Лук’янець О.О.

Про проведення міжнародної конференції “Молекулярні механізми внутрішньоклітинної кальцієвої сигналізації”

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Міжнародна конференція “Молекулярні механізми внутрішньоклітинної кальцієвої сигналізації» («Molecular mechanisms of intracellular calcium signalling») відбулась 11-13 жовтня 2009 р. у Києві в Інституті фізіології ім. О.О. Богомольця НАН України. Проведена за підтримки Національної академії наук України, Інституту фізіології ім. О.О. Богомольця, Міжнародного центру молекулярної фізіології НАНУ, Українського фізіологічного товариства та міжнародного Товариства з нейронаук (SFN). Організатори конференції – академіки НАНУ П.Г. Костюк, О.О. Крishталь та професор О.О. Лук’янець. Проведення конференції збігалося зі святкуваннями 75-річного ювілею Інституту фізіології ім. Богомольця та 85-річчя академіка П.Г. Костюка. Відкрив урочисте засідання співголова конференції академік Кришталь О.О. і привітав учасників із відкриттям форуму. Професор Оле Петерсен – Генеральний секретар міжнародного союзу фізіологічних наук (IUPS), який також займав посади Президента фізіологічного товариства Великобританії та Віцепрезидента Королівського товариства Великобританії відзначив вагомий внесок співробітників Інституту фізіології та особисто Костюка П.Г. у розвиток міжнародної фізіологічної науки. Академік РАН Островський М.А., який є головою Російського фізіологічного товариства ім. І.П. Павлова у своєму виступі відмітив значний внесок Костюка П.Г. у розвиток фізіологічної науки на теренах СРСР і сучасної України та вручив «Пам’ятну медаль Фізіологічного товариства ім І.П. Павлова» Російської Федерації. Також академік Островський повідомив, що Президіум РАН присудив «Золоту медаль імені І.М. Сеченова» академіку РАН Костюку П.Г. за цикл робіт «Кальцієва сигналізація в нервовій клітині», про що сповіщається в російській газеті «Поиск» №41 (1063) від 9 жовтня 2009 р. На відкритті Конференції також виступив виконавчий директор Державного фонду фундаментальних досліджень (ДФФД) Кияк"Богдан Романович, який розповів про плодотворну роботу ДФФД і голови Ради Фонду академіка Костюка П.Г. у сприянні підтримки фундаментальних досліджень в Україні та науковому міжнародному співробітництву ДФФД із академічними інституціями світу і вручив академіку П.Г. Костюку медаль «За внесок в науку» від імені фонду.

Академік РАН Угрюмов М.В., який є радником Президії РАН з питань міжнародної наукової діяльності, заступником голови наукової ради програми Президіуму РАН «Фундаментальні науки – медицині», віце-президентом Російського фізіологічного товариства ім. І.П. Павлова від імені президента Російської академії Наук академіка РАН Осіпова Ю.С. та віце-президента РАН академіка Григорьєва А.І. поздоровив академіка Костюка П.Г. із ювілеєм. Було відмічено, що академік Костюк П.Г. – приклад вченого – беззавітно відданого науці, який зробив величезний внесок у розвиток світової нейрофізіології і створив наукову школу європейського і світового масштабу. Також у привітанні сказано, що учні академіка П.Г. Костюка займають ключові позиції в галузі нейрофізіології в багатьох країнах «далекого зарубіжжя» та країнах СНД.

