A Comparitive Study of Mouse Ovarian Alkaline Phosphatase Activity in Normal and Psuedopregnancies

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Abstract

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Introduction: The purpose of this study was to determine alkaline phosphatase (ALP) activity of ovary after ovarian induction using pregnant mare serum gonadotropin (PMSG) and human chorionic gonadotropin (hCG) during implantation periods.

Material and Methods: A total of 240 female NMRI mice aged 6-10 weeks were selected and divided into control and hyperstimulated. These mice were rendered normal or pseudopregnant. Five mice per each group were sacrificed by cervical dislocation at the first to sixth day of natural or pseudopregnancy. For biochemical assay the samples were obtained from the ovary, then were hemogenated using Tris HCl buffered salin (pH=8.3) and centrifuged with 14000 g. The activity of enzyme was determined using paranitrophenyle phosphate as substrate. Then specific activity of enzyme was calculated according to the total protein. The data were evaluated with Mann whitney test. For histochemistry the samples were cryosectioned (5µm thickness) and the ALP activity was determined by azo-coupling technique using alphanaphtole phosphate as substrate.

Results: The pattern of ALP activity in the biochemical and hisotchemical study was the same in each group. The activity of the ovarian ALP was increased during early pregnancy in the control and hyperstimulated natural pregnant groups. There were significant differences between these groups in every days except on the first and fourth day of pregnancy ($p<0.05$). The ovarian enzyme activity was increased in pseudopregnancy control until 4^{th} day and in the pseudopregnant hyperstimulation groups until 2^{nd} day of pseudopregnancy then it was decreased. The daily patterny of these alterations were significantly different ($p<0.05$) comparing the above-mentioned groups. ALP activity was increased in every day of pregnancy ($p<0.05$) in normal pregnant hyperstimulated group in comparison with the psuedopregnant hyperstimulated group.

Conclusion: Thus ovarion hyprstimulation alters the ovarian ALP activity during early pregnancy. These alteration may be due to esteroidogenesis activity of ovarian cells. However more investigation with complementary technique is needed.

Key words: Alkaline phosphatase enzyme, Ovarion hyperstimulation, Ovary, Pseudopregnancy

Immunolocalization of Na^+, K^+- ATPase and Ionocytes in the Gills of Catfish, *Silurus glanis*

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Abstract

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Introduction: The regulation of the body fluid content (osmoregulation) of an aquatic animal, is performed by several organs. In fish, osmoregulatory mechanisms are based on the function of specialized cells (ionocytes) located in various tissues and organs including gills. Na^+, K^+-ATPase is one of the main osmoregulatory enzymes enabling the use of ATP as a source of energy for ion transport through a Na^+, K^+ exchange pump cross epithelial membranes of ionocytes. The aim of this study was localization of this enzyme and ionocytes in the gills of *S. glanis*.

Material and Methods: For light microscopic observation, samples were fixed in Buin for 24 h dehydrated with ethanol, and embedded in paraffin. Serial sections, 5 µm thick, was stained by the Haematoxylin, Eosin and Methyl green.

Immunolocalization of the Na^+, K^+-ATPase was performed by immunofluorescence light microscopy with a Mouse Monoclonal Antibody $IgG\alpha_5$ raised against the α-subunit of the Chicken Na^+, K^+-ATPase (Developmental Studies Hybridoma Bank, University of Iowa , USA) and a Mouse Anti-fluorescein Antibody FITC (Jackson Immuno Research , USA).

Results: In longitudinal sections of the gill, two series of lamellae were observed on both sides of the filaments. Gill filaments and lamellae were lined by special cells, contained pavement cells, mucous cells and ionocytes. The ionocytes were located in the basal parts of lamellae, inter-lamellar regions and in the apical parts of gill filaments.

The ionocytes showed a ovoid-spheroid shape with a strong immunofluorescence of Na^+, K^+-ATPase in the baso-lateral regions.

Conclusion: In catfish, *S. glanis,* the ionocytes was found to be distributed on filaments and mainly in inter-lamellar regions. Na^+, K^+-ATPase enzyme with a highly consistent immunoreactivity was observed in the baso-lateral parts of the ionocytes. These findings show that, in *S. glanis*, the filaments appear as the main site of osmoregulation and the gill lamellae are mainly devoted to respiration.

Key words: Na^+,K^+-ATPase, Ionocyte, *Silurus glanis*, Immunohistochemistry Osmoregulation

Evaluation of the Best Condition for ex vivo Expansion of Hematopoietic Stem Cells for the Propose of Cord Blood Transplantation

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Abstract

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Introduction: Umbilical cord blood (CB) has been identified as a rich source for hematopoietic stem cells (HSCs), and has provided an alternative to bone marrow transplantation. The use of ex vivo expanded cells has been suggested as a possible means to accelerate the speed of engraftment in cord blood (CB) transplantation. The main aim of our study is to find the best culture media and condition to increase number of $CD34^+/CD38^-$ hematopoietic stem cells in cord blood for transplantation.

