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РЕЦЕПТОРНЫЕ СИСТЕМЫ, ИОННЫЙ И ВЕЗИКУЛЯРНЫЙ ТРАНСПОРТ

THE RECEPTOR SYSTEMS, IONIC AND VESICULAR TRANSPORT

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THE ACTION OF BILE ACIDS AND THEIR RESPECTIVE CONJUGATES IN NEONATAL CARDIOMYOCYTES IS MEDIATED BY GI PROTEIN, MUSCARINIC RECEPTORS AND TGR5

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In cholestatic disorders deranged bile acid homeostasis results in accumulation of these molecules in the circulation and this affects peripheral organs. In the heart, in particular in neonatal and fetal hearts elevated bile acids are known to trigger arrhythmia, however there is limited knowledge about the underlying mechanisms. We set upon to delineate mechanisms underlying fetal heart rhythm disturbances in neonatal rat and mouse cardiomyocytes exposed to bile acids.

The level of cAMP was measured by FRET microscopy in cells isolated from transgenic mice expressing a FRET sensor pEPAC1-cAMPs. The contraction rate of myocytes isolated from wild type and TGR5KO mice was manually recorded. Mitochondrial toxicity was measured using membrane-bound dye. Acute stimulation for 15 minutes with 100μM bile acids and specific TGR5 agonist, INT777 was used.

The unconjugated bile acids CDCA, DCA and UDCA and, to a lesser extent, CA were found to be relatively potent agonists for the GPBAR1 (TGR5) receptor and elicit cAMP release, whereas all glyco- and tauro- conjugated bile acids are weak agonists. The bile acid-induced cAMP production does not lead to an increase in contraction rate, and seems to be mediated by the RI isoform of adenylate cyclase, unlike adrenaline-dependent release which is mediated by the RII isoform. In contrast, bile acids elicited slowing of neonatal cardiomyocyte contraction indicating that other signalling pathways are involved. The conjugated bile acids were found to be partial agonists of the muscarinic M2, but not sphingosin-1-phosphate-2, receptors, and act partially through the Gi pathway.

High level of cAMP produced in response to unconjugated bile acids is mainly attributed to TGR5, but does not translate into an elevated contraction rate (unlike adrenaline), which suggests different signalling compartmentation. Contraction slowing effect of unconjugated bile acids at higher concentrations may occur due to cytotoxicity.

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CELL SURFACE DETECTION OF LRRC8A SUBUNIT OF THE ANION CHANNELS VRAC IN NATIVE LIVING HUMAN LYMPHOMA CELLS U937 BY FLOW CYTOMETRY

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Volume regulated anion channels (VRAC) play significant role in cell biology. Much progress has been made in recent years in studying their molecular structure and electrophysiological properties. The regulation of VRAC at the cellular level in long-term scale in biological processes such as proliferation, differentiation and apoptosis remains poorly explored. Estimation of the abundance of the channels at the cell membrane in living cells is a necessary step in this work. VRAC subunit proteins were detected until now mostly in cells transfected with the somehow tagged VRAC subunits, by immunoblotting of the solubilized entire cells or cell membranes or by immunoimaging of the fixed cells. We explored whether this problem can be solved in native living cells using flow cytometry and commercially available antibody against an extracellular loop of the LRRC8A subunit known as obligatory for operating VRAC heteromeric channel. Human lymphoid cells U937 were used as an object and two types of cell alteration were tested which are supposed to be followed by an increase in chloride permeability of the cell membrane: an induction of apoptosis with staurosporine (STS) and cell water balance disturbance in hypotonic medium. It is found that endogenous LRRC8A subunits are detected and their abundance at the cell membrane of the native living U937 cells can be evaluated using flow cytometry and applied Alomone antibody. It appeared, however, that cell treatment for 1 h with either STS, or with hypotony does not cause visible change in the number of LRRC8A subunits expressed at the cell surface. We conclude that an increase in the entire chloride permeability of the cell membrane under considered conditions is not due to an increase in number of channels exposed at the cell membrane but alteration of channel properties.

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A TOOL FOR COMPUTATION OF CHANGES IN NA^+, K^+, CL^- CHANNELS AND TRANSPORTERS DUE TO APOPTOSIS BY DATA ON CELL ION AND WATER CONTENT ALTERATION

