6th International Symposium on Cell/Tissue Injury and Cytoprotection/Organoprotection

- Focus on GI Tract -

October 12-14, 2011
Park Inn
Pribaltiyskaya Hotel
St. Petersburg, Russia

PROGRAM
and
ABSTRACTS
OBJECTIVES AND SCOPE

The objectives of this international symposia series are to provide a lively forum for experts to present and integrate new findings on the mechanisms, manifestations, diagnosis, and consequences of cell and tissue injury. One of the goals is to review emerging concepts, e.g., that reversible or irreversible injury to a few or numerous cells not only that it does not necessarily lead to tissue damage, but actually results in mucosal protection (e.g., in stomach “cytoprotection” or gastroprotection). A further goal is to apply the new knowledge about cell and tissue injury to pharmacologic prevention of tissue damage in organ systems. Our specific areas of focus at this symposium include new developments in the molecular mechanisms of cell and tissue injury, especially in the gastrointestinal (GI) tract.

The scope of the symposium will involve primarily molecular and cell biologic aspects of cell injury and protection, with emphasis on biochemical and genetic mechanisms of both damage and protection. A major emphasis will be given to pharmacologic methods of cytoprotection and organoprotection as well as to morphologic manifestations of cell and tissue injury, mechanisms of mucosal protection and ulcer healing.

FORMAT

The format of minisymposia will be used again: The major presentations are complemented by short presentations selected from submitted abstracts. A poster session is also organized.

HISTORY

This symposia series is the product of international cooperation and interdisciplinary communication between basic science and clinical investigators who demonstrated that controversies and scientific disputes are best resolved by open communication and display of new data.

Actually, the series grew out of disputes over the role of intracellular calcium and phospholipase activation (Farber vs. Orrenius) in cell injury, and of prostaglandins vs. antioxidant sulfhydryls in cytoprotection (Robert vs. Szabo).

The fifth member of the initial Standing Committee has been K.H. Usadel who was the first clinical investigator to demonstrate the (initially controversial) protective role of somatostatin in pancreatitis and hepatic damage. After the passing of Andre Robert, Andrzej Tarnawski (first modern investigation of sucralfate and antacids in gastroprotection) joined the Standing Committee.

The present meeting is twenty-fifth anniversary celebration of this symposia series. The first formal meeting was held in 1986 at the University of Heidelberg in Germany, the second symposium was organized in 1989 at Harvard Medical School in Boston, MA, the third in Long Beach, CA in 2000, the fourth in Long Beach, CA in 2006, the fifth in Yalta (Crimea, Ukraine) in 2008.
**Scientific PROGRAM**

**6th International Symposium**

*On Cell/Tissue Injury and Cytoprotection/Organoprotection - Focus on GI Tract -*

12-14 October 2011

*Park Inn Pribaltiyskay Hotel, St. Petersburg*

## PROGRAM OVERVIEW BY SESSION

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<td>Special lecture “ANDRE ROBERT and the origins of these symposia” by Drs. S. Szabo, Y. Tache &amp; A. Tarnawski</td>
<td>Stress: dual action on GI tract</td>
<td>Pavlov Institute of Physiology: “AM Ugolev: the discovery of membrane digestion”</td>
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<td>Plenary lecture by Drs. B. Zhivotovsky &amp; S. Orrenius</td>
<td>Novel insights into the mechanisms of GI mucosal injury and protection</td>
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<td>Special presentations by Dr. S. Okabe Dr. D. Chen</td>
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<td>Welcome coctail in Pribaltiyskay Hotel</td>
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Wednesday, October 12, 2011
Park Inn Pribaltiyskaya Hotel, Hall Red 11

9.00 – 18:00 REGISTRATION

09.30 – 15:00 Excursion to the Institute of Exp. Medicine, Pavlov Institute of Physiology, Pavlov Apartments Museum

16.00 – 18:00 OPENING CEREMONY

Chairs: L. Filaretova and S. Szabo

16.00 – 16:10 Welcoming note by L. Filaretova

16.10 – 16:30 Special lecture
S. Szabo\(^1\), Y. Tache\(^2\) & A. Tarnawski\(^1\)
ANDRE ROBERT AND THE ORIGINS OF THESE SYMPOSIA
1-VA Long Beach Healthcare System, University of California-Irvine, Long Beach, CA and 2-VA Greater Los Angeles Health Care System, UCLA, Los Angeles, CA, USA

16:30 – 17:10 Plenary lecture
Boris Zhivotovsky & Sten Orrenius
MOLECULAR MECHANISMS OF CELL DEATH AND IMPLICATION IN DISEASE
Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Stockholm, Sweden

17:10 – 18:00 Special presentations
S. Okabe\(^1\), K. Kodama\(^1\), C.M. Zhao\(^1\), D. Chen\(^1\), T. Okamoto\(^2\), C. Morimoto\(^2\), H. Matsui\(^3\)
CYTOREDUCTIVE EFFECTS OF ACETIC ACID ON MOUSE GASTRIC CANCER, HUMAN AND RAT GASTRIC CANCER CELLS
1 - Department of Cancer Research & Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway; 2 - Institute of Medical Science, University of Tokyo, Tokyo, Japan; 3 - Division of Gastroenterology, Comprehensive Human Science, University of Tsukuba, Tsukuba, Japan

D. Chen
GI PHARMACOLOGY: THE IMPORTANCE OF BENCH-TO-BEDSIDE- AND-BACK CONCEPT
Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway

18:00 – 20:00 WELCOME COCTAIL IN PRIBALTIYSKAY HOTEL
Thursday, October 13, 2011
Park Inn Pribaltiyskaya Hotel, Hall Red 11

Morning Sessions

Session: Mechanisms of NSAID-induced tissue injury (Part 1)

Chairs: K. Takeuchi and J. Wallace

9:00 – 9:20  **J.L. Wallace**
NSAID GASTROPATHY AND ENTEROPATHY: DISTINCT PATHOGENESIS LIKELY NECESSITATES DISTINCT PREVENTION STRATEGIES
Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, Ontario, Canada

ROLES OF PROSTAGLANDIN EP4 RECEPTORS IN DEVELOPMENT AND HEALING OF NSAID-INDUCED SMALL INTESTINAL DAMAGE
Kyoto Pharmaceutical University, Department of Pharmacology and Experimental Therapeutics, Kyoto, Japan

9:40 – 10:00  **H. Satoh**¹, K. Amagase¹, S. Ebara², Y. Akiba³, K. Takeuchi¹
DUAL CONTROL OF DUODENAL MUCOSAL PROTECTION BY CYCLOOXYGENASE (COX)-1 AND COX-2 IN CATS
1 - Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Kyoto, Japan
2 - Department of Anatomy, Meiji University of Integrative Medicine, Kyoto, Japan
3 - CURE/UCLA Medical School, Los Angeles, CA, USA

SYNERGIC EFFECT OF NSAIDS AND ACID ON SUPEROXIDE-ANION PRODUCTION IN A GASTRIC EPITHELIAL CELLS
Division of Gastroenterology, University of Tsukuba, Tsukuba, Japan

10:20 – 10:40  **COFFEE BREAK**
Session: **Mechanisms of NSAID-induced tissue injury (Part 2)**

_Chairs: T. Mizushima, K.D. Rainsford_

10:40 – 11:00  
**T. Mizushima**  
DEVELOPMENT OF NEW TYPE OF NSAID WITH LOWER GASTRIC SIDE EFFECTS  
*Keio University, Tokyo, Japan*

11:00 – 11:20  
**K.D. Rainsford**  
NSAID-MUCOSAL INJURY: ROLES OF DRUG CHEMISTRY IN PATHOGENESIS  
*Sheffield Hallam University, Biomedical Research Centre, Sheffield, UK*

11:20 – 11:40  
**B.A. Callingham**¹, A. Maini¹, F. Mahood¹, M. Munnawwar¹, A.S. Milton¹, C. Rhodes¹, K.D. Rainsford²  
EFFECTS OF IBUPROFEN, AND SOME ANALOGUES, ON THE REACTIVITY OF FALLOW DEER DIGITAL ARTERY SEGMENTS  
¹ - Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge, UK  
² - Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK

11:40 – 12:30  
**Poster Session – I**  
_Chairs: H. Satoh and P. Sikiric_

12.30 – 14.00  
**LUNCH**
Thursday, October 13, 2011
Park Inn Pribaltiyskaya Hotel, Hall Red 11

**Afternoon Sessions**

Session: **Stress: dual action on GI tract**

*Chairs: Y. Tache and J. Wood*

14:00 – 14:20  **Y. Tache**, M. Larauche, A. Mulak  
**CHRONIC OR ACUTE EXTEROCEPTIVE STRESS-INDUCED VISCERAL ANALGESIA OR HYPERALGESIA IN RODENTS**  
Center for Neurobiology of Stress, Digestive Diseases Division, and VA Greater Los Angeles Health Care System, UCLA, Los Angeles, USA

14:20 – 14:40  **J.D. Wood**  
**NEUROBIOLOGY OF CRF IN THE ENTERIC NERVOUS SYSTEM DURING STRESS**  
The Ohio State University, Department of Physiology, Columbus, Ohio, USA

14:40 – 15:00  **L.P. Filaretova**, T.R. Bagaeva, O.Yu. Morozova, M.A. Mayzina  
**CRF MAY PROTECT THE GASTRIC MUCOSA IN STRESS THROUGH INVOLVEMENT OF GLUCOCORTICOIDS**  
Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia

15:00 – 15:20  **V.I. Ovsyannikov**, T.P. Berezina  
**THE PATHOGENIC POTENTIAL OF PSYCHOGENIC STRESS IN GASTRIC MUCOSAL INJURY**  
Institute of Experimental Medicine of the North-West Branch of the Russian Academy of Medical Sciences, Department of Visceral Systems Physiology, St. Petersburg, Russia

15:20 – 15:40  BREAK
Session: Novel insights into the mechanisms of GI mucosal injury and protection  
Chairs: K. Gyires and S. Szabo

15:40 – 16:00  
S. Szabo1,2, X.M. Deng1,2, A. Tolstanova1,2, T. Khomenko1,2, S.W. French3, A. Tarnawski1,2, Zs. Sandor1,2  
NEW MECHANISTIC AND TIME SEQUENTIAL PATHOGENESIS OF CELL/TISSUE INJURY LEADING TO EXPERIMENTAL ULCERATIVE COLITIS  
1VA Long Beach Healthcare System and 2University of California-Irvine, Long Beach, CA; 3Harbor-UCLA Medical Center, Los Angeles, CA, USA

16:00 – 16:20  
R.H. Hunt  
ADVANCES IN UNDERSTANDING INFLAMMATION IN UPPER GI DISEASES  
Department of Medicine, Division of Gastroenterology & Farncombe Family Digestive Disease Research Institute McMaster University Health Science Centre, Hamilton, Ontario, Canada

16:20 – 16:40  
K. Gyires1, Z.S. Zadori1, L. Hunyady2  
ANALYSIS OF THE EFFECTS OF DIFFERENT NEUROPEPTIDES IN GASTRIC MUCOSAL DEFENSE INITIATED CENTRALLY  
1 - Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary  
2 - Department of Physiology, Semmelweis University, Faculty of Medicine, Budapest, Hungary

16:40 – 17:00  
A. Yanaka  
SULFORAPHANE ENHANCES PROTECTION AND REPAIR OF GASTROINTESTINAL MUCOSA AGAINST OXIDATIVE STRESS VIA NRF2-DEPENDENT MECHANISMS  
Tokyo University of Science, Clinical Pharmacology, Noda, Japan

17:00 – 17:20  
O. Zayachkivska, A. Filipskyy, I. Titko-Pshyk, M. Gzhegotsky  
PHYSIOPATHOLOGY OF ESOPHAGEAL INFLAMMATION, ULCEROGENESIS AND REPAIR BY GLICOCONJUGATE PROFILE  
Lviv National Medical University, Physiology Department, Lviv, Ukraine

17:20 – 17:35  
E. Gregor, B. Slater, I. Osapay, T. Khomenko, X.M. Deng, A. Tarnawski, A. Ahluwalia, N. Hoa, Zs. Sandor, S. Szabo  
COMPARATIVE IN VITRO TOXICITY OF ULCEROGENIC CHEMICALS TO CULTURED ENDOTHELIAL, EPITHELIAL AND FIBROBLAST CELLS  
VA Long Beach Healthcare System, Long Beach, and University of California, Irvine, and 2 - VA Long Beach Medical Center and Departments of Pathology & Pharmacology, University of California-Irvine, Long Beach, CA, USA

19:00 – 24:00  
SYMPOSIUM DINNER & NIGHT BUS CITY TOUR
Morning Sessions

Session: Advances in GI pharmacology (Part 1)

Chairs: D. Chen and R.H. Hunt

9:00 – 9:20  R. H. Hunt
THE EVOLUTIONARY IMPACT OF LACTOBACILLI ON H. PYLORI AND GASTRIC ACID SECRETION: DID A CENTURY OF DIETARY CHANGE ALTER THE GASTRIC MICROBIOTA?
Department of Medicine, Division of Gastroenterology & Farncombe Family Digestive Disease Research Institute McMaster University Health Science Centre, Hamilton, Ontario, Canada

9:20 – 9:40  T. Brzozowski¹, S. Kwiecien¹, M. Pawlik¹, D. Drozdowicz¹, R. Olszanecki², R. Korbút², S.J. Konturek¹, W.W. Pawlik¹
ROLE OF RENIN-ANGIOTENSIN SYSTEM METABOLITES IN GASTROPROTECTION AND ULCER HEALING. INVOLVEMENT OF MAS RECEPTOR, PROSTAGLANDINS AND CAPSAICIN SENSITIVE NERVES
1 - Department of Physiology Jagiellonian University Medical College, Cracow, Poland; 2 - Department of Pharmacology Jagiellonian University Medical College, Cracow, Poland

9:40 – 10:00  P. Sikiric¹, S. Seiwerth², R. Rucman¹, B. Turkovic¹, D. Stancic Rokotov¹, L. Brcic², M. Sever¹, R. Kliece¹, B. Radic¹, D. Drmic¹, S. Ilie¹, D. Kolenc², H. Vrcie¹, B. Sebecie¹
NATURALLY OCCURRING PEPTIDES FOR GASTROPROTECTION.STABLE GASTRIC PENTADECAPEPTIDE BPC 157
1 - Medical Faculty, University of Zagreb, Department of Pharmacology, Zagreb, Croatia
2 - Medical Faculty, University of Zagreb, Department of Pathology, Zagreb, Croatia

10:00 – 10:20  Y. Nagasaki¹, V.B. Long¹, T. Yoshitomi¹, H. Matsui²
PROTECTIVE AND THERAPEUTIC EFFECT OF REDOX POLYMER NANOPARTICLES FOR ULCERATIVE COLITIS MODEL MICE
1 - Department of Materials Science, Graduate School of Pure and Applied Sciences, University of Tsukuba, Tsukuba, Japan
2 - Department of Gastroenterology, Graduate School of Comprehensive Human Sciences, Tsukuba, Japan

10:20 – 10:40  COFFEE BREAK
Session: **Advances in GI pharmacology (Part 2)**

*Chairs: S. Okabe and T. Brzozowski*

10:40 – 11:00  **C.M. Zhao**, R.A. Vigen, T. Viset, R. Fossmark, H. Waldum, M. Kidd, I. Modlin, D. Chen
AUTOPHAGY OF GASTRIC ECL CELLS, CARCINOIDS AND ADENOCARCINOMA
*Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway*

11:00 – 11:20  **A. Kawabata**
ROLES OF THE HYDROGEN SULFIDE/T-TYPE CALCIUM CHANNEL SYSTEM IN SOMATIC AND VISCERAL PAIN PROCESSING
*Kinki University School of Pharmacy, Division of Pharmacology and Pathophysiology, Higashi-Osaka, Japan*

11:20 – 11:35  **D. Zelena**, L. Filaretová
AGE-DEPENDENT ROLE OF VASOPRESSIN IN SUSCEPTIBILITY OF GASTRIC MUCOSA TO INDOMETHACIN-INDUCED INJURY
1 - Hungarian Academy of Sciences, Institute of Experimental Medicine, Behavioral and Stress Studies, Budapest, Hungary;
2 - Russian Academy of Sciences, Pavlov Institute of Physiology, Laboratory of Experimental Endocrinology, St. Petersburg, Russia

TRANSCRIPTION FACTOR EGR-1 IS CRITICAL FOR OXIDATIVE STRESS-INDUCED INJURY IN ULCERATIVE COLITIS PATHOGENESIS
1 - Kiev National Shevchenko University, Kiev, Ukraine
2 - VA Long Beach Medical Center and Departments of Pathology & Pharmacology, University of California-Irvine, Long Beach, CA, USA

11:50 – 12:30  **Poster Session – II**

*Chairs: A. Kawabata and G. Tolstanova*

12.30 – 14.00  **LUNCH**
Friday, October 14, 2011
Park Inn Pribaltiyskaya Hotel, Hall Red 1

Afternoon Sessions

14:00 – 14:20  Session: **Pavlov Institute of Physiology**
A. A. Gruzdkov
A. M. UGOLEV: A DISCOVERY OF THE MEMBRANE DIGESTION
Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia

14:20 – 14:40  Presentation Sponsored by TNK SILMA Ltd
O.V. Federova, E.N. Fedulova, O.A. Tutina
CYTOPROTECTIVE EFFECT OF ENTEROSORPTIVE THERAPY OF CHILDREN WITH INFLAMMATORY INTESTINAL DISEASES (IID)
FGU R&D Institute of Children’s Gastroenterology, Nizhniy Novgorod, Russia

14:40 – 15:00  BREAK

15:00 – 17:00  Session: **Mucosal defense by taste and visceral nutrient sensors**
(Sponsored by Ajinomoto Co., Inc.)

