Mechanisms of nitric oxide influence on the membrane structures of the cell

Brazhe N.A., Maksimov G.V.

Biophysics department, Biological faculty, Moscow State University, 1/12, Leninskie gory, Moscow, Russia 119991; e-mail: nadiya.brazhe@gmail.com

Change of membranes properties is one of the main principles of the modulation of the nerve cell function. Nitric oxide (NO), being a hydrophobic molecule, is known to easily penetrate into the cellular membranes and hydrophobic compartments. In the present work we show that NO influences various neuronal and axonal membrane structures and thus causes different time-scale changes of nerve cell properties. Using conventional and confocal fluorescent microscopies, patch-clamp and extracellular electrodes, we studied effect of NO on the amount of bound Ca^{2+}, mitochondria function, ion channel activity and excitability of neurons and nerve fibres. By means of Raman spectroscopy we estimated fluidity of axonal plasma memrane and fluidity of intercellular neuronal membranes. Our preparations were neurons and nerve fibres of the medical leech *Hirudo medicinalis* and nerves of the grass frog *Rana temporaria*. As NO donors we used spermine/NO and sodium nitroprusside.

We have shown, that exogenous NO firstly activates voltage-dependent K-channels, decreases nerves excitability and then evokes desorption of Ca^{2+} from the plasma membrane (Ca^{2+}_{mb}). Change of Ca^{2+}_{mb} amount was depended on the K-channels activity and in its turn altered membrane surface charge [1]. Besides, NO brought to the decrease in the plasma membrane fluidity [2]. In the cytoplasm NO caused the decrease in the FAD^{+}/FADH ratio relating to the dicrease in succinate-dehydrogenase activity in respiratory chain, and long-lasting depolarization of mitochondria bringing them to release Ca^{2+} into the cytoplasm. For the first time using interference microscopy [3] we showed that NO influences neurons' refractive index due to the cooperative processes evoked by NO in the plasma membrane and cytoplasm. We estimated characteristic frequencies of the refractive index variations in the plasma membrane and cytoplasm regions and tried to correlate them with certain cellular processes. Our results showed that variation of the refractive index with 1 Hz and 15-26 Hz frequencies correspond to changes of the membrane potential and reorganisation of organelles position and transport, respectively.

We suggest that prolonged changes of membranes and organelles properties induced by NO may be among mechanisms that provide signal transduction.

[1] *Ul'ianova N.A., Maksimov G.V., Churin A.A., Rubin A.B.,* Effect of nitric oxide on the viscosity of nerve cell membranes, *Biofizika* **50**, 289-296 (2005).
[2] *Brazhe N.A., Erokhova L.A., Churin A.A., Maksimov G.V*., Investigation of different-scale membrane processes under nitric oxide influence, *Journal of biological Physics* **31**, 533-546 (2005).
[3] *Sosnovtseva O.V., Pavlov A.N., Brazhe N.A., Brazhe A.R., Erokhova L.A., Maksimov G.V., Mosekilde, E.,* Interference microscopy under double-wavelet analysis: a novel approach to studying cell dynamics, *Phys. Rev. Lett.***94**, 218103-1-4 (2005).