SESSION 14

Biological response to mechanical stress

Organized by J. Stępiński
Mechanical stretch and activation of muscle repair

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The roles of hepatocyte growth factor (HGF) and nitric oxide (NO) in activation of satellite cells in passively stretched rat skeletal muscle were examined. A hind limb suspension model was developed in which the vastus, adductor and gracilis muscles were subjected to stretch for 0 to 2 hr. Satellite cell activation was assayed by injecting rats with 5-bromo-2'-deoxy-uridine (BrdU) following stretch, and 16 hr later satellite cells were harvested and cultured for 30 hr. BrdU labeling index in cultures reflected activation of satellite cells and entry into the cell cycle in vivo. A significant increase in BrdU incorporation in vivo was observed after 30 min of stretch and increased to 2 hr. Extracts from stretched muscles were found to have the ability to stimulate BrdU incorporation in freshly isolated control rat satellite cells in a concentration-dependent manner. Furthermore, extracts from stretched muscles were found to contain the mature form of HGF, by immunoblot analysis. Immunoneutralization experiments revealed that the in vitro satellite cell activating ability of stretched muscle extracts could be neutralized by incubation with anti-HGF antibody. The involvement of NO was investigated by administering L-NAME, an inhibitor of nitric oxide synthase, or the inactive enantiomer D-NAME, prior to stretch treatment. In vivo activation of satellite cells in stretched muscle was inhibited by the presence of L-NAME, but D-NAME injection permitted satellite cell activation in response to stretch. Stretched muscle extracts from rats treated with L-NAME or D-NAME were assayed for satellite cell activation activity in vitro; stretched muscle extract (SME) from D-NAME treated rats stimulated satellite cell activation to a level similar to SME from un-injected rats. Stretched muscle extracts from L-NAME injected rats, however, did not stimulate satellite cell activation above extracts from the contralateral un-stretched control muscle. The addition of HGF to SME from L-NAME injected rats stimulated satellite cell activation, indicating that the extracts were not inhibitory. Finally, nitric oxide synthase (NOS) activity in stretched and un-stretched muscle was assayed immediately following 2 hr stretch treatment. NOS activity in stretched muscle, with or without D-NAME, was elevated above normal control rat muscle or contralateral un-stretched muscles. In rats injected with L-NAME prior to stretch treatment, there was no significant increase in NOS activity in stretched muscle. Results from these experiments agree with previously reported experiments with isolated satellite cells stretched in culture in that stretching muscle or satellite cells appears to liberate HGF which can bind to and activate satellite cells. Evidence in vitro and in vivo indicates that the process is dependent on NO production in response to stretch.

Mechanical stress and glucose modulate glucose transport in podocytes

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In diabetic kidney, high glucose and mechanical strain resulting from capillary hypertension are relevant factors affecting glomerular cells. In podocytes, both these factors were shown to modulate the cell morphology and expression of various proteins. We have shown recently that in podocytes, differentiated glucose transporter system is expressed, in which GLUT2 and GLUT4 transporters play a dominant role. In the present study we checked the effect of mechanical stress on glucose transport in these cells. Podocytes were cultured for 15–20 days in the plates with flexible bottom. Twenty-four hours prior to stretching, the culture medium was replaced by the glucose-free RPMI1640 supplemented with low (LG, 2.5 mM), normal (NG, 5.6 mM) or high (HG, 30 mM) glucose. The podocytes were stretched for 1–4 hours in the presence of 0.5 μCi/well [3H]deoxy-D-glucose (3H-DG) and accumulated radioactivity was measured in scintillation counter. For assessment of GLUT distribution, the cells were stretched for 4 hours in the standard RPMI 1640, trypsinized, stained with antibodies to GLUT2 and GLUT4 and analyzed by the flow cytometry. For
the first 2 hours, the glucose uptake in LG and NG media was similar in stretched and non-stretched cells but after 4 hours, the stretched podocytes showed 2-fold higher glucose uptake than the control cells. In the cells stressed in HG, glucose uptake exceeded the control value already after 1 hour, and was 3.5-fold higher after 4 hours (0.67 ± 0.13 vs. 0.20 ± 0.03 micromol/mg prot, P<0.01). Mechanical stress induced a 4-fold increase of GLUT4 and 60% increase of GLUT2 in the cell membranes. Intracellular GLUT2 decreased whereas expression of intracellular GLUT4 increased by 54% (P<0.05). We conclude that mechanical stress stimulates redistribution of GLUTs and increases the glucose transport into podocytes. High glucose augments this process. Additionally, stretch-induced de novo synthesis of GLUT4 can not be excluded.

