANDERHEYDEN[1], Y. MICHOTTE[1], I. SMOLDERS[1] ([1]VUBrussel, [2]University of Melbourne, Parkville, Australia).
Anticonvulsive effects of insulin-regulated aminopeptidase deletion in a pentylenetetrazol mouse seizure model are not mediated by the accumulation of somato-statin-14.

3. J. PORTELLI[1], N. AOURZ[1], L. VER DONCK[2], Y. MICHOTTE[1], I. SMOLDERS[1] ([1]VUBrussel, [2]Janssen Pharmaceutica Beerse).
Anticonvulsant effects of ghrelin receptor ligands against pilocarpine-induced seizures.

4. M. VARCIN, H. YUAN, B. MERTENS, Y. MICHOTTE, S. SARRE (VUBrussel).
Apomorphine induced neuroprotection in an animal model of Parkinson's disease.

5. R. EL TAHRY, R. RAEDT, V. DE HERDT, A. VAN DYCKE, T. WYCKHUYS, A. MEURS, J. DELBEKE, K. VONCK, W. WADMAN, P. BOON (UGent).
In vivo measurements of vagus nerve compound action potential in rats.

6. T. WYCKHUYS[1], P. BOON[1], R. RAEDT[1], B. VAN NIEUWENHUYSE[1], K. VONCK[1], W. WADMAN[1,2] ([1]UGent, [2]Univ. Amsterdam NL.).
Hippocampal deep brain stimulation in an animal model for temporal lobe epilepsy.

7. R. RAEDT[1], L. PAULSON[2], A. PARSSON[3], A. VAN DYCKE[1], G. KUHN[3], P. BOON[1], E. BEN-MENACHEM[3], K. VONCK[1] ([1]UGent, [2]Univ. Oslo Norway, [3]Göteborg Univ. Sweden)
Effect of levetiracetam on hippocampal protein expression and cell proliferation in rats.

8. B. VAN NIEUWENHUYSE[1], T. WYCKHUYS[1], S. STAELENS[1], S. DELEYE[1], H. HALLEZ[1], K. VONCK[1], W. WADMAN[2], P. BOON[1] ([1]UGent, [2]Univ. Amsterdam NL.).
Evaluation of hippocampal deep brain stimulation by µSPECT.

9. V. COLOMBARO, V. VOISIN, A.E. DECLEVES, L. GIORDANO, I. HABSCH, B. FLAMION, N. CARON (FUNDPNamur).
Analysis of renal phenotype of hyaluronidase-1 of hyaluronidase-2 knockout mice.

10. V. HUBERT, V. VOISIN, A.E. DECLEVES, L. GIORDANO, I. HABSCH, N. CARON (FUNDPNamur).
Temporal evolution of inflammation and oxidative stress in the post-ischemic rat kidney.

11. I. KOPLJAR, A.J. LABRO, E. CUYPERS, J. RAINIER, J. TYTGAT, D.J. SYNDERS (UAntwerpen).
A novel binding site in voltage gated potassium channels revealed by the marine toxin gambierol.

12. M. BOL, M. DE BOCK, E. DE VUYST, N. WANG, E. DECROCK, M. VAN MOORHEM, B. VANHEEL, L. LEYBAERT (UGent).
Hemichannels contribute to Ca^{2+} dynamics in smooth muscle cells in acutely isolated small mesenteric arteries.

13. E. DECROCK[1], M. VAN MOORHEM[1], E. DE VUYST[1], N. WANG[1], M. DE BOCK[1], M. VINKEN[2], V. ROGIERS[2], C. ERNEUX[2], L. LEYBAERT[1] ([1]UGent, [2] VUBrussel).
Cell death communication via connexin channels investigated in a C6 glioma cell model.

14. N. WANG, E. DE VUYST, M. DE BOCK, E. DECROCK, M. VAN MOOORHEM, L. LEYBAERT (UGent).
Inhibition of connexin 43 hemichannel responses with high cytoplasmic calcium concentrations is mediated by mechanisms different from calcium activation of hemichannel responses.

15. E. DE VUYST[1], M. DE BOCK[1], N. WANG[1], E. DECROCK[1], M. VINKEN[2], M. VAN MOORHEM[1], V. ROGIERS[2], C.C. NAUS[3], W.H. EVANS[4], L. LEYBAERT ([1]UGent, [2]VUBrussel, [3]Univ. British Columbia Vancouver Canada, [4] Cardiff University U.K.).
Calcium regulation of connexin-43 hemichannel-mediated ATP release in glial cells.

16. E. MAS DEL MOLINO[1,2], X. GRANDES[1], L. BAHIMA[3], M. MARTIN SATUE[1,2], R. PUCHAL[4], L.C. BARRIO[5], J. MARSAL[1,2], L. LEYBAERT[6], C. SOLSONA[1,2], ([1]Univ. Barcelona, [2]BIERNED Barcelona, [3]Univ. California San Diego USA, [4]Hospital Univ. de Bellvitge, Llobregat, Spain, [5] Hospital Ramon y Cajal, Madrid Spain, [6]UGent).
Connexin 32, ATP release and X-linked Charcot Marie Tooth disease.

17. R. MACIANSKIENE[1,2,3], A. GWANYANYA[3], G. GESSNER[4], S.H. HEINEMANN[4], K. MUBAGWA[3], J.G. STARKUS[1] ([1]Univ. Hawai USA, [2] Kaunas Univ., Lithuania, [3]KULeuven, [4]Friedrich Schiller Univ. Jena, Germany).
Acceleration of hERG1 channel activation and deactivation by the amiodarone derivative KB130015.

18. A. SCHALLIER[1], A. MASSIE[1], E. LOYENS[1], D. MOECHARS[2], Y. MICHOTTE[1], I. SMOLDERS[1] ([1]VUBrussel, [2] Johson & Johnson, Beerse).
Decreased expression of vGLUT1 increases vulnerability to seizures.

19. A. MASSIE[1], S. GOURSAUD[2], A. SCHALLIER[1], K. VERMOESEN[1], L. ARCKENS[3], C. MESHUL[4], E. HERMANS[2], Y. MICHOTTE[1] ([1]VUBrussel, [2]UCLouvain, [3]KULeuven, [4]Oregon Health & Science Univ. Portland, USA).
Altered striatal glutamate transporter functioning could explain aberrant glutamatergic neurotransmission in striatum of hemi-Parkinson rat model.

20. K. VERMOESEN, I. SMOLDERS, A. MASSIE, Y. MICHOTTE, R. CLINCKERS (VUBrussel).
The control of kainic acid-induced status epilepticus.

21. H. DEMAEGDT[1], A. LUKASZUK[1], E. SZEMENYEI[1], G. TOTH[2], Y. MICHOTTE[1], D. TOURWÉ[1], G. VAUQUELIN[1] ([1]VUBrussel, [2]Hungarian Acad. Sciences, Szeged, Hungary).
[^{3}H]AL-11, a new stable and selective ligand for the IRAP/AT_4 receptor in both membranes and intact cells.

22. A. PACKEU[1], M. WENNERBERG[2], A. BALENDRAN[2], G. VAUQUELIN[1] ([1]VUBrussel, [2]AstraZeneca R&D, Mölndal, Sweden).
Simplified method for estimating dissociation rates of unlabelled ligand-receptor complexes.

23. S. STEURBAUT, S. ONS, T. LEYSEN, E. DE BAERE, L. LEEMANS, T. METS, A.G. DUPONT (VUBrussel).
Medication discrepancies at hospital discharge of elderly patients.

24. P. CORNU, S. STEURBAUT, L. LEEMANS, L. HUYGHENS, A.G. DUPONT (VUBrussel).
Reconciliation failure of chronic medication due to ICU admission.

25. S. HANON, D. SCHUERMANS, W. VINCKEN, H. VAN PARIJS, V. VINH-HUNG, G. STORME, S. VERBANCK (VUBrussel).
Abnormal small airways function in breast cancer patients.

26. M. BARAKA, L. LEEMANS, D. COOMANS, S. STEURBAUT, M. LAUBACH, E. JANSEN, A.G. DUPONT (VUBrussel).
Ethnicity and use of iron & folate during pregnancy.

27. I. REMORY, J. POELAERT (VUBrussel).
Isoflurane anesthesia inhibits firefly luciferase in a dose-dependent way: a pitfall for in vivo bioluminescence imaging.

28. D. SCHALLIER, F. TRULLEMANS, C. FONTAINE, L. DECOSTER, J. DE GREVE (VUBrussel).
Tyrosine Kinase Inhibitor (TKI)-induced macrocytosis.

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A B S T R A C T S

O-01

CLINICAL ADDED VALUE OF MEG IN PATIENTS WITH REFRACTORY PARTIAL EPILEPSY AND NON-LOCALIZING CONVENTIONAL PRESURGICAL EVALUATION

Carrette E.[1], De Tiège X.[2], Vonck K.[1], Meurs A.[1], Goossens L.[1], De Herdt V.[1], Van Dycke A.[1], El Tahry R.[1], Raedt R.[1], Thiery E.[1], Deblaere K.[1], Op De Beeck M.[2], Bourguignon M.[2], Goldman S.[2], Van Roost D.[1], Van Bogaert P.[2], Boon P.[1]
[1]Ghent University Hospital, Reference Center for Refractory Epilepsy, Ghent, 9000, Belgium
[2]ULB-Hopital Erasme, Brussels, 1000, Belgium

Introduction: Following conventional non-invasive presurgical evaluation (CNPE) for pharmacoresistant partial epilepsy, approximately half of patients are considered poor surgical candidates due to unclear localization of the epileptogenic zone. This study assesses the clinical added value of magnetoencephalography (MEG) in the presurgical evaluation of these patients.
Patients and methods: Thirty-two patients (mean age: 35 y;18 M) with refractory epilepsy and non-localizing CNPE results were included in this study. CNPE included clinical and neurological examination, neuropsychological evaluation, one week of video-electroencephalography (EEG) monitoring, optimized 3 Tesla structural brain magnetic resonance imaging (MRI) and [^{18}F]-fluorodeoxyglucose positron emission tomography (FDG-PET). Results of CNPE were considered non-localizing when no or multiple ictal onset zone(s) were identified during video-EEG monitoring (14 patients), when no or multiple lesions were identified on 3T MRI (5 patients) or when both investigations were non-localizing (13 patients). In 8/32 patients no interictal epileptiform discharges (IED) were observed during the video-EEG monitoring. All patients underwent 1-hour MEG recording using the whole-head 306-channel Elekta Neuromag® system installed into a lightweight magnetically shielded room (Maxshield, Elekta Neuromag Oy). Interpretable MEG data were visually screened for the presence of IED by 2 independent investigators (EC, XDT). Equivalent current dipoles (ECD, g/% >80%) were fitted in the patients' spherical head model and then superimposed on their co-registered MRI. ECD localisation was classified as either clustered or scattered. The added value of MEG was then evaluated by comparing the suitability of the patient for epilepsy surgery before and after adding the MEG results to the conventional investigations.
Results: Good signal-to-noise ratio was obtained in 27/32 patients (84%). IEDs were identified in 21/27 patients (78%) with interpretable MEG data. In 4/8 patients without IEDs on video-EEG monitoring, IEDs were observed in MEG data. ECDs were clustered in 16/21 patients and scattered in 5/21. MEG results changed patients' management in 7/21 patients (33%) either by preventing immediate RS without invasive video-EEG monitoring (IVEM, 1 patient), leading to a hypothesis for IVEM (2 patients) or by adjusting the electrode implantation scheme for the planned IVEM (4 patients).
Conclusion: This study highlights the clinical added value of MEG in the presurgical evaluation of refractory epilepsy patients when CNPE does not result in unambiguous localisation of the epileptogenic zone. Indeed, in 33% of patients with abnormal MEG and non-localizing CNPE, adding MEG to the presurgical evaluation changes the surgical management either by leading to IVEM or by adjusting the electrode implantation for the planned IVEM.