Професор Пьотр Брежестовський із Середземноморського Інституту нейробіології (Франція) заглибився в історію Інституту фізіології; відзначивши значний його внесок у міжнародну науку. Також Брежестовський привітав Платона Григоровича з ювілеєм від імені фізіологів Пущинського наукового центру РАН, відмітивши, що він стояв у витоків електрофізіологічної науки в Радянському Союзі, а тепер Інститут фізіології ім. О.О. Богомольця – один із світових лідерів в галузі фізіології клітини. Пьотр Брежестовський подарував картину відомої російської та французької художниці Світлани Богатир, яка зобразила П.Г. Костюка та Науку у вигляді сплетіння чисел, що символізують інтелект. У своєму слові член-кореспондент РАН, член бюро відділення біологічних наук РАН, заступник голови Президіуму Казанського наукового центру РАН Нікольський привітав учасників конференції від імені Казанського наукового центру РАН, що є великим комплексом академічних інститутів, широко відомих своїми науковими школами хімії, ядерного резонансу, молекулярної фотохімії; теплоенергетики, фізіології та біохімії рослин і біофізики. Нікольський передав вітання від віце-президента Російського фізіологічного товариства ім. І.П. Павлова члена-кореспондента РАМН Зефірова А.Л., який є Головою Татарського відділення Фізіологічного товариства Російської Федерації. У привітанні відзначається, що Інститут фізіології ім. О.О. Богомольця на чолі з його директором академіком Костюком П.Г. дуже шановані всією численною спільнотою фізіологів, біофізиків і лікарів Росії, України та інших країн, оскільки Платон Григорович – засновник школи дослідників в галузі нейрофізіології, клітинної та молекулярної фізіології, біофізики, яка відома в усьому світі. Нікольський Є.Є. подарував академіку Костюку П.Г. національний татарський одяг – зелені чапан і тубатай, які є символом мудрості у татар.

Академік П.Г. Костюк виступив на пленарній сесії конференції із лекцією «Кальцієва сигналізація – Київська історія», в якій нагадав що кальцієва сигналізація була вперше визначена в стінах Інституту. Так, уперше в світі співробітниками Інституту фізіології ім. О.О. Богомольця була відкрита кальцієва провідність мембрани та встановлена гетерогенність кальцієвих каналів мембрани. В міжнародній конференції брали участь 79 делегатів, що представляли, крім Інституту фізіології ім. О.О. Богомольця, інші наукові установи України (Києва, Львова, Донецька, Дніпропетровська), а також закордонні – Росії, Білорусі, Вірменії, Польщі, Чехії, Словаччини, Данії, Німеччини, Великобританії, Італії, Франції, Японії, США. В конференції представили доповіді відомі закордонні вчені – Оле Петерсен (Великобританія), Еміліо Карбоне (Італія), Норіо Акайке (Японія), Михаіл Угрюмов, Лев Магазанік та Євгеній Нікольський (Росія), Пьотр Брежестовський (Франція), Франц Гофман (Німеччина), Владимір Штрбак (Словаччина), Павел Мареш (Чехія) та багато інших. Серед них були також учні Платона Григоровича, що зараз працюють за кордоном та мають свої лабораторії – Алла Фоміна (США), Сергій Міронов (Німеччина), Дмитрій Русаков (Великобританія) та інші закордонні та українські вчені. Під час конференції відбувалося обговорення представлених результатів досліджень. В тому числі відбулася значна постерна сесія, в якій брали участь і молоді вчені. За відгуками закордонних вчених конференція пройшла на Європейському рівні. В період роботи конференції для іноземних вчених було організовано відвідання лабораторій Інституту фізіології та екскурсія по місту Києву.

На закритті міжнародної конференції П.Г. Костюк підсумував роботу та відмітив активну участь молодих учених, вказавши, що більшість постерів молодих учених свідчить про високий теоретичний, методичний, практичний рівень і комплексний підхід до багатьох сучасних проблем кальцієвої сигналізації. Істотну увагу було приділено

питанням, пов’язаним дослідженням патологічних станів, а також розглянуті молекулярно-генетичні та біохімічні аспекти досліджень. Академік П.Г. Костюк, професор Лук’янець О.О. а також голова Комісії конференції з нагородження кращих постерів молодих вчених професор П. Брежестовський відзначили кращі доповіді за результатами висновків журі. Переможцями конкурсу на кращий постер молодого вченого стали Д.В. Самігуллін (Казань, Росія) з роботою «Кальцієвий транзиєнт у різних частинах нервово-м’язового з’єднання жаби у відповідь на нервовий імпульс» (1-ше місце), Котлярова А. (Львів, Україна) зі стендовою доповіддю «Кальційзалежні механізми індукованої радіацією ксеростомії» (2-ге місце) та Саврасова А. (Київ, Україна) із роботою «Ефект блокатора нейроамінідази на нейрональну активність та формування гіпокампальних синапсів» (3-тє місце). Кращим молодим доповідачам були вручені дипломи, біографічна книга «Над океаном часу», підписана академіком Костюком П.Г., та грошові премії.

