tyrosine phosphorylation of the receptor are two independent mechanisms. Examining the role of the 5-HT_{2A}R C-terminus in c-Src interaction, we found that c-Src was able to co-IP only with wild type 5-HT_{2A}R but not with a truncated mutant lacking the C-terminus indicating that 5-HT_{2A}R C-terminus carries the interaction site(s) to associate with c-Src. Furthermore, the purified recombinant 5-HT_{2A}R C-terminus pulled down purified c-Src demonstrating their direct interaction. In conclusion, 5-HT_{2A}R directly interacts with c-Src via the C-terminal end of the receptor explaining their tight functional coupling. Supported by NIH.

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Intracellular detection of Reactive Oxygen Species using single lanthanide nanoparticle imaging: application to vascular signaling

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Reactive oxygen species (ROS) in low concentrations mediate a variety of physiological processes. In the vascular system, Endothelin-1 (ET-1) and Platelet Derived Growth Factor (PDGF) regulate contraction and migration, respectively, by producing intracellular H_2O_2. How these signaling cascades sharing the same second messenger can lead to different physiological effects is still an open question. It possibly relies on a fine regulation of amplitude, timing and location of H_2O_2 production and thus requires an adequate sensor.

We here propose a novel method based on lanthanide nanoparticles, $Y_{1-x}Eu_xVO_4$, for the quantitative, dynamic and local detection of H_2O_2 generation in living cells. $Y_{1-x}Eu_xVO_4$ nanoparticles are photostable probes presenting a continuous emission due to fluorescence of Eu^{3+} ions. We demonstrated in vitro that photoreduction and chemical oxidation by H_2O_2 causes a fluorescence modulation. We identified the temporal response of these nanoprobes submitted to an oxidative signal and proposed a method to determine the H_2O_2 concentration based on the particle fluorescence for concentrations down to 1 μM, relevant to cell physiology, with temporal resolution down to 10-30 s. In addition, the capability of single-particle detection allows spatial resolution.

Imaging of these nanoparticles loaded in vascular smooth muscle cells by pinocytic influx revealed the production of H_2O_2 under stimulation (C_{PDGF}=7 μM, C_{ET1}=13 μM) and a notable timing difference between the two pathways. This points to a method for the cell to integrate distinct signals sharing secondary messengers. Pharmacological treatments, moreover, revealed that H_2O_2 production is partly due to rapid transactivation of EGF receptors. Such cross-talk between pathways is essential for the signal transduction.

These results constitute the first quantitative, time-resolved monitoring of H_2O_2 production and open new perspectives for the deciphering of complex signaling pathways in a variety of biological systems.

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Study of Electrophysiology of Thermal Shock in Higher Plants using High Speed Data Acquisition

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Vascular plants such as the soybean plant, the *Aloe vera* plant, and the *Mimosa pudica* plant have developed mechanisms in order to respond quickly to external stimuli. Throughout the history of plant electrophysiology research, several scientists have attempted to measure the speeds of electrical signal propagation in higher plants in response to thermal stress and other stressors. Several earlier researchers produced signals which were erroneously reported as 0.1 mm/s to 20 cm/s, much slower than actual propagation speeds. These incorrect signaling data resulted from aliasing effects of antiquated data acquisition systems. In this research study, new high-speed data acquisition systems were used to obtain accurate speeds for electrical signals in higher plants. Our results show solitary waves in response to localized thermal stress, with speeds of propagation measured from a few meters per second to approximately 105 m/s [1,2]. In this study, possible mechanisms for electrical signal propagation in response to heat shock are also introduced.

[1] Lang, R.D., A.G. Volkov (2008). Solitary waves in soybean induced by localized thermal stress. *Plant Signal Behav 3*, 224-228.
[2] Volkov, A.G., R.D. Lang, M.I. Volkova-Gugeshashvili. (2007). Electrical signaling in *Aloe vera* induced by localized thermal stress. *Bioelectrochem 71,* 192-197.