O-02

ANGIOTENSIN II TYPE 2 RECEPTOR STIMULATION DECREASES TYROSINE HYDROXYLASE ACTIVITY IN THE RAT STRIATUM

Mertens B. , Vanderheyden P., Michotte Y., Sarre S.
Department of Pharmaceutical Chemistry and Drug Analysis, Research Group Experimental Neuropharmacology, Vrije Universiteit Brussel, Brussels, 1090, Belgium

A relationship between the renin angiotensin system and the dopaminergic system has been described in the striatum. Until now, the role of the angiotensin II type 2 (AT_2) receptor in this interaction remained to be elucidated. The present study examined the outcome of direct AT_2 receptor stimulation on dopamine (DA) release and synthesis by means of the recently developed non-peptide AT_2 receptor agonist, compound 21 (C21). The effects of AT_2 receptor agonism on the release of DA and its major metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) and on the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine biosynthesis, were investigated using *in vivo* microdialysis. Local administration of C21 (0.1 and 1 µM) resulted in a decrease of the extracellular DOPAC levels, whereas extracellular DA concentrations remained unaltered, suggesting a reduced synthesis of DA. This effect was mediated by the AT_2 receptor since the decrease could be blocked by the AT_2 receptor antagonist PD123319 (1 µM). Interestingly, the effects could be reproduced by local (10 nM) as well as systemic (0.3 and 3 mg/kg i.p.) administration of the AT_1 receptor antagonist, candesartan. TH-activity as assessed by accumulation of extracellular levels of L-DOPA after inhibition of amino acid decaroboxylase with NSD-1015, were also reduced after local administration of C21 (0.1 and 1 µM) and candesartan (10 nM). Together, these data suggest that the AT_2 receptor in the striatum is involved in the modulation of DA synthesis rather than DA release, possibly via interaction with the AT_1 receptor.

O-03

THE PHARMACODYNAMIC PROFILE OF ARIPIPRAZOLE IS NOT ALTERED IN A MODEL OF STRIATAL DOPAMINERGIC HYPERSENSITIVITY

Koener B., Goursaud S., Van De Stadt M., Calas A.G., Jeanjean A.P., Maloteaux J-M., Hermans E.
Laboratoire de Pharmacologie Expérimentale, Université Catholique de Louvain,
1200 Brussels, Belgium

The switch from antipsychotic to aripiprazole can be challenging, as the classical receptor theory predicts that the intrinsic activity of partial agonists is altered when the density of target receptors is modified. Therefore, we hypothesized that the partial agonist properties of aripiprazole would be more easily revealed on hypersensitive rat striatal tissues. A model of chronic treatments of rodents with typical antipsychotics, known to lead to an up-regulation and a hypersensitivity of dopamine D_2 receptors was used. Therefore, the functional response to aripiprazole and other full and partial agonists, obtained by [^{35}S]GTPγS binding was compared in striatal membranes from naïve rats or those exposed for three weeks to haloperidol. Experiments were performed in classical binding conditions, and in conditions known to facilitate the detection of the functional responses to low intrinsic activity partial agonists (the substitution of NaCl by NMDG). The partial agonist properties of aripiprazole were not revealed in different binding conditions, either on naïve nor hypersensitized striatal rat membranes. However, changing the binding conditions helped to detect the partial agonist profile of (-)-3-PPP, which was even enhanced on hypersensitized tissue. The difference observed between responses induced by full and partial agonists in distinct binding conditions could underline the functional selective profile at the level of the D_2 receptor. However, further experiments investigating the second-messenger cascades should be performed so as to establish the functional properties of aripiprazole and understand the mechanism underlying the switching issues in clinic.

O-04

LITHIUM CHLORIDE INDUCES MACROPHAGE APOPTOSIS IN RABBIT ATHEROSCLEROTIC PLAQUES

De Meyer I.[1], Martinet W.[1], De Meyer G.R.Y.[1]
[1]University of Antwerp, Wilrijk, 2610, Belgium

Macrophages play a key role in atherosclerotic plaque destabilization and rupture, whereas smooth muscle cells (SMCs) contribute to plaque stability. Therefore, selective depletion of macrophages from plaques may be a promising strategy to stabilize the structure of the plaque. Lithium chloride (LiCl) has been shown to induce cell death of cultured macrophages. Here, we investigated whether LiCl could induce macrophage death without affecting the viability of SMCs.

In vitro studies showed that 30 mM LiCl led to cell death of cultured J774A.1 macrophages, whereas SMCs were highly resistant. Cell death was characterized by externalization of phosphatidylserine, caspase-3 cleaving and DNA fragmentation, all indicative of apoptosis. Although LiCl can exert osmotic effects, this was only seen from 100 mM onward, similar to 100 mM NaCl. Subsequently, we investigated whether LiCl-induced apoptosis was mediated via inhibition of inositol monophosphatase (IMPase). LiCl reduced myo-inositol-1,4,5-triphosphate (IP_3) levels in macrophages. Moreover, the specific IMPase inhibitor L-690,330 as well as IMPase gene silencing induced apoptosis of macrophages, similar to LiCl. These findings strongly indicate that LiCl-induced cell death was mediated by IMPase inhibition. *In vitro* treatment of atherosclerotic rabbit carotid artery rings with 30 mM LiCl resulted in induction of macrophage death, whereas SMC viability was unaffected. Local *in vivo* administration of LiCl via osmotic minipumps to collared rabbit atherosclerotic carotid arteries reduced the macrophage content in the plaques through apoptosis, as shown by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL). Vascular reactivity studies showed that LiCl did not affect the functionality of SMCs and endothelium.

In conclusion, LiCl selectively decreased the macrophage load in rabbit atherosclerotic plaques without affecting SMC viability.

O-05

HYPOXIA ENHANCES THE RELAXING INFLUENCE OF PERIVASCULAR ADIPOSE TISSUE

Maenhaut N., Boydens C., Van de Voorde J.
Department of Pharmacology, Ghent University, Ghent, Belgium

Aims. Recent studies propose a paracrine role for perivascular adipose tissue in the regulation of vascular tone. Adipose tissue from different species releases a factor lowering tone of isolated arteries. This factor is called the “adipocyte-derived relaxing factor” (ADRF). The potential influence of hypoxia on this relaxing influence was investigated using isometric tension recording of isolated mice aorta with or without adherent fat tissue.
Methods and Results. Aorta from male Swiss mice with or without adherent adipose tissue were mounted in a wire myograph for isometric tension recording. Hypoxia (bubbling with 95% N_2, 5% CO_2) relaxed precontracted (NOR, 5 µM) aorta with adipose tissue while only a minimal vasorelaxing effect was observed in arteries without adipose tissue. This effect was also seen after precontraction with prostaglandin $F_{2\alpha}$ (30 µM) or U-46619 (10 nM). Precontraction with 60 and 120 mM K^+, incubation with tetraethylammoniumchloride (3 mM) and glibenclamide (30 µM) significantly impaired the hypoxic response. Lactate (10 nM to 1 mM) did not induce vasorelaxation of preparations with or without adipose tissue. Only the vasorelaxing effect of high concentrations of NaHS was diminished by glibenclamide (30 µM). 8-(p-sulfophenyl)theophylline (0.1 mM), zinc protoporphyrin IX (10 µM), 1 H-[1, 2, 4]oxadiazolo[4,3- A]quinoxalin-1-one (10 µM) and removal of the endothelium did not influence the hypoxic relaxation.
Conclusions. Our findings indicate that hypoxia has a relaxing influence on mice aorta that is dependent on the presence of adherent adipose tissue. This relaxation is at least in part mediated by opening K_{ATP} channels and independent of the endothelium and sGC. Neither lactate, adenosine, CO or H_2S seem to be involved in this hypoxic response. However, the involvement of the as yet unidentified "adipocyte-derived relaxing factor" (ADRF) can not be excluded.

O-06

ACTIVITY OF SUNITINIB IN ADVANCED MALIGNANT MELANOMA AND ITS CORRELATION WITH POTENTIAL PREDICTIVE BIOMARKERS.

Decoster L.[1], Vande Broek I.[1], Anckaert E. [1], Declerq D.[1], De Mey J. [1], Majois F.[2], Baurain JF.[2], Denys H.[3], Neyns B. [1], De Grève J. [1]

[1]VUB 1090 Brussel, Belgium, [2]UCL 1348 Louvain-la-Neuve, Belgium, [3]UGent 9000 Gent, Belgium

Background:

Sunitinib is approved for the treatment of renal cell carcinoma and GIST tumours. It is a small molecule that inhibits members of the split-kinase domain family of tyrosine kinase receptors, including VEGFR, PDGFR, c-KIT and RET kinases. These kinases are important for neoangiogenesis, tumour cell proliferation and survival.

Treatment options for advanced melanoma after dacarbazine-based chemotherapy are limited. We report our results with sunitinib in advanced melanoma patients, whose disease failed at least one line chemotherapy.

Methods:

Patients with locally advanced or metastatic melanoma were enrolled in a Belgian academic multi-centre phase II trial, following a Simon's two-stage design. Patients received treatment with sunitinib in 6 weekly cycles comprising of a 50 mg once daily dosing for 4 consecutive weeks, followed by a 2-week off-treatment period. The primary end point of the study was RECIST-defined objective response.

Angiogenic biomarkers were collected to study their potential predictive value for response. Peripheral blood was drawn every 2 weeks during the first treatment cycle and serum VEGF, VEGFR-1, VEGFR-2 and PlGF levels were determined by ELISA. The number of circulating endothelial cells was enumerated weekly by FACS during the 1st cycle and at day 1 of following cycles.

Results:

Thirty four patients (28 evaluable) with metastatic melanoma have been enrolled in the study. Six patients were not evaluable for response because of early discontinuation of sunitinib, due to adverse events or inability to swallow medication. Two patients (7.2 % of evaluable) demonstrated a partial response as best response with a mean duration of 6.3 months, 9 had stable disease (27.8%) with a mean duration of 4.4 months and 17 (60,7%) had progressive disease (32%). Most frequent toxicities were asthenia (59%), anorexia (41%), nausea (32.3%), stomatitis (29.4%) thrombopenia (67.6%) and anemia (41.2%). Preliminary biomarker analysis shows a trend for correlation of response and outcome with baseline level of angiogenesis parameters and magnitude of early angiogenic response. The tumour genomic analysis is ongoing.

Conclusion:

In this phase II trial in advanced melanoma antitumor activity of sunitinib was detected, with an overall clinical benefit rate of 28%, although the primary endpoint (3 responses) was not met.
Further evaluation of this treatment should be in enriched patient populations.

O-07

NURSING STUDENTS's PHARMACOLOGICAL KNOWLEDGE AND CALCULATION SKILLS

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Objectives: Medication care is a main task of nurses. to prevent medication errors, nurses need pharmacological knowledge and skills. a nursing certificate should guarantee that the student has obtained those competences.
the goal of this study was to evaluate nursing students' pharmacological knowledge and calculation skills before graduation and to assess their feeling of readiness to deliver safe medication care in practice.
Method: All flemish schools with a nursing department, either a bachelors' (n=18) or a diploma degree (n=20), were asked to let their graduating students participate in a cross-sectional survey. depending on the preferences of the school, students completed the survey on paper or online. all data were collected in february or march 2009. students were asked to fill in some personal characteristics, to judge 25 statements on pharmacology, to solve 5 medication calculation questions and to score their feeling of readiness to deliver safe medication care in practice on a 10 point scale. the test was developed by a registered bachelor nurse and a registered master nurse and evaluated by a clinical pharmacist, a statistical expert experienced in nursing research and a medical doctor and pharmacologist. using spss 15.0, differences in discontinuous variables were defined by chi square tests and continuous data were analyzed with non-parametric statistics (mann-whitney u, kruskal wallis, spearman rank). a p-value <0.05 was considered significant. students results were anonymously collected and analyzed.
Results: participation levels were 16 schools for bachelor students and 9 schools for diploma students, with an average student response rate of 45% [24%-100%] per school, resulting in 404 bachelor students and 209 diploma students who completed the survey. the mean score on the pharmacology test was 54%. when limiting the statements to those seven who were in the core of nurses responsibility, the mean score was 59%. including only students who completed at least one exercise, the mean score on the calculation test was 62%. on a scale ranging from 1 to 10 for the readiness to deliver safe medication care in practice, students gave themselves an average score of 6.4.
Discussion and implications: only a short time before graduation, nursing students' pharmacological knowledge and calculation skills were rather limited. the mistakes in the knowledge test and the calculations could lead to major mistakes in practice, putting patients at risk. also students themselves were not convinced to be able to deliver safe medication care in practice.
Pharmacological education for nurses should be focused on the expected competences of nurses in practice and make sure students have these competences when graduating in order to improve patient safety. within this context, an evaluation of the educational content and strategies might be advisable in some schools.