*Chairs: K. Takeuchi and Y. Tache*

THE PHYSIOLOGICAL ROLE OF THE GPRC6A AMINO ACID RECEPTOR
University of Copenhagen, Department of Medicinal Chemistry, Copenhagen, Denmark

**E. Nakamura**, H. Uneyama
GASTRIC MUCOSAL DEFENSE WITH AMINO ACIDS
Frontier Lab., Institute for Innovation, Ajinomoto Co., Inc., Kawasaki, Japan

**V.A. Zolotarev**
GASTRIC EXOCRINE REGULATION WITH FREE DIETARY GLUTAMATE
Pavlov Institute of Physiology of the Russian Academy of Sciences, Department of Physiology of Digestion, Saint-Petersburg, Russia

**Y. Akiba**, J.D. Kaunitz
DUODENAL MUCOSAL DEFENSES AND GUT NUTRIENT SENSORS
CURE/UCLA, WLA VAMC, BBRI, Los Angeles, CA, USA

PROPHYLACTIC EFFECT OF MONOSODIUM GLUTAMATE AGAINST NSAID-INDUCED ENTEROPATHY IN RATS
1 - Kyoto Pharmaceutical University, Department of Pharmacology and Experimental Therapeutics, Kyoto, Japan
2 - Physiology & Nutrition Group, Institute of Life Sciences, Ajinomoto Co., Inc, Japan

17:00 – 17:15  **Concluding remarks.**
Invitation by Dr. K.Takeuchi to the Satellite Symposium of IUPHAR-GI Section, Tokyo, July 12-14, 2012 (in conjunction with the 14th ICUR)
POSTER SESSIONS

The best poster will be awarded by the Organizing Committee

Poster Session – I

Chairs: H. Satoh and P. Sikiric


2. Bezpalko L., Zayachkivska O., Havryluk O., Gzhegotsky M. THE ROLE OF NUTRITION RELATED METABOLIC STRESS IN PRENATAL PERIOD ON HEPATOCYTOPLASMIC ORGANIZATION (EXPERIMENTAL RESEARCH). Lviv National Medical University. Lviv, Ukraine

3. Hrytsevych N., Zayachkivska O., Gzhegotsky M. ROLE OF ENDOGENOUS COX AND LOX PATHWAYS IN THE HEALING OF STRESS-INDUCED ESOPHAGEAL LESIONS DURING EXPERIMENTAL GLUCOZA DISREGULATION (GD). Lviv National Medical University, Lviv, Ukraine


5. Gyires K.1, Zadori Z.S.1, Feher A.1, Shujaa N.1, Al-Khrasani M.1, Lacko E.1, Brancati S.B.2, Lutz H.3 ANALYSIS OF THE ROLE OF IMIDAZOLINE RECEPTORS IN THE REGULATION OF GASTRIC MOTILITY IN A2-ADRENOCEPTOR DEFICIENT MICE. 1 - Department of Pharmacology and Pharmacotherapy, Semmelweis University, Faculty of Medicine. 2 - Department of Pharmaceutical Sciences, Pharmacology Section, University of Catania, Catania, Italy. 3 - Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg, Germany

6. Filaretova L.P., Bagaeva T.R., Morozova O.Yu., Zelena D.2 THE HEALING OF NSAID-INDUCED GASTRIC LESION MAY BE FOLLOWED BY SMALL INTESTINAL AND CARDCIOVASCULAR SIDE EFFECTS: TELEMETRY STUDY IN FREELY MOVING CONSCIOUS RATS. 1- Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia. 2 - Institute of Experimental Medicine, Budapest, Hungary

7. Klicek R., Amic F., Patrlj L., Seiwerth S., Sikiric P. THE HEALING OF COLOCUTANEOUS FISTULAS AND DICLOFENAC OVERDOSE. THE ROLE OF PENTADECAPEPTIDE BPC 157. Medical Faculty, University of Zagreb, Department of Pharmacology and Department of Pathology, Zagreb, Croatia

8. Nasadyuk C.M., Sklyarov A.Y. THE CYTOPROTECTIVE ACTION OF IMUNOFAN IN EXPERIMENTAL GASTRIC LESIONS IN RATS IS MEDIATED BY ITS INHIBITORY EFFECT ON INDUCIBLE NO-SYNTHASE. Lviv National Medical University. Lviv, Ukraine
9. Sever M., Klicek R., Ilic S., Drmic D., Breic L., Breic I., Kolene D., Bojic D., Cvjetko I., Boban Blagaic A., Jelaska A., Becejac T., Zoricic I., Rasic Z., Stancic Rokotov D., Seiwerth S., Sikiric P. STABLE GASTRIC PENTADECAPEPTIDE BPC 157 PROTECTS AGAINST PERTINENT ISCHEMIC CHALLENGE IN RATS. Medical Faculty, University of Zagreb, Department of Pharmacology and Department of Pathology, Zagreb, Croatia

10. Sikiric P., Kavaja F., Klicek R., Drmic D., Seiwerth S. PENTADECAPEPTIDE BPC 157 REDUCES THE COMPLICATIONS OF ILEOILEAL ANASTOMOTIC HEALING FOLLOWING THE DICLOFENAC INTOXICATION. Medical Faculty, University of Zagreb, Zagreb, Croatia

11. Sklyarov A.Y., Zhuromskyi V.S. NITRIC OXIDE-SYNTHASES AND CYCLOOXYGENASE-2 ACTIVITY IN THE MECHANISMS OF GASTROPROTECTIVE ACTION OF VITAMIN C. Lviv National Medical University, Lviv, Ukraine

12. Yarushkina N.I., Bagaeva T.R., Filaretova L.P. CORTICOTROPIN-RELEASED FACTOR-INDUCED EFFECTS ON PAIN SENSITIVITY MAY BE MEDIATED BY GLUCOCORTICOIDS. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia

Poster Session – II

Chairs: A. Kawabata and G. Tolstanova

13. Bagaeva T.R., Bobryshev P.Yu., Filaretova L.P. GLUCOCORTICOIDS PARTICIPATE IN GASTROPROTECTIVE EFFECT OF ISCHEMIC PRECONDITIONING IN RATS. Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg, Russia


15. Drmic D., Grgic T., Klicek R., Boban Blagaic A., Seiwerth S., Sikiric P. THE PENTADECAPEPTIDE BPC 157 PROMOTES HEALING OF COLOVESICAL FISTULAS IN RATS. Medical Faculty, University of Zagreb, Department of Pharmacology and Department of Pathology, Zagreb, Croatia


17. Klicek R., Sever M., Patrlj L., Situm A., Seiwerth S., Sikiric P. THE PENTADECAPEPTIDE BPC 157 HEALS CHRONIC COLOCUTANEOUS FISTULAS. Medical Faculty, University of Zagreb, Department of Pharmacology and Department of Pathology, Zagreb, Croatia

18. Lensman M.V.¹, Zolotarev V.A.¹, Artemjeva A.I.¹, Silin L.V.¹, Wei E.T.², Polenov S.A.¹ HEALING EFFECT OF CRF ON ETHANOL-INDUCED GASTRIC LESIONS IN RATS.

20. **Sever M.**, Barisic I., Radic B., Klicek R., Ilic S., Bilic V., Berkopic L., Dobric I., Petrovic I., Filipovic M., Breic L., Breic I., Kolenc D., Lovric Bencic M., Romic Z., Seiwerth S., Sikiric P. PROLONGED SEVERE HYPERCALCEMIA INDUCES AN ACUTE PANCREATITIS IN RATS, THE EFFECT OF BPC 157 (PL14736). *Medical Faculty, University of Zagreb, Department of Pharmacology and Department of Pathology, Zagreb, Croatia*

21. **Sikiric P.**, Becejac T., Cesarec V., Kolaric K., Djakovic Z., Franjic S., Olujic D., Drmic D., Breic L., Stancic Rokotov D., Seiwerth S. PENTADECAPEPTIDE BPC 157 AS A THERAPY FOR CORROSIVE MUCOSAL LESIONS IN RATS. *Medical Faculty, University of Zagreb, Zagreb, Croatia*

22. **Sikiric P.**, Cesarec V., Becejac T., Djakovic Z., Olujic D., Drmic D., Stancic Rokotov D., Seiwerth S. PENTADECAPEPTIDE BPC 157 AND THE ESOPHAGEAL-CUTANEOUS FISTULA HEALING, AND A THERAPY FOR FISTULA HEALING. *Medical Faculty, University of Zagreb, Zagreb, Croatia*

DUODENAL MUCOSAL DEFENSES AND GUT NUTRIENT SENSORS
Y. Akiba, J.D. Kaunitz
CURE/UCLA, WLA VAMC, BBRI, Los Angeles, CA, USA
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The duodenal mucosa is exposed to gastric acid, followed by high CO$_2$ and foodstuffs during the post-prandial period. In order to protect the mucosa from injury, the duodenal mucosa rapidly responds to luminal contents and enhances mucosal defense mechanisms including HCO$_3^-$ and mucus secretion and hyperemic response. Addition to acid sensing via epithelial-afferent nerve pathway, luminal nutrients and chemicals are also sensed via the mucosal nutrient sensors, accompanied by the release of gut hormones.

We have studied the effects of luminal umami substances on the duodenal defense mechanisms. Umami receptor, consisting of heterodimer T1R1/T1R3, was expressed in the enteroendocrine cells in the duodenum. Luminal perfusion of umami receptor ligand L-glutamate (L-Glu) with 5'-inosine monophosphate (IMP) synergistically increased duodenal HCO$_3^-$ secretion, one of most-studied duodenal defense factors. We have found that perfusion of L-Glu/IMP increased glucagon-like peptide-2 (GLP-2) release into portal vein (PV), and that L-Glu/IMP-induced HCO$_3^-$ secretion was inhibited by the inhibition of GLP-2 receptors, VIP receptors or a nitric oxide synthase. Furthermore, perfusion of L-Glu/IMP increased VIP release into PV and NO release into the perfusate. Capsaicin pretreatment or atropine iv had no effect, whereas indomethacin treatment partially inhibited L-Glu/IMP-induced HCO$_3^-$ secretion. Taken together, our study suggests that luminal L-Glu/IMP stimulates umami receptor, increases GLP-2 release, which activates GLP-2 receptors on myenteric neurons, and then VIP and NO are released to increase HCO$_3^-$ secretion. Therefore, luminal amino acid sensing enhances mucosal defenses via GLP-2 release.

Since GLP-2 as well as an incretin GLP-1 is rapidly degraded by dipeptidyl peptidase IV (DPPIV), we further hypothesized that DPPIV inhibition potentiates umami receptor activation-GLP-2 pathway. A DPPIV inhibitor NVP728 iv enhanced L-Glu/IMP-induced HCO$_3^-$ secretion. Furthermore, the effect of L-Glu/IMP was further enhanced by the activation of a bile acid receptor TGR5, which is also expressed in L cells, accompanied by the enhanced GLP-2 release.

These results suggest that luminal nutrient sensors and chemosensors in the duodenal mucosa help regulate mucosal defenses during the post-prandial period. Combination of luminal nutrients, chemosensor agonists and DPPIV inhibitor may enhance mucosal protection and repair as well as glucose metabolism.

GLUCOCORTICOIDS PARTICIPATE IN GASTROPROTECTIVE EFFECT OF ISCHEMIC PRECONDITIONING IN RATS
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The present study was designed to investigate whether glucocorticoid hormones participate in a realization of the protective effects of ischemic preconditioning in gastric mucosa. Rats were exposed to prolonged gastric ischemia-reperfusion (30 min occlusion of celiac artery followed by 3 h of reperfusion) with or without preliminary ischemic-reperfusion preconditioning (two 5 min episodes of occlusion of celiac artery followed by 10 min reperfusion). The experiments were carried out: 1) in sham-operated or adrenalectomized rats (with or without corticosterone replacement); 2) in rats pretreated by metyrapone (Alirdich, Germany), the inhibitor of glucocorticoid synthesis, or in control group. Both short and prolonged ischemia-reperfusion induced plasma corticosterone rise and prolonged ischemia-reperfusion also produced gastric lesions. The short ischemia-reperfusion (preconditioning) decreased the gastric lesions caused by prolonged ischemia-reperfusion and this gastroprotective effect was prevented by both adrenalectomy or metyrapone pretreatment. An acute corticosterone replacement mimicking corticosterone rise protected the gastric mucosa of adrenalectomized animals against the prolonged ischemia-reperfusion. These results suggest that glucocorticoids released during ischemia-reperfusion preconditioning contribute to the protective effect of the preconditioning on gastric mucosa against gastric lesions induced prolonged ischemia-reperfusion. Thus, glucocorticoid hormones participate in a realization of the protective effects of ischemic preconditioning in gastric mucosa. Supported by RFBR-11-04-01088, BScIM RAS - 2010, 2011; DBSci RAS - 2010, 2011.

NEW MOLECULAR MECHANISM OF STRESS-INDUCED GASTRIC ULCER: TRANSCRIPTION FACTOR EGR-1
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Stress is one of the main factors of ulcer disease pathogenesis. Transcription factor Egr-1 is an immediate early gene which is activated by stress exposure and regulated by mitogen-activated protein kinases (Erk1/2, p38). It was demonstrated that Egr-1 is a key gene and transcription factor in experimental duodenal ulceration (Khomenko T et al. Am J Physiol...
Gastrointest Liver Physiol. 2006;290(6):G1211-8). In present study we tested the hypotheses that transcription factor Egr-1 may play a role in the molecular mechanisms of stress-induced gastric ulcers. Methods: Gastric ulcers were induced in male rats (150-190 g) by immobilization stress (IMO) for 6, 12 and 24 hr duration or immobilization stress combined with water-immersion (IMO-WI) for 20 min, 1 and 3 hr duration. Rats were killed immediately after stress exposure. Size and number of erosions/ulcers were estimated. Levels of Egr-1, Erk1/2, pErk1/2, p38, p-p38 in gastric mucosa were determined by Western blot. Results: IMO-WI stress induced rapid up-regulation of Egr-1 levels by 1.6- and 1.5-folds (p<0.05) at 20 min and 1 h before ulcers developed and down-regulation - at 3 hr when gastric ulcers were detected. Up-regulation of Egr-1 was associated with increased phosphorylation of Erk1/2 by 2.2; 2.6; 2.5-folds at 20 min, 1 and 3 hr (<0.01), respectively. Erk2 was the major isoform expressed and phosphorylated. Levels of p38 and its phosphorylated form were changed insignificantly.Unlike IMO-WI stress, IMO stress is characterized by slow ulcers development with appearance of small hemorrhage at 6 hr and ulcers at 24 hr. IMO stress induced up-regulation of Egr-1 levels at 6 hr (2.1-folds, p<0.05) with gradual down-regulation by 24 hr. Conclusion: 1) Expression of Egr-1 is increased in early stages of stress-induced gastric ulcer development; 2) Erk1/2 protein kinase might regulate Egr-1 activity in pathogenesis of stress-induced gastric ulcer disease.

OXIDATIVE MODIFICATION OF PROTEINS IN THE LIVER CELLS UPON LONG-TERM GASTRIC HYPOACIDITY IN RATS

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Introduction Over the past years it was shown that prolonged use of the proton pump inhibitors, e.g. omeprazole, can cause changes hormones secretion: hypergastrinemia and cholecystokinin secretion decrease. Last may lead liver cells dysfunctions. But to date there is insufficient information on the effects of prolonged use of omeprazole in rat liver. Proteins are key molecules in the functioning of cells that provide structural, regulatory and other functions. Thus, the normal state of protein molecules is essential for any cell.

Aim The goal of study was to determine the rate of oxidative modification of proteins in the liver cells of rats in conditions of long-term hypoacidity in rats.

Materials and methods Non-leaver male rats were used in our experiments. The control group of animals received the injections with 0,2ml of dH2O for 28 days. The rats of the experimental group was given omeprazole («Sigma», USA) (14 mg/kg, i.p.) during 28 days.

The extraction of liver cells was made by standard non-fermentative assays. To determine the rate of oxidative modification of proteins we used the method by Dubinina et al. Enzyme activities were determined by colorimetric methods: catalase activity according to Koroluk et al and Xanthine oxidase activity according to Bergmeyer H.U. The H2O2 levels were determined by Lapkin.

Statistical analysis was done by t-Student test.

Results It was established that in a day after 28 days treatment of omeprazole to rats the content of protein oxidative modification products in the cells of rat liver was enhanced. The level of neutral aldehyde (max absorbance at 346 nm) and neutral ketone (370 nm) products in liver cells increased in 1,72 folds (p<0.05) and in 2,12 folds (p<0.05) in comparison to control. At the same experimental conditions the amount of alkaline aldehyde (430 nm) and ketone (530 nm) products enlarged even more dramatically – in 2,14 folds (p<0.05) and in 1,86 folds (p<0.05) respectively.

The increase of the protein oxidation products level upon long-term hypoacidity evoked by omeprazole can indicate on the activation of free radical processes in the liver.

The study have shown, that xanthine oxidase activity by 31,4% (p<0.05) and H2O2 levels in 2,85 fold (p<0.05) were increased in experimental rats, besides catalase activity of liver cells decreased by 32,0% (p<0.05) in rats with prolonged hypoacidity comparing with control animals.

Conclusion It is evident that long-term hypoacidity induce oxidative stress in liver.

THE ROLE OF NUTRITION RELATED METABOLIC STRESS IN PRENATAL PERIOD ON HEPATOCellular ORGANIZATION (EXPERIMENTAL RESEARCH)

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Recent evidence indicates that the incidence of nonalcoholic fatty liver disease (NAFLD) has rise dramatically over the past several decades, resulting in 28-36% of Europeanians that are currently overweight or obese. Accumulating data from basic and clinical studies demonstrate that the etiopathogenetic mechanisms leading to the development of hepatic steatosis remain poorly understood, it is often characterized by excess hepatic lipid accumulation, hepatic injury, inflammation, dyslipidemia and finally liver fibrosis. However, studies in this area have suggested that fatty livers are highly vulnerable to liver residential and by recruited inflammatory cells and secondary insults, such as those mediated by alimentary oxidative stress, which could accelerate the progression of hepatic steatosis toward more debilitating and advanced stages of NAFLD. Thus, we tested hypothesis that the prenatal programming of metabolic status, as presence of prenatal stress will play an important role in development of metabolic-related hepatocellular reorganization. This study was designed to determinate influence of modification of metabolic status during the prenatal period on hepatic cell organization and
comparative analysis of the hepatocellular reorganization and the histological changes in liver at the terms of the different models of premetabolic syndrome. Histomorphological researches of liver were estimated in nonlinear rats males (n=80, by mass of 180±30 g), control group (intact animal) and offsprings of mothers, that during pregnancy induced metabolic stress after next models: 1 group - chronic introduction of 30% solution of saccharose with a drinking-water (by Kozar, et al., 2009); 2 group - high-calorific feed with prevailing of fats (to 45% kcal by A. Lintermans, et al., 2009); 3 group - a binary influence of those marked metabolic factors. To estimate the degree of the hepatocellular lesion the videoanalysis of liver microsections by semiquantitative intensity score system of the degree of hyperplasia, intensity of inflammation and modification of microangiarchitectonic was performed on the licensed system of the videotape recording of AVerMedia. Results. Liver of the animals from 1 group had ordinary histological structure. In the second group of animals liver changes were insignificant: signs of swelling, lobular disorganization in some hepatocytes and wrong accumulation of glycogen. In the 3 group appeared such liver changes as edema and infiltrations of leukocytes. We concluded that prenatal period is the important stage of forming the hepatocellular resistance in ontogenesis. Prenatal metabolic stress initiates cellular reorganization of liver, that potentially is the foundation for development of NAFLD. The nature of these changes by additional biomarkers of inflammation remains to be investigated.

