1st symposium on
“Novel Frontiers in Physiology and Pharmacology of the Cholinergic System”

Organizing institution:
Department of pharmacology and toxicology
Faculty of Pharmacy
Comenius University

4 – 6 November 2011
The High Tatras
SUMMARY

The objective of the symposium is to discuss the latest discoveries and progress in the field of physiology and pharmacology of the cholinergic system, presented by young researchers, postgraduate students and undergraduate students.

Anna Hrabovska

REVIEWERS: Anna Hrabovska
Eric Krejci
Jan Kyselovic

Editor: Peter Musil
Issued by: Faculty of Pharmacy
Comenius University in Bratislava; 2011

MAIN FINANCIAL SUPPORT
“Novel Frontiers in Physiology and Pharmacology of the Cholinergic System”

INTERNATIONAL ADVISORY BOARD

Hrabovska, Anna
Department of pharmacology and toxicology,
Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Krejci, Eric
Centre d’Etude de la sensorimotricité, CNRS UMR 8194,
Université Paris Descartes, Paris, France

Kyselovic, Jan (Slovakia)
Dean of Faculty of Pharmacy,
Comenius University, Bratislava, Slovakia

Myslivecek, Jaromir
Institute of Physiology,
1st Faculty of Medicine, Charles University, Prague, Czech Republic

PRESENTERS

Dingova, Dominika*
Farar, Vladimir*
Kucera, Matej*
Mlynarova, Jana*
Mrvova, Katarina*
Obzerova, Lucia*
Valuskova, Paulina*

OTHER PARTICIPANTS

Matus, Marek*
Musil, Peter*
Klimas, Jan*

*Dept. of Pharmacol and Toxicol, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia; ^Inst. of Physiol., 1st Faculty of Medicine, Charles University, Prague, Czech Republic; CESem, CNRS UMR 8194, Université Paris Descartes, Paris, France
All contributions were presented in form of an oral presentation followed by discussion. There was no time limit for presentations or discussion.

PREFACE

The objective of the symposium was to discuss the latest discoveries and progress in the field of physiology and pharmacology of the cholinergic system, presented by young researchers, postgraduate students and undergraduate students.

The cholinesterases, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), are the key modulator of the cholinergic function. During the past 20 years, much progress was obtained by using recombinant enzyme to understand how the catalysis is so efficient to cleave acetylcholine (ACh) at the bottom of a deep gorge. Recombinant cellular systems were used to understand how AChE and/or BChE associated in the intracellular compartment to generate several hetero-oligomers with ColQ or PRiMA, and probably other derivative peptide containing proline rich sequence. Recombinant systems were used to identify the domain of each protein that is involved and how it may associate into the stable molecular forms.

During the past ten years, the mouse mutants have started to reveal expected and unexpected observations. The discussions and presentations of this symposium deal with the study and utilization of mouse mutants with a partial deficit in cholinesterases in order to better understand the function of the cholinergic system.

For new reasons, we need to quantify the cholinesterases as they do exist in the tissues particularly in the mutants with partial deficits. This is an old problem. The Ellman’s method is commonly used to determine the enzymatic activity. How the method could be improved to allow the quantification of very low amount of AChE and BChE? How the compounds used for the efficient
extraction do affect the enzymatic properties? The BChE mutant was used to generate new monoclonal antibodies that can be used to study the polymorphism in human population, which links to obesity and cardiovascular disease. Dominika, Paulina, Katarina and Lucia have presented the complementary results of their Master and PharmD. studies.

The brain is obviously a major target for cholinergic function. A mouse mutant, in which the gene encoding a small transmembrane protein called PRiMA is deleted, does not have mature AChE at the cell surface of the neurons in the brain. Surprisingly this mutant does not have the symptoms of AChE mutant attributed to altered brain function. Vlado Farar has presented the latest results obtained during his PhD project.

The heart function is an organ in which ACh plays important roles. In this complex tissue, several molecular forms of cholinesterase are expressed. How does the heart beat when one form is specifically removed? How has the system adapted this repertory of receptors and how is the beating affected after the inhibition of cholinesterases? Matej Kučera has presented the first promising results of his PhD project.