O-08

NO EVIDENCE FOR INCREASED ACTIVITY OF THE L-ARGININE/NO PATHWAY IN MIGRAINE PATIENTS

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Objectives: To assess whether migraine patients display a chronic nitric oxide synthase (NOS) hyperactivity by comparing the nitric oxide (NO) production before and following a loading dose of L-arginine between migraineurs (interictally) and healthy controls. *Methods:* Twenty healthy subjects and migraineurs participated in a 2-period, randomized, double-blind, placebo-controlled study. They received either 30 g of L-arginine hydrochloride or placebo. Biomarkers associated with the L-arginine-NO pathway (i.e. exhaled/nasal NO (eNO/nNO), plasma citrulline and urinary excretion of nitrite/nitrate and cGMP) were assessed before and for 6 hours following the administration. *Results:* At baseline, eNO and nNO were higher in migraineurs than healthy subjects: 15.9 (8.8, 23.0) ppb versus 10.8 (7.0, 14.5) ppb for eNO (P = 0.04) and 76.3 (61.2, 91.4) versus 61.6 (51.2, 72.0) ppb for nNO (P =0.03), respectively. The AUC_{0-6} in ppb for eNO and nNO following L-arginine and saline infusion did not differ between both groups. The increase in L-citrulline following L-arginine infusion was slightly lower in migraine patients (15 (13,18) µmol/L) compared to healthy volunteers (19 (16,23) µmol/L) (P = 0.046). In healthy subjects, both nitrate and cGMP excretion were higher following L-arginine compared to placebo infusion: 132.63 (100.24, 165.02) versus 92.07 (66.33, 117.82) µmol $mmol^{-1}$ creatinine for nitrate (P = 0.014) and 50.53 (42.19, 58.87) versus 39.64 (33.94, 45.34) nmol $mmol^{-1}$ creatinine for cGMP (P = 0.0003), respectively. In migraineurs, excretion of these biomarkers was similar following L-arginine and saline infusion. *Conclusion:* The results of the present study do not support the idea of a generalized increase in NO synthase activity in migraine patients outside of a migraine attack.

O-09

INCREASED CEREBRAL TYPE 1 CANNABINOID RECEPTOR AVAILABILITY IN MIGRAINE PATIENTS

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Objective: To compare the in vivo type 1 cannabinoid receptor (CB1R) availability between healthy women and female migraine patients using positron emission tomography (PET) with ^{18}F-MK9470, a CB1R PET tracer and relate changes to disease characteristics.
Methods: 20 female migraine patients and 18 healthy women, matched for age and BMI, were investigated with ^{18}F-MK9470 PET and T1 MPRAGE MRI. Parametric standardized uptake value (SUV) images reflecting receptor availability were calculated. For regional analysis, SUV values were normalized on the individual global grey matter SUV. On these CB1R PET images, statistical parametric mapping (SPM2; $p_{height}<0.005$, uncorrected; $p_{cluster}<0.005$, corrected) was performed.
Results: Globally, cerebral CB1R availability was significantly increased in the migraine patients (average difference +16%). But the increase was not correlated with severity or frequency of migraine attacks. Increases in CB1R availability were most pronounced in the cingulo-frontal cortex and limbic system regions of the brain known to be involved in pain modulation
Conclusion: The results of the present study suggest that there are changes in cannabinoid signalling in the brain of migraine patients as manifest by an increase in CB1R availability. Future studies are therefore warranted into the role of this important endogenous pain modulatory system in predisposition and the pathophysiology of migraine that could lead to pharmacological advances in its treatment.

O-10

PHARMACOKINETIC STUDY OF CHEMOTHERAPEUTIC AGENTS IN PREGNANT WOMEN

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Background: Physiological changes during pregnancy affect major pharmacokinetic processes. However, data on pharmacokinetics of chemotherapeutic agents during pregnancy are scant.

Methods: In pregnant and non-pregnant patients receiving the same chemotherapy schemes, pharmacokinetic parameters were determined based on serial plasma concentrations over the first 48 h after administration. Drug levels were calculated using high performance liquid chromatography (HPLC) (doxorubicin, epirubicin and paclitaxel) or platinum with atomic absorption spectrometry (AAS) (carboplatin). Area under the curve (AUC), maximal plasma concentration (C_{max}), terminal half life ($t_{1/2}$), drug clearance and distribution volume (V_d) were determined using a non-compartimental pharmacokinetic analysis with WinNonLin Software. Bone marrow toxicity was used as a surrogate marker for tissue toxicity.

Results: Sixteen (16) and 11 cycles were available from pregnant and non-pregnant patients, respectively. Numbers of pregnant/non-pregnant patients tested for paclitaxel, doxorubicin, epirubicin and carboplatin were respectively as follows: 5/2, 7/5, 4/4, 2/2. During pregnancy V_d and clearance were increased and the AUC and C_{max} decreased. Changes were consistent among all drugs. In addition, a lower reduction in platelet count and hemoglobin was seen during pregnancy.

Conclusion: These data support the hypothesis that the physiological changes of pregnancy alter plasma levels of chemotherapeutic agents. In particular the reduced tissue toxicity warrants further investigation and long term follow up of women who needed chemotherapy during pregnancy.

O-11

TRPV1 ACTIVATION BY ALLYL ISOTHIOCYANATE

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Allyl isothiocyanate (mustard oil, MO) is a highly reactive electrophilic compound known to cause irritation, pain and inflammation. These effects are thus far though to be mediated by activation of TRPA1, a Transient Receptor Potential (TRP) cation channel expressed in nociceptive neurons. Recent research has shown that TRPV1, the heat and capsaicin receptor, can be also activated by reactive compounds such as allicin and leek and onion extracts. Here, we show that both human and mouse TRPV1 are activated by MO, at concentrations at which TRPA1 undergoes fast desensitization and block. In Ca^{2+} imaging experiments of intact HEK293 cells, MO induces an increase of the intracellular Ca^{2+}, which was not present when Ca^{2+} was omitted in the bath solution. Activation of TRPV1 by MO is dose-dependent and is caused by a shift of the voltage dependence of channel activation to more negative potentials, similar to the activation of TRPV1 by capsaicin. Stimulation of TRPV1 by MO can be observed in inside-out patches, indicating a membrane-delimited mechanism of activation. Notably, MO was able to stimulate a large population of sensory neurons isolated from *Trpa1* KO mice. This population was significantly reduced in *Trpa1/Trpv1* double KO mice, indicating the physiological importance of TRPV1 activation by MO. The response observed in the double KO mice is consistent with an activation of TRPM8. WT, *Trpa1* and *Trpv1* KO mice displayed significantly stronger aversion to MO than double KO mice in forced drinking and open field exploration assays. The identification of TRPV1 and TRPM8 as novel targets of MO is essential for the full understanding of the mechanisms of action of this compound *in vivo* and prompts to re-evaluate the results of previous research, in which MO was used as specific activator of TRPA1.

O-12

COMBINED EXPERIMENTAL AND COMPUTATIONAL APPROACHES TO STUDY THE ACTION OF BLOCKERS OF SMALL CONDUCTANCE CALCIUM-ACTIVATED POTASSIUM (SK) CHANNELS

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Small conductance calcium-activated potassium channels (SK) are widely expressed throughout the central nervous system (CNS) and underlie medium duration afterhyperpolarizations in many types of neurons. Three subtypes of SK channels, SK1, SK2 and SK3, have been identified so far in different parts of the brain. Blocking SK channels might be beneficial in the treatment of several CNS disorders such as depression, Parkinson's disease and cognitive disorders.
Until now, the precise site of interaction between these channels and their blockers has not yet been elucidated. In this context, molecular modeling is a theoretical approach that can quickly provide ideas on the binding mode of SK blockers. We first performed homology modeling of the S5-H5-S6 portion of the channels on the basis of the crystal structure of the KcsA potassium channel (Zhou et al. Nature. 2001, 414, 43-48). The binding sites of *N*-methyl-laudanosine (NML) (Scuvée-Moreau et al. J. Pharmacol. Exp. Ther. 2002, 302, 1176-83), a non-selective and non-peptidic ligand, and apamin (Blatz et al. Nature. 1986, 323, 718-20), an octadecapeptide with a preference for the SK2 subtype, were subsequently explored by docking analysis. Different amino-acids were suggested to interact with the two blockers. The docking of NML revealed a binding site in the turret region, far from the pore. The docking of apamin identified a very large binding site that includes a portion of the site of NML. In order to confirm the predicted binding sites, site-directed mutagenesis was used. The first mutant channels tested in electrophysiological experiments by the patch clamp technique validated some of the theoretical data. Using this strategy, we hope to get a better understanding of the mechanism of action of SK blockers and eventually find strategies to obtain subtype-selective blockers.

O-13

CONNEXIN CHANNELS CONTRIBUTE TO ENDOTHELIAL CA^{2+} DYNAMICS AND ALTER BLOOD-BRAIN BARRIER FUNCTION

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Endothelial cytoplasmic calcium ($[Ca^{2+}]_i$) is an important factor determining blood-brain barrier (BBB) permeability but little is known on the influence of $[Ca^{2+}]_i$ dynamics on BBB function. Here, we applied several conditions that trigger intercellular Ca^{2+} waves or intracellular oscillations and determined the involvement of connexin (Cx) channels and consequent effects on BBB function, making use of RBE4 and primary capillary endothelial cells. Exposure to low extracellular Ca^{2+} or bradykinin (BK) respectively triggered Ca^{2+} waves or oscillations that increased BBB permeability in a $[Ca^{2+}]_i$-dependent manner. Both Ca^{2+} waves/oscillations and BBB alterations were inhibited by the Cx mimetic peptide Gap27. Ca^{2+} wave propagation involves gap junctional communication and opening of hemichannels but hemichannels did not contribute as a BBB permeability-increasing pathway. Ca^{2+} oscillations were inhibited by Cx37/43 knockdown and involved hemichannel opening as well as autocrine purinergic signaling, but hemichannels were, as for Ca^{2+} waves, not the pathway of increased BBB permeability. Exposure to ATP triggered, like BK, Ca^{2+} oscillations but in contrast, these were not affected by Gap27 and did not disturb BBB function. We conclude that Cx channels and purinergic signaling contribute to endothelial $[Ca^{2+}]_i$ dynamics and are essential in altering BBB function.

O-14

MILD HYPOTHERMIA AFTER A TRANSIENT FOCAL CEREBRAL ISCHEMIA IN RATS: INFLUENCE ON CYTOKINES

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Hypothermia is a promising neuroprotective strategy to improve functional outcome after stroke. Cytokines are produced shortly after the insult and contribute to the resulting damage. Therefore, this study investigated the effect of mild hypothermia on pro- and anti-inflammatory cytokines after a transient focal cerebral ischemia. Endothelin-1 (Et-1), a vasoconstrictor, was infused in the vicinity of the middle cerebral artery to elicit a transient focal cerebral ischemia in male Wistar rats. Rats subjected to two hours of mild hypothermia (34°C) starting 20 minutes after Et-1 injection, were compared to a normothermic group (37°C). The parameters assessed were functional outcome (neurological deficit score), infarct volume and the concentrations of inflammatory (Interleukin-1 beta, Tumor Necrosis Factor alpha) and anti-inflammatory (Transforming Growth Factor beta) cytokines at different time points after stroke. Hypothermia significantly reduced the infarct volume compared to normothermia. This reduction correlated with behaviour as hypothermic rats showed a better outcome after the insult. However, even in normothermic rats, neurological deficit improved in time. Stroke induced an increase in pro-inflammatory cytokines in the core and the penumbra that peaks at 24 hours after the insult. Application of hypothermia lead to increased levels of Tumor Necrosis Factor alpha at 8 hours after the insult and decreased levels of the pro-inflammatory cytokines at 24 hours. In contrast, hypothermia had no effect on the stroke induced increase in Transforming Growth Factor beta. These results suggest that beneficial effects of hypothermia on infarct volume and functional outcome may be, at least in part, mediated by modulation of pro-inflammatory cytokine expression.

