Effect of pulsed radiofrequency on nitroxidergic system in a model of neuropathic pain in rat

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Pulsed radiofrequency (PRF) has been ascribed among the most promising non-invasive methods for the treatment of neuropathic pain (Sluijter 1998), nevertheless its mechanism of action has not been still clarified. Nitric oxide is involved in pain modulation both at peripheral and central nervous system (Rodella 1998; Cizkova 2002).

The aim of this work was to monitor the effect of PRF on nitroxidergic system in DRGs, spinal cord and PAG (periaqueductal grey matter) in a neuropathic pain model.

Experiment was carried out on 18 male Sprague-Dawley rats.

The animals were subdivided into two groups: 1) non-operated animals; 2) operated animals, in which the left sciatic nerve was tied (chronic constriction injury - CCI) according to Bennett and Xie (1998). The half of the animal of each group was treated with PRF, whereas the others were used as an untreated control. PRF was performed at 7th post-operative day and monitored at 14th post-operative days. The animals were killed and the DRGs, lumbar spinal cord (L4-L6) and midbrain were removed, frozen and then processed for nNOS immunohistochemistry.

In operated (CCI) animals we observed a significant increase in nNOS immunostaing intensity in the small neurons of DRGs; an increase of nNOS- positive neurons at spinal cord level and a decrease of nNOS-immunostaining in dorsolaterl area of the PAG. In the animals treated with PRF, the patter of nNOS was similar to the control group.

Our data showed that PRF modulates nNOS both in peripheral and central nervous system suggesting a direct effect of PRF on nitroxidergic system.

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Caveolae mediated endocytosis in HepG2 cells: caveosomes or lysosomal degradation

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Nowadays it is generally accepted that, under special conditions, caveolae can take part in ligand internalization. Endocytosis via caveolae is a slow, highly regulated process exists as alternative endocytic machinery parallel to clathrin-dependent endocytosis. Along the caveolar pathway, caveosomes were described as intermediate organelles, characterized only by the presence of caveolin-1 at their limiting membrane. Ligands endocytosed by clathrin-coated pits, however, were never detected in caveosomes. At present, there is no evidence indicating or excluding the potential communication between caveosomes and the organelles of the classical endocytic pathway.

In our work we were especially interested in what can be the further intracellular fate of caveolin-1 and caveosomes. To

answer this question we followed the route of caveolae/caveolin-1 in HepG2 cells by immunocytochemistry on ultrathin frozen sections and Western blot analysis of purified membrane fractions under the inductive effect of albumin.

We found that the number of caveolae at the plasma membrane strongly depended on the presence of albumin. As it was expected albumin induced the internalization of caveolae. To study whether caveolar endocytotic machinery can join to the classical endocytotic pathway, late endosomes/lysosomes and caveolae were labeled with anti-CD63 (LIMP-1), and anti-caveolin-1 antibodies on ultrathin frozen sections respectively. Long term (1 and 3 hours) albumin treatment resulted in the appearance of albumin containing caveolae in special multicaveolar complexes and caveosome-like structures. Numerous late endosomes/multivesicular bodies were characterized by CD63 (LIMP-1) contained caveolin-1 suggesting that caveolin-1 entered the degradative pathway. Our Western blot analysis showed that albumin uptake resulted in a significant decrease of caveolin-1 in the cytoplasmic membranes (including late endosomes and lysosomes) providing further evidence about the degradation of caveolin-1. Inhibition of the endosomal and lysosomal fusion by monensin has not changed the level of caveolin-1 present in the cytoplasmic membranes. Cycloheximide treatment blocked the appearance of caveolin-1 on the plasma membrane indicating that protein synthesis is necessary for new caveolae formation.

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The adaptability, flexibility and versatility of haematopoietic stem cells

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Stem cell populations are not characterized by the possession of distinctive morphological features but can be defined operationally using their ability to maintain self-renewal while providing an appropriate output of precursors to one or more maturation compartments. They must therefore be capable of generating new stem cells and strictly speaking a transplanted stem cell population should be capable of restoring a depleted population in a primary recipient and subsequently in a secondary recipient. The immediate progeny of stem cells are termed "progenitor cells", which provide an appropriate output of precursors to one or more lineages. Progenitor cells lose the ability to maintain self-renewal but are distinguished by their enormous clonogenic capacity. The ability of a stem cell population to respond to variable demands thus depends upon the susceptibility of its progenitor cell output to regulatory mechanisms. This is correctly termed "stem cell plasticity", a term frequently used to designate the versatility of self-maintaining cell populations, although It does not differentiate between their versatility, flexibility and adaptability. The adaptability of a stem cell population can be defined as the ability to adjust its output of precursors to a single maturation compartment, which can for instance enable the rate of proerythroblast production to be increased in response to hypoxia. Adaptability can usefully be distinguished from flexibility, the ability of a multipotent stem cell population to regulate the distribution of such adjustments between two or more maturation compartments and versatility, the ability of a stem cell population to contribute to the production of previously unexpected progeny. The concept of stem cell versatility has been generated during the past decade or so by the demonstration of donor specific markers and the concurrent expression of cell specific markers in transplanted bone marrow-derived cells, which appear to have been assimilated into several populations of host cells derived from each of the three germ layers. The initial contention that versatility can be attributed to the trans-differentiation of transplanted cells has subsequently been endorsed. The use of host specific markers, in addition to donor specific markers and cell specific markers, has however revealed the formation of hepatocytes, skeletal muscle fibres and neurons in which heterokaria that have derived markers from both the donated cells and the cells of the host reflect the fusion of donor cells with host cells. It has thus become evident that while versatility may depend upon trans-differentiation, apparent versatility may result from cell fusion. These alternatives may both be important during development and regeneration as well as in cell replacement therapy. In some instances the replacement of damaged host cells by donor cells, with or without trans-differentiation, may be the only available option but in others the modification of viable but imperfect host cells by fusion with donor cells may be infinitely preferable. Thus following the destruction of irradiated blood cell precursors replacement is essential, whereas the modification of liver cells deficient in a single enzyme is a far more elegant alternative, if the polyploid heterokaria and the reprogrammed genes generated following fusion are not