Bratislava, November 6th 2011

Eric Krejci
Hearth remodeling is the process by which size, shape, and function are regulated by mechanical, neurohormonal, and genetic factors. Gene expression studies are necessary for better understanding of pathophysiological changes in metabolism connecting with remodeling cardiac cell in pathological conditions. The heart and arteries are surrounded by layers of adipose tissue, exerting vasocrine and paracrine control of the subtending tissues. We start prospective clinical study aimed to the remodeling after myocardial infarction and to the gene expression in cells of the epicardial fat.

Jana Mlynarova has presented first set of experiments focused on the selection of the best reference genes for the correct evaluation of gene expression in epicardial and subcutaneous adipose tissue.

Bratislava, November 8th 2011

Jan Kyselovic
OPTIMIZED ELLMAN'S ASSAY – A BETTER TOOL TO STUDY KINETICS OF CHOLINESTERASES

DOMINIKA DINGOVÁ, ANNA HRABOVSKÁ

Dpt. of Pharmacol and Toxicol, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Introduction: Ellman's assay (EA) is a commonly used method, especially in biochemistry. The method enables quantification of the free SH-groups, which is used as well to determine the activity of cholinesterases. EA has few limitations particularly when used to study kinetics of cholinesterases. Namely, it is instability of Ellman's reagent (DTNB) or high nonspecific binding in biological samples. Because of those, it is impossible to follow very low activities or to follow activity during a prolonged time interval.

The aim of this project is to follow the effect of different buffers on EA limitation parameters and thus provide better conditions to study cholinesterase kinetics.

Method: Stability tests of DTNB alone and in the presence of the substrate (BTC; 0,5mM and 1mM) were performed in phosphate buffer (0,1M), HEPES buffer (5mM), TRIS buffer (5mM), Kolthoff-Vleeschhouwer buffer and Palitzch buffer at pH values 7,0; 7,5; 8,0 and 8,5 up to 5 days. Moreover, effect of methanol was studied. Nonspecific signal with DTNB in plasma (2,5ul, 5ul and 10ul) was followed in different buffers for up to 5 days. Impact of the studied buffer on the enzyme kinetics was studied with butyrylthiocholine jodid (1mM) as a substrate and purified recombinant human butyrylcholinesterase as a model enzyme in presence of 0,5mM DTNB.

Results: Stability of DTNB and DNTB with BTC decreases with increasing pH value in all tested buffers, while the highest stability was detected in TRIS and HEPES buffers and the lowest one in the phosphate buffer. DTNB is extremely stable in methanol. The intensity of nonspecific signal in plasma depends on the amount of plasma and incubation time. The product formation (wavelength peak or velocity) is not effected by type of buffer and pH.

Conclusion: Based on our results, TRIS and HEPES are the buffers of choice for measurement of cholinesterase activity by EA.

Supported by APVV grants SK-CZ-0028-09 and SK-FR-0031-09.
THE ROLE OF PRIMA IN CHOLINERGIC TRANSMISSION IN BRAIN AND SELECTIVITY OF CENTRALLY ACTING ANTICHOLINESTERASE DRUGS

VLADIMÍR FARÁR1,6, FRANCIZSKA MOHR2, JOCHEN KLEIN2, MARIE LEGRAND1, VERONIQUE BERNARD1, ANNA HRABOVSKÁ5, JÁN CENDELIN2, MARIE-PASCALE MARTRES4, JAROMÍR MYSLIVEČEK6,7 AND ERIC KREJCI1

1Cesem, UMR 8194 CNRS, Université Paris Descartes, Paris, France; 2Dpt. Pharmacology, Coll. of Pharm., Goethe Univ, Frankfurt am Main, Germany; 3Dpt. Pathophysiology, Fac. of Med., Pilsen, Czech Republic; 4PMSCN, CNRS UMR7224, Université Pierre at Marie Curie, Paris, France; 5Dpt. Pharmacol and Toxicol, Fac. of Pharm. of Comenius Univ., Bratislava, Slovakia; 6Inst. of Physiol., Charles Univ., Prague, Czech Republic; 7Inst. of Health studies, Liberec, Czech Republic

Introduction: Cholinesterases (ChE), especially acetylcholinesterase (AChE) are believed to constitute essential part of cholinergic synapse where they terminate the action of acetylcholine (ACh). Within the brain ChE are present as tetramers of catalytic subunits attached to the surface of neurons and glias by transmembrane protein PRiMA (proline rich membrane anchor). As apposed to the brain, in the periphery at neuromuscular junction the major form of ChE are tetramers associated with anchoring protein ColQ (collagen tail).