O-15

A REDUCTION OF GLUCOSE-INDUCED BURSTING FREQUENCY IN PANCREATIC ISLETS CORRELATES WITH DECREASED INSULIN RELEASE AND IMPAIRED GLUCOSE TOLERANCE IN *TRPM5*$^{-/-}$ MICE

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Glucose homeostasis is critically dependent on insulin release from pancreatic beta cells, which is strictly regulated by glucose-induced simultaneously oscillations in membrane potential (V_m) and cytosolic calcium concentrations $[Ca^{2+}]_{cyt}$. We propose that TRPM5, a Ca^{2+}-activated monovalent cation channel, is a positive regulator of glucose-induced insulin release. Micro-array screening and immunostaining reveal high and selective expression of TRPM5 in pancreatic islets. Whole cell current measurements demonstrate a Ca^{2+}-activated non-selective cation current with a bell-shaped dependency on intracellular Ca^{2+} in WT pancreatic islet cells. This current is significantly reduced in *Trpm5*$^{-/-}$ cells. Ca^{2+}-imaging and electrophysiological analysis show that glucose-induced oscillations of V_m and $[Ca^{2+}]_{cyt}$ have a reduced frequency in *Trpm5*$^{-/-}$ islets, due to a lack of fast oscillations. Fast oscillations in V_m show a shorter burst interval, due to a higher slope of depolarization towards the threshold potential for burst initiation. Our results indicate that TRPM5 accelerates the depolarization during the interburst interval, initiating rapid oscillations and higher insulin release. As a consequence, glucose-induced insulin release from *Trpm5*$^{-/-}$ pancreatic islets is significantly reduced, resulting in an impaired glucose tolerance in these mice. Pharmacological modulation of TRPM5 activity may represent a novel means to adjust insulin release in diabetic patients.

O-16

EXPRESSION AND LOCALIZATION OF NA^+-HCO_3^- COTRANSPORTER (NBCE1) IN THYROID AND ITS REGULATION BY TSH

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The purpose of this study was to determine the expression of Na-bicarbonate-cotransporter NBCe1 in rat and human thyroid. It was demonstrated both at the mRNA and protein levels in thyroid using RT-PCR, immunohistofluorescence, and Western blotting.. Three variant specific primers were designed but only two NBCe1 variant transcripts (NBCe1-B and NBCe1-C but not NBCe1-A variants) were detected in rat and human thyroid. The localization of NBCe1 in rat and human thyroid was determined using a commercial antibody against a region common to the three NBCe1 variants. By immunofluorescence, only the basolateral membrane was labeled in rat thyrocytes, while both apical and basolateral membranes were immunostained in human thyroid. By western blot analysis on human thyroid primary cultured cells and rat thyroid cell line PC Cl3 cells, a ~130 kDa band was detected, and its expression increased with TSH or forskolin stimulation of both human primary culture cells and rat PC Cl3 cells. To test whether this also occurs *in vivo*, four groups of rat were studied for up to 4 weeks: 1) control rats, 2) oral treatment with methimazole (MMI) or 3) perchlorate (Per) and, 4) thyroxine (T_4), each of them given in the drinking water. The animals were sacrificed; their thyroïd gland was removed and used for immunocytochemistry and western blot analysis. Using these methods, we observed an up-regulation of NBCe1 in the stimulated thyroid of MMI and Per treated groups but a down-regulation in the resting thyroid of the T_4 treated group. Thus NBCe1 expression is positively regulated by TSH.

P-01

BODY SIZE AFFECTS MORPHOLOGICAL ECHOCARDIOGRAPHIC PARAMETERS IN THE EQUINE SPECIES

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In various species including human beings and small animals, it has been shown that echocardiographic parameters are affected by physiological factors such as sex, age, breed, training or body size. The objective of this study was to evaluate the effect of body size on morphological echocardiographic parameters in the equine species.

Bidimensional and M-mode echocardiographic measurements (systolic and diastolic right and left ventricular and left atrial internal diameter, interventricular and left ventricular free wall thickness, and aortic and pulmonary internal diameter) were performed in 163 healthy horses. The correlation between those measurements and body weight, body surface area, body volume, height at withers, body length and thoracic circumference of the horses were studied using a simple linear regression test.

All echocardiographic parameters showed a strong (r^2 : 0.62-0.92) and significant ($p < 0.05$) correlation with all body size parameters, and the correlation obtained with each body size parameter was quite comparable. However, for most echocardiographic parameters, the correlation was stronger with the thoracic circumference than with the other body size parameters.

In conclusion, this study suggests that (1) whatever the parameter used to evaluate the body size, it significantly affects morphological echocardiographic parameters in the equine species, and (2) the thoracic circumference could be superior to evaluate the body size effect on echocardiographic parameters in this species.

P-02

ANTICONVULSIVE EFFECTS OF INSULIN-REGULATED AMINOPEPTIDASE DELETION IN A PENTYLENETETRAZOL MOUSE SEIZURE MODEL ARE NOT MEDIATED BY THE ACCUMULATION OF SOMATOSTATIN-14

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Rationale: The peptide angiotensin IV (Ang IV) has been shown to be antiepileptogenic in a mouse model of pentylenetetrazol (PTZ) kindling. It was suggested that one of the mechanisms by which Ang IV exerts these effects is by inhibiting insulin-regulated aminopeptidase (IRAP) and consequently prolonging the half-life of anticonvulsive substrate neuropeptides in the brain. Indeed, Stragier *et al.* found that the Ang IV-mediated inhibition of pilocarpine induced seizures in rats was prevented by a somatostatin-2 receptor antagonist, suggesting the involvement of somatostatin-14 (sst-14).

Methods: To unequivocally unravel the involvement of IRAP in seizure generation, IRAP knock-out (KO) mice and their wild-type (WT) littermates were subjected to an intravenous tail infusion of PTZ, which is an established acute model of generalised seizures. Via *ex vivo* enzymatic studies using the synthetic substrate L-leucine-p-nitroanilide, the aminopeptidase activity in cortical homogenates of IRAP WT and KO mice was measured. The degradation profile of labelled sst-14 in these homogenates was determined at different time intervals (0, 30, 60, 120 and 180 minutes) by using radio immuno assays (RIA).

Results: Compared to male WT mice, male KO mice showed significantly increased PTZ thresholds for myoclonic twitch, clonus without loss of reflexes and clonus with loss of reflexes. Female KO mice did not show any differences in PTZ thresholds compared to female WT littermates. However, the PTZ doses needed to induce the different behavioural manifestations were similar in male KO en female WT and KO mice. Enzymatic studies in brain cortex membranes of WT mice, using the synthetic IRAP inhibitor AL-11 and the synthetic AP-N inhibitor 7B, showed that 70% of the aminopeptidase activity was represented by IRAP. The remaining 30% was aminopeptidase-N (AP-N), non-IRAP and non-AP-N. In agreement, AP-N, non-IRAP and non-AP-N were the only active aminopeptidases in KO mice. Subsequently, these brain cortex membranes were incubated with exogenously added sst-14. RIA data indicate a time-dependent degradation of sst-14 which was similar in male WT and KO mice. Female KO mice, however, showed 25% less degradation of sst-14 in comparison with female WT.

Conclusions: This study shows that IRAP is involved in seizure generation, since male IRAP KO mice are less sensitive to the development of generalized seizures following PTZ administration. Unfortunately, the mechanism by which IRAP inhibition leads to anticonvulsive effects remains elusive as our comparable study between males and females showed that it is not due to the accumulation of sst-14.

P-03

ANTICONVULSANT EFFECTS OF GHRELIN RECEPTOR LIGANDS AGAINST PILOCARPINE-INDUCED LIMBIC SEIZURES.

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*Rationale***:** Ghrelin is a 28 amino acid-containing bioactive peptide that plays a significant role in various functions, such as in the modulation of appetite, energy and glucose homeostasis. Studies are also implicating this peptide in the pathogenesis of many diseases, namely growth hormone deficiency, obesity and myopathy. Ghrelin is a peptide that is predominantly produced in the stomach, however it is also produced in the brain. This hormone is known to be a natural ligand of the growth hormone secretagogue (GHS) receptor type 1a (GHS-R1a) of which the mRNA is abundantly expressed in various brain structures such as the hypothalamus and hippocampus. The role of ghrelin in the mechanisms of epileptic seizures is not well explored to date. There are currently two studies showing that ghrelin has an inhibitory effect on seizures (Obay et al., 2007; Aslan et al., 2009), while another study illustrates that ghrelin protects against hippocampal neuron cell death following pilocarpine-induced status epilepticus (Jingjing et al., 2009). No studies have been performed to date to study the role of ghrelin receptor agonists in limbic seizures and to unravel whether the GHS-R1a receptor is the primary receptor involved in the possible anticonvulsant effects. To this end, two selective GHS-R1a antagonists were used. *Methods:* Freely moving Wistar rats underwent a 2 hour intrahippocampal microperfusion of the selective ghrelin receptor agonist capromorelin (0.5-1-10-20µM), the ghrelin receptor antagonist D-Lys^3-GHRP-6 (40µM) or the ghrelin receptor antagonist A-778139 (10-25-50µM) via a stereotactically implanted microdialysis probe. Ten mM pilocarpine was subsequently co-administered intrahippocampally for 40 min. Behavioural changes that were indicative of seizure activity were scored following the initiation of pilocarpine administration. *Results:* Capromorelin dose-dependently suppressed pilocarpine-induced limbic seizures. Surprisingly, both ghrelin receptor antagonists D-Lys^3-GHRP-6 and A-778139 also showed anticonvulsant properties. *Conclusions:* Our results suggest that activation of an unknown ghrelin receptor, other than GHS-R1a, results in the attenuation of locally provoked limbic seizures since both the agonist and GHS-R1a antagonists reproduce the same anticonvulsant effect.

P-04

APOMORPHINE INDUCED NEUROPROTECTION IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

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We investigated the neuroprotective effects of the dopamine (DA) receptor agonist R-apomorphine (R-APO) [10mg/kg/day, subcutaneously (s.c.) for 11 days] in the striatal 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease (PD). The treatment was started either 15' before or 24h after lesioning. R-APO was also administered to intact rats. Two weeks after lesioning, the neuroprotective effects were evaluated using behavioural, neurochemical and histological tests. The *in vivo* salicylate trapping technique was used to investigate the radical scavenging properties of R-APO administered s.c. on 6-OHDA-induced hydroxyl (•OH) radical formation. We also studied the effect of R-APO on the neurotrophic factor fibroblast growth factor 2 (FGF-2) mRNA expression in the striatum of intact rats. Both in the striatum and the substantia nigra pars compacta (SNpc), 6-OHDA induced a lesion of about 50%. The treatment significantly reduced the amphetamine-induced ipsiversive rotations, the size of the lesion at the level of the SNpc and ventral tegmental area (VTA), and the 6-OHDA induced striatal DA depletion. The number of cells in the VTA significantly increased in intact rats, suggesting a neurotrophic action of R-APO. We observed that R-APO had no effect on the 6-OHDA-induced •OH radical formation. Preliminary data show an increase of the striatal FGF-2 mRNA levels. We conclude that R-APO has a neuroprotective and possibly a neurotrophic effect in our rat model. This may, at least in part, be mediated by an up-regulation of FGF-2.

