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Editing of the Abstract Book Marco Capolupo, Silvia Franzellitti, and Gunaalan Kuddithamby (University of Bologna)

chain length. Structural modeling of Cx36 protein docking with hexanol and isoflurane that stimulated as well as nonanol and carbenoxolone that inhibited the conductance of Cx36 GJs revealed their multiple common docking sites and a single pocket accessible only to hexanol and isoflurane. The pocket is situated in the vicinity of three unique cysteine residues, namely C264 in the fourth, and C92 and C87 in the second transmembrane domain of the neighboring Cx36 subunits. To examine the hypothesis that disulfide bonding might be involved in the stimulatory effect of hexanol and isoflurane, we generated cysteine substitutions in Cx36 and demonstrated by a dual whole-cell patchclamp method that in HeLa and N2A cells these mutations reversed the stimulatory effect of hexanol and isoflurane to inhibitory one, typical of other tested Cxs (Cx26, Cx30.2, Cx31, Cx43, Cx45 and Cx47) that lack respective cysteines and/or a specific docking pocket for these compounds. Our findings suggest that the stimulatory effect of hexanol and isoflurane on Cx36 GJ conductance could be achieved by re-shuffling of the inter-subunit disulfide bond between C264 and C92 to the intra-subunit one between C264 and C87.

PP.215

Induction of the apoptotic volume decrease (AVD) under normotonic conditions in HeLa cells exposed to Trolox

Giordano ME, **Caricato R**, **Schettino T**, **Lionetto MG**

Department of Biological and Environmental Sciences and Technologies, Salento University, Lecce, Italy

Previous observations showed that Trolox, a synthetic analog of vitamin E, widely used as antioxidant standard in a number of bioassays, can exert a pro-oxidant behavior at higher concentrations (>40 μM) on HeLa cells exposed for 24h, producing an isotonic cell shrinkage. A number of cellular events are known to be triggered by oxidative stress, including impairment of ion transport mechanisms and alteration of cell volume homeostasis. This work aims to investigate the possible mechanisms through which Trolox at high concentrations acts on cell volume homeostasis alteration in HeLa cells. The study was carried out by 1) spectrofluorimetric determination of intracellular oxidative stress in cells charged with CM-H_2DCFDA, 2) morphometric analysis of cells observed under optical microscopy for cell volume determination, and 3) spectrofluorimetric and confocal analysis of cells charged with Annexin V/Propidium Iodide for apoptotic induction. HeLa cells exposed for 24h to high Trolox concentrations showed a significant dose-dependent isotonic reduction of cell volume associated to intracellular oxidative stress. The observed isotonic shrinkage was accompanied by apoptosis induction, as demonstrated by Annexin V/Propidium Iodide and was ascribed to Apoptotic Volume Decrease (AVD). The isotonic shrinkage appearance was demonstrated to occur early (after 2h) during the exposure to high Trolox concentrations. It was completely inhibited by pretreatment of the cells with a Cl- channel blocker SITS (0.5 mM). These results indicate that treatment of HeLa cells with high Trolox concentrations induces the activation of volume-regulatory Cl^- channels, most likely by an increase in endogenous ROS production, which in turn is able to generate AVD.

PP.216

Effect of O-GlcNAcylation of ICln in the regulation of cellular volume

Remigante A[1,2], **Costa R**[1], **Civello D**[1], **Morabito R**[2], **Nagy T**[3], **Marino A**[2], **Dossena S**[1]

[1]Institute of Pharmacology and Toxicology, Paracelsus Medizinische Privatuniversität, Salzburg, Austria; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy; [3]Department of Laboratory Medicine, University of Pécs, Hungary.

O-GlcNacylation (O-GlcNAc) is the post-translational conjugation of N-acetylglucosamine to serine or threonine residues of cellular protein. O-GlcNAc is known to be elevated in diabetes mellitus, but the pathophysiological role of this finding is not fully elucidated. Recently, the protein ICln, which is crucial in the activation of a chloride conductance ($I_{Clswell}$) after anisosmotic cell swelling, has been found to be O-GlcNacylated. Mass spectrometry and bioinformatics analysis of the amino acid sequence of ICln show several O-GlcNAc modification sites, of which the effect on ICln function is unknown. To elucidate this point, ICln wild type (WT) and different mutant forms have been expressed in human kidney cells and characterized by patch-clamp in conditions of normal or elevated O-GlcNAc levels. Moreover, the protein levels were assessed by western blot. The findings show that: O-GlcNAc elevation suppresses the ICln-induced current; IClnT223A is functional and sensitive to O-GlcNAc elevation; IClnS193X loses most of its activity, even though the residual current is sensitive to O-GlcNAc elevation; IClnS67A is functional but insensitive to O-GlcNAc elevation; IClnS67T is hypofunctional, insensitive to O-GlcNAc elevation, and, accordingly, its protein levels are reduced compared to the WT. Overall, these results indicate that O-GlcNAcylation of ICln at the level of Serine 67 leads to suppression of the ICln-induced current and may disclose the mechanism by which O-GlcNAc elevation affects the regulation of cellular volume. These findings