THE PHYSIOLOGICAL ROLE OF THE GPRC6A AMINO ACID RECEPTOR

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In 2004 we reported the cloning and tissue expression of a novel human family C G protein-coupled receptor, termed GPRC6A [1]. Measurement of Ca2+-dependent chloride currents in Xenopus laevis oocytes facilitated the deorphanization of GPRC6A and identification of L-alpha-amino acids as agonists. The most active agonists were basic L-alpha-amino acids, L-Arg, L-Lys and L-ornithine, suggesting that these may function as modulators of intracellular calcium and inositol phosphate levels in mammalian cells, which have been used to characterize a range of commercially available L-Arg and L-Lys analogs and allosteric modulation by divalent cations [4]. In order to study the physiological function of the receptor we have developed a GPRC6A knock-out mouse by ablation of exon 6 coding the 7-transmembrane domain. The mice show no obvious phenotype on regular chow [5], but do show a phenotype related to exercise/metabolism in more complex scenarios. Most recently, we have also developed a GPRC6A specific antagonist using a chemogenomic approach [6], which will likewise be used to elucidate the function of the receptor. Here we will present this compound series and mutational analyses of the binding site which has been shown that the compounds bind in the 7TM domain.

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ROLE OF RENIN-ANGIOTENSIN SYSTEM METABOLITES IN GASTROPROTECTION AND ULCER HEALING. INVOLVEMENT OF MAS RECEPTOR, PROSTAGLANDINS AND CAPSAICIN SENSITIVE NERVES

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Angiotensin II, the central product of renin-angiotensin system was shown to induce vasoconstriction, oxidative stress and inflammation but the inhibition of angiotensin converting enzyme (ACE) or the blockade of angiotensin AT-1 receptors prevents the gastric damage induced by ethanol and ischemia/reperfusion. We assessed the metabolism of angiotensin in the gastric mucosa and we studied whether angiotensin 1-7 (Ang1-7), the downstream peptide of angiotensin I pathway could influence gastric lesions induced by water immersion restraint (WRS). Rats were pretreated 30 min before the WRS with either A) Ang 1-7 (5 -150 μg/kg), B) Ang II (10 – 100 μg/kg i.p.) C) Prindopril (5 mg/kg i.p.), the long-lasting ACE inhibitor and 4) Losartan (5 mg/kg i.p.), the AT1 receptor antagonist. In separate group of rats, the Ang1-7 was given with or without the co-treatment with 1) A779, the selective Mas receptor antagonist (50 μg/kg); 2) AVE0199 (50 μg/kg), the agonist of Mas receptor, 3) the non-selective (indomethacin; 5 mg/kg i.p.) or selective COX-1 (560, 5 mg/kg i.p.) and COX-2 inhibitors (celecoxib 30 mg/kg i.g.), and 2) blockade of sensory nerves by capsaicin (125 mg/kg s.c.) or inhibition of vanilloid receptor (VR-1) by capsazepine (10 mg/kg i.g.). The number of gastric lesions was measured by planimetry, the gastric blood flow (GBF) determined by H2-gas clearance technique and COX-1-, COX-2-, cNOS, IL-1β-, and TNF-α mRNAs and proteins were assessed RT-PCR and Western Blot. Mass spectrophotometry determinations revealed that Ang1-7 is the most abundant metabolite of Ang-1 within gastric mucosa. WRS induced mucosal hemorrhagic lesions

5. Gloriam, Wellendorph, Johansen et al. Submitted.

METABOLITES IN GASTROPROTECTION AND ULCER HEALING. INVOLVEMENT OF MAS RECEPTOR, PROSTAGLANDINS AND CAPSAICIN SENSITIVE NERVES

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Angiotensin II, the central product of renin-angiotensin system was shown to induce vasoconstriction, oxidative stress and inflammation but the inhibition of angiotensin converting enzyme (ACE) or the blockade of angiotensin AT-1 receptors prevents the gastric damage induced by ethanol and ischemia/reperfusion. We assessed the metabolism of angiotensin in the gastric mucosa and we studied whether angiotensin 1-7 (Ang1-7), the downstream peptide of angiotensin I pathway could influence gastric lesions induced by water immersion restraint (WRS). Rats were pretreated 30 min before the WRS with either A) Ang 1-7 (5 -150 μg/kg), B) Ang II (10 – 100 μg/kg i.p.) C) Prindopril (5 mg/kg i.p.), the long-lasting ACE inhibitor and 4) Losartan (5 mg/kg i.p.), the AT1 receptor antagonist. In separate group of rats, the Ang1-7 was given with or without the co-treatment with 1) A779, the selective Mas receptor antagonist (50 μg/kg); 2) AVE0199 (50 μg/kg), the agonist of Mas receptor, 3) the non-selective (indomethacin; 5 mg/kg i.p.) or selective COX-1 (560, 5 mg/kg i.p.) and COX-2 inhibitors (celecoxib 30 mg/kg i.g.), and 2) blockade of sensory nerves by capsaicin (125 mg/kg s.c.) or inhibition of vanilloid receptor (VR-1) by capsazepine (10 mg/kg i.g.). The number of gastric lesions was measured by planimetry, the gastric blood flow (GBF) determined by H2-gas clearance technique and COX-1-, COX-2-, cNOS, IL-1β-, and TNF-α mRNAs and proteins were assessed RT-PCR and Western Blot. Mass spectrophotometry determinations revealed that Ang1-7 is the most abundant metabolite of Ang-1 within gastric mucosa. WRS induced mucosal hemorrhagic lesions
accompanies the fall in GBF but Ang1-7 dose-dependently attenuated WRS-induced gastric lesions, while producing a significant increase in GBF, the dose inhibiting WRS lesions by 50% being 38 μg/kg. The protective and hyperemic effects of Ang 1-7 were similar to those exhibited by prinidopril, Losartan and AVE0199. Ang 1-7 protection was significantly reversed by the A779 and significantly attenuated by indomethacin and celecoxib, but only slightly altered by SC-560. Co-administration of 16.16 dm PG_E2 (5 μg/kg-d i.p.) with Ang 1-7 restored the protective activities of these peptides. Capsaicin denervation or capsazepine also significantly reduced the gastroprotective effects of Ang 1-7 and the addition of exogenous CGRP (10 μg/kg s.c.) restored the protection and mucosal hyperemia of this peptide in capsaicin-denervated rats. The Ang 1-7 increased expression of AT1 receptor and COX-2 mRNA and downregulated IL-1β and TNF-α expression and plasma levels of these cytokines enhanced by WRS. We conclude that Ang 1-7 exerts gastroprotective activity via mechanism involving activation of PG-COX-2 and NOS/NO system, sensory nerves and suppression of the proinflammatory cytokines.

**EFFECTS OF IBUPROFEN, AND SOME ANALOGUES, ON THE REACTIVITY OF FALLOW DEER DIGITAL ARTERY SEGMENTS**

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Therapy with non-steroidal anti-inflammatory drugs (NSAIDs) can lead to gastrointestinal complications, including dyspepsia, erosions and ulcers, etc. Their adverse effects in the GI tract are thought to be related to lowering the production of COX-1-derived prostaglandins. One attempt to reduce the gastric toxicity of NSAIDs has been to exploit the protective properties of nitric oxide (NO), which includes mucosal vasodilation to increase the rate of absorption of the NSAID. In the course of a study of the vascular reactivity of isolated segments of fallow deer common digital artery, nitroxybutyl esters of some NSAIDs were found to be effective at reducing the tension responses to applied vasoconstrictors and electrical stimulation. The tension responses of vessel segments, of approximately 3 mm in length, in water-jacketed organ baths at 37°C, were recorded. Resting tension was maintained at 3 g. The vessel segments were contracted, either with single or graded concentrations of either phenylephrine (PHE) or 5-hydroxytryptamine (serotonin, 5-HT). Square-wave, electrical stimulation was applied through field electrodes. Nitroxybutyl-aspirin (NO-aspirin) effectively reduced the contractile responses of vessel segments, produced by increasing concentrations of 5-HT, whereas aspirin and its butyl ester were without effect. When the effects produced by ibuprofen and flurbiprofen were compared with their respective nitroxy-derivatives, all four compounds reduced responses to stimulation electrical stimulation, with no significant difference in effect between the NO-derivatives and their parent compounds. With electrically-stimulated segments it was found that (+)-ibuprofen itself produced a reversible relaxation of vessel segments, pre-contracted with 3 x 10^{-6} M PHE, down to a tension of 16.5 ± 15% of control by itself. Use of 1 x 10^{-5} M of the soluble guanylate cyclase (sGC) inhibitor, 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) reduced the effect of the ibuprofen to control values indicating that stimulation of sGC was involved. Diclofenac also caused a reversible relaxation to the resting tension at 3 x 10^{-4}M.

These experiments also demonstrated that the R(-)-ibuprofen produced a significantly larger relaxation than the S-(+)enantiomer is of relevance because, in vivo, R(-)-ibuprofen is converted into S-(+)-ibuprofen, which is the much more effective NSAID. Thus, for prolonged use, a preparation consisting predominantly of R(-)-ibuprofen might be less damaging as it should pass through the gastric mucosa more rapidly than the S-(+)ibuprofen, which is the much more powerful inhibitor of COX enzymes.

**GI PHARMACOLOGY: THE IMPORTANCE OF BENCH-TO-BEDSIDE-AND-BACK CONCEPT**

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The concept of bench-to-bedside-and-back, the so-called translational research, can be defined as “The process of applying ideas, insights and discoveries generated through basic scientific inquiry to the treatment or prevention of human disease”(NIH). It also accommodates “Taking ideas from clinical research back into experimental settings”. Cases in point can be traced back to the early 19th century when William Beumont (surgeon) performed his clinical research and published a book entitled “Experiments and observation on the gastric juice and the physiology of digestion” in 1833. Discovery of the role of vagus in regulation of gastric acid secretion by Ivan Pavlov (physiologist) in the late 19th century led Lester R. Dragstedt (physiologist and surgeon) to perform the first truncal vagotomy in a patient with chronic duodenal ulcer in 1943. Furthermore, discoveries of H2 receptor antagonist cimetidine by James W. Black (pharmacologist) in 1976 and H. pylori in the stomachs of patients with gastritis and stomach ulcers by Barry J. Marshall (physician) in 1982 exemplify the translational research. Hence, the lessons learned illustrate the importance of the right concept for the basic research to identify the right target and test the right compound in the right model; and for the clinical research to identify the right disease-relevant phenotypes and the right endpoints. The emerging challenge in GI pharmacology
is to shorten the time it takes to translate bench discoveries into new medical treatments or preventions.

THE PENTADECAPEPTIDE BPC 157 PROMOTES HEALING OF COLOVESICAL FISTULAS IN RATS

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Introduction. A colovesical fistulas present an important clinical problems. They usually occur as a complication of malignant process or IBD. The pentadecapeptide BPC 157, in clinical trials for IBD therapy, has already shown the effectiveness in healing colocutanous fistulas (J. Pharmacol Sci 2008), gastrocutaneous fistulas (Dig Dis Sci 2009). Therefore we suggest it as a possible therapy of colovesical fistulas.

Materials and methods. 20 male Wistar Albino rats were randomly assigned into two groups. The colovesical fistulas were created at 5 cm from anocutanous borderline, with the diameter of 5 mm. The surgical procedure was performed according to rules brought by the Local Ethical Committee. Half of them received saline (5 mL/kg, i.p., control group), while the others received the pentadecapeptide BPC 157 (10 µg/kg,i.p.). The treatment was once daily, the first dose was applied immediately after the operation and the animals received the last dose 24h before sacrifice. At the end of each experimental period (2 days, 6 days) the animals were sacrificed and the biomechanical, functional, macroscopic and microscopic assessment were performed.

Results. Seven days following the surgery the diameters of fistulas were larger in controls then in those treated with pentadecapeptide BPC 157. Until the 14th postoperative day all BPC 157 treated animals urinated through urethral orifice, while at the majority of controls urinating was through the anus the same as the first postoperative day. The fistulas in control animals were still opened, while the ones in BPC 157 treated animals shown even more intensive diameter reduction.

Conclusion. According to these results the pentadecapeptide BPC 157 could present a new possible pathway in therapy of colovesical fistulas.

CYTOPROTECTIVE EFFECT OF ENTEROSORPTIVE THERAPY OF CHILDREN WITH INFLAMMATORY INTESTINAL DISEASES (IID)

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IID are stipulated by chronic nonspecific autoimmune inflammation of the intestine wall with development of endogenous intoxication, evidence of which is stipulated by severity of the process and depends on the nature and location of the lesion. In accordance with standards complex therapy of the given children’s diseases includes enterosorption. Comparative characteristic of modern enterosorbents has shown that the preparation to be chosen shall be selective national enterosorbert Enterosgel (reg.no.003719/02). The given preparation has selective sorption and detoxication properties, thus reducing toxic load on natural detoxication systems and the risk of development of infectious and autoimmune complications. Enterosgel does not reduce absorption of vitamins, microelements and Ca. In comparison with sorbents of other nature for children with IID the given preparation has a whole series of undisputable advantages. It has an organic nature and high biocompatibility with intestine tissues. It is rapidly withheld from the gastrointestinal tract together with toxic substances and does not penetrate into the internal environment of the organism through the intestinal barrier, it has hydrophobic surface. Enterosgel is completely harmless and in contract to other sorbents does not cause damages to the mucous membrane of the gastrointestinal tract and may be administered protractedly (within several months) without causing atony of the intestine. It protects cell surface of the mucous membrane of the stomach against aggressive attacks and provides absorption of ulcerative destruction products, which prevent adequate epithelization and improvement of morphofunctional state of the intestine wall. Enterosgel has adhesive properties as to pathogenic germs, destructs pathogenic and conditionally pathogenic microflora, Candida fungi, viruses (including cytomegalovirus) and their toxic products, reduces excessive microbe contamination of the small intestine. The preparation does not inhibit saprophytic microflora (lactobacteria, bifidobacteria, etc.) Consequently Enterosgel creates comfortable conditions for development of the normal microflora and improvement of the microbiological landscape of the intestine. Cytoprotective properties of the preparation are stipulated by restoration of the intestinal barrier accompanied by increase of sIgA secreted by cells of the mucous membrane of the intestine, restoration of the structure of the microcirculatory race and positive morphologic dynamics.

Apparent clinic efficiency with the distinct cytoprotective effect and comfortable pharmaceutical form (paste, gel) enable to recommend Enterosgel for wide application with children with IID for the purpose of sorption effherent therapy.

CRF MAY PROTECT THE GASTRIC MUCOSA IN STRESS THROUGH INVOLVEMENT OF GLUCOCORTICOIDS

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The results of our previous investigations suggest that glucocorticoids released during stress act as gastroprotective hormones, and not as ulcerogenic
agents as has been generally accepted for a long time. In the present study we investigated whether corticotropin-releasing factor (CRF) may protect the gastric mucosa against stress-induced gastric injury through involvement of glucocorticoids. CRF administration (1.25 mg/kg, i.p., 30 min before onset of stress) markedly increased plasma corticosterone level and significantly suppressed the occurrence of gastric erosion induced by 3-h cold-restraint stress (at 10°C) in control rats. To estimate the role of glucocorticoids in CRF-induced gastroprotection the effect of CRF on the stress-induced gastric erosion was studied after acute reduction of corticosterone release by metyrapone (30 mg/kg, i.p., 30 min before CRF) or occupation of glucocorticoid receptors by the antagonist RU-38486 (20 mg/kg, i.p., 2 h before CRF). Metyrapone injected shortly before CRF administration caused a fast inhibition of corticosterone response to CRF and prevented its protective effects on the gastric mucosa against the stress-induced erosion formation. The gastroprotective effect of CRF was also eliminated by the pretreatment rats with glucocorticoid receptor antagonist RU-38486. The results obtained suggest that CRF may protect the gastric mucosa against stress-induced gastric injury through involvement of glucocorticoids. This study was supported by grants from RFBR-10-04-00605; FNM RAS-2010-2011; DBS RAS-2011.

THE HEALING OF NSAID-INDUCED GASTRIC LESION MAY BE FOLLOWED BY SMALL INTESTINAL AND CARDIOVASCULAR SIDE EFFECTS: TELEMETRY STUDY IN FREELY MOVING CONSCIOUS RATS

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Background/Aim: Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most commonly used kinds of medications, however, adverse effects, seriously complicate their use. Beside the most widely known side effect in the upper gastrointestinal (GI) tract, NSAIDs may also induce adverse effects in the small intestine and cardiovascular system. However, the side effects are investigated separately, which impedes progress in understanding pathogenic mechanisms. The purpose of the present study was to verify the hypothesis that the healing of indomethacin-induced gastric erosion may be followed by small intestinal and cardiovascular side effects. Methods: During a telemetry study on freely moving conscious male rats we investigated effects of indomethacin, injected at a gastric ulcerogenic dose (35 mg/kg, SC), on intestinal mucosal integrity, circadian heart rate, body temperature and locomotion cycles. The rats underwent operation for intraperitoneal implantations with radiotelemetry transmitters under ketamin-xylazine-pipolphen anaesthesia (50-10-5 mg/kg, IP) and six days later (after preliminary 24 h fasting) were administered indomethacin or its vehicle. Four hours after the indomethacin or vehicle administration the animals were fed and the experiment was continued for another 3 days. To explore indomethacin-induced gastric injury and its healing, a part of animals were decapitated in 4 h or 24 h after indomethacin administration. Results: Indomethacin produced hemorrhagic erosion that was visible in the glandular stomach 4 h after its administration and was almost completely healed one day later. However, in 3 days after indomethacin administration an intensive injury was discovered in the small intestine. The control rats’ heart rate, core body temperature and locomotion all agreed with a normal circadian rhythm (i.e. their maximum values at night and their minimum values during the day) 3 days after the vehicle injection. However, the circadian cycle of rats treated with indomethacin in one day after indomethacin administration was disrupted: their heart rate rose to it’s maximal level and their locomotion and core temperature values fell to their minimal levels. Conclusions: Our results suggest that the healing of gastric erosion induced by a single indomethacin injection may be followed by other pathological events outside of the stomach, among which there may be intestinal injury and a lost of a normal circadian cycle of heart rate as well as body temperature and locomotion. Our findings agree with a novel composite endpoint that measures damage to the entire GI tract - clinically significant upper and lower GI events (CSULGIE) - in patients with NSAID-induced GI damage. Moreover, our results emphasize the need to investigate NSAID-induced cardiovascular effects together with the measuring of damage dealt to the entire GI tract. This study was supported by grants from RFBR-10-04-00605; FNM RAS-2010-2011; DBS RAS-2011.