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Monovalent ions are involved in a vast array of cellular processes. Their movement across the cell membrane is regulated by numerous channels and transporters. Identification of the pathways responsible for redistribution of ions and cell water in living cells is hampered by their

strong interdependence. This difficulty can be overcome by computational analysis of the whole cell flux balance. Our previous computational studies were concerned with monovalent ion fluxes in cells under the conditions of balanced ion distribution or during transition processes after stopping the Na^+/K^+ pump. Here we analyze a more complex case — redistribution of ions during cell apoptosis when the parameters keep changing during the process. New experimental data for staurosporine-induced apoptosis of human lymphoma cells U937 have been obtained: the time course of changes in cellular K^+, Na^+, Cl^-, and water content, as well as Rb^+ fluxes as a marker of the Na/K pump activity. Using a newly developed computational tool, we found that alteration of ion and water balance was associated with a 55% decrease in the Na^+/K^+-ATPase rate coefficient over a 4-h period, with a time-dependent increase in potassium channel permeability, and a decrease in sodium channel permeability. The early decrease in $[Cl^-]_i$ and cell volume were associated with an ~5-fold increase in chloride channel permeability. The developed approach and the presented executable file can be used to identify the channels and transporters responsible for alterations of cell ion and water balance not only during apoptosis but in other physiological scenarios.

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FLUOROMETRIC INTRACELLULAR NA^+ MEASUREMENT AS COMPARED WITH FLAME EMISSION ASSAY: AN UNEXPECTED PROBLEM WITH GRAMICIDIN-BASED CALIBRATION

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Fluorescent probes are a popular and indispensable tool for monitoring sodium concentration in living cells in situ. Calibration of fluorescent probes inside cells commonly uses ionophores to equilibrate intracellular and external ion concentrations. Here we test this calibration method using in parallel classical flame emission assay. Suspension human lymphoma cells allow both flow cytometry fluorometric study and flame emission assay. The most sensitive Na^+ fluorescent probe ANG-2 and the most common ionophores were tested. Cellular Na^+ was altered for calibration in three different ways: by stopping the sodium pump with ouabain, by inducing of apoptosis with staurosporine, and by gramicidin or amphotericin B treatment. We found that ANG-2 fluorescence in cells treated with gramicidin or amphotericin was about two fold lower than in the cells with the same sodium concentration but without ionophores. The equal fluorescence measured in the absence and in the presence of ionophores corresponds to different cell sodium concentrations. No effect of gramicidin on hydrolyzed ANG was observed in vitro. The mechanism, by which gramicidin decreases ANG fluorescence in cells is unlikely to be physical quenching and remains obscure. We conclude that ANG fluorescence does not display realistic cell Na^+ if fluorescence in cell is measured in ionophore absence while calibrated in its presence.

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ПРЕДВАРИТЕЛЬНАЯ ОБРАБОТКА ПЕРЕД ЗАМОРАЖИВАНИЕМ УВЕЛИЧИВАЕТ ЧИСЛО ЖИЗНЕСПОСОБНЫХ СПЕРМАТОЗОИДОВ БЫКОВ ПОСЛЕ РАЗМОРАЖИВАНИЯ

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PRELIMINARY TREATMENT BEFORE FREEZING INCREASES THE NUMBER OF VIABLE SPERMATOZOA OF BULLS AFTER THAWING

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Использование различных криосред способствует получению после замораживания жизнеспособных сперматозоидов. Однако их количество не превышает половины от изначально подвижной популяции [1]. Целью работы явилось изучение влияния предварительной инкубации сперматозоидов быков до замораживания на жизнеспособность клеток после размораживания. Долю клеток с различным функциональным статусом определяли с помощью красителя хлортетрациклин. Оценку сперматозоидов проводили с использованием микроскопа Zeiss с фазовым контрастом и эпифлуоресцентной оптикой. Инкубацию проводили в присутствии соединений, одно из которых стимулировало в сперматозоидах быков капацитацию (кофеин, 2 мМ), другое активировало акросомную реакцию (ПРЛ, 10 нг/мл и ГТФ, 10 мкМ). Совместное в течение 4 час действие ПРЛ и ГТФ приводило к увеличению количества акросома-реактивных клеток, тогда как инкубация в присутствии кофеина вызывала увеличение числа капацитированных сперматозоидов.

Жизнеспособность сперматозоидов оценивали с помощью красителя пропидиума иодида. Сперматозоиды делили на две группы, каждую из которых предварительно подвергали процедуре капацитации в течение 4 час, а затем одну группу замораживали, вторую — нет. В клетках, не подвергавшихся замораживанию, во всех вариантах экспериментов соотношение количества живых и мертвых клеток составляло примерно 2 к 1. Предварительная инкубация сперматозоидов быков перед заморозкой в присутствии кофеина и последующее размораживание приводили к увеличению количества жизнеспособных клеток. В то же время предварительная инкубация сперматозоидов в присутствии ПРЛ и ГТФ не приводила к росту числа жизнеспособных клеток после процедуры замораживания/оттаивания.

Таким образом, только предварительная инкубация сперматозоидов быков перед замораживанием в присутствии кофеина, активирующего рост числа капацитированных клеток, приводила к увеличению числа жизнеспособных клеток после размораживания.