The aim: To study the importance of PRiMA protein for central cholinergic system using PRiMA knockout (KO) mice.

Methods: Behavioural characterization of mice was done by Morris water maze (MWM), open field (OF), rotarod and catwalk. The distribution of AChE in the brain was assessed by immunohistochemistry. The level of ACh was determined by microdialysis in freely moving mice. Adaptation of receptor systems was studied by means of indirect autoradiography and direct radioligand studies. High affinity choline uptake (HACU) was determined in synaptosomal preparation by uptake of 3H choline and the activity of choline acetyltranferase (ChAT) was measured as production of 3H acetylCoA. Pharmacological studies were done using scopolamine, oxotremorine and donepezil.

Results: PRiMA KO showed almost the same performance in all behavioural tests except that they were worse in the first day of rotarod test and somewhat slower as revealed by catwalk.
PRiMA is the only anchor of AChE in all types of neurons, not only in cholinergic. The absence of PRiMA leads to 500 fold increase in basal levels of ACh in striatum in comparison to wild type mice. The increase in ACh amount is followed by downregulation of muscarinic receptors (MR) throughout the whole brain. Contrary to MR nicotinic receptors showed only minor modification and other receptor systems such as dopaminergic, glutamatergic and gabaergic is not changed. Similar no changes were observed in metabolism of ACh. Pharmacological intervention confirmed the changes in MR and revealed that cholinergic system is still functional. Surprisingly donepezil which is considered to be brain ChE selective inhibitor is still active in PRiMA KO mice.

**Conclusion:** PRiMA is the only anchor of AChE in the brain and PRiMA KO represents unique model of selective absence of ChE solely in the brain. The results suggest that our classical view of ChE and their inhibition in the brain has to be revised.

*Supported by GAUK111409, GACR309/09/0406, MEB0810127/ APVV grant SK-CZ-0028-09.*
AGING OF MR IN PRIMA KO

VLADIMÍR FARÁR¹,4, ANNA HRABOVSKÁ², VLADIMÍR RILJAK⁴,⁵, ERIC KREJCI¹ AND JAROMÍR MYSLIVEČEK⁴,⁵

¹Cesem, UMR 8194 CNRS, Université Paris Descartes, Paris, France; ²Dpt. Pharmacology and Toxicology, Fac. of Pharm. of Comenius Univ., Bratislava, Slovakia; ³Universite Rene Descartes, Paris, France; ⁴Inst. of Physiol., Charles Univ., Prague, Czech Republic; ⁵Inst. of Health studies, Liberec, Czech Republic

Introduction: PRiMA knockout mice (KO) with absence of functional form of cholinesterases (ChE) solely in the brain adapt to the increased level of acetylcholine (ACh) mainly by downregulation of muscarinic receptors (MR). The decrease in MR assessed in the homogenates prepared from the whole brains is 50 %. However it is not known when in the course of mice development the change occurs and if the change has always the same extent.

The aim: To determine the aging of MR in the course of mouse development and to compare wild type mice with PRiMA KO.

Methods: The density of MR at 6 different ages ( E18.5, P0, P9, P30, P 120, P425) were determined by direct radioligand labeling of MR using MR nonselective antagonist 3H QNB in the homogenates prepared from the whole brains. The activity of AChE at each age was assessed by Ellmans method.

Results: The density of MR rapidly increases in the first 3 weeks of development. This increase correlates with increase of AChE activity. Even in the absence of AChE activity in PRiMA KO the density of MR increases in the same manner as in wild type mice. The difference between the two genotypes in MR levels differ at different ages. While at E 18.5 there is no difference, at P0 decrease is 12 %, at P9 18%, at P30 42 % at P120 and P425 48%.

Conclusion: Our results suggest that gradual adaptation of MR to the absence of PRiMA contributes to the survival and almost normal phenotype of PRiMA KO.