COMPARATIVE IN VITRO TOXICITY OF ULCEROGENIC CHEMICALS TO CULTURED ENDOTHELIAL, EPITHELIAL AND FIBROBLAST CELLS

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Our previous in vivo studies demonstrated that injury of mucosal vascular endothelial cells precedes development of ethanol-induced gastric erosions and iodoaceticamide-induced ulcerative colitis in rats (Gastroenterology, 1985 and 1987; Lab Invest, 2011). This study aimed to examine in vitro the susceptibility of cultured mucosal endothelial, epithelial cells and fibroblasts to injury by ulcerogenic chemicals, i.e., gastrotoxic ethanol, duodenal ulcerogen cyssteamine and ulcerative colitis-inducing iodoaceticamide. Methods: Rat intestinal epithelial cells (IEC-6), rat gastric microvascular endothelial cells and mouse (3T3) fibroblasts were exposed to: A) 2, 4, 8, 10 or 12% of ethanol for 0.5 hr; B) 0.1, 1, 5, 10 mM of cysteamine for 3 hr or C) 10, 50, 100, 150, 200 μM of...
A discovery of membrane digestion has led to reappraisal of many classic concepts, and has solved a number of serious contradictions in physiology of digestion. In particular, strikingly high rates of food digestion in the body under the normal conditions, and very efficient coupling between hydrolytic and absorptive processes have received their explanation. Many aspects of membrane digestion have been characterized in the investigations performed under A.M.Ugolev's supervision: the levels of organization of membrane hydrolysis, its spatial topography along the small intestine and apical-cryptal axis, organization and regulation of poly-substrate processes in the small intestine.

On the base of novel views about digestion and nutrition, A.M.Ugolev presented physiological grounds for the problem of creation and use of artificial food, and was a leader and participant in development of many new diagnostic methods and techniques, generally accepted in clinics. He gave an explanation of etiology and pathogenesis of some forms of gastrointestinal pathology on the ground of the membrane digestion conception, made a significant contribution in the problem of productivity of agricultural animals.

The membrane digestion theory has become a new area of physiology, vital for understanding and studying of the gastrointestinal tract functioning. For the creation of the modern theory of digestion and nutrition A.M.Ugolev was awarded the I.P. Pavlov (1963) and I.M. Sechenov (1986) Prizes of the USSR Academy of Sciences.

### FUNCTIONAL CHARACTERISTICS OF THE RAT SMALL INTESTINE AT THE DIFFERENT TIMES AFTER DEXAMETHASONE INJECTIONS

The aim is to evaluate absorptive capacities of the rat small intestine and activities of some digestive enzymes at various time intervals after the injection of dexamethasone.

Methods. In the 1st experiment we were recording for 5-6 hrs the rates of free consumption of glucose solution (200 g/L) by Wistar rats, who were trained to drink the solution after a preliminary 20-hrs fasting. The animals were then divided into two groups with close average rates of glucose consumption. Three days later, in the 2nd experiment, the rates of consumption of the solution by fasted rats were recorded after the injections of propylene glycol (gr.1, control) or dexamethasone (gr.2) 24 hrs before the experiment. Seven days later, the 3rd experiment was performed on the same rats without any injections. In the 4th experiment (four days later) a consumption of glucose solution was recorded after injections of propylene glycol (gr. 1) or dexamethasone (gr.2) 1 hour before the experiment. After the 2nd, 3rd and 4th experiments, three rats from each group were
neuropeptides, e.g. TRH, amylin, adrenomedullin, showed that central administration of several integrity has been intensively studied, and the results nervous system (CNS) in maintaining gastric mucosal documented. In the last 15 years the role of central involved in gastric mucosal protection are well Background: 

by central mechanism (Shujaa et al., 2009) AG/) and synthetic cannabinoid derivatives (WIN 55 2006; Brozowska et al., J. Phys. Pharm. 2009) induced gastric mucosal protection in experimental ulcer models. 

It is concluded that the effect of dexamethasone on functional characteristics of the small intestine (regarding the rates of free consumption of glucose solution by rats as indicators of the small intestinal capacity for glucose absorption) varies depending on the time elapsed since its injection. 

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ANALYSIS OF THE EFFECTS OF DIFFERENT NEUROPEPTIDES IN GASTRIC MUCOSAL DEFENSE INITIATED CENTRALLY 

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Background: Peripheral factors and mechanisms involved in gastric mucosal protection are well documented. In the last 15 years the role of central nervous system (CNS) in maintaining gastric mucosal integrity has been intensively studied, and the results showed that central administration of several neuropeptides, e.g. TRH, amylin, adrenomedullin, β-endorphin, leptin, nociceptin, nocistatin, ghrelin, melatonin (Tache et al., J. Gastroent. Hepatic. 1994; Guidobono et al., Br. J. Pharm. 1998; Kaneko et al., Am. J. Physiol. 1998; Gyires et al., J. Pharm. Exp. Ther. 2001; Brzozowski et al., J. Phys. Pharm. 2001; Zádor et al., Peptides 2008; Brzozowski et al., J. Phys. Pharm. 2006; Brzozowska et al., J. Phys. Pharm. 2009) induced gastric mucosal protection in experimental ulcer models. Besides neuropeptides endogenous cannabinoids (anandamide, 2-arachidonoylglycerol /2-AG/) and synthetic cannabinoid derivatives (WIN 55 212-2, ACEA) also induced gastric mucosal protection by central mechanism (Shujaa et al., 2009). Recent findings showed that activation of angiotensin AT1 receptors leads to activation of co-expressed cannabinoid CB1 receptors in CHO cells (Turu et al., J. Biol. Chem. 2007). Aims: To study whether activation of AT1 receptors by angiotensin II (AngII) may elicit gastric mucosal protection by a cannabinoid-dependent pathway. Methods: Gastric mucosal damage was induced by oral administration of acidified ethanol in Wistar rats as well as in wild type and CB1 receptor KO mice; the gastric lesions were evaluated 60 min (rat) and 30 min (mice) later. The compounds were given intracerebroventricularly (icv.) 10 min before ethanol administration. Results: 1. AngII inhibited the ethanol-induced gastric mucosal lesions in a dose-dependent manner (0.012-0.19 nmol/10 icv). The effect was antagonised by both the AT1 receptor antagonist candesartan (16 nmol) and the CB1 receptor antagonists SR 141716A (22 nmol) and AM 251 (1.8 nmol). 2. Tetrahydrolipstatin (THL) (0.2 nmol), inhibitor of DAG lipase, which enzyme is reponsible for the formation of 2-AG, inhibited the gastroprotective effect of AngII (0.19 nmol). 3. AngII (0.19 nmol) exerted gastroprotective effect also in wild type mice, however, it was ineffective in CB1 receptor KO mice. Conclusion: AngII exerts centrally initiated gastric mucosal protection by activation of the endogenous cannabinoid system. This is the first in vivo evidence for the AT1 receptor-induced activation of cannabinoid CB1 receptors. 

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ANALYSIS OF THE ROLE OF IMIDAZOLINE RECEPTORS IN THE REGULATION OF GASTRIC MOTILITY IN A2-ADRENOCEPTOR DEFICIENT MICE 

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Aims: Several studies suggest that some effects of clonidine and structurally related α2-adrenoceptor agonists are mediated by imidazoline receptors, instead of α2-adrenoceptors. However, analysis of imidazoline receptors can raise several difficulties, since most imidazoline agonists possess reasonable affinity for α2-adrenoceptors as well. The aim of our study was to test different imidazoline ligands (moxonidine, rilmenidine, AGN 192403) in α2-adrenoceptor KO mice in order to clarify, whether they can inhibit gastric contractions in mice, and if so, α2-adrenoceptors or imidazoline receptors mediate their effect. 

Methods: Wild type, α2x-α2y and α2c-KO C57BL/6 mice were used. For analysis of gastric motor activity, mice were killed by cervical dislocation, their stomachs

sacrificed to measure activities of intestinal enzymes (maltase, saccharase). Results. The rates of glucose consumption were almost constant in the interval 90–360 min after the beginning of each experiment, and during the 1st experiment did not differ in gr.1 and gr.2 (13.1±0.2 and 12.4±0.3 mg/min respectively). In the 2nd experiment the rate of glucose consumption in gr.1 (13.0±0.4 mg/min) was significantly higher as compared with gr.2 (9.1±0.5 mg/min, P<0.02). Seven days later, the rates of glucose consumption differed insignificantly in the two groups of the animals (10.4±0.8 against 12.1±0.8 mg/min). Injection of dexamethasone 1 hr before the experiment resulted in an increase of glucose consumption (16.1±1.3 mg/min as compared with the control: 7.8±0.8 mg/min, P<0.01). Maltase activity in the small intestinal mucosa after the dexamethasone injection 24 hrs before the experiment increased (against the control) being calculated per g of tissue, but remained unchanged being calculated per whole length of the small intestine, while saccharase activity decreased in the both ways of calculation. It is concluded that the effect of dexamethasone on functional characteristics of the small intestine (regarding the rates of free consumption of glucose solution by rats as indicators of the small intestinal capacity for glucose absorption) varies depending on the time elapsed since its injection. 

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were removed and fundus stripes were suspended between two electrodes in 5 ml organ baths containing 37°C Krebs solution, then EFS was applied. Drugs were added in a cumulative manner. Results: 1) Both oxamoxindone and rilmenidine (1-10000 nM) inhibited the EFS-induced contractions in a concentration dependent manner in wild type, α2B- and α2C-KO mice. 2) The effect of both drugs was antagonized by the non selective α2-adrenerceptor and imidazoline antagonist idoxazan (10000 nM) and by the selective α2A- adrenerceptor antagonist BRL 44408 (10000 nM), but not by the α2Hc-adrenerceptor antagonist ARC 239 (10000 nM). 3) Neither oxamoxindone, nor rilmenidine inhibited the gastric contractions in α2A-KO mice. 4) The selective 11-agonist AGN 192403 (1-10000 nM) failed to affect the EFS-induced contractions in wild type mice. Conclusion: Our results obtained from genetically engineered mice strongly suggest that α2A- adrenerceptors, and not imidazoline receptors mediate the inhibitory effect of imidazoline ligands on the gastric motility. The work was supported by ETT 341/2009 from the Scientific Health Council, Hungary, and by TAMOP-4.2.1/B-09/1/KMR from the National Development Agency.

ROLE OF ENDOGENOUS COX AND LOX PATHWAYS IN THE HEALING OF STRESS-INDUCED ESOPHAGEAL LESIONS DURING EXPERIMENTAL GLUCOSA DISREGULATION (GD)

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The increased incidence of GD, as prediabetes, defined as the presence of elevated fasting glucose, abnormal glucose tolerance or both, which associated with risk for type 2 diabetes (DM), and prevalence of type 1 DM are a global trend. Gastro-intestinal (GI) disorders associated with GD are actual for translational medicine. Recent clinical observation suggest that GD associated to COX/LOX pathways, which are involved in cell proliferation, differentiation and protection against apoptosis, have been implicated in esophageal adenocarcinoma development. The therapeutic approach of modification COX/LOX activity whereby enhanced esophageal healing are incompletely understood. Leukotrienes are moreover pro-inflammatory and induce chemotaxis of leucocytes in the inflamed tissue and increase microvascular permeability, increases superoxide generation and proinflammatory cytokines production. Previous studies from our laboratory demonstrated that nitrosative stress involved in diabetic esophagopathy (JPP, 2008, V.59(2):77-89) and we hypothesized that selective inhibition of COX-2 alone may lead to a shunt of arachidonic acid metabolism towards the LOX pathway, and therefore the inhibition of both COX-2 & 5-LOX may produce a better pro-inflammatory response. This study examined further the role of COX/LOX pathways in healing of stress-associated esophagitis (SAE) during experimental GD and compare the effects of different COX/LOX inhibitors, as a novel dual block acting COX-2 & 5-LOX agent darbufelone (DFL) which was generated in LNMU. Were enrolled rat groups without/with GD induced by metabolic stress (MS) model by Kozar, 2009 & streptozotocin-induced DM model (SIDM) which randomly divided into 4 groups: control (intact) and treated by week either with 1) vehicle 2) celecoxib (10 mg/kg) 3) DFL (10mg/kg). SAЕ was induced in all animals by model of water immersion and restraint stress by Takagi, 1964. Survival rate, destruction, size and the quality SAЕ by histological score index (HSI) was investigated. GD was confirmed daily glucometeria. Esophageal mucosal expression of iNOS/cNOS activity were determined by Sumbaeva, 2004 methods. Results: iNOS and eNOS activity was expressed basally in esophageal mucosa. SAΕ was developed in all GD animals, HIS of SAЕ in SIDM was higher than in MS rats followed by increase in 3,2 fold of iNOS expression. DFL, celecoxib significantly reduced HIS of SAЕ and reversed the fall of eNOS activity and decreased iNOS in DM group to compare to vehicle group. Higher activity eNOS was observed in MS DFL-treated vs to celecoxib-treated animals. Conclusion: COX-2 & 5-LOX play an important role for in esophageal lesions linked to GD. Novel findings related to disproportion of iNOS/eNOS activity and possible associations with GI-GD are also presented. Antulcerogenic and anti-inflammatory potential of DFL on SAΕ-related to GD was revealed.

THE EVOLUTIONARY IMPACT OF LACTOBACILLI ON H. PYLORI AND GASTRIC ACID SECRETION: DID A CENTURY OF DIETARY CHANGE ALTER THE GASTRIC MICROBIOTA?

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Since the mid 1980s, it has been accepted that Helicobacter species are the only organisms capable of surviving the hostile mammalian gastric environment. Here we present the evidence that Helicobacter is not the only bacterial genus able to colonize the gastric mucosa. Lactobacillus species, which have recently been shown to colonize the human stomach, are probably a natural probiotic that commonly co-existed with Helicobacter pylori until about 150 years ago. Lactic acid produced by Lactobacilli can impact the survival of Helicobacter pylori as well as modulate gastric physiology as a natural antisecretory agent. Helicobacter species with its potential detrimental effects and the Lactobacillus species with potential beneficial effects acting as a natural gastric antisecretory probiotic, have co-existed in the stomach throughout human evolution, serving as a good example of self-regulating bacterial co-existence. Changes in the gastric microbiota with the emergence of the industrial evolution have subsequently lead to an increasing
gastric secretory capacity, dominance and acquisition of pathogenic genes by *Helicobacter pylori* in humans, resulting in the subsequent emergence of the “modern” acid related diseases. We propose that the diminished prevalence and loss of *Lactobacillus spp.* over the past century as a result of the modernization of our diet and environment have contributed to a dominance of *H. pylori*–induced inflammation, hyperchlorhydria and subsequently the increase of peptic ulcer disease and GERD over this time. Thus, *Lactobacilli* should be explored as a normal organism of the gastric microbiota and as such positively impact *Helicobacter pylori*-induced inflammation and potentially acid-related diseases.

**ADVANCES IN UNDERSTANDING INFLAMMATION IN UPPER GI DISEASES**

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Erosive esophagitis (EE) and non-erosive reflux disease (NERD) are key components of reflux disease. Esophageal acid exposure initiates symptoms and induces or perpetuates mucosal damage. Diagnosis is based on typical reflux symptoms, endoscopic findings, pH monitoring, and histological changes (Vakil 2006, Modlin 2009).

The pathophysiology is complex involving acid, pepsin, bile and pancreatic secretions in the refluxate; failure of anti-reflux mechanisms and esophageal mucosal defense mechanisms. Weakly acidic reflux (pH 4-7) contributes to symptom generation but the precise role of acid in NERD remains unclear (Wang 2008). Mechanoreceptor-mediated pathways and different types of receptors are involved in symptom generation in NERD and peripheral and/or central hypersensitivity mechanisms contribute to symptoms (Modlin 2009).

Esophageal sensory control is governed via vagal and spinal pathways. Neurally mediated effects of reflux and inflammation in the mucosa and submucosa can lead to neural changes including remodelling (Shaker 2007). Mucosal changes (erosions / breaks) are assessed by endoscopy and the apparently “normal” esophagus may be neither normal nor healthy. The introduction of novel endoscopic and ultrastructural techniques indicate, minimal changes as potentially specific findings (Modlin 2008).

Histologic markers of reflux-induced mucosal injury are seen in most patients with NERD but have been considered of limited diagnostic value. Minimal changes, include basal cell hyperplasia, papillary elongation, neutrophil / eosinophil intraepithelial infiltration and dilatation of intercellular spaces (DISs). (Vieth 2008a, Zentilin 2005) which are responsive to PPIs, supporting their clinical relevance (Vieth 2008b).

New categories of M (minimal) and Grade N are proposed modifications to the LA system. DISs occur in 41 - 100% of NERD patients vs. 0 to 30% of controls (Tytgat 2008). Acidification of intercellular spaces can trigger symptoms, suggesting a mechanism for enhanced perception of acid reflux in NERD patients (Caviglia 2007). Acid-sensing ion channels (ASICs) in neurons are proton-gated cation channels in peripheral sensory / central neurons, playing an important role in physiological and pathological conditions (Xiong 2008). Deleting the ASIC3 channel in a rodent model prevents gastritis induced acid hyper-responsiveness of the stomach-brainstem axis (Wultsch 2008).

The inflammation in GERD is complex and cytokine profiles may explain the different outcomes in reflux disease, EE, NERD, Barrett’s metaplasia etc. (Rieder 2010). Cytokine-mediated mechanisms are related to acid / peptic injury. Acidified bile salts significantly increased secretion of IL-8 and interleukin-1β by squamous cells and media from these cells increased migration of T cells and neutrophils (Souza 2009). Interleukin (IL)-1β and IL-8 expression correlates with histo-morphological changes in the esophageal mucosa of patients with EE and NERD and a stepwise increase of DISs and basal cell hyperplasia from controls, NERD towards EE. Gene expression of both cytokines correlated with histology (Monkemuller 2009). The transient receptor potential vanilloid-1 (TRPV1) receptor is implicated in acid induced inflammation and nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF) are associated with up-regulation of TRPV1 receptors in patients with esophagitis (Shieh 2010). Proteinase-activated receptor-2 (PAR-2) is elevated in patients with NERD and EE and induces pro-inflammatory and neuro-inflammatory effects altering trans-epithelial resistance and mediating visceral hypersensitivity (Kandulski 2010). Esophageal inflammation may be induced by PAR2 activation by the induction of NFKappaB- and AP-1-dependent IL-8 production (Yoshida 2007). Moreover, luminal bacteria in the esophagus are potently important. Alterations in the esophageal microbiome are associated with inflammation and intestinal metaplasia of the distal esophagus and a type II pattern contains a greater proportion of gram-negative anaerobes / microaerophiles primarily correlated with EE and BE (Yang 2009).