Підсумовуючи результати конференції, можна констатувати, що вона сприяла плідній роботі всесвітньовідомих учених і наукової молоді, реалізації її творчого потенціалу і зародженню нових ідей, розширила кругозір українських та іноземних дослідників, познайомила їх з актуальними проблемами в галузі кальцієвої сигналізації, сприяла встановленню нових наукових зв’язків і можливостей для міжнародної наукової співпраці. Матеріали конференції представлені нижче.

Учасники конференції

LECTURES

CALCIUM SIGNALLING - KIEV HISTORY

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Historically it happened that Kiev became a very comfortable city for the origin and development of cellular biophysics. Although Kiev University and Bogomoletz Institute of physiology buildings were damaged during the war, they were rapidly restored and delivered excellent conditions for experimental work. Not less important was presence of D.S. Vorontsov who initiated our experimental programs. The origin and development of investigations of ionic processes in single cells by microelectrode technique started in Kiev in a small laboratory belonging to Kiev State University – not in the main research institute of NASU, the Bogomoletz Institute of Physiology; 75th anniversary of which foundation is celebrated this year. Only in 1956 the department of electrophysiology was organized in this institute. This opened for us a way to this institute.The main topic of our investigations this time was a comparative analysis of the function of Ca^{2+} -selective voltage-operated ion channels in different excitable cells which form the main pathway for transmembrane calcium currents. An important technical step was the creation of a special technique allowing the recording of calcium currents separately from other types of currents (sodium, potassium) - the intracellular perfusion or dialysis Kostyuk, et al., (1975, 1977). By using this technique it became possible to characterize precisely calcium channels in different types of cells. In our group the presence of two main subtypes of such channels was detected; they were separated into low- and high-voltage activated ones with quite different functional characteristics Veselovsky and Fedulova (1983). This separation has been confirmed later in other laboratories. The introduction of intracellular perfusion technique opened for us the way for intensive investigations of the biophysical properties of calcium channels and intracellular mechanisms determining the function of Ca^{2+} ions entering the cytoplasm ("calcium signals"). Calcium signals produced by activation of ligand-activated channels are another important research topic of our group. In this respect our interest concentrated specially on purinoreceptors activated by extracellular ATP – discovered in neuronal tissue by Krishtal, et al., (1983). An extensive study of these receptors on different neurons demonstrated important role of calcium influx through corresponding channels in modulation of synaptic transmission. For the continuation of this topic a search for the sources of extracellular ATP is important. The fate of calcium ions which enter the cell is the next important question extensively studied in many laboratories. Our interests concentrated in the determination of the expression of these mechanisms in different parts of the cell and different cell types. An important finding was age-dependence of this mechanism. A reduction of the amplitude of calcium signals was found in aged neurons, accompanied by prominent deceleration of the recovery of the cytoplasmic Ca^{2+} level – an alteration of its extrusion mechanisms. Retardation of calcium accumulation by endoplasmic reticulum has been demonstrated as one of the reasons for such changes. Such alterations may be responsible for the increased vulnerability of the aged brain (Verkhratsky et al., 1994). The role of mitochondria in the determination of the fate of intracellular Ca^{2+} has also been extensively analyzed by our group.
Keywords: microelectrodes, cell perfusion, calcium channels, mitochondria, endoplasmic reticulum