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Mechanical stress decreases activity of particulate guanylyl cyclase in cultured podocytes

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Function of podocytes is largely based on their cytoarchitecture, including a well-developed contractile apparatus. Receptors for numerous vasoactive hormones were found in these cells. Mechanical stress induces a reorganization of the actin cytoskeleton (Endlich et al. JASN, 2001) and alters expression of numerous genes in podocytes (Endlich et al. FASEB J, 2002). The present study was designed to characterize the response of podocytes to vasorelaxing agents stimulating cGMP synthesis and to check the effect of mechanical stress on this response. Immortalized mouse podocytes were cultured in RPMI1640 supplemented with antibiotics, 10% foetal bovine serum (FBS) and 10 U/ml gamma-interferon, at 33°C. Sub-confluent cells were shifted to 37°C and cultured for 5–6 days without interferon. Quiescent cells were seeded to the 6-well plates with flexible bottom and exposed for 3 days to cyclic up- and downward motion induced by an apparatus (StretchCo, Edingen, Germany) inside the incubator. Control cells remained motionless. 60 min prior to stimulation, medium was replaced by RPMI1640 containing papaverine, a phosphodiesterase inhibitor. Cells were stimulated with 0.1 micromol/L atrial (ANP), C-type (CNP) natriuretic peptides or 100 micromol/L sodium nitroprusside (SNP). For receptor characteristics, HS-142-1, guanylyl cyclase-bound natriuretic peptide receptor antagonist was used. Cyclic GMP in lysed cells was determined by enzymatic immunoassay (EIA). RT-PCR analysis showed that podocytes express mRNA for CNP and its receptor, NPR-B. Mechanical stress decreased by half the cGMP response to both ANP and CNP (p<0.05). Response to CNP was markedly higher than to ANP, whereas SNP, a soluble guanylyl cyclase stimulator, had no effect on cGMP synthesis, both in control and stretched cells. In conclusion, mechanical stress decreases ability of podocytes to produce cGMP in the presence of natriuretic peptides. CNP seems to be an autocrine hormone, with higher than ANP potency to generate cGMP in these cells.

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Effect of gallic acid and its lauryl derivative on physical state of cell membrane lipids and cell cycle of human osteosarcoma 143B cell line

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The effect of gallic acid and its lauryl gallate on human osteosarcoma 143B cell line was studied using spin labeling of human osteosarcoma 143B cell line. The cells were treated with gallic acid and its lauryl derivative at the concentration of 1 μM and 10 μM, next labeled with 5-doxylstearic acid and electron paramagnetic resonance (EPR) spectra as well as cell cycle were analyzed. An increase of membranes fluidity (a decrease of microviscosity) was observed as a result of gallic acid interaction with the membranes. This was probably due to significant increase in fluidity of weakly ordered regions within membrane, where rotation correlation...
time is reduced by gallic acid by up to 40%. The effect of gallic acid on highly ordered regions was small — the correlation time in those regions decreased by about 10%. Concentration of the spin labels was similar in those two (weakly and highly ordered) regions of the membrane. Similar effect was observed for lauryl ester of gallic acid. However, in this case the increase of the membranes fluidity was observed not only in weakly ordered regions but also in those highly ordered, with the decrease in correlation time down to 60% and 40% respectively. The concentration of spin labels in ordered regions of the membranes was about 25% lower than in weakly ordered regions.

Long-term cell cultures in complete medium supplemented with fetal calf serum (up to 24 h) in the presence of those compounds exhibited unusual concentration dependent pleiotropic mechanism involving cell cycle arrest at two different points only by the long chain derivative. 1 μM lauryl gallate inhibited cell cycle at G2/M phase while 10 μM lauryl gallate caused G1 phase arrest. Flow cytometric analysis revealed that cell cycle was not perturbed at any specific phase by 1 μM and 10 μM concentration of gallic acid. Structure-activity analysis suggests that lauryl gallate induced antiproliferative effects derived from the structure of molecule penetrating cell membrane more effectively and perturbing physical state of different membrane microdomains.

### Oral Presentation

**Localized changes within membrane of neuroblastoma SN56 cells induced by amyloid β (25–35) peptide**

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Alzheimer’s disease (AD) is one of the age-related neurodegenerative disorders. There is increasing evidence of an association between amyloid β (Aβ) and AD.

Amyloid β (Aβ) may alter membrane lipid dynamics, the activity of membrane-bound proteins and may form ion channels. Hence, the interaction of Aβ with the cell membrane is probably the part of its neurotoxic action. The primary amino-acid sequence of amyloid β peptide 25–35 suggests superficial membrane localization of charged lysine (28) residue while seven nonpolar amino-acids of carbonyl terminus, namely Gly-Ala-Ile-Gly-Leu-Met should extend into the hydrocarbon core of the membrane bilayer. Such extension of hydrophobic tail of amyloid β peptide can affect membrane fluidity. Fatty acids with bounded doxyl group localized at the defined carbon atom provide information on highly localized changes in microfluidity sensed at various depths of membrane hydrocarbon core.

In our study, the 5- and 7-doxyl-stearic acid methyl-esters (5-, 7-DSAME) were used as molecular sensor to investigate the localized changes of the membrane fluidity caused by Aβ (25–35). Aggregated Aβ (25–35) at concentrations of: 1, 2 and 5 μM was added to neuroblastoma SN56 culture for 72 h. Next the cells were suspended in phosphate buffer, pH 7.4 and spin labeled using 5-DSAME or 7-DSAME. The alteration of membrane fluidity caused by Aβ (25–35) was found to be concentration dependent, and also depth dependent as different fluidity was detected for each of the two localized regions of the membrane.

Our results have shown for the first time that it is the deeper localized region of the membrane hydrocarbon core that is stiffened by amyloid. The degree of this alteration of fluidity was maximal for Aβ (25–35) concentration equal to 2 μM. On the other hand the more superficial region sensed by 5-DSAME was only weakly stiffened by amyloid concentration of 1 μM but fluidized at higher concentrations.