Vieth M. Best Pract Res Clin Gastroenterol. 2008;22(4):625-38. (a)
Vieth M. Digestion. 2008;78 Suppl 1:24-30. (b)
Hydrogen sulfide (H₂S), a gasotransmitter, is formed from L-cysteine by enzymes including cystathionine-γ-lyase (CSE) in the mammalian body, playing various roles in the mammalian body including the gastrointestinal (GI) tract. H₂S targets multiple molecules including ATP-sensitive K⁺ (K_ATP) channels and TRPA1 channels. A series of our studies have shown that H₂S enhances T-type Ca²⁺ channel-dependent membrane currents, and that H₂S causes sensitization/activation of nociceptors in a manner dependent on Ca₃.2 among three isoforms of T-type Ca²⁺ channels, leading to peripheral hyperalgesia. Interestingly, the acceleration of Ca₃.2 signaling by CSE-derived H₂S is involved in the maintenance of neuropathic pain. We have also found that the H₂S/Ca₃.2 system participates in signaling of visceral pain through the CSE and subsequent increase in endogenous H₂S generation. In the colon, stimulation of sensory nerves by the H₂S/Ca₃.2 system might exert mucosal cytoprotection in rats with TNBS-induced colitis. Together, the CSE/H₂S/Ca₃.2 cascade appears to play emerging roles in processing of somatic and visceral pain signaling and also in the GI mucosal cytoprotection.

**THE HEALING OF COLOCUTANEOUS FISTULAS AND DICLOFENAC OVERDOSE.**

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Introduction. Until now there were only a few studies analyzing the impact of NSAID overdose onto the healing organic tissues. We analyzed the impact of diclofenac overdose onto the healing using the model of colocutaneous fistulas which comprises both the healing of anastomosis and fistula. The overdose of diclofenac impaires healing of colocutaneous fistulas despite the impairment caused by diclofenac intoxicification.

**THE PENTADECAPEPTIDE BPC 157 HEALS CHRONIC COLOCUTANEOUS FISTULAS.**

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Introduction. The inflammatory bowel disease is often associated with the appearance of fistulas as common complication. The therapy of complications presents a challenge for itself. To date there was no efficient conservative therapy found. The surgical procedure is often the only choice in treating of such cases. The pentadecapeptide BPC 157 has been proved efficient in clinical trials for IBD (phase II). The numerous studies has shown its effectiveness in healing different organic lesions including colocutaneous fistulas (J Pharmacol Sci 2008). In this study we assessed its activity onto the chronic lesions which cannot heal spontaneously. On the other hand we performed a 16 months long follow up in order to estimate the incidence of possible recurrences.

Materials and methods. 40 male Wistar Albino rats under deep anesthe5sis underwent surgical procedure. A colocutaneous fistula has been created (J Pharm Sci 2008). During operation 30animal received an overdose of diclofenac (6.25 mg/kg, i.p.). The animals were randomly assigned into two groups; half of them were in control group that was treated with saline (5mL/kg, i.p.), while the other received pentadecapeptide BPC 157 (10µg/kg). The treatment was once daily, the first dose was applied immediately after the operation and the animals received the last dose 24h before sacrifice. At the end of each experimental period (2 days, 5 days, 7 days) the animals were sacrificed and the biomechanical, functional, macroscopic and microscopic assessment were performed.

Results. In animals that received diclofenac the healing of anastomosis was impaired in comparison to those that did not receive the diclofenac overdose. On the second postoperative day the majority of animals treated with the pentadecapeptide BPC 157 started to defecate through anus, while the control animals defecate through fistula. At the fifth postoperative day the control were able to sustain only a small volume of water without leakage through fistula, while the animals treated with BPC 157 sustained larger volume. A week after surgery the animals treated with pentadecapeptide BPC 157 had reduced diameters of fistula, both external and internal, the same as reduced inflammatory answer according to morphologic assessment.

Conclusion. The overdose of diclofenac impaires healing of colocutaneous fistulas. The pentadecapeptide has shown the healing effect onto both skin and colonic defect of colocutaneous fistula despite the impairment caused by diclofenac intoxicification.
Non-steroidal anti-inflammatory drugs (NSAIDs) often cause gastrointestinal complications such as gastric ulcers and promoted vagal secretion of $\text{HCO}_3^-$.

**Results.** The animals treated with the pentadecapeptide BPC 157 from the 3rd posttreatment day started to expose the morphological and functional recovery. Through 2 weeks of treatment the fistulas were closed and animals started to defecate through anus only. In control animals the fistula remained during the whole period of study and animals defecate through fistula. After the treatment ended the animals treated with pentadecapeptide did not develop recidivisms.

**Conclusion.** According to these results the pentadecapeptide BPC 157 could present the encouraging new pathway in healing IBD complications.

**EFFECT OF PROTON PUMP INHIBITORS ON GASTRIC SECRETION OF BICARBONATES AND PEPsinogen INDUCED BY CHEMICAL IRRITATION OF THE GASTRIC MUCOSA**

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Proton pump inhibitors are able to modify sensitivity of the gastric mucosa to chemical irritation by suppressing of the back-diffusion of protons and by enhancing of the gastric epithelium permeability. Furthermore, inhibition of acid secretion leads to reduction of the alkaline tide and probably exhausts resource of bicarbonates in the submucosa. The aim of the study was to evaluate the effect of omeprazole on gastric secretions of bicarbonates and pepsinogen induced by irritation of the gastric mucosa with hyperosmotic acidic solution. Studies were performed with male Sprague-Dawley rats. In conscious gastric fistula rats or in anesthetized rats, the stomach was luminaIIy perfused with solutions of different osmotic pressure and acidity. Concentrations of $\text{H}^+$, $\text{HCO}_3^-$, and pepsinogen were measured in perfusate draining from the stomach.

In both conscious and anesthetized rats, continuous irritation of the stomach mucosa with hyperosmotic acidic solution (500 mMol NaCl, pH 2.0) elevated base secretion of bicarbonates and suppressed acid secretion but had no effect on the output of pepsinogen. Pretreatment with omeprazole promoted the further increase of the irritant-stimulated base secretion of $\text{HCO}_3^-$ and induced the base output of pepsinogen. Elevation of the base output of $\text{HCO}_3^-$ and pepsinogen was abolished by pretreatment with indomethacin. In anesthetized rats, mild irritation of the gastric mucosa potentiated $\text{HCO}_3^-$ and pepsinogen secretions caused by electrical stimulation of the subphrenic vagus. Pretreatment with omeprazole attenuated irritant-stimulated base secretion of $\text{HCO}_3^-$. The last effect of the proton pump inhibitor did not depend on production of prostaglandins. The obtained results show that inhibition of the proton pump affects gastric secretion of bicarbonates and pepsinogen induced by chemical irritation of the gastric mucosa interacting with mechanisms involving prostaglandin synthesis. A possible role of the reduced alkaline tide is discussed.

**HEALING EFFECT OF CRF ON ETHANOL-INDUCED GASTRIC LESIONS IN RATS**

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Neuropeptide corticotrophin-releasing factor (CRF) mediates the wide spectrum of physiological responses, acting both centrally and peripherally. Peripheral applications of CRF have been shown to reduce inflammation, vascular leakage, and edema formation after a variety of injuries (Thomas et al., 1993). At the periphery, CRF seems to act as an antagonist to a number of known inflammatory mediators such as histamine and substance P (Wei et al., 1993), having a specific anti-inflammatory action on skeletal muscle (Wei, Gao, 1992) and skin (Wei, Thomas, 1993). Additionally, local injection of CRF has been shown to reduce significantly the development of skin injury in rabbits.

In this study we tested the possible healing effect of CRF on the ethanol-induced gastric mucosal damage. Absolute ethanol (1 ml) administered intragastrically (i.g.) produced severe gastric mucosal lesions in all rats tested. Three hours later human/rag CRF (100 mcg in 1 ml) was administered intragastrically, control rats received vehicle. Animals were sacrificed 6, 12, 24 and 48 hours after CRF or vehicle. Macroscopically, hemorrhagic lesions occupying 30-50% of oxyntic mucosa were seen in all rats in four control groups. In 50% of CRF treated rats, mucosal hemorrhagic lesions were absent, in the other 50% of CRF treated rats mucosal lesions were less pronounced both macro- and microscopically at all time points (6, 12, 24 and 48 h) as compared to vehicle.

Hence, CRF is capable of accelerating healing of the ethanol-induced gastric mucosal damage in rats.

**SYNERGIC EFFECT OF NSAIDS AND ACID ON SUPERoxide-ANion PRODUCTION IN A GASTRIC EPITHELIAL CELLS**

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**Background and Aims:** Non-steroidal anti-inflammatory drugs (NSAIDs) often cause gastrointestinal complications such as gastric ulcers and...
erosions. Recent studies on the pathogenesis have revealed that NSAIDs induce lipid peroxidation in gastric epithelial cells by generating superoxide anion (O$_2^-$) in mitochondria, independently with cyclooxygenase-inhibition and the subsequent prostaglandin deficiency. Although not clearly elucidated, the impairment of mitochondrial oxidative phosphorylation, or uncoupling, by NSAIDs is associated with the generation of O$_2^-$. Moreover, gastric hydrochloric acid (HCl) has been regarded as an inciting factor of gastric mucosal injuries, and reportedly induced cellular lipid peroxidation in vitro. We hypothesized that both gastric acid and NSAIDs treatment synergistically induces O$_2^-$ production in mitochondria to cause cellular injury in gastric epithelial cells. **Methods:** We pretreated RGM1 cells with the acidic solutions for 30 min and after that, we treated them with each NSAIDs for 18hrs, and examined cellular injury with MTT assay. We also investigated lipid peroxidation and mitochondrial membrane potential using fluorescent probes, DPPP and JC-1 respectively. We confirmed the kind of reactive oxygen species using an electronic paramagnetic resonance apparatus with a probe CYPPO. Moreover, we investigated the effect of a manganese superoxide dismutase (MnSOD) on NSAIDs/ acid-induced cellular injuries using RGM-MnSOD which is a stable clone to overexpressing MnSOD after gene transfection technique. **Results:** The pretreatment with acidic condition accelerated NSAIDs-induced cellular injuries in accordance with the HCl concentrations. Moreover, the NSAIDs and acid treatment aggravated the value of both lipid peroxidation and mitochondrial membrane potential decrease in comparison with a NSAIDs-alone treatment and /or an acid-alone treatment. Both acid and NSAIDs treatment derived O$_2^-$ production from mitochondria. Overexpression of MnSOD significantly attenuated NSAIDs/ acid-induced cellular injuries, lipid peroxidation, membrane potential decrease and O$_2^-$ production. **Conclusion:** Both NSAIDs and acid synergically inhibited mitochondrial function to generate O$_2^-$ to derive cellular injury via lipid peroxidation. The overexpression of MnSOD protected these injuries.

**DEVELOPMENT OF NEW TYPE OF NSAID WITH LOWER GASTRIC SIDE EFFECTS**

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Non-steroidal anti-inflammatory drugs (NSAIDs) are a useful family of therapeutics. The anti-inflammatory actions of NSAIDs are mediated through their inhibitory effects on cyclooxygenase (COX) activity and resulting decrease in prostaglandins (PGs). On the other hand, NSAID use is associated with gastrointestinal complications. Although PGs have a strong protective effect on gastrointestinal mucosa, the inhibition of COX by NSAIDs is not the sole explanation for the gastrointestinal side effects of NSAIDs. We examined the COX-independent mechanism involved in NSAID-induced gastric lesions. Using DNA microarray analysis, we found that CHOP, a transcription factor with apoptosis-inducing ability is induced by NSAIDs. We also found that NSAIDs have membrane permeabilization activity. Furthermore, intracellular Ca$^{2+}$ chelator inhibited the NSAID-induced apoptosis. Results showed that NSAID-induced apoptosis is mediated by permeabilization of cytoplasmic membranes, increase in the intracellular Ca$^{2+}$ levels and induction of CHOP. In vivo, we obtained pharmacological evidences showing that both COX inhibition at gastric mucosa and direct gastric mucosal cell damage (such as induction of apoptosis) by NSAIDs are required for the production of gastric lesions, suggesting that NSAIDs without membrane permeabilizing activity have reduced gastrointestinal side effects. We screened such NSAIDs and found that loxoprofen sodium (LOX) has lower membrane permeabilization activity than other NSAIDs. LOX has been used clinically for many years as a standard NSAID in Japan. We synthesized a series of LOX derivatives and examined their membrane permeabilization. We selected one compound, fluoro loxoprofen (F-LOX), which has much lower membrane permeabilization activity than LOX. F-LOX showed IC$_{50}$ values for COX-1 and COX-2 that are similar to LOX.

In vivo, oral administration of F-LOX produced fewer gastric lesions than LOX in rats. A rat carrageenan-induced footpad edema assay showed that F-LOX has anti-inflammatory activity equivalent to LOX.

Results in this study are chemical evidences in support of our proposal that the membrane permeabilization activity of NSAIDs is involved in their induction of gastric lesions. Furthermore, F-LOX showed very low gastric lesion-inducing activity, although they have no apparent selectivity for COX-2. Thus, we consider that F-LOX likely to be therapeutically beneficial NSAIDs in terms of gastrointestinal safety.

**PROTECTIVE AND THERAPEUTIC EFFECT OF REDOX POLYMER NANOPARTICLES FOR ULCERATIVE COLITIS MODEL MICE**

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Inflammatory bowel disease (IBD), included Crohn's disease (CD) and ulcerative colitis (UC), affects million patients over the world. Since the pathogenesis and mechanism of IBD are not clearly understood, it is recognized as intractable disease and there is no drug possessing completely efficiency to treat this disease. It is reported that excess reactive oxygen species (ROS) are also generated within both small intestine and colon during IBD. Recent evidences have suggested that ROS are critical mediators of inflammation, tissue damage during IBD occurrence and they may contribute to the pathogenesis of both inflammation and cancer. Excessive ROS generation leads to imbalance of
antioxidant system in gastrointestinal tract, which is believed that critical cause of intestinal injury in UC patients. To suppress the ROS generation and inflammation, several medicines such as aminosalicylates, corticosteroids and immunosuppressants have so far been used, however, no drug can work effectively. In addition, these medications cause significant side effects. To obtain therapeutic efficiency and suppress unwanted effects, the innovative strategy must be required. Nitroxide radical is one of promising compounds as an antioxidative drug. However, it has not been employed clinically because of several issues, e.g. preferential renal clearance, anti-hypertensive effect and toxicity. To utilize this nitroxide radical in vivo, we have developed nitroxide radical-containing nanoparticle (RNP), prepared by a self-assembling amphiphilic block copolymers. It has been confirmed that RNP significantly prevents oxidative stress damage in an cerebral ischemia-reperfusion injury model in rats, renal ischemia-reperfusion injury model in mice and a neuron cell line used as a model for Alzheimer’s disease, in which excess ROS is generated. Systemic intravenous administration of RNP scavenged ROS generated by these injuries well. Thus, RNP is promising candidate for novel nanotechnology based therapy. Oral drug administration is one of the most important pathways for a medical treatment. However, gastrointestinal tract has severe barriers such as strong acidity in stomach, numerous digestive enzymes, bacteria and so on. Nanoparticle is one of the interesting strategies to vary efficiency of drugs. The drug was compartmentalized from the environmental conditions to prevent decomposition and dispersion. Interestingly, nanoparticles are known to accumulate in specific regions because of the changes in the specific vascular microenvironment such as inflammation site. Therefore, we hypothesized that RNP may be used as a nanoparticle therapy in UC treatment. In this study, we demonstrate that protective and therapeutic effect of RNP on suppressive dextran sodium sulfate (DSS)-induced colitis compared with that of low-molecular-weight TEMPOl and mesalamine, a commercial anti-ulcer drug.

GASTRIC MUCOSAL DEFENSE WITH AMINO ACIDS
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Backgrounds & Aims: Glutamate is the most abundant amino acid that composes body proteins. Once free glutamate (an umami substance) is ingested with the diet, it supports multiple functions as an energy source in the GI tract during nutrient absorption. We have previously reported that glutamate supplemented in the diet reduces Helicobacter pylori (HP)-induced gastric mucosal damages in gerbils. To clarify the mechanisms of the glutamate protection against HP, we examined the expressions of excitatory amino acid transporters (EAAT) in gastric epithelial cells and then characterized the functional role of the transporter in the gastric epithelial protection by glutamate against ammonia (one of the main toxin produced HP).

Materials & Methods: Isolated rat gastric epithelial cells and RGM1 (rat gastric epithelial cell line) cells were used in this study. Cell viability was determined using crystal violet staining method. NH4Cl was used as an ammonia donor, which mimics ammonia concentration in the gastric juice of HP-infected patients. Glutamate transport into the cells were determined using [3H]glutamate radioisotope tracing in the absence/presence of selective transporter inhibitors. The expression of EAAT mRNAs was examined using RT-PCR. Immunohistochemical analysis was performed using EAAT1 antibody.

Results: Glutamate protected gastric epithelial cells against NH4Cl-induced cell death. In both cells, the transport of [3H]glutamate were Na⁺-dependent with high affinity. The transport was completely inhibited by DL-TBOA (a selective non-transportable inhibitor of EAATs), TFB-TBOA (a high affinity EAAT1 and EAAT2 blocker) but not Way213613 (a potent, non-substrate EAAT2 inhibitor). Both cells expressed EAAT1 transcripts. Immunohistochemical analysis showed that EAAT1 was expressed in both primary and RGM1 cells. Cell viability analysis revealed that glutamate protection against NH4Cl-induced cell death was blocked by TFB-TBOA, indicating the contribution of EAAT1 in the protection.

Conclusion: From these results, in gastric epithelial cells, EAAT1 mediates glutamate uptake and contributes to the glutamate protection against ammonia. Thus, we speculate that, in daily life, free glutamate in the diet would contribute to the gastric epithelial protection via EAAT1 against HP.