Supported by GAUK111409, GACR309/09/0406, MEB0810127/ APVV grant SK-CZ-0028-09.
CENTRAL CHOLINERGIC RECEPTOR SYSTEM IN DIFFERENT MODELS OF CHE DEFICIENCY

VLADIMÍR FARÁR¹,⁴, ANNA HRABOVSKÁ², VLADIMÍR RILJAK⁴,⁵, ERIC KREJCI¹ AND JAROMÍR MYSLIVEČEK⁴,⁵

¹Cesem, UMR 8194 CNRS, Université Paris Descartes, Paris, France; ²Dpt. Pharmacology and Toxicology, Fac. of Pharm. of Comenius Univ., Bratislava, Slovakia; ³Universite Rene Descartes, Paris, France; ⁴Inst. of Physiol., Charles Univ., Prague, Czech Republic; ⁵Inst. of Health studies, Liberec, Czech Republic

Introduction: In the past ten years different models of cholinesterase deficit have been developed by the means of genetical techniques to address the importance of presence of broad range of ChE molecular forms and their tissue and cell specific distribution. AChE knockout is completely devoid from AChE, BChE KO from butyrylcholinesterase, PRiMA KO have absence of functional ChE forms in the brain, ColQ KO at the neuromuscular junction, AChE del56 lacks functional forms of AChE both in the brain and at NMJ, AChE del1 does not have AChE in skeletal muscles only. The impact of selective impairment of ChE on the brain cholinergic receptors have only been studied in AChE KO.

The aim: To study and compare muscarinic receptors in different brain areas in: PRiMA KO, ColQ KO, BChE KO, AChE del56, AChE del1 and wild type mice.

Methods: The density of MR receptors were assessed in coronal brain sections by indirect autoradiography. 3H QNB was used to target overall levels of MR, 3H pirenzepine to target M1 MR and 3H AFDX-384 to label M2 MR.

Results: The central deficit of ChE in PRiMA KO and AChE del56 leads to profound decrease in MR. Surprisingly the deficit of AChE in the periphery (ColQ KO and AChE del1) triggers brain region specific MR upregulation. The observed brain region upregulation of MR in BChE KO is in an agreement of previously published decreased sensitivity of BChE KO to MR agonists.

Conclusion: Both central and periphery complete or partial deficit of ChE triggers changes in central cholinergic system.

Supported by GAUK111409, GACR309/09/0406, MEB0810127/ APVV grant SK-CZ-0028-09.
PARTICIPATION OF DIFFERENT MOLECULAR FORMS OF CHOLINESTERASES ON SOME PHYSIOLOGICAL PARAMETERS OF HEART

MATEJ KUČERA¹, MAREK MÁŤUŠ¹, VLADIMÍR FARÁR², EVA KRÁLOVÁ¹, TATIANA STANKOVIČOVÁ¹, ANNA HRABOVSKÁ¹.

¹Dpt. of Pharmacol and Toxicol, Faculty of Pharmacy, Comenius University, Bratislava, Slovakia; ²Dpt. of physiology, 1st Faculty of Medicine, Charles University, Prague, Czech Republic

Introduction: Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) exist in many molecular forms. Cholinesterases can be either soluble or anchored to the cell surface by the anchoring proteins. Two basic anchoring proteins are known so far: collagen Q (ColQ) and Proline-Rich Membrane Anchor (PRiMA). Both enzymes (AChE and BChE) and both anchoring proteins (ColQ and PRiMA) are present in heart tissue. BChE activity in heart is higher than AChE activity. The level of ColQ mRNA is higher in heart tissue than in skeletal muscle. To these days, the exact role of different molecular forms of cholinesterases and their anchoring proteins ColQ and PRiMA on heart physiology is not known.

The aim of this project was to study the effect of individual molecular forms of cholinesterases on the heart physiology by following the basic physiological parameters in mutant mice strains with impairment of the molecular forms.

Method: Three lead ECG, catheterization of right Arteria carotis, Langendorff heart perfusion with Aorta canulation and radioligand receptor binding study were performed in the study. As the model mice, knock-outs for ColQ, PRiMA, BChE, AChE exon 5 and 6; AChE enhanceosome region in the first intron were used. applied in our research. We used ColQ (ColQ -/-) and PRiMA (PRiMA -/-) deficient mice on ECG, hemodynamics and Langendorff perfusion experiments. Besides these we used also BChE -/-, Del E 5+6 -/- and Del i1RR -/- mutant strains on radiolabeled receptor binding study. Tribromoethanol was used as an anesthetic. Effect of neostigmine (3μM) on heart physiology was tested in Langendorff experiments. Muscarinic and beta adrenergic receptor numbers were determined in left ventricle, right ventricle and septum.
**Results:** ECG measurement revealed that ColQ and PRiMA deficient mice had higher heart rate and hence shorter cardiac cycle than wildtype littermates. Duration of PQ interval and QRS complex were did not differ but we observed tendency of shortened QT interval in both, PRiMA-/- and ColQ-/- mutant strains in comparison to wildtypes. Hemodynamics measurement showed that systolic and diastolic blood pressures are higher in PRiMA-/- and ColQ-/- than in wildtypes. In Langendorff experiments, we observed no changes between genotypes in heart rate, left ventricle developed pressure and speed of contraction and relaxation. Continual application of neostigmine leads to a significant decrease in heart rate of all studied genotypes, while the extend of the change was a mutant specific. In terms of radioligand receptor binding study, we observed significantly increased muscarinic receptors in PRiMA-/- left ventricle, Del 5+6 -/- left ventricle and septum and BChE-/- left and right ventricles and septum in comparison to wildtype littermates. Beta receptors showed no changes between genotypes.