P-05

IN VIVO MEASUREMENTS OF VAGUS NERVE COMPOUND ACTION POTENTIALS IN RATS.

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Rationale: Nerve fiber activation may be a useful biological marker for understanding vagus nerve stimulation (VNS) mechanism of action and optimalisation of VNS parameter choice in clinical practice.This study was performed to evaluate whether compound action potentials (CAP) of the vagus nerve could be measured in a chronic fashion after implantation of a stimulation/recording cuff electrode around the cervical vagus nerve of rats.

Method: Male Wistar rats (N=11) were implanted with a cuff electrode, composed of two rectangular (3x1 mm) platinum stimulation poles, spaced by 1mm and one square (1x1 mm) platinum recording contact at 2 mm rostral of the cathode. Stimulation pulse (bipolar rectangular, 500µsec pulsewidth) varied from 0 to 200 µA.

Intra-operative CAP's were recorded and in/output (I-O) curves, illustrating the relationship between stimulus intensity (input) and the size of the compound evoked potential (output), were determined. All measurements resulted from recording of the 1x1 mm contact in the cuff electrode compared to a ground, which consisted of a EEG electrode in right occipital skull. CAP measurements were performed every week during 4 weeks after implantation. I-O data were fitted to a Boltzmann sigmoidal function. From this curves we deduced stimulation intensity needed to activate a 50% response (=b) and steepness of the curve (=c), which indicates capacity of nerve activation, i.e. intensity needed to achieve maximal stimulation.

Results: In 11 rats, CAP's were measured intra operatively. Mean value for b intra operatively was 68µA +/- 25µA. At week one, mean b value was 55 µA +/- 12 µA, at week two 43 µA +/-15 µA, at week three 67 µA +/- 30 µA and finally at week four 75 µA +/- 16 µA.

Intra operatively mean c values were 10,3 µA+/- 4,3 µA, at week one 3,4 µA +/-0,9 µA, at week two 4,4 µA+/- 2,6 µA, at week three 3,8 µA +/-1,1 µA and finally at week four 2,8 µA+/- 0,3 µA. Intensity needed to activate a 50% response and steepness of the curve did not change significantly over time compared to day of surgery.

Conclusions: Intra operative recording of rat vagus nerve CAP with an implantable cuff electrode is feasible. In 6/11 rats CAP's were measurable and I-O curves of remained stable over time. Moreover, our CAP data illustrate principle of 'all or nothing' of vagus nerve activation: c values were very small, which indicate the existence of a small window between threshold en maximal activation intensity.

Finally, CAP measurements can be useful tool as biological marker of stimulation in all future VNS experiments.

P-06

HIPPOCAMPAL DEEP BRAIN STIMULATION IN AN ANIMAL MODEL FOR TEMPORAL LOBE EPILEPSY

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Temporal lobe epilepsy is one of the most difficult to treat forms of epilepsy. One third of the patients is or becomes refractory to anti-epileptic drugs, emphasizing the need for new therapeutic strategies, among which hippocampal deep brain stimulation (DBS). In this study we compare the efficacy of two stimulation paradigms: Poisson distributed stimulation (PDS) is compared with High Frequency Stimulation (HFS) in the kainic acid model, a validated model for human temporal lobe epilepsy.

Status epilepticus (SE) was induced by intraperitoneal injection of kainic acid, leading to epileptogenesis. After fifty days, rats with spontaneous seizures were implanted with depth stimulation and recording electrodes in the hippocampus. After 15 days of continuous baseline EEG monitoring, 13 rats received continuous PDS (mean frequency 130 Hz) and 11 received regular HFS (fixed frequency of 130 Hz) during the following 10 days. The maximum stimulus intensity at which rats could be stimulated without experiencing EEG and/or behavioral side effects was significantly lower for PDS than for HFS ($p<0.02$). Seizure frequency and seizure duration were continuously monitored before, during and after PDS and HFS.

Seven out of 13 rats (54%) treated with PDS and 5 out of 11 rats (45%) treated with HFS experienced a significant reduction in seizure frequency and were considered responders. In them seizure frequency was reduced with 67% from baseline ($p<0.01$) during PDS and with 50% from baseline ($p<0.01$) during HFS. None of the stimulation modalities affected mean seizure duration. After termination of the stimulation, the effect induced by PDS faded away in days restoring seizure frequency to its pre stimulus levels. The other 12 non-responder rats did not demonstrate any reduction in seizure frequency.

We conclude that continuous hippocampal PDS with a mean frequency of 130 Hz is an interesting new stimulation paradigm, which significantly reduces spontaneous seizure frequency in a large fraction of the epileptic rats. Its efficacy, even at a lower stimulus intensity, is larger than that of the equivalent regular HFS at 130 Hz.

P-07

EFFECT OF LEVETIRACETAM ON HIPPOCAMPAL PROTEIN EXPRESSION AND CELL PROLIFERATION IN RATS.

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Introduction

Levetiracetam (LEV) is a broad-spectrum anti-epileptic drug (AED). The precise mechanism of action (MOA) of LEV remains unclarified. To further unrafel the potential MOA pathways of LEV this study investigated altered protein expression and cell proliferation in rat hippocampal tissue during LEV administration.

Methods

On day 1 of the experiment, rats (n=20) were randomly assigned to a treatment group (LEV, 600 mg/kg/day, n=10) and a control group (saline, n=10). On days 2 and 3 rats were injected with BrDU (50mg/kg, i.p., BID). On day 7 the right brain hemisphere was processed for histology. The left hippocampus was dissected and frozen for proteomic analysis. Proteins were extracted from the tissue and two-dimensional gel electrophoresis was performed.

Results

Treatment with levetiracetam did not influence cell proliferation in the hippocampus. Multivariate analysis of differential protein expression, determined by proteomic analysis, revealed a significant clustering of the control and treatment groups. Twenty of the most up- and down-regulated proteins, correlating to the dependent variable, were identified by mass spectroscopy.

Discussion

Although levetiracetam does not influence hippocampal cell proliferation, it does have a significant effect on the expression of proteins involved in a variety of physiological processes. Further work needs to be done to identify which of these proteins are involved in the anti-epileptic effects of levetiracetam.

P-08

EVALUATION OF HIPPOCAMPAL DEEP BRAIN STIMULATION BY µSPECT

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Rationale: Deep Brain Stimulation (DBS) is a promising experimental approach to treat various neurological disorders. Hippocampal DBS has recently been successfully used to treat refractory Temporal Lobe Epilepsy patients. However, the precise mechanism of action of hippocampal DBS and the affected pathways are unknown and may possibly hamper its therapeutic potential. Neuro-imaging by means of Single Photon Emission Computed Tomography (SPECT) is a non-invasive manner of evaluating regional cerebral blood flow (rCBF) changes, which are assumed to reflect changes in neural activity. In this study, rCBF changes induced by different stimulation paradigms of hippocampal DBS were evaluated by means of subtraction analysis following small animal SPECT (µSPECT) in the rat brain.

Methods: Rats (n=7) were implanted with a multi-contact DBS electrode in the right hippocampus. After recovery from surgery, rats received 10mCi HMPAO-$Tc99^{m}$ every day for several days either during application of hippocampal DBS (various stimulation paradigms) or during sham stimulation. Consequently, µSPECT scans of the brain were manually co-registered with 3T-MRI images of the same rat. Co-registered images were evaluated by means of subtraction analysis.

Results: Hippocampal DBS with bipolar Poisson distributed stimulation caused a significant decrease in rCBF, both in the ipsi- ($p<0.01$) and contralateral hippocampus ($p<0.001$) as well as in commissura hippocampalis ($p<0.01$). The spatial extent of the area of decreased perfusion was correlated with the intensity of hypoperfusion ($p<0.001$). Other stimulation paradigms also induced hypoperfusion in these structures, but the rCBF-changes were less prominent.

Conclusions: Small animal SPECT allows us to draw conclusions on the location, the spatial extent and the intensity of the rCBF changes induced by hippocampal DBS. Depending on the stimulation paradigm used, significant hypoperfusion was observed in the ipsi- and contralateral hippocampus as well as the commissura hippocampalis. Our results promote further research on stimulation parameters for DBS using µSPECT in rats.

P-09

ANALYSIS OF RENAL PHENOTYPE OF HYALURONIDASE-1 OR HYALURONIDASE-2 KNOCKOUT MICE

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Hyaluronan (HA), a glycosaminoglycan involved in many biological processes (i.e. cell migration and differentiation, regulation of extracellular matrix organization, inflammation) is synthesized by hyaluronan synthases and degraded by hyaluronidases (Hyals), mainly Hyal1 and Hyal2 in somatic tissues. Since intrarenal HA is mainly restricted to the inner medulla, where HA has been hypothesized to play a role in water handling, our aim was to determine to what extent the contribution of Hyal1 and Hyal2 in HA metabolism could modulate urine concentration. To do so, the renal phenotype of *Hyal1*$^{-/-}$ and *Hyal2*$^{-/-}$ mice was analysed in baseline conditions, during 24 hours water deprivation (WD) or after acute water loading (2 ml sterile water i.p.). Our results indicated that, compared to wild-type littermates (n=20), *Hyal1*$^{-/-}$ mice (n=20) are characterized by a significant lower urine output (ΔUV= -25%), a higher urine osmolarity (ΔUosm=15%) and lower Na^+ and K^+ excretion rates (Δ= -27% and -34%, respectively). In contrast, baseline renal parameters were similar in *Hyal2*$^{-/-}$ (n=16) vs *Hyal2*$^{+/-}$ mice (n=20). After WD, a significant reduction in UV was observed, which was more pronounced in *Hyal1*$^{+/+}$ mice (Δ= -65 vs -40%, P=0.02, n=9), and the expected increase in Uosm only occurred in *Hyal1*$^{+/+}$ mice. In *Hyal2*$^{+/-}$ and *Hyal2*$^{-/-}$ mice, changes induced by WD were similar in both strains, averaging -60% and +65% for ΔUV and ΔUosm, respectively. In response to the test of acute water loading, all *Hyal1*$^{+/+}$ mice (n=9) started to excrete water during the first hour, whereas none of the *Hyal1*$^{-/-}$ mice (n=9) produced any urine, as it was also the case in *Hyal2*$^{+/-}$ (n=10) and *Hyal2*$^{-/-}$ mice (n=10). Nevertheless, the total excreted volume of water was similar in the four groups, averaging 1200 µl after 6 hours. In summary, these observations could indicate that *Hyal1*$^{-/-}$ mice present some impairment in the urine concentration mechanisms whose underlying processes have to be further investigated.

P-10

TEMPORAL EVOLUTION OF INFLAMMATION AND OXIDATIVE STRESS IN THE RAT POST-ISCHEMIC KIDNEY

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It has been previously reported that the Wistar-Furth (WF) rats are protected against chronic renal failure induced by several models, showing preserved renal nitric oxide (NO) production compared to control rats. In the present study, our aim was to evaluate the morpho-functional responses to renal ischemia-reperfusion (IR) in WF and Wistar-Hanover (WH) rats. To do so, we analysed the temporal evolution of renal function, oxidative stress and inflammation. Renal excretory capacities were evaluated throughout a 14-days period post-IR in conscious rats placed in metabolic cages. Inflammation was assessed by measuring intrarenal monocyte-chemoattractant protein-1 (MCP-1) levels at different time-points after IR, in correlation with immunostaining of ED1-positive cells. Oxidative stress markers were measured in urine, i.e. hydrogen peroxide (H_2O_2), malonedialdehyde (MDA), as well as NO metabolites (NOx). Our results indicated that the increase in urine output, associated with a lowered osmolarity observed during the first 5 days post-IR, were more pronounced in WF rats, compared to WH rats. Simultaneously, the early increase in urinary excretion of NGAL, a new biomarker of renal injury, was attenuated in WF rats. Intrarenal MCP-1 levels were characterized by significant peak values at 24h post-IR, with no difference between the two strains, while the interstitial accumulation of ED1-positive cells was maximal at day 7. As for oxidative stress, H_2O_2 and MDA urinary excretion rates were significantly enhanced as soon as 24h post-IR, and thereafter returned towards baseline at day 7, with a similar temporal pattern in both strains. NOx urinary excretion rates were similarly decreased in WF and WH rats at 48h post-IR and returned to baseline at day 7. In summary, these preliminary results seem to indicate that the functional difference observed between WF and WH rats could not be related to differences in NO production, nor inflammation or oxidative stress, and therefore need to be further investigated.