THE CYTOPROTECTIVE ACTION OF IMUNOFAN IN EXPERIMENTAL GASTRIC LESIONS IN RATS IS MEDIATED BY ITS INHIBITORY EFFECT ON INDUCIBLE NO-SYNTHASE
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Background. Recent studies showed the high gastroprotective activity of some olygopeptides, mainly glyprolines, the Croatian compound BPC 157 and hexapeptide Imunofan of the chemical structure of Arg-Alfa-Asp-Lys-Val-Tyr-Arg (“Bionox”, Russia). The positive effects of olygopeptides on gastric mucosa (GM) could be explained by their multidirectional influence on homeostasis, intercell communication, receptors of γ-aminobutyric acid, the systems of dopamine, NO, prostaglandins [Ilic S. et al. 2009; Samonina G. et al. 2008; O. Orlovsky, 2007], although all these mechanisms leave much to be elucidated.

Aim of research was to study the cytoprotective action of Imunofan in interaction with the systems of NO-synthases (NOS), cyclooxygenases (COX) and lipoxygenases (LOX) in experimental gastric lesions (GL) in rats.
Methods. GL in rats were induced by intraperitoneal application of epinephrine (2 mg/kg). The experimental animals 10 minutes before the introduction of epinephrine were pretreated with Imunofan (1μg/100g) administered alone and in combination with COX/LOX blocker identical to darbufelone (20 mg/100g) and iNOS blocker aminoguanidine (20 mg/kg).

Results: The rats introduced to epinephrine developed severe GL, accompanied by increase of the activity of total NOS (by 133%) in GM in particular due to the increase of iNOS activity (6-fold), increase of NO (by 50%) and decrease of the level of L-arginine in plasma (by 37%). Pretreatment with Imunofan caused 50% decrease of the area of GL, 56% decrease of the activity of total NOS and 62% decrease of iNOS, 31% decrease of the content of NO in GM as well as tendency to increase of the level of L-arginine in plasma. Under conditions of iNOS blockage with aminoguanidine in rats introduced to epinephrine Imunofan provided decrease of the damaged area of the stomach by 61% compared to its isolated action, enhanced blockage of iNOS, decrease of NO in GM and increase of the level of L-arginine in plasma. The administration of COX/LOX blocker darbufelone on the background of Imunofan in animals introduced to epinephrine did not provide significant cytoprotection but showed tendency to increase of the activity of eNOS.

Conclusions: Imunofan provides cytoprotection in experimental GL in rats in particular via the modulation of the activity of NOS. The cytoprotective effect of Imunofan is more visible under conditions of iNOS blockage with aminoguanidine and is not enhanced by the dual COX/LOX blocker darbufelone.

CYTOREDUCTIVE EFFECTS OF ACETIC ACID ON MOUSE GASTRIC CANCER, HUMAN AND RAT GASTRIC CANCER CELLS

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Background and aim: Gastric cancer is the second leading cause of cancer-related death in the world. Topical application of acetic acid induces chronic gastric and duodenal ulcers in experimental animals. The aim of this study was to examine the cytoreductive effects of acetic acid on gastric cancer in vivo and in vitro. Methods: The so-called INS-GAS transgenic mice that develop spontaneously gastric cancer (highly differentiated glandular cancer) at 10-14 months of age were used. After the male and female mice were deprived of food, but not water, overnight, the stomach was exposed through a midline abdominal incision under isoflurane anesthesia. These mice were subjected to 60% or 100% acetic acid (0.1ml) that was topically applied to the serosal side or luminal side of the stomach using a metal cylinder (4 mm ID) with an aperture in the side for 60 sec. Gastric mucosa was evaluated by visual inspection and histology. Gastric cancer cell lines (human Kato III cells and rat RGK1 cells) were grown in 96 well culture plate for 24h, and then exposed to acetic acid (0.01%-1%) for 10 min. Cytotoxicity were determined by MTT assay. Results: In INS-GAS mice, gastric cancer was found in the corpus of both the anterior and posterior walls in all mice. The pH of gastric contents of several mice ranged from 11 to 13. There were severe necrosis of the cancer tissue in the region exposed to acetic acid after 30 or 60 min, and ulcer formation after 1-7 days. The ulcer depth extended occasionally to the muscularis mucosa and muscle layers. In vitro, acetic acid suppressed the survival of human Kato cells by 63%, 33% or 0% at a concentration of 0.1%, 0.3% or 0.5%, respectively. It also suppressed the survival of rat RGK1 cells by 87%, 84% or 41% at 0.1%, 0.3% or 0.5%, respectively. Conclusions: Acetic acid topicaly applied at 60 or 100% concentration induced promptly the necrosis of tumor in the mouse model of gastric cancer. Acetic acid applied in the culture medium at less than 0.5% concentration for only 10 min suppressed the survival of human and rat gastric cancer cells. Thus, we may suggest the potential of this simple method, using either endoscopy or laparoscopy, for cytoreductive treatment of gastric cancer.

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THE PATHOGENIC POTENTIAL OF PSYCHOGENIC STRESS IN GASTRIC MUCOSAL INJURY

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It is widely accepted that stress is a risk factor for gastric ulceration, but our understanding of mechanisms constituting the pathogenic potential of stress, is incomplete. We previously showed that psychogenic stress induced by rigid fixation of the rabbit to a frame was accompanied by the inhibition of contractile activity (CA) in the antral and pyloric portions of the stomach and simultaneous increase of CA in duodenum. Contraction of the duodenum under conditions of open pyloric sphincter can cause duodenogastric bile reflux. The latter is considered as a pathogenic factor in the development of the gastric mucosal injury (erosions and subsequent ulcers). So the above mentioned motor discoordination in the gastroduodenal zone may be considered as a way of realization of the pathogenic potential of stress. Prevention of the duodenogastric dyskinesia and the bile reflux might be useful for the gastric mucous protection in conditions of stress. For realization of the idea it is necessary to know what mechanisms are responsible for the inhibition of gastric and activation of duodenal CA under stress. In our experiments the stress-induced suppression of gastric
Gastric erosions were responsible, at least partly, for the transformation of this stress factor (supposedly corticotropin-releasing hormone) on nonadrenergic inhibitory neurones of the enteric nervous system. We revealed different mechanisms of stress-induced intensification of CA in the proximal and distal portions of duodenum. Against the background of blockade of muscarinic and nicotinic cholinergic receptors and β1/β2-adrenoceptors the increase of CA was persisted in postpyloric portion of the duodenum. This change of CA was resulted from the direct action of a hormonal stress factor (supposedly corticotropin-releasing hormone) on the smooth muscle of this portion of the duodenum. The blockade of muscarinic and nicotinic cholinergic receptors and β1/β2-adrenoceptors abolished the stress-induced increase of CA in the distal third of the duodenum. The intensification of the duodenal CA in this case was mediated by the action of circulatory catecholamines on the excitatory β-adrenoceptors located on cholinergic neurones of the enteric nervous system.

PROTECTIVE ACTION OF CORTICOSTERONE AGAINST INDOMETHACIN-INDUCED GASTRIC ULCERATION: DOSE- AND TIME-DEPENDENT STUDY

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Glucocorticoids may have dual action on the gastric mucosa: physiological gastroprotective and pathological proulcerogenic one. We demonstrated previously that manifestation of protective or deleterious effects of dexamethasone injected at the same dose on the gastric mucosa may depend on the time interval between the hormonal administration and onset of ulcerogenic stimulus. Single injection of dexamethasone at a dose of 1 mg/kg attenuated or aggravated indomethacin-induced gastric erosions depending on the duration of its action before indomethacin injection. Short-lasting (1-12 h) action of dexamethasone attenuated indomethacin gastric erosions, however, the further increase in the time interval caused transformation of this gastroprotective action of dexamethasone to proulcerogenic effect. In the present study we investigated whether corticosterone, a natural glucocorticoid in rats, may cause similar dual action on the gastric mucosa. Methods: Gastric erosions were induced by indomethacin (35 mg/kg, sc) in male S-D rats after 24 h fasting. Dose- and time-dependent effects of corticosterone on the gastric erosions as well as blood corticosterone and glucose levels and somatic parameters were examined after single injection of the hormone. In the dose-dependent study, the animals were given a single injection of corticosterone (at a dose one from 5, 10, 25, 50 or 100 mg/kg, ip) 1 h before the subsequent decapitation of rats 4 h after indomethacin or its vehicle administration. Results: Corticosterone at the doses of 25, 50, 100 mg/kg decreased the gastric erosion area dose dependently in the case of its injection 1 h before indomethacin administration. Further increase in the time interval resulted in a disappearance of the gastroprotective action but did not cause a transformation of this protective action of corticosterone to its proulcerogenic effect. Conclusion: The results obtained in dose- and time dependent study demonstrate that corticosterone causes only protective effect on the gastric mucosa as opposed to both gastroprotective and proulcerogenic action of dexamethasone. Dexamethasone-induced long-lasting corticosterone deficiency may be responsible, at least partly, for the transformation of gastroprotective effect of dexamethasone to its proulcerogenic action.

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NSAID-MUCOSAL INJURY: ROLES OF DRUG CHEMISTRY IN PATHOGENESIS

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While extensive studies have been performed on the pathogenesis of gastro-intestinal (GI) injury from non-steroidal anti-inflammatory drugs (NSAIDs), there has been relatively little attention to the chemical features of the drugs which cause cellular injury. Furthermore, much interest has been shown in the development of nitric-oxide donating NSAIDs (CINODS), without consideration of the chemical and consequent pharmacokinetic (PK) limitations of these drugs in achieving gastro-protection.

It has been established that the chemical properties of most NSAIDs as organic acids accounts for their irritant properties which are achieved by their uptake through mucosal membranes as non-ionized acids and subsequent entrapment in mucosal cell in their ionized state. Our studies (Bjarnason et al., 2007; Alim Pharmacol Ther, 26:95) have shown that there is a direct relationship between pKa of the drugs and their Lanza scores of human gastric mucosal injury, with less of a component attributed to lipophilicity (LogP). Further data are presented here from studies in a rat model of ulcer formation showing similar relationships between pKa and Log P (of Log D). However, both sets of data do not explain how the chemical properties of individual NSAIDs differ considerably in their ulcerogenicity. Evidence is presented that additional factors particularly...
the steric, electronic and quantum chemistry of NSAIDs markedly influences their ulcerogenic potential. These factors play an important role in determining the potential of pro-drugs to be less ulcerogenic than their parent NSAIDs. We have shown that nitro-butoxyl esters of drugs (NO-NSAIDs) such as naproxen, indomethacin and aspirin are in some cases no less irritating to the gastric mucosa than their butyl-ester analogues. This is probably due to the relatively rapid rate of hydrolysis of the NO-NSAIDs as reflected in HPLC assays of the mucosal and plasma concentrations of the NO- and acidic NSAID. Understanding of the reason for this and other pro-drugs having limited mucosal protection comes from their basic chemical properties and especially the roles of electron-withdrawing activity across aromatic and alkyl ester groups. These factors may help in the development of less ulcerogenic drugs in the future.

**DUAL CONTROL OF DUODENAL MUCOSAL PROTECTION BY CYCLOOXYGENASE (COX)-1 AND COX-2 IN CATS**

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**Backgrounds/Aim:** It is reported that simultaneous inhibition of both cyclooxygenase (COX)-1 and COX-2 is necessary to induce damage in the stomach and small intestine by NSAIDs. Though NSAIDs often cause ulcers in the duodenum in humans, the role and necessity of inhibition of the COX isoforms in NSAID-induced duodenal ulcers has not been elucidated. In the cats, we examined (1) the localization of COX isoforms in the duodenum, (2) effect of feeding on expression of COX isoforms and prostaglandin (PG) levels in the duodenum, (3) ulcerogenic effects of selective inhibitors of COX-1 (SC-560, ketorolac) and COX-2 (celecoxib, meloxicam), and a non-selective COX inhibitor (indomethacin) on the duodenal mucosa, and (4) effects of those drugs on duodenal motility and distribution of PAS-positive mucus cells in the duodenum. **Methods:** COX inhibitors were administered after the morning meal in cats once daily for 3 days. Localization of COX isoforms in the duodenum, and venules and basal granulated cells. (2) Feeding increased the expression of both COX isoforms and PGE2 levels in the duodenum, and the effects were markedly inhibited by pretreatment with cimetidine. (3) Selective COX-1 or COX-2 inhibitors alone, as well as indomethacin, produced ulcers in the duodenum. (4) Both selective COX-1 inhibitors and indomethacin, but not COX-2 inhibitors, increased duodenal motility and decreased the number of PAS-positive goblet cells in duodenal villi.

**Conclusions:** Both COX isoforms were present in the duodenum and the expressions were up-regulated by feeding, COX-1 and COX-2 positive responses were observed in different cells, and inhibition of either COX-1 or COX-2 alone caused ulcers in the duodenum, suggesting that COX-1 and COX-2 differently protect the duodenal mucosa in cats.

**STABLE GASTRIC PENTADECAPEPTIDE BPC 157 PROTECTS AGAINST PERTINENT ISCHEMIC CHALLENGE IN RATS**

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We focused on the stable gastric pentadecapeptide BPC 157 and long lasting ischemic conditions after ligation of left gastric artery in rats. The suggestion about a particular importance of BPC 157 in further therapy of upper GI tract based on the evidence that this pentadecapeptide given alone in different species, without carrier, is effective when given parenterally, perorally and locally (i.e., cream). With a special structure (GEPPPGKPPADDAGLV, MW 1419) (non degraded in human gastric juice more than 24h), BPC 157 may be particularly important as a small anti-ulcer peptide in the whole GI tract, liver and pancreas lesion, efficient in inflammatory bowel disease trials (PL 14736) and various wound treatment, no toxicity reported. Grossly, after left gastric artery ligation the mucosal surface of prepyloric area became (very soon, i.e., 3 days) flattened and very thin, involving more than 30% of total glandular area of rat stomach. The lesions appear to be even more prominent in more prolonged periods (i.e., 14 days, 2 months, 1 year (Fig. 1, control (C), BPC 157 (B)). These were along with microscopic data, thinner gastric wall, pronounced decrease of both mucosa and muscle layer. Decrease of muscle layer is more prominent at 14 days while mucosa decrease is permanent one. Given in drinking water 10 ug/kg, BPC 157 maintained normal gross appearance during all intervals while regularly preserving thickness of mucosa, gastric wall thickness and eventually led to an increase of muscle thickness. Thus, these findings are consistent with the expectation that the cytoprotective
effect may be an important advantage of BPC 157 activity that may have both cytoprotective and adaptive cytoprotective activity even in condition of pertinent ischemic challenge such as left artery ligation in rats.

**PROLONGED SEVERE HYPERCALCEMIA INDUCES AN ACUTE PANCREATITIS IN RATS, THE EFFECT OF BPC 157 (PL14736)**

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**Background.** Prolonged severe hypercalcemia induces an acute pancreatitis in rats, and shortening of QTc interval and prolongation of PQ interval. For counteraction, we propose the stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419), an anti-ulcer peptide efficient in trials for inflammatory bowel disease (PL 14736, Pliva) and various wound treatment, no toxicity reported) that recovered acute pancreatitis as well as arrhythmias in rats (Dig Dis Sci, 1996, J Pharm Sci, 2003). **Materials and methods:** Rats received CaCl2 solution (200 mg or 400 mg/kg i.p.). ECG was continuously recorded, K, Na, Ca, Cl, amylase, CK, LDH serum values assessed at 5, 10, 15, 25, and 60 min, and acute pancreatitis at 60 min (as described (Dig Dis Sci, 1996, J Pharm Sci, 2003, 2007 (in press)). BPC 157 (10 mcg/kg, i.p.) was given (i) prophylactically (30 min before CaCl2).

**Results:** Severe hypercalcemia was sustained throughout shortening of QTc interval and prolongation of PQ interval and raise of serum enzymes values (QT, interval, PQ interval, Ca, amylase, CK, LDH(5 min - 40±2 msec, 50±2 msec, 4,75 mmol/L, 2605 U/L, 10130 U/L, 3500 U/L), (10 min - 40±2 msec, 50±1 msec, 5,95 mmol/L, 2810 U/L, 12480 U/L, 4110 U/L), (15 min - 35±2 msec, 75±3 msec, 6,2 mmol/L, 3330 U/L, 14140 U/L, 5250 U/L), (25 min - 30±3 msec, 80±1 msec, 6,5 mmol/L, 3480 U/L, 15530 U/L, 5580 u/L), (60 min - 30±1 msec, 100±2 msec, 7,2 mmol/L, 3620 U/L, 19900 U/L, 6480 U/L)). BPC 157, prophylactically. Severe hypercalcemia was modified, abolished shortening of QTc interval and prolongation of PQ interval and decreased raise of serum enzymes values (QTc interval, PQ interval, Ca, amylase, CK, LDH (5 min - 46±2 msec, 58±2 msec, 3,06 mmol/L, 2110 U/L, 8010 U/L, 1800 U/L), (10 min - 48±3 msec, 60±2 msec, 4,12 mmol/L, 1900 U/L, 8100 U/L, 2200 U/L), (15 min 40±6 msec, 58±4 msec, 5,2 mmol/L, 2650 U/L, 8500 U/L, 3900 U/L), (25 min 45±2 msec, 63±2 msec, 5,8 mmol/L, 2750 U/L, 9100 U/L, 4100 U/L), (60 min - 47±3 msec, 60±4 msec, 6,2mmol/L, 2880 U/L, 10500 U/L, 5700 U/L)). At 60 min acute pancreatitis appeared in control rats with raised Ca, amylase values. In BPC 157 rats acute pancreatitis was markedly reduced, along with decreased raise of Ca and serum amylase values.

**Conclusion:** Acute CaCl2 bolus induces prolonged hypercalcemia that causes acute pancreatitis along with shortening of QT, interval and prolongation of PQ interval, increased serum enzymes values (i.e., amylase, CK, LDH). BPC 157 medication modified Ca raise after acute CaCl2 bolus and reduced acute pancreatitis, reduced amylase, CK, LDH serum values, and prevented ECG disturbances.