EFFECTS OF A2 TYPE BOTULINUM TOXIN ON SPINAL EXCITATORY AND INHIBITORY TRANSMISSION

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We observed the effects of newly developed A2 type botulinum toxin (A2NTX) on the spontaneous miniature and evoked transmitter release from inhibitory (glycinergic or GABAergic), and excitatory (glutamatergic) nerve terminals, by use of 'synaptic bouton' preparations, under voltage clamp condition. A2NTX (0.1-1 pM) initially augmented and then decreased the amplitude and frequency of the spontaneous miniature release of glycine or GABA (mIPSCs), dose-dependently, without affecting those of glutamate (mEPSCs) in the presence of TTX. However, at an increased concentration (1-10 pM), A2NTX gradually suppressed the amplitude of glutamatergic mEPSCs. The rank order of the inhibitory effects was glycinergic > GABAergic >> glutamatergic synapses. Focal electrical stimulation of inhibitory or excitatory 'synaptic boutons' elicited eIPSC or eEPSC with larger amplitude and low failure rate (Rf). A2NTX

(0.01–1 pM) initially enhanced the amplitude or decreased the failure rate of eIPSC or eEPSC, and then almost completely abolished the generation of eIPSC or eEPSC. The evoked release was more sensitive to A2NTX comparing to those of spontaneous miniature release of glycine, GABA and glutamate. The rank order of the inhibitory effects on the amplitude or Rf was glycinergic eIPSC ? GABAergic eIPSC > glutamatergic eEPSCs. Excess extracellular 30 mM K^+ or 10 mM Ca^{2+} (excess $[K^+]_o$ or $[Ca^{2+}]_o$), and 300 мM 4-AP restored glycinergic, GABAergic or glutamatergic mIPSCs supressed by A2NTX. 4-AP also restored the evoked glycine release suppressed by A2NTX. We conclude that A2NTX inhibits spontaneous miniature release at 0.1–10 pM and evoked release at 0.01–1 pM from spinal inhibitory and excitatory 'synaptic boutons', and the inhibition was much efficient in the evoked release rather than the spontaneous miniature release. Excess $[K^+]_o$, 4-AP and excess $[Ca^{2+}]_o$, which raise the intracellular Ca concentration, rescue the transmission suppressed by A2NTX poisoning, suggesting the transmitter release machinery became less sensitive to intracellular Ca level in A2NTX poisoned 'synaptic boutons' to trigger the exocytosis.
Keywords: botulinum toxin, A2NTX, rat spinal neuron, excitatory and inhibitory transmission

PHYSIOLOGY AND PATHOPHYSIOLOGY OF CALCIUM SIGNALLING IN ELECTRICALLY NON-EXCITABLE CELLS

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The pancreatic acinar cell continues to be a very useful object for Ca^{2+} signalling studies due to its highly polarized structure and the relative ease of isolation. High-resolution confocal and two-photon studies of the dynamics of $[Ca^{2+}]$ in the cytosol, all major organelles and the immediate extracellular environment of these cells have provided a detailed understanding of physiological (Petersen & Tepikin *Annu Rev Physiol* 70, 273-299, 2008) and pathological (Petersen et al *Cell Calcium* 45, 634-642, 2009) Ca2+ signalling processes. The most important information will be reviewed together with very recent data about the role of IP3 receptors in pathophysiology (Gerasimenko et al *PNAS* 106, 10758-10763, 2009) and the roles of STIM1 and Orai1 in regulating Ca^{2+} entry (Lur et al *Curr Biol* 2009 in press).
Keywords: calcium, calcium signalling, physiology, pathophysiology