P-11

A NOVEL BINDING SITE IN VOLTAGE GATED POTASSIUM CHANNELS REVEALED BY THE MARINE TOXIN GAMBIEROL

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Abstract

Gambierol is a marine polycyclic ether toxin belonging to the group of ciguatera toxins. It does not activate voltage gated sodium channels (VGSC), but inhibits Kv1 potassium channels by an unknown mechanism. While testing whether Kv2, Kv3 and Kv4 channels also serve as targets, we found that Kv3.1 was inhibited with an IC_{50} of 1.2 ± 0.2 nM while Kv2 and Kv4 channels were insensitive to 1 µM gambierol. Onset of block was similar from either side of the membrane and gambierol did not compete with internal cavity blockers. The inhibition did not require channel opening and could not be reversed by strong depolarization. Using chimeric Kv3.1-Kv2.1 constructs, the toxin sensitivity was traced to S6, in which T427 was identified as a key determinant. In Kv3.1 homology models, T427 and other molecular determinants (L348, F351) reside in a space between S5 and S6 outside the permeation pathway. In conclusion, we propose that gambierol acts as a gating modifier binding to the lipid exposed surface of the pore domain thereby stabilizing the closed state. This site may be the topological equivalent of the neurotoxin site 5 of VGSCs. Further elucidation of this novel binding site may explain why most ciguatoxins activate VGSCs, while others inhibit Kv channels. This novel Kv neurotoxin site may have wide implications not only for our understanding of channel function at the molecular level, but also for future development of drugs to alleviate ciguatera poisoning or to modulate electrical excitability in general.

P-12

HEMICHANNELS CONTRIBUTE TO CA^{2+} DYNAMICS IN SMOOTH MUSCLE CELLS IN ACUTELY ISOLATED SMALL MESENTERIC ARTERIES

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Intracellular Ca^{2+} mediates a variety of vascular endothelial and smooth muscle cell functions. Smooth muscle cells (SMC) respond to biological activators with oscillatory and propagating rises in $[Ca^{2+}]_i$ that are highly organized in both time and space. Gap junctions (GJs) play a crucial role in the communication between vascular cells and in the synchronization of Ca^{2+} signals thereby tightly controlling the level of vasoconstriction. Before being incorporated into GJs, connexin (Cx) hemichannels reside in the plasma membrane in a closed state. Recent evidence suggests that hemichannels can be opened by various messengers and conditions, thereby forming a pore that allows the passage of ATP and ions. Using confocal microscopy and the Ca^{2+} sensitive dye Fluo-3, we examined the role of hemichannels in dynamic Ca^{2+} responses of SMC in intact acutely isolated small rat mesenteric arteries. Norepinephrine (3 µM) induced Ca^{2+} oscillations that were reduced in frequency by 98.4 % ($p< 0.05$) when exposed to carbenoxolone (50 µM), a none specific Cx channel inhibitor. Gap27 (200 µM), a Cx mimetic peptide that mainly blocks hemichannel responses (assayed by ATP release and dye uptake) after short incubation, reduced the spiking frequency by 96.4 % ($p< 0.05$). Suramin (200 µM) and PPADS (75 µM), two P2Y receptor antagonists, decreased the spiking frequency by 90.5 % ($p<0.05$) and 96.4% ($p<0.01$) respectively. Apyrase (5 U/ml), an enzyme that rapidly degrades extracellular ATP, reduced the spiking frequency by 71.4% ($p<0.01$). None of these agents affected the amplitude of the Ca^{2+} oscillations. Our results suggest a role for Cx hemichannels and purinergic signaling in Ca^{2+} oscillations of SMC stimulated with norepinephrine. Further work will be directed to assess the involvement of these signaling partners in contractile responses of these vessels.

P-13

CELL DEATH COMMUNICATION VIA CONNEXIN CHANNELS INVESTIGATED IN A C6 GLIOMA CELL MODEL

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A characteristic feature of astrocytes is their high level of intercellular communication mediated by connexin (Cx) channels, i.e. gap junction channels (GJs) connecting the cytoplasm of adjacent cells and hemichannels (hemi-GJs; HCs) forming a paracrine conduit between the cytoplasm and the extracellular environment. This type of network organization is not only of major importance for their neuronal supportive function but may have detrimental consequences as well, contributing to exacerbation of injury. The present study was set up to determine the contribution of both Cx channels in the communication of apoptosis towards surrounding cells.

We used an *in situ* electroporation technique to load a localized area of an adherent C6 glioma cell culture, stably transfected with Cx43 (C6Cx43), with the apoptotic agent Cytochrome C (CytC) and found that healthy surrounding cells underwent apoptotic transformation. Further work with wild type cells, inhibitors of GJs and/or HCs and Cx43 gene silencing showed that GJs contribute to the spread of apoptosis in a zone next to where apoptosis was triggered while HCs also promoted cell death beyond this area. Application of the calcium chelator BAPTA-AM and an inositol trisphosphate (IP_3) degrading phosphatase reduced the cell death mediated by GJs as well as HCs, suggesting that calcium and IP_3 are involved in both processes.

We conclude that Cx43 HCs can function as an entry route or a leakage pore for pro-apoptotic messengers, thereby playing a role in communicating cell death messages in concert with their GJ counterparts.

P-14

INHIBITION OF CONNEXIN 43 HEMICHANNEL RESPONSES WITH HIGH CYTOPLASMIC CALCIUM CONCENTRATIONS IS MEDIATED BY MECHANISMS DIFFERENT FROM CALCIUM ACTIVATION OF HEMICHANNEL RESPONSES

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Connexin 43 (Cx43) hemichannel (HC) responses can be activated by $[Ca^{2+}]_i$ changes according to a bell-shaped response curve with activation of the responses at moderate $[Ca^{2+}]_i$ transients (peak below ~500 nM) and disappearance of the responses (further called inactivation) with $[Ca^{2+}]_i$ transients peaking above ~500 nM. We further explored the mechanisms of $[Ca^{2+}]_i$-dependent inactivation. $[Ca^{2+}]_i$–triggered HC responses have been demonstrated to involve an intermediate step of calmodulin (CaM) and arachidonic acid (AA) signaling (see abstract & poster of De Vuyst E. et al.). ATP release assays on HC responses triggered in C6 glioma cells stably transfected with Cx43 cells by Ca^{2+}-independent CaM activation with the peptide CALP 1 demonstrated an S-shaped concentration-response curve, indicating that high $[Ca^{2+}]_i$ related inactivation was absent with this trigger. Dye uptake assays (PI and EtBr) with AA-triggered responses revealed a similar S-shaped concentration-response behavior (half-maximal activation at ~330 µM). We next triggered HC dye uptake with AA and combined this trigger with various concentrations (6-12 µM) of the Ca^{2+}-ionophore A23187 to induce large (µM) $[Ca^{2+}]_i$ transients. These experiments demonstrated concentration-dependent suppression of AA-triggered HC responses, confirming that high $[Ca^{2+}]_i$ levels inhibit HC responses. In a next step, we investigated whether the $[Ca^{2+}]_i$- dependency of HC responses was related to Cx43 phosphorylation. Western blot analysis showed that A23187 concentrations activating HC responses reduced the degree of non-phosphorylated Cx43 protein (presumably due to the Ca^{2+}-activated kinases) and that higher A23187 concentrations, that cause HC inactivation, restored the degree of non-phosphorylated Cx43. A possible explanation for this observation may reside in the activation at high $[Ca^{2+}]_i$ of a Ca^{2+}-dependent serine/threonine protein phosphatase such as calcineurin. Inhibition of phosphatase type 2B with 2 µM cyclosporin A indeed reduced the high $[Ca^{2+}]_i$-induced dephosphorylation and furthermore prevented inactivation of the HC responses probed with dye uptake. Our results demonstrate that the modulation of HC responses by $[Ca^{2+}]_i$ is a bi-modal process each governed by distinct signaling mechanisms.

P-15

CALCIUM REGULATION OF CONNEXIN-43 HEMICHANNEL-MEDIATED ATP RELEASE IN GLIAL CELLS

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Connexin (Cx) hemichannels are closed before being incorporated into gap junctions but can be opened by various stimuli, thereby forming a release pathway for small paracrine messengers. Here, we investigated hemichannel-mediated ATP release in response to changes of intracellular Ca^{2+} ($[Ca^{2+}]_i$) making use of C6 glioma cells stably transfected with Cx43 and primary glial cells isolated from rat cortex. The involvement of hemichannels was studied with gja1 gene silencing experiments and the exclusion of other release mechanisms. Hemichannel-related ATP responses appeared when $[Ca^{2+}]_i$ changes, brought about by exposure of the cells to the Ca^{2+} ionophore A23187, were in the 500 nM range while these responses disappeared with larger $[Ca^{2+}]_i$ transients. Ca^{2+}-triggered responses induced by A23187 and also glutamate activated a cascade sequentially involving calmodulin (CaM), calmodulin-dependent kinase II (CaMK-II), p38 mitogen activated kinase (MAPK), arachidonic acid (AA), reactive oxygen species (ROS) and NO. This cascade was also activated by stimulation at intermediate points such as activation of CaM with the Ca^{2+}-like peptide (CALP1) or exogenous application of AA, and was also operational in primary glial cell cultures (astrocytes in majority) isolated from rat cortex. The disappearance of hemichannel responses with high $[Ca^{2+}]_i$ was independent of CaM signaling. We conclude that ATP release via Cx43 hemichannels can be invoked by $[Ca^{2+}]_i$ via an intermediate signaling axis that is combined with distinct OFF-mechanisms that help to limit and control the release process.

P-16

CONNEXIN 32, ATP RELEASE AND X-LINKED CHARCOT MARIE TOOTH DISEASE.

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Connexins are the proteins responsible of forming gap junctions. Six subunits are necessary to form a hemichannel or connexon anchored in the plasma membrane. Two hemichannels of adjacent cells form a gap junction. At present, there are, at least, 20 isoforms of connexins described. Particularly, mutations in Connexin 32 (Cx32) have been associated with the X-linked form of Charcot-Marie-Tooth disease (CMTX), a neurodegenerative illness affecting the peripheral nervous system. There is growing evidence that ATP is being released, under some stimulus, through hemichannels constituted either by connexins or pannexins. Using immunofluorescent methodology we have localized Connexin 32 (Cx32) in Schwann cells (SCs) of teased nerve fibers from mice sciatic nerve, concretely in the paranodal zones of nodes of Ranvier and in the Schmidt-Lantermann incisures. We have assayed to detect the release of ATP from mechanical and electrical stimulated nerve fibers through the Luciferin-Luciferase bioluminescent reaction using the Orca II and the ImagEM digital cameras (Hamamatsu). Under these conditions we found the maximal release of ATP in the paranodal zones of Schwann cells. Because Schwann cells express some forms of P2X and P2Y receptors, we hypothesize that, in physiological conditions, Schwann cells release ATP which would act as an autocrine signal.

We have expressed the human Cx32 (hCx32) in *Xenopus laevis* oocytes and we have measured simultaneously the electric currents supported by Cx32 and the release of ATP. We have recorded that hCx32 is permeable to ATP when is forming hemichannels. Five mutations of Cx32 have been tested for ATP release and in all mutations tested (*hCx32 S26L; hCx32 P87A; hCx32 Del111-116; hCx32 D178Y* and *hCx32 R220X*) the ATP release was inhibited or completely abolished

In view of our results**,** we suggest that mutations of Cx32 described in CMTX disease may be due to an impairment of autocrine purinergic signalling, which should be a key process to maintain SCs in healthy conditions.