**Conclusion:** Our results suggest that BChE and anchored molecular forms of AChE have important role in cholinergic transmission in heart and play a significant role in heart physiology in general.

*Supported by APVV grants SK-CZ-0028-09 and SK-FR-0031-09.*
NFAT3, TBX18, WT1, CALN, EDN1 AND ENDOGENOUS REFERENCE GENES EXPRESSION ANALYSIS IN EPICARDIAL AND SUBCUTANEOUS ADIPOSE TISSUE.

J. MLYNÁROVÁ1, G. DÓKA1, M. HULMAN2, V. HUDEC2, P. MUSIL1, E. GONÇALVESOVÁ2, J. KYSELOVIČ1

1Dpt. of pharmacol and toxicol, Faculty of Pharmacy, Comenius University, Bratislava
2The National Institute of Cardiovascular Diseases in Bratislava

Introduction: Calcineurin (CALN) and nuclear factor of activated T-cells (NFAT3, a transcription factor) are implicated in adipogenesis and adipocyte differentiation. T-box 18 (TBX18) is a transcription factor expressed in the epicardial cell layer of the heart and its progenitors. This gene is expressed with WT1 in epicardial progenitors which were found to have cardiomyogenic potential. Endothelin 1 (EDN1) stimulates lipolysis in adipose tissue. It’s expression is higher in subcutaneous than omental adipose tissue. Reference genes are genes stably expressed in tissues under every condition. Selecting a wrong reference gene for the experiment can show false results. It is popular to use one the most stably expressed reference gene. Several studies prefer using multiple reference genes for target analysis.

The aim of this work is to select the best reference genes for the correct evaluation of gene expression in epicardial and subcutaneous adipose tissue and analyze the expression of NFAT3, TBX18, WT1, CALN and EDN1 in these adipose tissues.

Materials and Methods: We selected 11 commonly used reference genes (β-actin, HPRT1, RPL13, B2M, TFR1C, RP II, GUSB, GAPDH, PPIA, LRP10, PGK1) for expression analysis in epicardial (EAT) and subcutaneous adipose tissue (SAT). We collected samples of EAT and SAT during CABG from 3 patients. We collected also EAT from failing hearts during heart transplantation of 20 patients immediately after the heart explantation. We used the RT-PCR method to analyze gene expression levels. We used multiple statistical methods including algorithms as GeNorm, Normfinder and Bestkeeper for the selection of the most stable reference genes. We used ΔΔCt method with one or three reference genes and geometric mean of three most stable genes for evaluation of the expression of nuclear factor of activated T-cells (NFAT3), Wilms Tumor 1 (WT1), T-box 18 (TBX18), calcineurin (CALN) and endothelin 1 (EDN1) target genes.
Results: Taken together all statistical methods for the evaluation of the 11 commonly used reference genes, B2M, RPL13, PPIA and HPRT1 can be selected as the four most stable genes in EAT and SAT. We selected B2M, RPL13 and HPRT1 for further analysis of target genes expression. Using the $\Delta\Delta$Ct method with three or one reference gene we observed a significantly higher expression of NFAT3 and TBX18 in EAT of failing hearts compared to EAT of ischemic hearts and in EAT from failing hearts compared to SAT of patients undergoing coronary artery bypass grafting (CABG) respectively. We similarly observed a significant elevation of NFAT3 expression in EAT of failing hearts when using $\Delta\Delta$Ct method with one reference gene. We did not observe any other significant changes in expression of other target genes with any evaluation method used.