NEURONAL CALCIUM CHANNELS AS PACEMAKERS AND SECRETION-CONTROLLER IN CHROMAFFIN CELLS

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Chromaffin cells express different densities of high-voltage-activated (HVA) Ca^{2+} channels (L, N, P/Q, R) that control, with nearly equal efficacy, the Ca^{2+}-dependent release of catecholamines. With brief stimulatory pulses exocytosis is fast, linearly correlated to the quantity of Ca^{2+} charges and independent of the type of Ca^{2+} channel controlling the Ca^{2+} entry. Even "low voltage-activated" T-type channels, which are not normally expressed in physiological conditions but can be recruited under pathological stimuli, are able to control the release of catecholamines with the same Ca^{2+} efficacy of L-type and other HVA channels. Recent works from our group (Marcantoni et al., *Cell Calcium*, 2007; Marcantoni et al., *Pflьgers Archiv*, 2009) suggest that Ca^{2+} channels do not only support the Ca^{2+} and voltage-dependent vesicular release of catecholamines but do also regulate the firing frequency of spontaneously active rat and mouse chromaffin cells. Pacemaking of spontaneously firing cells is mainly controlled by L-type channels which activate at relatively low voltages (-50 mV in 2 mM Ca^{2+}) and display little sign of inactivation during long depolarizing pulses. Nifedipine (3 mM) fully blocks most of the firing cells and Bay K 8644 increases the action potential frequency. Spontaneous firing is lost in a large fraction of mouse chromaffin cells isolated from the Cav1.3 KO mouse, suggesting a dominant role of this L-type channel isoform in the regulation of action potential shape and interspike intervals. An action potential-clamp study reveals that firing is controlled by the strong coupling of Cav1.3 to fast inactivating BK channels, while Cav1.2 and non-inactivating BK channels play a less critical role. Our studies highlight the new roles of Cav1.3 and Cav3.2 as pacemaker channels and controllers of hormone release in the

chromaffin cells of adrenal medulla. These properties are likely to be shared by other neuroendocrine cells and brain neurons.
Keywords: Cav1.3 and Cav3.2 voltage-gated calcium channels, BK channels, Action potential firing, Catecholamine secretion

P2X3 RECEPTORS: MYSTERIES OF USE-DEPENDENT HIGH AFFINITY BINDING

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$P2X_3$ purinoreceptors expressed in mammalian sensory neurones are involved in nociception, mechanosensory transduction and temperature sensation. Due to their high Ca^{2+} permeability $P2X_3$ receptors serves as one of the main sources of Ca^{2+} in sensory neurones. Slow recovery from desensitization of $P2X_3$ receptor makes quite problematical the understanding of its physiological function. We found that the recovery from desensitization of $P2X_3$ receptor is speeding up by decrease in external pH and increase in temperature. On the contrary, the onset of desensitization is independent of these influences. Such unusual combination of temperature sensitivity/insensitivity allows receptor to function near normal body temperature even at low nanomolar concentrations of ATP. Slight acidosis and increased temperature are typical for inflammation, so we can conclude that at inflammatory conditions the function $P2X_3$ receptor is upregulated.
Keywords: P2X3 receptors, temperature-sensitivity, pH-sensitivity, gating, sensory neurons.

DIFFERENT ROLE OF CALCIUM IN CELL SWELLING-INDUCED INSULIN SECRETION FROM PANCREATIC ISLETS AND TUMOR CELL LINE INS-1E

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Cell swelling evokes exocytosis of proteins and peptides stored in secretory vesicles from various types of cells. Dynamics of this type of hormone secretion is indistinguishable from that induced by specific secretagogue. Peculiarities of swelling-induced secretion indicate an involvement of the unique signalling pathway. Hyposmotic stimulation of insulin secretion from isolated pancreatic islets is independent from the extra- and intracellular Ca^2, does not involve other intracellular mediators of glucose stimulation, and could not be inhibited by noradrenaline.
We compared response of freshly isolated rat pancreatic islets and INS-1 and INS-1E tumour cell lines to high glucose, 30 % hypotonic medium and 20 % hypertonic medium. In Ca^{2+}-containing medium glucose induced insulin release in all three cell types. Hypotonicity induced insulin secretion from islets and INS-1 cells but not from INS-1E cells, in which secretion was even inhibited despite similar increase in cell volume. Surprisingly, perifusion with Ca^{2+}-depleted medium either with or without BABTA/AM revealed distinct secretory response of INS-1E cells to stimulation with hypotonicity. Similar response to hypotonicity was also seen if INS-1E cells were perifused with calcium channel blockers either separately or in combination (10 mM nifedipine, 100 nM agatoxin, 10 mM mibefradil). Hypertonic medium inhibited glucose-induced insulin secretion from islets but not from tumor cells. Noradrenaline (1 ммol/l) inhibits glucose-induced but not swelling-induced insulin secretion. In contrast to glucose stimulation, swelling-induced insulin secretion is resistant to N-ethylmaleimide thus suggesting the presence of a separate pool of secretory granules available for swelling-induced but not glucose-induced exocytosis. In Ca^{2+} containing medium response either to glucose or hypotonicity was inhibited by 20 mM tetanus toxin. Interesting enough, in presence of combination of Ca^{2+} channel blockers hypotonicity-induced insulin secretion from INS-1E cells persisted despite inhibition of SNARE proteins by tetanus toxin. Conclusion: Ca^{2+} can determine either positive or negative response to hypotonicity with opposite effect in INS-1 and INS-1E cell lines. Cell swelling-induced secretion of insulin includes peculiar features not involved in glucose-stimulated exocytosis.
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Keywords: cell swelling, exocytosis, calcium signalling,