**NATURALLY OCCURRING PEPTIDES FOR GASTROPROTECTION, STABLE GASTRIC PENTADECAPEPTIDE BPC 157**

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Stable gastric pentadecapeptide BPC 157 is an anti-ulcer peptidergic agent, safe in inflammatory bowel disease clinical trials (GEPPPGKPADDAGLV, M.W. 1419, PL 14736) and wound healing, stable in human gastric juice and has no reported toxicity. It was suggested to be solution of Pavlov’s concept and novel mediator of Robert’s cytoprotection. We focused on BPC 157 as a therapy in peridontitis, esophagus, stomach, duodenum, intestine, liver and pancreas lesions. Particularly, it has a prominent effect on alcohol-lesions (i.e., acute, chronic) and NSAID-lesions (interestingly, BPC 157 both prevents and reverses adjuvant arthritis). In rat esophagitis and failed function of both lower esophageal sphincter (LES) and pyloric sphincters (PS), BPC 157 increased pressure in both sphincters till normal and reduced esophagitis. However, in healthy rats, it may decrease (PS) or increase (LES) the pressure in sphincters. It has strong angiogenic potential, it acts protectively on endothelium, prevents and reverses thrombus formation after abdominal aorta anastomosis, affects many central disturbances (i.e., dopamine and 5-HT system), the NO-system (either L-arginine and L-NAME effects), endothelin, acts as a free radical scavenger (counteracting CCl4-, paracetamol-, diclofenac-injuries) and exhibits neuroprotective properties. BPC 157 successfully heals the intestinal anastomosis, gastrointestinal fistulas and colonic fistulas in rats, as well as interacting with the NO-system. Interestingly, the fistula closure was achieved even when the BPC 157 therapy was postponed for one month. In short-bowel syndrome escalating throughout 4 weeks, the constant weight gain above preoperative level. Interestingly, BPC 157 both prevents and reverses adjuvant arthritis. In rat esophagitis and failed function of both lower esophageal sphincter (LES) and pyloric sphincters (PS), BPC 157 increased pressure in both sphincters till normal and reduced esophagitis. However, in healthy rats, it may decrease (PS) or increase (LES) the pressure in sphincters. It has strong angiogenic potential, it acts protectively on endothelium, prevents and reverses thrombus formation after abdominal aorta anastomosis, affects many central disturbances (i.e., dopamine and 5-HT system), the NO-system (either L-arginine and L-NAME effects), endothelin, acts as a free radical scavenger (counteracting CCl4-, paracetamol-, diclofenac-injuries) and exhibits neuroprotective properties. BPC 157 successfully heals the intestinal anastomosis, gastrointestinal fistulas and colonic fistulas in rats, as well as interacting with the NO-system. Interestingly, the fistula closure was achieved even when the BPC 157 therapy was postponed for one month. In short-bowel syndrome escalating throughout 4 weeks, the constant weight gain above preoperative level.
PENTADECAPEPTIDE BPC 157 AS A THERAPY FOR CORROSIVE MUCOSAL LESIONS IN RATS

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Aim. Ingestion of a chemical agent (absolute alcohol) was used for model of Robert’s cytoprotection. A safe stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419, stable in human gastric juice, LD1 not achieved) may be the novel mediator of Robert’s cytoprotection, and thereby, may counteract all corrosive mucosal injuries. Successful in inflammatory bowel disease trials, it counteracts esophagitis, sphincters failure, gastrointestinal ulcer and skin ulcer, and fistulas in rats (1-5).

Methods. Corrosive mucosal injuries in anesthetized rat were induced by 1 ml of 96% alcohol at tongue, whilst BPC 157 (10 µg, 10 ng/kg) or saline (5 ml/kg) were given intraperitoneally immediately after. Tongue, esophagus, stomach and duodenal mucosal defects, lower esophageal sphincter (LES) and pyloric sphincter (PS) pressure were evaluated at 30 sec, 1, 5, 15, 30, 60, 120 minutes, and 24 hours

Results. Control. Mucosal defects after 30 sec: tongue (lesions area, mm²: 52.0±2.8), esophagus (110.5±43.1), stomach (125.0±84.9) and duodenal (9.0±1.4). Mucosal defects after 24 h showed progression: 24.7±21.3 (tongue), 174.6±111.4 (esophagus), 271.3±76.9 (stomach), 30.9±48.7 (duodenum). Sphincter pressure remained continuously low i.e., 46.2±11.3 cm H2O (LES 24 h); 34.0±13.4 cm H2O (PS 24 h).

BPC 157. BPC 157 ug-regimen initially exhibited smaller mucosal defects on tongue (18.5±9.2), esophagus (110.5±43.1), stomach (125.0±84.9) and duodenal (9.0±1.4). Mucosal defects after 24 h showed progression: 24.7±21.3 (tongue), 174.6±111.4 (esophagus), 271.3±76.9 (stomach), 30.9±48.7 (duodenum). Sphincter pressure remained continuously low i.e., 46.2±11.3 cm H2O (LES 24 h); 34.0±13.4 cm H2O (PS 24 h).

Conclusion. BPC 157 could be used as a therapy for corrosive mucosal lesions.


PENTADECAPEPTIDE BPC 157 AND THE ESOPHAGEAL-CUTANEOUS FISTULAS HEALING, AND A THERAPY FOR FISTULAS HEALING

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Aim. There is generally lack of pharmacotherapy for esophagocutaneous fistulas. It is also a particular challenge for surgical therapy. Therefore, we tested a safe stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, MW 1419), LD1 not achieved, since successful in inflammatory bowel disease trials, and counteracts esophagitis, sphincters failure, gastrointestinal ulcer and skin ulcer, gastro- or colo-cutaneous fistulas in rats (1-5).

Methods. Cervical esophagocutaneous fistula (4 mm diameter esophagus and skin defect), esophagitis (scored 0-4), lower esophageal sphincter (LES) and pyloric sphincter (PS) pressure was validated macro/microscopically and biomechanically at day 1, 2, 3 and 4, whilst BPC 157 was given in drinking water (10 µg, 10 ng/kg, 12 ml/rat/day).

Results. Control. Weight loss was along with severe esophagitis, sphincteric failure (49.0±2.0 cm H2O i.e., pyloric) and both defects (largest diameter, mm, 4.0±0.1, skin; 3.0±0.5, esophagus) observed and did not restore till day 4. Also, mortality rate was 70%, BPC 157. Both defects (i.e., day 1, 1.5±0.1 (ug), 1.7±0.1 (ng) skin; 1.5±0.2 (ug), 2.0±0.1 (ng) esophagus) started to heal in short period. There was no mortality at day 4 (vs. Control p<0.05); no esophagitis in any animal during whole experiment. Also, higher PS pressure was observed (65.0±2.0 (ug), 62.0±1.0 (ng) cm H2O). Likewise, failure of LES pressure (60.0±2.1 cm H2O) was restored in BPC 157 rats (69±2.1 cm H2O).

Conclusion. BPC 157 improved the esophagocutaneous fistulas healing, and thereby it should be considered as a possible therapy for fistulas healing.

PENTADECAPEPTIDE BPC 157 REDUCES THE COMPLICATIONS OF ILEOILEAL ANASTOMOTIC HEALING FOLLOWING THE DICLOFENAC INTOXICATION

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Introduction. Several studies express the ulcerative effect of diclofenac. In addition some studies have proved the impact of the diclofenac overdose onto the raised incidence of anastomotic leakage and dehiscence. Therefore we analyzed the effect of the pentadecapeptide BPC 157 onto the healing the ileoileal anastomosis accompanied with the diclofenac overdose.

Materials and methods. Under deep anesthesia 50 male Wistar Albino rats underwent the surgical procedure of creating an ileoileal anastomosis (Surgery Today, 2007). The animals were assigned randomly into control groups treated with saline (5mL/kg, i.p.), while the other half received pentadecapeptide BPC 157 (10 µg/kg, i.p.). During operation 30 animals (15 BPC 157 and 15 controls) received an overdose of diclofenac (12,5 mg/kg,i.p.). The treatment was once daily, the first dose was applied immediately after the operation and the animals received the last dose 24h before sacrifice. At the end of each experimental period (2 days, 6 days) the animals were sacrificed and the biomechanical, functional, macroscopic and microscopic assessment were performed. Results. In animals that received diclofenac the healing of anastomosis was impaired in comparison to those that did not receive the diclofenac overdose. The animals treated with the pentadecapeptide BPC 157 shown the signs of recovery (larger volume of water needed to cause the anastomotic leakage, lower rate of mortality and obstruction) even at those animals that received the overdose of diclofenac. The raised values of serum AST, ALT were lower in those animals that did not receive the diclofenac overdose, accompanied by more significant decrease of the degree of GM damage, and NO2− content (by 23%) versus the effect of vitamin C alone and eNOS activity was decreased (by 24%). At the background of iNOS and COX-2 blockage, vitamin C caused insignificant reduction of the activity of NO-synthases and lipoperoxidation processes that gives evidence of a predominant effect of vitamin C.

Conclusion. Gastroprotective action of vitamin C in experimental GL in rats is attributed to its inhibiting effect on the activity of iNOS and lipoperoxidation processes. The gastroprotective effect was enhanced under conditions of combined action with L-arginine, accompanied by more significant decrease of the degree of GM damage, and NO2− content (by 24%) versus the effect of vitamin C alone and eNOS activity was decreased (by 24%). At the background of iNOS and COX-2 blockage, vitamin C failed to affect considerably the activity of NO-synthases. Thus, obtained data show that non-antioxidant effects of vitamin C is due to its inhibiting effect on proinflammatory enzymes.

NEW MECHANISTIC AND TIME SEQUENTIAL PATHOGENESIS OF CELL/TISSUE INJURY LEADING TO EXPERIMENTAL ULCERATIVE COLITIS

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After our previous demonstration that subepithelial microvascular injury precedes gastric & duodenal mucosal lesions in rats, we hypothesized that similar
mechanistic sequence may occur in the very early stages of ulcerative colitis (UC) development. Namely, we were surprised that vascular endothelial growth factor (VEGF) aggravated, while anti-VEGF markedly improved experimental UC (Tolstanova et al., JPET 2009;328:749-57). We then initiated time-sequence studies in 4 models of UC & demonstrated that increased vascular permeability (VP) & early vascular injury preceded colonic mucosal erosions & ulcers. Methods: We used 2 chemically induced rat models of UC, i.e., caused by intracolonic administration of either SH alkylator 6% iodoacetamide (IA) or by ingestion of 5% dextran sulfate sodium (DSS) in drinking water, as well as 2 spontaneously developing UC models in Ga-i2 KO & IL-10 KO mice. VP was measured by colonic extravasation of Evans blue (15 min after i.v. injection) & epithelial permeability (EP) by absorption of intragastrically administered FITC-dextran (4 hr after gavage). Blood levels of histamine, colonic concentrations of VEGF & myeloperoxidase (MPO) activity (representing infiltration of leukocytes) were measured after IA enema. We also used electron microscopy in time course experiments with the chemically induced UC models as well as light microscopic histology of colonic sections from both chemical & genetic models of UC. Results: Plasma levels of histamine significantly increased in the very early stages (15 & 30 min) after IA, while VEGF (also known as vascular permeability factor – VPF) was significantly elevated later at 1 – 6 hr after IA. MPO activity significantly increased starting at 4 hr after IA. Pretreatment with mast cell stabilizer doxantrazole or H1 receptor antagonist mepyramine significantly reduced colonic VP in a dose-dependent manner. Electron microscopy showed in subepithelial microvessels endothelial damage, platelet aggregation & extravasation in subepithelial capillaries, followed by edema of lamina propria and submucosa in the very early stages of UC development, e.g., 15 min after IA and 12 hr after DSS in rats, while the epithelial tight junctions were still intact. We also found that increased VP (15 min after IA) was followed by hypoxia (in 30 min) in colonic mucosa, sequentially occurred earlier than elevation of colonic VEGF (1 hr after IA), all preceding the increased EP. Conclusions: 1) This is the first demonstration that vascular injury & increased VP preceded epithelial barrier dysfunction & mucosal lesions in experimental UC. 2) Histamine initiated the early increase in VP before VEGF/VPF elevation. 3) The subsequent increase in VEGF exaggerated the already increased VP, resulting in mucosal edema & inflammatory cell infiltration. 4) Thus, during development of experimental UC, histamine released from colonic mast cells may initiate the early increase in VP, causing colonic mucosal hypoxia, VEGF release & enhanced synthesis, colonic edema, inflammatory cell infiltration & colonic ulcers.

Schematic illustration of the new time-sequance in the development of UC focusing on vascular factors

CHRONIC OR ACUTE EXTEROCEPTIVE STRESS-INDUCED VISCERAL ANALGESIA OR HYPERALGESIA IN RODENTS
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Dual actions of stress on pain have been better characterized in the somatic pain field where stress-induced analgesia (SIA) has been early one described while less is known in the visceral field. We studied the influence of the exteroceptive stress of water avoidance (WAS) applied acutely or chronically on visceral pain response in rodents. Colorectal distension (CRD) applied at nociceptive range (40-80 mmHg) results in autonomic and behavioral pseudoaffective reflexes (changes in arterial pressure and heart rate, passive avoidance behaviors, and contraction of abdominal musculature) in rodents. Monitoring the contraction of abdominal muscles or visceromotor response (VMR) is the most commonly used index of visceral pain response in rats and mice. In conscious animals, it can be directly assessed by electromyographic (EMG) signals monitored via surgically-implanted recording electrodes into the external or internal abdominal muscle, where the electrode device is either externalized through the skin. We recently developed an alternative non-invasive method to study visceral sensitivity to CRD in conscious rodent, using a commercially-available miniaturized pressure catheter to record intraluminal colonic pressure (ICP). Briefly, a PE50 catheter was taped 2 cm below the pressure sensor of a miniaturized pressure transducer catheter (SPR-524 Mikro-Tip catheter; Millar Instruments, Houston, TX). A custom-made balloon (1-cm width x 2-cm length) prepared from a polyethylene plastic bag was tied over the catheter at 1 cm below the pressure sensor with silk 4.0. Ligature points were covered with parafilm to prevent any air from leaking. To validate the technique, we monitored the VMR to graded phasic CRD by simultaneously recording ICP and EMG signals in mice chronically implanted with electrodes. We found an excellent correlation between signals from ICP and EMG during consecutive ascending phasic distensions between 15 to 60 mmHg when recorded simultaneously in the same mice. We also showed that the colonic pain pressure threshold to CRD detected by both methods were
similar (about 32 mmHg). As colonic pressure could be altered following abdominal contractions and/or contractions of the colonic wall proper, we assessed the effects of atropine, a muscarinic blocker known to inhibit colonic motility in mice, on the VMR to CRD monitored by ICP in naïve mice. We found that atropine did not significantly modify the phasic CRD-associated ICP changes, while inhibiting distal colonic motility measured by ICP changes in conscious mice maintained under similar recording conditions. In addition, with the use of the non-invasive method, we confirmed that buprenorphine, a partial agonist for mu-opioid receptors, inhibits visceral sensitivity to graded phasic ascending CRD which is consistent with opioid-induced reduction of basal visceral response to CRD in both rats and mice. Mice that had undergone surgery for the placement of EMG electrodes on the abdominal wall and were subsequently singly housed to avoid deterioration of the implanted electrodes by cage-mates, developed visceral hyperalgesia to CRD in response to repeated WAS (1h/day for 10 consecutive days), while mice tested for visceral pain to CRD using the non-invasive solid-state ICP recording and were kept group housed, developed a strong visceral analgesia under otherwise similar conditions of repeated intermittent WAS. In male Wistar rats exposed to WAS for 1 h had a reduced VMR to CRD at 40 and 60 mmHg by 28.7 ± 6.0% to 32.4 ± 15.2% respectively. The days of repeated WAS (1h/day)-induced reduction of VMR occurred only at 40 mmHg (36.2 ± 17.8%) while in rats fed with prebiotic (4% enzyme-treated rice fiber ERF), the VMR was lowered at 20, 40 and 60 mmHg by 64.9 ± 20.9%, 49.3 ± 11.6% and 38.9 ± 7.3% respectively. The visceral analgesia was still observed on day 11 in ERF- but not in standard diet-fed rats 24 h after the last session of WAS. While in the ERF group VMR was lowered at 20, 40 and 60 mmHg by 64.9 ± 20.9%, 49.3 ± 11.6% and 38.9 ± 7.3% respectively. WAS stress-induced analgesia in rats was naloxone independent. By contrast, a hyperalgesic response to CRD can be induced after the intraperitoneal injection of cortagine, a selective corticotropin releasing factor receptor subtype 1 (CRF1) agonist, known to act directly on the CRF1 receptor expressed in the colon in rats. Collectively, these data demonstrate that the state of the animal tested (naïve vs exposed to surgery), its social environment (group housing vs single housing), the methods used to record visceromotor responses (EMG requiring surgery and antibiotic post surgery vs manometry not requiring surgery/antibiotic) can significantly affect the analgesic response to exteroceptive stressors. Based on recent clinical findings demonstrating that IBS patients have a compromised engagement of the inhibitory descending pain modulation systems, gaining a deeper understanding of the mechanisms involved in the expression of stress-induced visceral analgesia, or lack thereof, are promising avenues to be explored and may lead to new therapeutic targets for IBS. Therefore the use of non-invasive methods of monitoring VMR that allows the unraveling of the analgesic influence of stress on visceral pain represents a step forward to gain insight into the underlying mechanisms, in particular the neural substrates and neurochemistry of stress-related analgesia as established in the somatic field.