Conclusion: In this study, we analyzed the expression of 11 commonly used reference genes in EAT and SAT of patients undergoing CABG or heart transplantation. According to multiple statistical methods, we selected B2M, RPL13, and HPRT1 as the best reference genes in EAT and SAT. Using different evaluation methods and their results confirmed the importance of the correct selection of reference genes.

Supported by Biomakro 2- ITMS 26240120027 and FaF UK 33/2011.
THE STUDY OF INTER-INIDIVIDUAL VARIABILITY OF BUTYRYLCHOLINESTERASE ACTIVITY IN HUMANS

KATARÍNA MRVOVÁ, ANNA HRABOVSKÁ

Dpt. of pharmacol and toxicol, Faculty of Pharmacy, Comenius University, Bratislava

Introduction: Butyrylcholinesterase (BChE) is a serum protein that hydrolyzes acetylcholine and detoxifies several foreign compounds. BChE activity observed in plasma is highly variable in humans. This variability may arise from different catalytic properties and/or different quantity of protein. Here we introduce a novel ELISA assay to determine the specific activity of captured BChE by using recently generated antibody against human BChE. By comparing specific activity and total activity, we will be able to suggest the origin of the inter-individual variability in the activity of this protein in humans.

Method: We measured the activity of BChE in the same plasma volume and the activity of the same amount of protein captured from the plasma samples. In the first step we determined the conditions for the ELISA assay - titrated the primary antibody, human plasma dilution and substrate concentration in the secondary antibody coated plates (1ug/well). BChE activity in the same plasma volume was determined by Ellman’s assay with substrate (1mM butyrylthiocholine iodide), DTNB (0,5 mM) and 5 mM HEPES buffer, pH =7,5. Number of the active sites of the captured protein was determined by quenching fluorescence of coumarine with p-nitrophenol and quenching fluorescence of tacrine.

Results: Based on our results, studied samples could be divided into two groups: (1) plasma samples with different BChE activity showed the same specific activity (i.e., the same value when identical amount of protein was captured in the plate); (2) BChE activity remained different even in ELISA. Results in the group 1 suggest different BChE protein levels in human plasma samples. Results obtained in group 2 propose different catalytic properties (or inhibition) of the enzyme among individuals.

Conclusion: Our results suggest that an inter-individual variability in human is caused by both, variable protein level and different catalytic properties of the enzyme.

Supported by APVV grants SK-CZ-0028-09 and SK-FR-0031-09.
STUDY OF ANTIBODIES PREPARED BY NEW METHOD OF IMMUNIZATION OF UNIQUE KNOCK-OUT MICE

LUCIA OBŽEROVÁ, ANNA HRABOVSKÁ
Dpt. of pharmacol and toxicol, Faculty of Pharmacy, Comenius University, Bratislava

Introduction: Antibodies (AB) are essential tools for modern scientific research. Their ability to bind specific antigens is used to analyze, identify, quantify and detect various antigens, markers or other peptides of interest. However, generation of selective and specific AB can be a difficult even impossible task for some antigens. Butyrylcholinesterase (BChE) represents such an antigen. But novel and efficient method for AB production has been developed recently which has enabled the generation of a set of new anti-BChE AB.

The aim of this study is to characterize anti-BChE AB produced by the novel method and determine conditions for their use in our future research.

Method: In this study, six different AB (4 anti-mouse and 2 anti-human BChE) were investigated by ELISA. The conditions for this method were determined before in our laboratory (see abstract Mrvova and Hrabovska). Each AB was titrated with human or mouse plasma BChE, with regard to the antigen that the AB was raised against. Previously characterized anti-human BChE AB (11D8) was used as a control. BChE activity in the plasma was measured by Ellman's assay with conditions established in our laboratory before (see abstract Dingova and Hrabovska).

Results: Based on our results, two of the investigated AB against mouse BChE gave strong signal in ELISA, while they differed in the affinity. In comparison to 11D8, none of the studied anti-human AB recognized human BChE. This is probably due to the degradation during the storage or random mutantions during the sub-clonning steps of AB generation.

Conclusion: To conclude our findings, novel AB production strategy provided one very selective and specific anti-human BChE AB and two efficient anti-mouse BChE AB, which however require further characterization. We have ruled out the dogma that mouse BChE is not an immunogenic protein and that it is impossible to raise AB against this protein.