BRAIN-DERIVED SIGNALING MOLECULES: ROLE IN THE REGULATION OF THE DEVELOPMENT OF THE BRAIN AND THE WHOLE ORGANISM

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This lecture is devoted to the evaluation of the author's and literature data on the neuron differentiation and the role of neuron-producing signaling molecules (SM) (neuropeptides, classical neurotransmitters, growth factors, etc.) in the regulation of the development of the brain and the whole organism. It has been demonstrated that the neurons begin to secrete SM shortly after their genesis from the progenitor cells and long before the establishment of interneuronal relations and synaptogenesis. Most of SM contribute to the autocrine and paracrine regulations of the target neuron differentiation as morphogenetic or transcriptional factors. They act during strictly predetermined critical periods providing long-lasting effects on the neuron differentiation and expression of specific phenotype. Indeed, SM contribute to the autocrine and paracrine regulations of: (a) the proliferation of the progenitor cells and thereby of neuronogenesis; (b) the migration of differentiating neurons from the place of their origin to the place of final settling in the brain; (c) the expression of specific genes and syntheses (neuropeptides, growth factors, enzymes of synthesis of classical neurotransmitters, membrane transporters, vesicular transporters, receptors); (d) the guided growth of the axons and dendrites; (e) the development of specific interneuronal relations (synapses, etc.). Taking into account that the differentiating neurons begin to secrete SM long before the development of specific interneuronal relations and establishment of the blood brain barrier, we hypothesized that the neurons in the early ontogenesis operate like secretory cells and the brain - as a multipotent endocrine gland. It means that the brain should contribute to the endocrine regulations of the peripheral organs and the brain itself (autoregulation) over the ontogenetic period from the onset of the neuron functioning as a secretory cell to the establishment of the blood brain barrier. Indeed, it has been proven that only over this period of ontogenesis the brain-derived neuropeptides and classical neurotransmitters are delivered to the general circulation. The concentration of circulating brain-derived SM occurred to be high enough for providing the regulatory influence on the target cells. Furthermore, at that time the peripheral and brain target cells express the receptors to these SM. Thus, the neuron-derived SM provide the autocrine and paracrine regulations of the neuron differentiation in the developing brain as well as the endocrine regulation of the development of the peripheral target cells and the brain neurons lasting until the establishment of the blood brain barrier.
Keywords: Brain, development, neuron differentiation, neuropeptides, classical neurotransmitters, growth factors

TRANSDUCTION OF NEURONAL CALCIUM SIGNALS BY CALMYRIN PROTEINS

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Calmyrins sustain a novel family of the Ca^{2+} binding proteins that coordinate Ca^{2+} in the EF-hand motives. Calmyrins (CaMy) are mostly similar to calmodulin and to the neuronal Ca^{2+} sensors. Calmyrin genes are evolutionary conserved from Nematoda; in human four genes encode calmyrins (CaMy1 – CaMy4). We have recently characterized CaMy1 and its closest homolog CaMy2 in human and rat brains. These studies revealed that Ca^{2+}-dependent features, localization in the brain, and putative Ca^{2+}-signaling functions of CaMy1 and CaMy2 in neurons are different.