**ROLES OF PROSTAGLANDIN EP4 RECEPTORS IN DEVELOPMENT AND HEALING OF NSAID-INDUCED SMALL INTESTINAL DAMAGE**

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The receptors of PGE2 are pharmacologically classified into 4 G protein-coupled subtypes, EP1 to EP4, and their distribution is considered to explain the multiple effects of PGE2 in various tissues including the gastrointestinal tract. We review our recent data on the importance of EP4 receptor in the protective and healing promoting effects of PGE2 on NSAID-induced small intestinal lesions. Indomethacin (10 mg/kg, s.c.) caused severe damage in the small intestine, but the lesions healed rapidly, decreasing to about 1/5 of their initial size within 7 days. PGE2 prevented indomethacin-induced small intestinal lesions, and this action was functional associated with inhibition of intestinal hypermotility and stimulation of Muc2 expression/mucus secretion as well as fluid secretion, resulting in suppression of enterobacterial invasion and the upregulation of iNOS expression. These effects of PGE2 were mimicked by the selective EP4 agonist and abrogated by prior administration of the selective EP4 antagonist. On the other hand, the healing of these lesions was significantly impaired by the repeated treatment of indomethacin (2 mg/kg) given once daily for 6 days. The healing impairment effect of indomethacin was mimicked by the EP4 antagonist AE3-208 given for 6 days and significantly reversed by the co-administration of PGE2 as well as the EP4 agonist AE1-329. The COX-2 expression in the intestinal mucosa was up-regulated after ulceration, persisting for 3 days, while the mucosal VEGF expression was increased from 1 day after ulceration, reaching a peak on day 3. Furthermore, the changes in COX-2 and VEGF expressions were in good parallel with those in mucosal PGE2 content. Indomethacin (2 mg/kg) down-regulated both the expression of VEGF and the number of microvessels in the mucosa, and these effects were significantly reversed by the co-administration of PGE2. The COX-2 expression in the intestinal mucosa was up-regulated after ulceration, persisting for 3 days, while the mucosal VEGF expression was increased from 1 day after ulceration, reaching a peak on day 3. Furthermore, the changes in COX-2 and VEGF expressions were in good parallel with those in mucosal PGE2 content. Indomethacin (2 mg/kg) down-regulated both the expression of VEGF and the number of microvessels in the mucosa, and these effects were significantly reversed by the co-administration of PGE2 and the number of microvessels in the mucosa. The protective action of PGE2 is brought about by alteration of various intestinal functions, including motility, mucus secretion and bacterial invasion, mediated by EP4 receptors, while the healing promoting action is associated with stimulation of angiogenesis via the upregulation of VEGF expression mediated by EP4 receptors.
PROPHYLACTIC EFFECT OF MONOSODIUM GLUTAMATE AGAINST NSAID-INDUCED ENTEROPATHY IN RATS
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Glutamate has been known as the “umami” substance in the diet. In the present study, we examined the effect of monosodium glutamate (MSG) on the development and healing of small intestinal lesions induced by loxoprofen, one of NSAIDs frequently used in Asian countries, in rats. Male SD rats without fasting were administered loxoprofen (60 mg/kg, p.o.) and killed various days (1, 2, 3, 5, 7 days) later to examine hemorrhagic lesions developed in the small intestine. MSG (0.1~5%) was given as powder food (10 g/rat/day) for 5 days before or after loxoprofen treatment. Mucus secretion was examined by PAS staining, while enterobacterial count in the mucosa was determined by a culture method. Mucosal expression of iNOS and Muc2 mRNAs was examined by RT-PCR, while that of VEGF was examined by western blotting. Loxoprofen (60 mg/kg) induced hemorrhagic lesions in the small intestine within 24 hr, accompanied by the down-regulation of Muc2 expression, the increase in bacterial invasion and MPO activity, and the up-regulation of iNOS expression. Pretreatment of the animals with MSG for 5 days dose-dependently and significantly prevented the development of loxoprofen-induced intestinal lesions, together with suppression of bacterial invasion and INOS expression as well as MPO activity. In addition, MSG treatment at 5% alone increased mucus secretion and reverted the decrease in mucus secretion and Muc2 expression after loxoprofen treatment. On the other hand, these intestinal lesions healed spontaneously within 7 days, but the process was impaired by the repeated administration of low-dose loxoprofen (30 mg/kg) for 5 days after the ulceration, with the decrease of VEGF expression and angiogenesis. The healing-impairing effect of loxoprofen was prevented by feeding 5% MSG for 5 days after the ulceration with the restoration of VEGF expression.

These results suggest that MSG not only prevents loxoprofen-induced small intestinal damage but also promotes a healing of these lesions; the former is functionally associated with the increase in Muc2 expression/ mucus secretion and the suppression of bacterial invasion and iNOS expression, while the latter is associated with the stimulation of VEGF expression/angiogenesis.

TRANSCRIPTION FACTOR EGR-1 IS CRITICAL FOR OXIDATIVE STRESS-INDUCED INJURY IN ULCERATIVE COLITIS PATHOGENESIS
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We and others showed the importance of hypoxia/oxidative stress in acute and chronic ulcerative colitis (UC)-induced injuries. Egr-1 is an early-response, redox sensitive transcription factor, which triggers expression of pro-inflammatory and pro-angiogenic factors. Now we tested the hypothesis that Egr-1 might play a pathogenic role in the molecular mechanisms of UC development. Methods: UC was induced by enema with 0.1 ml 6% SH-alkylator iodoacetamide (IA) in Sprague-Dawley rats or 0.05 ml 0.5% IA in wild type (WT) & Egr-1 (-/- and -/+ mice). Rats were killed in 0.5, 1, 2, 6, 24 hr and 3, 7 days; mice – in 3 days after IA enema. Body weight and diarrhea were recorded daily. Levels of Egr-1, Sp1, HIF-1α were assessed in colonic mucosa by Western blot and real time PCR. Egr-1 DNA-binding and its transcription interactions were assessed in nuclear extracts of colonic mucosa by TF-TF array I & II (Panomics, CA).

Results: Iodoacetamide enema induced rapid increase in Egr-1 protein and mRNA expression and its nuclear translocation as early as 0.5 hr (p<0.001) and were further elevated (p<0.001). Nuclear translocation was associated with increase in DNA binding of Egr-1 - 11.2-fold at 0.5 hr (p<0.001) and 9.2-fold at 6 hr (p<0.001) vs. control. Egr-1 shares similar consensus binding sites with transcription factor Sp1 and its transactivation effectiveness depends on displacement of Sp1. We found attenuation of Egr-1/Sp1 interaction and Sp1 DNA binding in nuclear extracts of colonic mucosa at 6 hr after IA-enema (p<0.001) which was associated with gradual downregulation of Sp1 total protein expression. IA enema strongly increased (70-fold vs. control, p<0.001) interaction between Egr-1 and NF-κB (strong pro-inflammatory regulator) in nuclear extracts of colonic mucosa at 0.5 and 6 hr. Expression of HIF-1α (central regulator of hypoxia adaptation) was significantly upregulated in colonic mucosa of rats at early and late stages of IA-UC. Egr-1 didn’t interact with HIF-1α in nuclear extracts of colonic mucosa during IA-UC. Deficiency of Egr-1 in Egr-1-/- and Egr-1+/ mice markedly improved morphological signs of IA-UC. Colonic lesion areas (mm²) were significantly decreased from 23.6±26.7 in WT to 1.3±2.7 in Egr-1-/- mice (p=0.12) and from 60.7±26.1 in WT to 25.3±17.0 in Egr-1-/- mice (p<0.05). Egr-1-/- mice had 43% of mortality vs. 12% in WT and 0% in Egr-1+/ mice.

Conclusions: 1) Egr-1 expression increased in experimental UC; 2) The enhanced Egr-1 transcriptional activity depended on reduction of Egr-1/Sp1 and upregulation of Egr-1/NF-κB interaction and didn’t depend on Egr-1/HIF-1α; 3) Deficiency of Egr-1...
markedly improved morphologic signs of IA-UC; 4) These data show the importance of Egr-1 in the pathogenesis of UC.

**NSAID GASTROPATHY AND ENTEROPATHY: DISTINCT PATHOGENESIS LIKELY NECESSITATES DISTINCT PREVENTION STRATEGIES**

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The mechanisms underlying the ability of nonsteroidal anti-inflammatory drugs (NSAIDs) to cause ulceration in the stomach and proximal duodenum are well understood, and this injury can largely be prevented through suppression of gastric acid secretion (mainly with proton pump inhibitors). In contrast, the pathogenesis of small intestinal injury induced by NSAIDs is less well understood, involving more complex mechanisms than those in the stomach and proximal duodenum. There is clear evidence for important contributions to NSAID-enteropathy of enteric bacteria, bile and enterohepatic recirculation of the NSAID. There is no evidence that suppression of gastric acid secretion will reduce the incidence or severity of NSAID-enteropathy. Indeed, clinical data suggest little, if any, benefit. Animal studies suggest a significant exacerbation of NSAID-enteropathy when proton pump inhibitors are co-administered with the NSAID. This worsening of damage appears to be linked to changes in the number and types of bacteria in the small intestine during proton pump inhibitor therapy. The distinct mechanisms of NSAID-induced injury in the stomach/proximal duodenum versus the more distal small intestine likely dictate distinct strategies for prevention.

**NEUROBIOLOGY OF CRF IN THE ENTERIC NERVOUS SYSTEM DURING STRESS**

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Stress-related signaling in the enteric nervous system (ENS) is comparable to corticotropin releasing factor (CRF) signaling in the brain. CRF’s role in changing colonic motility and mucosal function in response to restraint stress has become better understood. Neuronal actions are studied electrophysiologically with microelectrodes. Expression of CRF is studied with real time RT-PCR and immunofluorescence. Si-RNA for CRF and its receptors is used to suppress gene expression. Excitatory action of CRF in the ENS is mediated exclusively by the CRF₁ receptor in guinea pig enteric neurons. RT-PCR for isolated myenteric ganglia finds CRF mRNA expression in proximal and distal colon. Enteric neurons expressed CRF immunoreactivity (IR) in the myenteric and submucosal plexuses of the rat and guinea pig colon. CRF-IR neurons are 21.4±1.8% of the total myenteric population and 27.7±7.1% of the total submucosal population in the rat colon. Restraint stress evokes widespread c-fos expression in colonic myenteric and submucosal neurons. Double labeling finds that most of the c-fos positive neurons in stressed animals expressed either CRF-IR or CRF₁ receptor-IR. Intraperitoneal administration of the CRF₁ receptor antagonist, NBI27914, suppressed the effects of stress on c-fos expression. Intramural injection of si CRF RNA “knocks-down” basal CRF expression in the colon. Real-time RT-PCR shows expression of CRF mRNA to be decreased by about 60% four days after siCRF RNA. Immunoreactivity for CRF was undetectable or very low relative to siControl after injection. In controls, restraint stress significantly increases fecal output, baseline short-circuit current (Isc) measured in Ussing flux chambers and mucosal conductance compared with non-stressed controls. Restraint stress does not increase fecal output, baseline Isc or transepithelial conductance in siRNA CRF-treated animals as compared with non-stressed controls. The evidence supports the hypothesis that CRF-action in the ENS underlies stress-induced changes in intestinal transit and mucosal functions.

**SULFORAPHANE ENHANCES PROTECTION AND REPAIR OF GASTROINTESTINAL MUCOSA AGAINST OXIDATIVE STRESS VIA NRF2-DEPENDENT MECHANISMS**

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Both Helicobacter pylori infection and NSAIDs intake induce oxidative stress on gastrointestinal mucosa, thereby causing mucosal damage, retarding mucosal repair, and eventually inducing a variety of gastrointestinal diseases. Cells, however, can survive against oxidative stress by enhancing activities of antioxidant enzymes, thereby cells are protected from DNA damage. Recent studies have clearly shown that the genes encoding nrf2 (NF-E2 p45-related factor-2) and keap1 (Kelch-like ECH-associated protein 1) play an important role in the induction of antioxidant enzymes against oxidative stress. In this study, using nrf2−/− mice, we found the mechanisms by which the nrf2-keap1 system contributes to induction of a variety of antioxidant enzymes during exposure to oxidative stress. Secondly, we also found beneficial effects of a natural compound sulforaphane, an isothiocyanate family, rich in broccoli sprouts, on gastrointestinal mucosa from Helicobacter pylori-induced injury, via stimulating nrf2 gene-dependent antioxidant enzyme activities. Finally, we have recently found that sulforaphane not only protects small intestinal mucosa from NSAIDs-induced injury, but also mitigates colonic mucosa from DSS-induced injury. Our data suggest that sulforaphane affords cytoprotection of GI mucosa against various types of stresses.
Corticotropin-releasing factor (CRF) is involved in endocrine, behavioural, immune and visceral responses to stress. Stress-related gastrointestinal disorders result in the change of somatic sensitivity. Gastric ulcers or chronic inflammation in gastrointestinal tract may induce somatic hypersensitivity and hyperalgesia. CRF participates in somatic pain regulation during the stress. However, the underlying mechanisms remain to be determined. Our previous results demonstrated contribution of glucocorticoid hormones to maintenance and recovery of gastric mucosal integrity (Filaretova et al, 2004) as well as somatic pain regulation (Yarushkina, 2008). This point suggests that CRF-induced effects on somatic pain sensitivity may be mediated by glucocorticoids. The aim of study was to study and compare the participation of glucocorticoids in central and systemic CRF-induced effects on somatic pain sensitivity in rats under identical experimental conditions.

The participation of glucocorticoids was studied by pharmacological suppression of the hypothalamic-pituitary-adrenocortical (HPA) axis leading to the deficiency of glucocorticoid production as well as an occupation of glucocorticoid receptors by its antagonist RU 38486. Since CRF administration causes the release of β-endorphin from the pituitary, the opioid antagonist naltrexone was used to determine the contribution of opioid-dependent mechanism to both central and systemic CRF-induced analgesia. An electrical current threshold test was applied for measurement of somatic pain sensitivity in anesthetized rats.

Both central (2 µg/rat, i.c.v.) and systemic (40 µg/kg, i.p.) administration of CRF caused analgesic effects and an increase in plasma corticosterone level. Pretreatment with naltrexone did not change analgesic effects of central or systemic CRF. Pharmacological suppression of the HPA axis as well as pretreatment with RU 38486 attenuated both central and systemic CRF-induced analgesic effects. However, systemic CRF-induced effects were partially reduced while central CRF-induced effects were completely suppressed by these pretreatments.

The data suggest that the both central and systemic CRF-induced analgesic effects may be mediated by nonopioid mechanism associated with endogenous glucocorticoids in our experimental conditions. At the same time according to data obtained glucocorticoid-independent mechanisms may also contribute to systemic CRF--induced analgesic effects additionally to glucocorticoid-mediated mechanisms.

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Physiopathology of esophageal inflammation, ulcerogenesis and repair by glycoconjugate profile

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Symptoms suggestive of gastro-oesophageal reflux disease (GERD) have been mentioned in the literature since 4000 years but C. Rotkansky (1804-1878) was the first to suggest that acid was associated to the GERD, whereas he had noted a peptic ulcer of the lower oesophagus. Current prevalence of esophageal pathology in the world is 10 to 38%, whereas distribution of erosive esophagitis and non-erosive esophagitis are different in West and East parts of world. The types of esophageal lesions (EL) linked to GERD are Barrett esophagus and esophageal adenocarcinoma (EA), the incidence of which markedly increased in the past 30 years. This probably cannot be explained by the usual economic and cultural “westernization” of environmental factors. At the same time, presbyesophagus is common age-depended changes of esophagus and its pathogenesis in view of healthy ageing perspective has not been fully elucidated. Animal and human studies of EL demonstrated that old postulate of esophagus, as simple conduit with neuromuscular function, transformed to novel, as organ with specific defense system. Our previous study have shown the esophagoprotection is multiplexer process and involved various mucosal components (epithelial, endothelial, entero-endocrine and muscle cells, fibroblasts, and extracellular matrix, glycoconjugates microenvironment) and different players from local stress-limited system, as COX/LOX, NOS activities, cytokines, adhesion molecules, and the process of apoptosis. We developed an animal preclinical models of erosive and nonerosive esophagitis and investigated the quality of EL repair determined by proper interactions between growth factors (VEGF, EGF), cytokines (IL-1β, TNF-α) and mesenchymal, melatonin-produced entero-endocrine and epithelial cells. Using of lectin labeling during the histochemical investigation of EL was helpful method about mucus secretion and cellular migration. We evaluated that severity of inflammatory reaction is central to esophageal ulcerogenesis and repair manifestation and starting point for abnormal restituto ad integrum. NEU5AC network was essential for the initiation and regulation of immune responses and effective tool of representing injury-cell-associated inflammation. We concluded that NEU5AC profiles and inflammatory signaling contributed for the esophageal phenotype of cytoprotection and repair. NEU5AC interacted at a defence/receptor level in much the same fashion as pro-inflammatory cytokine in the regulation of esophageal repair. The results of age-related changes in EL repair suggest that they are declined and depended of dysfunction in angiogenesis and glycoconjugates production in esophageal mucosa and highest inflammatory reaction. Furthermore, each esophageal cellular and subcellular mechanisms often play both roles as effector and target functional mediators, and also serve as a back-up system.

Age-dependent role of vasopressin in susceptibility of gastric mucosa to indomethacin-induced injury

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The high prevalence of gastric ulceration underlines the importance of understanding the mechanisms. Based upon its multifactorial role vasopressin (VP) is supposed to be one of the contributory factors, however the data are contradictory. We intended to reevaluate the role of VP in development of gastric ulceration. Naturally VP-deficient Brattleboro rats were used and the indomethacin-induced gastric erosion model (35 mg/kg sc, 4 h) has been chosen because of its clinical importance. Since gastric mucosal vulnerability shows age-dependent alterations, we compared young (4 week old) rats to old ones (1 year old). The lack of VP resulted in attenuation of erosion formation in young rats, while aggravation in old ones. Four hours after indomethacin treatment adrenocorticotropin and corticosterone levels as well as blood glucose levels were higher in VP-deficient rats, independent of the age. Old VP-deficient Brattleboro rats had smaller thymuses. Our results demonstrate the age-dependent role of VP in susceptibility of gastric mucosa to indomethacin-induced injury. Probably the absence of VP per se is not the reason of the data obtained but also the hormonal and metabolic consequences of VP deficiency (e.g. short versus long-term corticosterone and blood glucose elevation as well as related immunodisturbances).

Autophagy of gastric ECL cells, carcinoids and adenocarcinoma

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The role of autophagy in normal cell survival as well as suicide is complicated, and even more so in tumorigenesis. Previously, we have shown that in response to long-term hypergastrinemia evoked by omeprazole treatment, exocytosis of the ECL cells is not able to keep up with the pace of formation of secretory organelles, and consequently the autophagic pathway takes over, i.e. the autophagic disposal of excess secretory organelles. Up to date, little is known about the role of autophagy in gastric tumorigenesis. The aim of the present study was to test our hypothesis that autophagic pathway is impaired during the tumorigenesis of gastric carcinoids and...
Molecular mechanisms of cell death and implication in disease

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In 1972 Kerr and colleagues described apoptosis as a basic biological phenomenon with wide-ranging implications in tissue kinetics and in recent years emerging evidence indicates that apoptosis indeed is a genetically controlled and evolutionarily conserved form of cell death of critical importance for normal embryonic development and for the maintenance of tissue homeostasis in the adult organism. Research during the past forty years has revealed that apoptosis is not the only cell death program involved in the regulation of tissue homeostasis and the removal of unwanted cells in biological organisms. Now we know that multiple cell death mechanisms operate in both uni- and multicellular organisms. While the molecular pathways of apoptosis and necrosis are now relatively well established, the precise mechanisms of other cell death modalities, and their cross-talk, require additional study. This is particularly important, since many human disorders can be attributed, directly or indirectly, to defective cell death mechanisms. In this presentation the characteristics and cross-talk between various modes of cell death and their role in cell death-related disorders will be discussed.

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