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Plant Electrical Memory

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Electrical signaling, memory and rapid closure of the carnivorous plant *Dionaea muscipula* Ellis (Venus flytrap) have been attracting the attention of researchers since the XIX century. We found that the electrical stimulus between a midrib and a lobe closes the Venus flytrap upper leaf in 0.3 s without mechanical stimulation of trigger hairs. As soon as the 8 μC charge for small trap or a 9 μC charge for large trap is transmitted between a lobe and midrib from the external capacitor, the trap starts to close at room temperature. At temperatures 28-36°C a smaller electrical charge of 4.1 μC is required to close the trap of the *Dionaea muscipula*. The Venus flytrap can accumulate small subthreshold charges, and when the threshold value is reached, the trap closes. The cumulative character of electrical stimuli points to the existence of short-term electrical memory in the Venus flytrap. We also found sensory memory in the Venus flytrap. When one sustained mechanical stimulus was applied to only one trigger hair, the trap closed in a few seconds. Prolonged pressing of the trigger hair generates two electrical signals, which stimulate the trap of *Dionaea muscipula* to close.

Membrane Transporters & Exchangers II

3530-Pos Board B577

Activation of the $Na^+/K^+/2\ Cl^-$-Cotransporter in Mammalian Skeletal Muscle

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Skeletal muscle expresses a functional active isoform of the $Na^+/K^+/2\ Cl^-$-Cotransporter (NKCC). This cotransporter is activated by an increase of extracellular osmolality. Previous studies show a linear relationship between the NKCC activity and osmolality [1]. This is contradictory to the fact, that the transport rate should saturate, when the NKCC is activated to its maximum. The aim of this study was to determine the activation curve of the NKCC activity of mammalian skeletal muscle. **Methods and results:** Activation of the NKCC has an impact on the resting membrane potential. Therefore, we measured membrane potentials of rat diaphragm and flexor digitorum brevis muscles at different extracellular osmolalities. Histogram plots of the data revealed a bimodal distribution of membrane potentials - one fraction with high (HP) and one with low (LP) membrane potentials. The means of the LP fractions at different osmolalities were depolarized to values between −50 and −60 mV, those of the HP fractions represented a sigmoidal shaped curve. A computer model of an excitable cell, in which a volume-dependent NKCC activity was incorporated, was fitted to the HP data. In rat diaphragm the NKCC is maximal activated at an extracellular osmolality of 340 mOsmol, in rat FDB this maximum occurs at an osmolality of 320 mOsmol. **Conclusion:** The apparent linear relationship between osmolality and membrane depolarization caused by NKCC activation is based on the invalid calculation of means under the assumption of a monomodal distribution. Accounting for the real bimodal distribution, we successfully revealed the assumed sigmoidal activation curve.

[1] van Mil HG et al., Br J Pharmacol. 1997, 120(1):39-44.

3531-Pos Board B578

Water Transport By The Sodium Glucose Cotransporter

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In addition to transepithelial water flow along an osmotic gradient, isosmolal fluid absorption and water transport against the osmotic gradient were also observed. Two putative mechanisms of solute-solvent flux coupling may explain these findings: (i) local osmosis due to increased solute concentration in the poorly mixed water layers adjacent to the basolateral membrane; and (ii) ''molecular water pumping'' by secondary active cotransporters. The present work aims to identify the water transport mechanism of human sodium glucose cotransporter. Thus, we stably transfected MDCK cells with the hSGLT1-EGFP fusion protein and measured their osmotic water permeability by laser scanning reflection microscopy. We also assessed water flux through confluent cell monolayers, both in the presence and absence of an osmotic gradient by detecting tiny concentration changes with scanning fluorescence correlation spectroscopy (FCS). Fitting the solution of the differential equations for the osmotic drift and for back diffusion to the experimentally determined dye distribution adjacent to the epithelial monolayer allowed calculation of the osmotic water permeability. Assessment of the single transporter permeability coefficient p_f required the simultaneous determination of hSGLT1 abundance in the plasma membrane by FCS. With 4.6×10^{-14} cm^3/sec p_f is close to the single channel permeability of aquaporin-1. Consequently, even small osmolyte concentration differences between the cytoplasm and the basolateral buffer solution are sufficient to drive a substantial water flux. Thus, the physiological importance of secondary active water transport is doubtful.