Material and Methods: Mononuclear cells (MNCs) were seperated from cord blood and cultured in RPMI1640 with 10% fetal calf serum (FCS) or 10% cord blood plasma (CBP) or serum free media (SF). Culture media contained 50ng/ml of Interlukin 6 (IL6), IL3, Thrombopoietin (TPO) Stem cell factor (SCF) and flt3-ligand. Cells were cultured for two weeks and number of $CD34^+/CD38^-$ cells and total MNCs measured at days 0, 7 and 14.

Results: At 14 days culture mean fold of expansion of $CD34^+$ and $CD34^+/CD38^-$ cells was 20.4 and 57.4 for FCS, 5.6 and 10.3 for SF and 10.8 and 4.7 for CBP culture media.

Conclusion: Due to efficacy and predictability of SF media for cell expansion and because of its better safety for allergic reactions and microbial contamination (in comparison to animal products containing media) and enough expansion for clinical applications, we suggest that SF media is better than CBP or SF from clinical points of view.

Key words: cord blood, expansion and hematopoietic stem cells.

Responsiveness of PGi Neurons to the Noxious Stimulus in Capsaicin Treated Morphine Dependent Rats

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Abstract

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Introduction: Nucleus reticularis paragigantocellularis (PGi) is one of the major components of the rostral ventromedial medulla (RVM), which is involved in nociceptive processing and pain modulation. The aim of this study was to examine the effect of C fiber destruction on responsiveness of nucleus reticularis paragigantocellularis (PGi) to formalin, as a noxious stimulus, in normal and morphine dependent rats.

Material and Methods: C-fiber destruction was induced by neonatal capsaicin (CAP) treatment (50mg/Kg, s.c.), in the second postnatal day. Extracellular single unit recording was used in control, capsaicin treated and morphine dependent, urethane (1.2-1.5g/Kg) anesthetized rats. After baseline recording (40 min), 100µl formalin (5%) was injected into the controlateral hind paw and recording was continued in the PGi, for 60 min after noxious stimulus.

Results: In control rats three types of neurons were detected, which were categorized as, increased (38.45%), decreased (23.1%) and neutral neurons (38.45%). In CAP treated rats the 3 types of neurons were observed, too. The duration of response (Baseline ± 2SD), in CAP treated rats was significantly shorter than that of controls. All recorded neurons in morphine dependent rats were neutral, but 30% of recorded neurons in CAP treated morphine dependent rats showed short increase in firing rate.

Conclusion: It was concluded that C-fiber destruction may just reduce the time course of changes in firing rate in response to peripheral noxious stimulus, while chronic morphine exposure may suppress the neuronal responsiveness, totally.

Key words: Nucleus reticularis paragigantocellularis (PGi), Single unit extracellular recording, Capsaicin treatment, Pain modulating pathways, noxious stimulus

Evaluation of the Efficiency of pIRES2-EGFP and pcDNA3-hBDNF-v5 Plasmids in Transfection of CCE ES Cells by the Electroporation Method

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Abstract

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Introduction: Unlimited self renewal and potential capacity of embryonic stem cells (ESCs) in differentiating into a wide variety of cell types has made the cells an attractive source of donor cells for developmental studies and cell therapy. The aim of the present study was the evaluation of the transfection efficiency of pIRES2-EGFP and pcDNA3-hBDNF-v5 plasmids in CCE ES cells by the electroporation method.

Material and Methods: The plasmids transformed into DH5α competent bacteria and propagated as maxi-prep. The plasmids then purified and transfected into ESCs by means of the electroporation method. To confirm the expression of the GFP and BDNF genes, invert fluorescent microscopy and RT-PCR were used. Expression of EGFP was confirmed by examining the transfected cells with fluorescent microscopy. In case of BDNF, total RNA extracted from stably transfected cells, were quantitatively evaluated by spectrophotometry and qualitatively by agarose gel electrophoresis. Then mRNAs were reverse transcripted and BDNF cDNA amplified by specific primers.

Results: The products of the PCR were separated and visualized on agarose gel electrophoresis. Both techniques revealed a successful transfection of CCE ES cells by both plasmids.

Conclusion: The obtained data indicated that the CMV promoter is active in undifferentiated mouse ESCs and a CCE cell line is an appropriate donor cell for cell-mediated BDNF gene transfer.

Key words: Embryonic Stem Cells (ESCs), Transfection, Electroporation, GFP, BDNF

Dose Dependent Effect of Memantine on Long-Term Potentiation in the CA1 Area of the Hippocampus

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Abstract

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Introduction: Generally, NMDA receptor antagonists inhibit learning and long-term potentiation (LTP). However, it has been suggested that direct tonic activation of NMDA receptors, in contrast to learning, may lead to an increase in synaptic "noise". Uncompetitive NMDA receptor antagonist memantine can paradoxically reverse deficits in learning and synaptic plasticity, and restore LTP impaired by tonic NMDA receptor activation.