P-17

ACCELERATION OF HERG1 CHANNEL ACTIVATION AND DEACTIVATION BY THE AMIODARONE DERIVATIVE KB130015

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KB130015 [2-methyl-3-(3,5-diiodo-4-carboxymethoxybenzyl)benzofuran] is an analogue of amiodarone, synthesized with the aim to keep beneficial but avoid side effects of the parent molecule. The drug was expected to have an action on various ion currents, especially a block of delayed rectifier currents as is the case with amiodarone. We have investigated the effect of KB130015 using the patch-clamp technique at 22℃ on isolated membrane patches of *Xenopus* oocytes heterologously expressing hERG1, and the whole-cell voltage-clamp at 35℃ on pig ventricular myocytes. In *Xenopus* oocyte membranes, 10 μM KB130015 had no effect when applied on the extracellular side of outside-out patches but markedly modified hERG1 currents when applied on the intracellular side of inside-out patches. In patches expressing wild-type channels, the drug increased the amplitude and shortened the time-to-peak of the hERG1 current elicited upon depolarization, and accelerated the rate of channel deactivation. In mutant S620T channels, which lack inactivation and were used to quantify the effect on activation, KB130015 accelerated the activation kinetics and shifted the steady-state voltage-dependent activation to more negative potentials. In contrast, amiodarone applied on the intracellular side of the patches only displayed a potent blocking effect. In cardiac cells exposed to extracellular KB130015, I_{Kr} was unchanged, whereas in cells dialyzed with 10 μM of the drug I_{Kr} deactivation tended to be faster. We conclude that KB130015 has novel effects on hERG1 channels, different from those of amiodarone. The drug may serve as a parent molecule for designing new compounds with stimulatory action on I_{Kr}, which could be useful for the treatment of hERG mutations that slow deactivation.

P-18

DECREASED EXPRESSION OF VGLUT1 INCREASES VULNERABILITY TO SEIZURES

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RATIONALE: Glutamate, the most abundant excitatory neurotransmitter in the central nervous system, is well known to be implicated in epileptic seizures. Vesicular glutamate transporters (vGLUTs) are responsible for loading synaptic vesicles with glutamate. There are 3 vGLUT isoforms molecularly identified in the mammalian brain: vGLUT1-3. Recent published data show up-regulated expression profiles of vGLUT1 in different models of epilepsy. Although these data might suggest an important involvement of this transporter protein in the process of epileptogenesis, the alterations might as well result from the hyperexcitability and/or presence of seizures in these animal models.
METHODS: In the present study, we investigated the seizure susceptibility of vGLUT1 heterozygous (HET) mice and their wild type (WT) littermates in the pilocarpine model for temporal lobe epilepsy and the pentylenetetrazol (PTZ) model for generalized epilepsy. This will allow us to investigate whether a severe reduction in vGLUT1 protein in the HET mice can affect seizure generation and thus whether the vGLUT1 protein is crucially involved in the origin of limbic and/or generalized seizure activity.
Seizure threshold for pilocarpine (i.v.; 24 mg/ml) and PTZ (i.v.; 7,5 mg/ml) were compared in vGLUT1 HET mice and vGLUT1 WT littermates.
RESULTS: The threshold doses for pilocarpine that induced rearing, falling, tonic hindlimb extension and death were significantly lower in vGLUT1 HET compared to vGLUT1 WT mice. Similarly, a tendency towards a decreased dose of PTZ was necessary to induce falling, tonic hindlimb extension and death in the vGLUT1 HET animals, compared with their WT littermates, pointing towards a lower resistance to convulsions provoked by PTZ in the vGLUT1 HET animals.
CONCLUSIONS: These data suggest, for the first time, that vGLUT1 is a transporter that could be involved in generating limbic and generalized seizures.

P-19

ALTERED STRIATAL GLUTAMATE TRANSPORTER FUNCTIONING COULD EXPLAIN ABERRANT GLUTAMATERGIC NEUROTRANSMISSION IN STRIATUM OF HEMI-PARKINSON RAT MODEL

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Important mediators in the pathogenesis of Parkinson's disease are oxidative stress and excitotoxicity. Increased glutamate concentrations can be linked to both processes. Extracellular glutamate concentrations are mainly determined by an interplay between vesicular glutamate transporters (VGLUTs), glial high-affinity Na^+/K^+-dependent glutamate transporters (GLAST and GLT-1) and the cystine/glutamate antiporter. Using semi-quantitative Western blotting, we studied the expression levels of VGLUT1, VGLUT2, GLAST and GLT-1 in the striatum of rats at different survival times (3, 5 and 12 weeks) after unilateral 6-OHDA injection into the medial forebrain bundle (hemi-Parkinson rat model). The significant bilateral increase in GLT-1 expression that we observed at 3 and 12 weeks post-lesion, was translated into an increased reuptake activity, as revealed by ex vivo D-[3H]-aspartate reuptake studies in striatal synaptosomes. To determine the origin of the increased striatal expression level of VGLUT1 protein at 3 weeks post-lesion and decreased expression at 12 weeks, in situ hybridization experiments were performed to visualize VGLUT1 mRNA expression levels throughout the brain of hemi-Parkinson rats. Our data suggest that the earlier observations by Meshul et al. (1999) of a biphasic change in extracellular striatal glutamate levels after 6-OHDA injection into the medial forebrain bundle, with increases 1 month post-lesion and decreases 3 months post-lesion, might be explained by an interplay of changes in the expression/functioning of all glutamate transporter families. Modulation of one or more of these transporters might help to normalize the extracellular glutamate levels and prevent further excitotoxic and oxidative damage.

P-20

THE CONTROL OF KAINIC ACID-INDUCED STATUS EPILEPTICUS

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Today preclinical epilepsy research is shifting towards the use of true epilepsy models to unravel the processes underlying epileptogenesis. One of the most widely used and thus best characterized animal models for temporal lobe epilepsy is the kainic acid-induced post-status epilepticus rat model. In rodents status epilepticus (SE) is followed by a latent phase during which the brain is rendered epileptic. Eventually the animals develop chronic spontaneous seizures. In order to generate reproducible conditions between rats, the induced SE should be interrupted after 90 minutes. There is consensus that this time interval is sufficient to initiate epileptogenesis and that this approach limits animal suffering. Most research groups use systemic administration of 10 mg/kg diazepam as a standard protocol to interrupt SE. Termination of behavioral seizure activity is currently used by many researchers as sole parameter to monitor the establishment of SE control. Using electrocorticographic (ECoG) monitoring we want to verify whether lack of behavioral seizure activity is indeed always correlated with full SE control. SE was induced in rats by consecutive intraperitoneal kainate injections (5 mg/kg) with a one hour interval. Using simultaneous video-ECoG monitoring establishment of SE was verified behaviorally and electrocorticographically. Diazepam (10 mg/kg) was administered to terminate SE after 90 minutes. SE was controlled in only 28% of the rats after diazepam administration (n=7). Behaviorally, all rats consistently appeared to be seizure free. However this was not confirmed by the ECoG analysis. In 72% of the rats ECoG activity showed continuous epileptic activity despite the lack of typical seizure-related behavioral activity. The present data clearly demonstrate the need to use ECoG monitoring to enable reliable assessment of SE control as the number of false positives is extremely high when SE control is only behaviorally assessed. Our data show that the standard protocol to control SE is not sufficient and needs further optimization.

P-21

[^{3}H]AL-11, A NEW STABLE AND SELECTIVE LIGAND FOR THE IRAP/AT$_4$ RECEPTOR IN BOTH MEMBRANES AND INTACT CELLS

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'AT$_4$ receptors' through which angiotensin IV (Ang IV) improves memory acquisition, were recently identified as insulin regulated aminopeptidase (IRAP). Radioligand binding studies have hitherto been performed with iodinated Ang IV in the presence of divalent cation chelators EDTA and 1,10-phenanthrolin. Hence, they take place at the non-physiological apo-form of IRAP. Presently, binding of [^{3}H]AL-11, a stable Ang IV analog, was characterized on Chinese hamster ovary (CHO-K1) cell membranes. In the presence of chelators, its high affinity sites showed the same pharmacological profile as for radiolabeled Ang IV binding. Without chelators, only for [^{3}H]AL-11 high affinity binding could be perceived. This pharmacological profile corresponded to catalytically active IRAP and was different from the one in presence of chelators. This confirms that the active and apo-forms of IRAP have a distinct pharmacological profile and that [^{3}H]AL-11 can specifically label both forms.
Moreover, we show that [^{3}H]AL-11 can also be used to detect IRAP in intact cells. While [^{3}H]AL-11 only binds with high affinity to IRAP, [^{3}H]Ang IV fails to do so. As an application, we also show that in adipocytes the translocation of IRAP to the cell surface, mediated by insulin, can be measured by the use of [^{3}H]AL-11.

P-22

SIMPLIFIED METHOD FOR ESTIMATING DISSOCIATION RATES OF UNLABELLED LIGAND-RECEPTOR COMPLEXES

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Background and purpose: The dissociation rate of unlabelled ligand-receptor complexes can be estimated indirectly by radioligand binding. To this end, we developed a simple to use and interpret "two-step competition" binding experiment.

Experimental approach: The experiment consists of pre-incubating the receptor-preparation with a wide range of ligand concentrations, washing off free ligand molecules, adding radioligand and monitoring its receptor binding after a fixed time. The ability of this approach to yield the unlabelled ligand's dissociation rate is evaluated both by simulations and experimentally.

Key results: When binding of both ligands is mutually exclusive, already bound unlabelled ligand molecules need to dissociate before radioligand binding can take place. Based on this principle, simulations suggest that the unlabelled ligand's dissociation rate can be estimated from the upward shift that the competition curve experiences after washing. Experimental confirmation is provided by comparing the dissociation rates of unlabelled D_2 dopamine-receptor antagonists according to this and alternative approaches. Additionally, the "two-step competition" approach could also disclose the ability of unlabelled ligands to partition in the cell membrane.

Conclusions: Compared to the previously published indirect approaches, the "two-step competition" approach gets around the discomfort of measuring radioligand binding at different time intervals and it stands out by the simplified analysis of the binding data.

P-23

MEDICATION DISCREPANCIES AT HOSPITAL DISCHARGE OF ELDERLY PATIENTS

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Background and Objective
Transition from hospital to community and primary care makes patients vulnerable to drug related problems. This is particularly true for geriatric patients who are often polymedicated.
The purpose of the study was to list changes in preadmission and discharge drug regimes, to look for medication discrepancies in hospital discharge letters and to identify opportunities for clinical pharmacists to improve the continuity of care.
Design
Retrospective study comparing clinical pharmacist-acquired medication histories with standard generated discharge medication lists, i.e. without prior review by clinical pharmacists.
Setting
The study was conducted at the geriatric ward (29 beds) of a Belgian university hospital.
Main outcome measures
Determination of the type and amount of medication discrepancies in hospital discharge letters as well as the number of patients with such discrepancies.
Results
Medication records and discharge letters of 96 geriatric patients were investigated. All together, patients took 787 drugs prior to hospital admission and were prescribed a total of 901 drugs at discharge. Of the former, 277 drugs (35,2%) were discontinued, whereas the latter consisted of 391 newly added drugs (43,4%), 129 drugs (14,3%) with at least 1 change with respect to the preadmission medication and 381 unchanged drugs (42,3%). The multitude of changes resulted in 237 discrepancies consisting of 137 (57,8%) discontinued drugs of which it was not clear whether they were omitted by mistake or intentionally, and 49 (20,7%) newly added drugs with unclear rationale. Although some changes in home medication were warranted, others were not. This is illustrated by 32 (13,5%) non-conducted resubstitutions although being standard hospital policy, and 14 (5,9%) doubtful changes in dose or posology, as well as 5 (2,1%) unnecessary galenic form changes. Medication discrepancies were found in the discharge letter of 80 patients (83,3%).
Conclusions
Correct and completely documented discharge letters are a prerequisite for a seamless transition between secondary and primary care. This study revealed several shortcomings with standard generated discharge medication lists. Review by clinical pharmacists improves the reconciliation between preadmission and discharge medication. This may help to reduce the number of potential adverse drug events.