Supported by APVV grants SK-CZ-0028-09 and SK-FR-0031-09.
OPTIMIZATION OF THE BEST CONDITIONS FOR EXTRACTION AND PURIFICATION OF CHOLINESTERASES FROM SERUM

PAULÍNA VALUŠKOVÁ, ANNA HRABOVSKÁ

Dpt. of pharmacol and toxicol, Faculty of Pharmacy, Comenius University, Bratislava

Introduction: Ellman’s assay is the most common method for measuring cholinesterase activity in biological samples used by investigators in research and clinical applications. The method is based on the hydrolysis of acetylthiocholine to thiocholine and acetate. The thiocholine reacts with DTNB and produces a compound whose color intensity reflects the activity of cholinesterases (ChE). Precise execution of this method and optimization of working protocol is crucial. High attention is usually paid to the sample preparation prior the measurements, however less attention is paid to the compounds used for the extraction which may remain in the solution and affect the determination of acetylcholinesterase (AChE) or butyrylcholinesterase (BChE) activities.

The aim: In our project we focused on the optimization of the conditions to follow ChE activity by Ellman’s method. Activity of AChE, as well as BChE, may be influenced by the solution composition which can contain different buffers, detergents and salts.

Method and Results: We compared 4 detergents which are commonly used: CHAPS, Triton X-100, Tween 20 and Tween 80 at the concentrations 0.5%; 1%; 2%. All detergents have significant inhibition effect on ChE activity. In all cases, we observed that % of inhibition increased with concentration. Among the studied detergents, CHAPS 1% interfered the least with the AChE and BChE measurements. Moreover, after removal of CHAPS, the activity was recovered up to 90% of the original activity value. We also compared an impact of different salts on ChE activity: NaCl, KCl, LiCl, KNO3, MgSO4.xH2O, MgCl2.6H2O, CaCl2 in five different concentrations in the range between 0-1M. NaCl, KCl and MgCl2.6H2O showed the lowest inhibition effect. Similarly to detergents, desalting in centrificon spin columns leads to the recovery of the activity of AChE and BChE up to 91%.

In conclusion the choice of detergent and its concentration and type of salt significantly influences the yield of ChE extraction. The best detergent for measuring ChE activity is CHAPS 1% and from salts NaCl, KCl and MgCl2.6H2O.

Supported by APVV grants SK-CZ-0028-09 and SK-FR-0031-09.
THE EFFECT OF VARIOUS DEGREE OF CHE DEFICIENCY ON LUNGS RECEPTOR SYSTEMS

PAULÍNA VALUŠKOVÁ, ANNA HRABOVSKÁ

Dpt. of pharmacol and toxicol, Faculty of Pharmacy, Comenius University, Bratislava

Introduction: Physiology of lungs is regulated by adrenergic and cholinergic systems which work in synergy but in opposite manner. Both systems are the targets of effective pharmacology, for example in the treatment of diseases such as asthma. The action of acetylcholine (ACh) in lungs is reputedly terminated by cholinesterases (ChE). Among ChE in lungs, butyrylcholinesterase (BChE) contributes in majority to the ChE activity. In addition to blood ChE the major molecular form of ChE in lungs seems to be tetramers associated with ColQ. Acetylcholinesterase (AChE) knock-out mice have altered respiration that is not originating from the CNS. Indeed receptors of both cholinergic and adrenergic systems are markedly changed in lungs of AChE knock-out mice.

The aim of our project was to test the hypothesis that different types of AChE deficiency and BChE deficiency may have a direct effect on receptor systems in lungs.

Methods: Several mutants with total or partial deficit of ChE were developed and are intensively studied also in relation to lung physiology. Using radioligand binding studies, we assessed the levels of muscarinic, α1 and β - receptors in lungs of all studied knock-out mice - PRiMA, ColQ, AChE del1, AChE del 5-6, BChE and were compared to the wild-type mice.

Results: Except for female BChE knock-outs, where we observed up-regulation of muscarinic receptors, other genotypes did not show any significant differences in receptor levels. The changes of α1 and β – receptors were low. In addition, male and female gender in both, adrenergic and cholinergic systems, did not differ significantly.

In conclusion we can add new data on adaptation mechanisms in ChE mutant mice, which suggest an important role of different receptor systems in order to cope with the increased level of ACh due to its impaired enzyme hydrolysis.

Supported by APVV grants SK-CZ-0028-09 and SK-FR-0031-09.