Material and Methods: Adult rats weighed 200 to 250g were used in this in vivo study. Stimulating Schaffer collaterals field excitatory postsynaptic potentials (fEPSPs) were evoked in neurons of the CA1 area of the hippocampus. For induction of LTP, high frequency stimulation was applied to the path. Pre- and post-tetanic fEPSPs were recorded extracellularly in the anesthetized rats. Test groups were administered intraperitoneally with memantine (10 mg/kg or20 mg/kg) and the control animals received equal volumes of saline.

Results: Our results express that the drug has no effect on the baseline EPSPs. The tetanic stimulation induced a pronounced LTP in the control group lasting at least 2 hours. The animals treated with 10mg/kg of memantine also displayed a significant LTP; however, the potentiation was lower than the controls. The high frequency stimulation under administration of 20mg/kg of memantine failed to induce LTP in the fEPSPs.

Conclusion: These findings point out a dose dependent attenuation of LTP by memantine. Comparison of the present data and those indicating the ability of memantine to restore LTP led us to conclude that, due to the activation level of the recording path, this moderate affinity NMDA receptor antagonist displays different effects on potentiation of hippocampal recordings.

Key word: Hippocampus, Memantine, Long-term potentiation, NMDA receptor

Evaluation of the Susceptibility of Dermatophytes to Garlic Extract

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Abstract

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Introduction: In this study, we evaluated the activity of garlic extract against Dermatophytes which is planned to be used instead of chemical durgs. Garlic has been widely used as medicine since ancient times for varieties of illnesses, including abdominal pain, parasitic infection, insect and snakebites, hemorrhoid and rheumatism. In the last decades, garlic has been reported to display antibiotic and antifungi activities.

Material and Methods: Garlic was obtained from Hamadan, Iran. Using the Mantis method, dry garlic bulbs were peeled and homogenized with two parts of distilled water in a blender and liquid garlic extract was obtained. Then the homogenized garlic extract was run through Amicon DIAF10 ultra-filtration system, using XM and PM membranes. The ultra-filtrated fractions were collected as Residue (R) 300, 100, 50, 30, 10 and filtrate (F) 10. The fractions were evaluated by SDS-PAGE, using 14 percent Acrylamide gel. Serial dilutions of fraction from 1/2 up to 1/32 were tested against each Dermatophyte in Sabourauds Dextrose agar and Minimum Inhibition Concentration (MIC) was obtained. The Dermatophytes tested included: Trichophyton mentagrophytes var. mentagrophytes, Triochophyton Mentagrophytes var. interdigitale, Tricophyton rubrum, Tricophyton tonsurans, Microsporum canis, Microsporum gypseum and Epidermophyton floccosum. An ointment was prepared with Fraction F_{10} as active ingrediant and was used for treatment of dermatophitosis of ginea pigs.

Results: The result showed that F_{10} inhibited growth of Microsporum canis, Epidermophyton floccosum, Tricophyton rubrum, Tricophyton tonsurans. MIC 1/4 was active against Microsporum gypseum and 1/2 against Triochophyton Mentagrophytes var, interdigitale. Trichophyton mentagrophytes var. Mentagrophytes was resistant to all dilutions. Also the ointment used for treatment of dermatophitosis of guinea pig showed a statistically significant inverse relation between the severity and diameter of lesions and the duration of treatment ($p<0.01$).

Conclusion: This research showed that F_{10} fraction, which contains nonprotein components, is the most effective treatment for dermatophitosis.

Key words: Garlic, Dermatophyte, Dermatophitosis

The Effect of N-nitro-L-arginine methyl Ester (L-NAME) on the Thickness and Number of Circular Smooth Muscle Cells of Pylor in Rat Embryo

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Abstract

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Introduction: The aim of this study is to investigate the effect of one nitric oxide inhibitor on the thickness and number of circular smooth muscle cells of pylor in rat embryo.

Material and Methods: To determine the influence of nitric oxide reduction on the muscular layer of pylor in rat embryo, in the present study, pregnant Sprague-Dawley rats received the NO synthase inhibitor N-nitro-L-arginine methyl ester (L-NAME). A dose of 80mg/kg of L-NAME solution in saline was injected to the rats intraperitoneally (IP) during the middle week and last week of the pregnancy period per day. The embryos were removed on the expected day of delivery. Their stomach and duodenum were dissected, fixed by Bouin solution and tissue processing was carried out. By using a rotary microtome 5µ serial cross sections were obtained and stained with Trichrom-Mason and Pop-Nicola. Then sections were evaluated for thickness and number of circular smooth muscle cells under a light microscope using a scaled lens and a checkered lens eye-piece.

Results: Statistical analysis (One-Way ANOVA- Duncan)of light microscopic findings indicated that 80mg/kg of L-NAME in the last week of pregnancy (the first trial group) results in pyloric hypertrophy and hyperplasia.

Conclusion: On the basis of these results we believe that reductions of nitric oxide production in the third trimester of pregnancy could be one of the reasons of pyloric stenosis in infants.

Key words: Nitric Oxide (NO), N-nitro-L-arginine methyl ester (L-NAME), Nitric Oxide Synthase (NOS), Circular smooth muscle cells of pylor.