P-24

RECONCILIATION FAILURE OF CHRONIC MEDICATION DUE TO ICU ADMISSION

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Background and Objective
Admission to an intensive care unit (ICU) may entail an elevated risk of drug discrepancies (DD) due to the focus of care on stabilization of the patient.
The objective of the study was to investigate whether an ICU stay leads to more DD's in particular with respect to chronic medication.
Design
Observational, prospective, controlled cohort study. At hospital admission, the medication history was documented by a pharmacist. For drugs administered during hospitalization and prescribed at discharge, physicians' medication records were consulted. For patients transferred from the ICU to another ward, and who were later discharged from the hospital, the medication list and the medication scheme in the discharge letter respectively, were compared with the pharmacist-acquired medication history with special focus on chronic drugs.
Setting
Two adult ICU's (study group) and one cardiologic care unit (control group) of a Belgian university hospital (UZ Brussel).
Main outcome measures
The percentage of patients with unintended DD and the incidence as well as the type of DD in chronic medication.
Results
The study group consisted of 24 patients and the control group of 12 patients. In the study group 67% of the patients had one or more discrepancies in the medication history vs. 83% in the control group. There was no statistically significant difference in the number of DD between the two groups (Mann-Whitney test; U=135,5; n_1=24; n_2=12; p=0,77; α=0,05). The most common discrepancy was omission of a chronic medication.
At the time of transfer from the ICU to another medical ward, 83% of the patients had one or more DD. The most common discrepancy was again omission of a chronic medication.
At hospital discharge, the percentage of patients with unintended DD was remarkably higher for the study group (81%) than for the control group (55%). There were significantly more DD in the study group than in the control group (Mann-Whitney test; U=63,5; n_1=21; n_2=11; p=0,036; α=0,05). The most common discrepancy was omission of a chronic medication in the discharge letter. The percentage of unintended DD at discharge due to the ICU stay was 36%.
Conclusions
The stay in an ICU leads to more unintended DD in chronic medication. Some discrepancies are solved during the further hospitalization, but an important number results in DD at hospital discharge. At transition periods, structural medication reconciliation is necessary to prevent drug discrepancies. The complete and accurate transfer of information between hospital health care providers, patients and health care providers at home is crucial to prevent drug related problems.

P-25

ABNORMAL SMALL AIRWAYS FUNCTION IN BREAST CANCER PATIENTS

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Introduction: In the framework of a clinical trial comparing pulmonary implications of hypofractionation with Tomotherapy® and conventional radiotherapy in post-operative breast cancer patients, lung function testing with an emphasis on small airways function was to be conducted at baseline and at various intervals post-radiotherapy. Baseline lung function appeared to show unexpected abnormalities, warranting a detailed analysis.
Methods: Baseline lung function data (collected shortly after breast cancer surgery and before radiotherapy had been started) were analyzed from 36 female breast cancer patients. We collected forced expiratory flow rate in 1 second (FEV_1) as a measure of overall airway function, Tiffeneau index (FEV_1/FVC) specific for overall airway obstruction, forced end-expiratory flow rate (FEF_{75}) for small airway function and diffusing capacity (DLco) for integrity of the gas exchanging lung zone. An independent inert gas test also provided two indices of small airways function in the conductive and acinar lung compartments (Scond and Sacin).
Results: The patients under study (56±12(SD)years) had negligible smoking history (0.7±1.6(SD)packyears) and no history of asthma. Predicted values for FEV_1 (109±18(SD)%pred) and DLco (87±14(SD)%pred) were normal. By contrast, FEF_{75} values were abnormally low (63±28(SD)%pred) and correlated with FEV_1/FVC ($r=0.89$; $p<0.001$). Average values for Sacin and Scond were also abnormal, with average values just beyond their respective limits of normal.
Conclusion: While overall lung function is preserved in post-operative breast cancer patients, a distinct malfunction is observed of small airways that are probably located around the entrance of the gas-exchanging lung zone. This is the first observation of abnormal lung function in breast cancer patients prior to radiotherapy, which needs to be further scrutinized and taken into account when evaluating any potential radiotherapy-induced lung function impairment.

P-26

ETHNICITY AND USE OF IRON & FOLATE DURING PREGNANCY

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Background and Objectives

Periconceptional folic acid use considerably reduces both the risk of neural tube defects and possibly other birth defects. Iron use also prevents anemia. Racial and ethnic disparities in general healthcare utilization have been related to numerous factors including the cultural competence of care providers, i.e. the extent to which they are aware of foreign cultural health beliefs, as well as the patient's social class, educational level, and language proficiency. The aim of this study is to compare the behavioral pattern relating to iron and folate use during pregnancy in three population groups of pregnant women: women from Western (Caucasian) origin, women from Arabic & Turkish origin, and women from other origins (referred to as 'mixed origin' group).

Design

A structured questionnaire was designed to collect information on demographic characteristics of the participants as well as on their iron and folate use pattern. Pregnant women who gave consent to enrollment in the study were asked to fill in the questionnaire in the third trimester of their pregnancy. If necessary complementary information could be collected from the patient's medical record.

Setting

The study was conducted at the UZ Brussel which is one of the medical referral centers in Brussels with a large number of immigrants.

Main outcome measures

Quantitative analysis of iron and folate use during pregnancy in different population groups. Data analysis was done using SPSS version 17.0.

Results

The analyses included 350 patients categorized into 3 groups according to origin. Regarding the use of iron, 63.8% of the pregnant women used iron during pregnancy with the highest use in the Western (Caucasian) group (66.8%), followed by the Arabic & Turkish origin group (60.0%) and the mixed origin group (59.0%). There was no statistically significant difference among the 3 groups (*P*=0.398). Regarding the use of folate, 59.2% of the pregnant women used folate during pregnancy. There was a significant difference in the use of folic acid among the 3 groups (*P*=0.003).The highest use was in the Western (Caucasian) group (66.8%), followed by the mixed origin group (51.3%) and the Arabic & Turkish group (47.6%). A significant difference exists between the Western (Caucasian) and the Arabic & Turkish group (*P*=0.001).

Higher levels of educational attainment were associated with a greater use of folate (*P*=0.000) and iron (*P*=0.017). Socio-economic status was associated with the use of folate (*P*=0.000) and with the use of iron (*P*=0.001) during pregnancy. There was a higher use in women belonging to families in which both partners are working.

Conclusion

There are some differences between Western (Caucasian) and Non-Western pregnant women in iron and folate intake behaviour but more collaborative studies are warranted to confirm the role of ethnicity. Maternal educational level and household income were associated with the use of iron and folate during pregnancy. There is a need to increase the awareness of pregnant women to use folate during pregnancy, especially among the low educated and immigrated women.

P-27

ISOFLURANE ANESTHESIA INHIBITS FIREFLY LUCIFERASE IN A DOSE-DEPENDENT WAY: A PITFALL FOR IN VIVO BIOLUMINESCENCE IMAGING

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Bioluminescence imaging (BLI) offers unique possibilities to study biological processes in intact organisms in a quantitative, non invasive and repeated approach. Rodents are anesthetized during imaging and BLI cameras are routinely provided with an isoflurane anesthesia unit. Isoflurane is animal- and user-friendly because of its fast induction and recovery. However a direct inhibition of the luciferase enzyme by anesthetics has been described and might influence in vivo quantification. *Aim:* To assess the impact of isoflurane anesthesia on the bioluminescent signal intensity in vitro and in vivo. *Methods:* For in vitro assessment, 10E6 Fluc+ R1M cells were plated in 25cm² flasks. Cells were exposed to O_2/isoflurane mixtures starting 10min before and continuously throughout BLI. D-luciferin was added to reach a 0.15mg/ml concentration. For in vivo analysis, a mouse bearing a sc Fluc+ R1M tumor was injected ip with 30mg/kg of D-luciferin under 2.5% isoflurane anesthesia. Kinetic BLI data were acquired using list mode acquisition while the amount of isoflurane was varied between 2.5 and 5%. *Results:* Cells incubated with 1.5% isoflurane showed a decrease in signal of 38.5±2.2% ($p<.001$) compared to control samples (100% 0_2). Isoflurane at 3% shows a dose-dependent decrease of 29.8±0.8% compared to the intensity at 1.5% isoflurane. No statistical difference was detected between different 0_2 levels (100-50-25% in N_2), confirming that the inhibition is not due to a decrease in oxygen but the increase in isoflurane.
In vivo imaging also shows the presence of isoflurane dose-dependency of the BLI signal (see fig). Preliminary results with desflurane and sevoflurane show similar in vitro effects. *Conclusion:* Isoflurane anesthesia significantly reduces the intensity of the BLI signal both in vitro and in vivo, indicating the presence of a direct inhibitory effect. Researchers should be aware of this impact on BLI quantification and should standardize the use of gas anesthetics thoroughly.

P-28

TYROSINE KINASE INHIBITOR (TKI)-INDUCED MACROCYTOSIS

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RATIONALE: Small molecule TKI's are routinely used in the clinic or are under clinical development in different cancer types. Treatment with sunitinib in patients (pts) with metastatic renal cell cancer (RCC) and with imatinib in pts with gastrointestinal stromal tumors (GIST) were shown to induce a significant increase of the mean corpuscular volume (MCV) of peripheral red blood cells(RBC)[1,2].The pathophysiological mechanism remains unknown but could involve the c-kit dependent signaling pathway in progenitor cells of the bone marrow. We analyzed the effect on MCV of sunitinib, imatinib and sorafenib, all agents targeting c-kit and of TKI's targeting the Epidermal Growth Factor Receptor (EGFR) such as erlotinib and BI 2992 in multiple cancer types and settings.

PATIENTS AND METHODS: The increase in MCV from baseline was studied in 10 pts treated with sunitinib (6 with RCC and 4 with MBC), in 6 pts treated with imatinib (6 with GIST), in 2 pts treated with sorafenib (1 with RCC and 1 with hepatocellular cancer) and in 6 and 5 pts treated with erlotinib and BI 2992 with non-small-cell lung cancer (NSCLC) respectively. All pts received treatment for more than 3 months (mo) at the respective recommended dose. In 4 pts showing the increase in MCV under sunitinib a bone marrow aspirate and serum levels of folate, vitamine B12 and thyroid hormones were determined.

RESULTS: Baseline values of MCV in groups treated with sunitinib and imatinib were not different. Sunitinib induced a larger increase in MCV versus baseline than imatinib (mean increase of 12.4%, 16.8%, 16.6% and 12.7% for sunitinib versus 0.7%, 5.6%, 5.9% and 5% for imatinib at 3, 6, 9 and 12 mo respectively; p-values of <0.005,<0.011, $p<0.031$ and $=0.06$ at 3, 6, 9 and 12 mo respectively). Folate, vitamine B12 and thyroid function remained normal in pts treated with sunitinib. Macrocytosis did not result in anemia, was self limiting and recovered completely within 3 to 6 month of drug withdrawal in both groups. Evaluation of the bone marrow in 4 pts under sunitinib showed *nonspecific dyserythrosis* .Sorafenib, erlotinib nor BI 2992 had any effect on MCV.

CONCLUSION: Sunitinib-induced macrocytosis was not limited to RCC cancer but also occurred in MBC. The increase with imatinib in GIST was significantly less than with sunitinib at all time points studied. Sorafenib was without significant effect on MCV which corroborates previous data[1] but only 2 patients were studied. TKI's targeting EGFR did not induce an increase in MCV. Sunitinib , imatinib and sorafenib were used at an effective pharmacodynamic dose (inhibition of c-kit) and sunitinib-treated patients often had toxicity related dose reductions. These data strongly suggest that inhibition of additional pathways targeted by sunitinib (on top of c-kit) are involved in the drug induced macrocytosis and implicate the VEGF, FLT3 and RET in normal RBC development. The induction of macrocytosis may compromise the blinding process in placebo-controlled trials with known or novel TKI’s targeting similar pathways.