Higher levels of CaMy1 are detected in neurons of cerebellum and low levels in neurons of hippocampus and cortex. We identified the microtubule-destabilizing factor SCG10 protein as a CaMy1-interacting ligand in the brain. CaMy1 binds directly to SCG10 in Ca^{2+}-dependent manner and modulates SCG10 activity as a microtubule destabilizing factor. Our results indicate that CaMy1 plays a role in coupling Ca^{2+} signals to SCG10 and to the dynamics of microtubules in neurons, especially in neuronal growth cones. CaMy2 protein is present mainly in neurons of the hippocampus. CaMy2 level in hippocampal neurons is controlled by NMDAR and Ca^{2+}. In NMDA-stimulated neurons, endogenous CaMy2 is associated with membranous fraction and is localized in neurites and the Golgi apparatus. Consistently, CaMy2 was found to interact with SNARE proteins that are essential for the vesicular traffic between the Golgi compartment and cell membrane, and for neurotransmitter release. Our results indicate CaMy2 involvement in Ca^{2+} signaling mediated by NMDA receptor activation and point to CaMy2 involvement in trafficking of vesicles.
Keywords: Calcium, calmyrin proteins, brain, microtubule-destabilizing factor

ACTIVITY-DEPENDENT CONTROL OF PRESYNAPTIC Ca^{2+} BY KAINATE AND CANNABINOID RECEPTORS AT INDIVIDUAL CENTRAL SYNAPSES

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Ca^{2+} -dependent release of neurotransmitters underlies fast information flow in the brain. Presynaptic kainate receptors (KARs) modulate transmission between granule cell axons (mossy fibres, MFs) and CA3 pyramidal neurons in the hippocampus, but the underlying mechanisms are poorly understood. We show that a single action potential in a single MF activates both presynaptic KARs and local Ca^{2+} stores, contributing to use-dependent synaptic facilitation. Application of kainate onto individual MF boutons facilitates evoked Ca^{2+} entry through a Ca^{2+} store-dependent mechanism. Local two-photon uncaging of IP3 confirms the presence of functional Ca^{2+} stores at presynaptic MF boutons. Analyses of unitary postsynaptic responses in CA3 pyramids are consistent with the KAR - Ca^{2+} store coupling acting as a synapse-specific, short-range autoreceptor mechanism. We also find that the CA3 - CA1 synaptic connection in the hippocampus is modulated by presynaptic CB1 cannabinoid receptors which inhibit presynaptic Ca^{2+} signals altering release probability at individual Schaffer collateral synapses. Preliminary data indicate that the mechanism involved differs from that of classical G-protein coupled receptors, such as GABAB receptors, and potentially provides for use-dependent modification of transmission by controlling the extent of multi-vesicular release.

Keywords: Calcium, cannabinoid receptors, pyramidal neurons, GABAB receptors

GENOMIC AND NON-GENOMIC REGULATION OF TRPM8 COLD RECEPTOR BY ANDROGENS

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In this report we describe two modes of the regulation of TRPM8 cold receptor/channel in the non-neural tissues and in the sensory dorsal root ganglion (DRG) neurons by androgens. The first mode, which is more specific to the non-neural, particularly prostate epithelial cells, involves genomic, androgen receptor(AR)-dependent regulation of the expression of two TRPM8 splice variants: truncated, endoplasmic reticulum-localized, $_{ER}$TRPM8, and full-size, plasma membrane-localized, $_{PM}$TRPM8. The expression of the $_{ER}$TRPM8 is little AR- and epithelial cell phenotype-dependent, whereas the expression of $_{PM}$TRPM8 is strongly dependent on functional AR, is determined by the differentiation status of prostate epithelial cells peaking in the fully differentiated apical/luminal phenotype and is further enhanced upon transition to the prostate cancer, which makes it a viable marker for the prostate cancer staging. In addition to the genomic mode of regulation of TRPM8 expression by androgens detected in the prostate epithelial cells, full-size TRPM8 in DRG neurons also appears to be the subject of the short-term, non-genomic inhibition by testosterone, which involves putative plasma membrane testosterone receptor coupled to the Gi protein – adenylate cyclase (AC) – cAMP – protein kinase A (PKA) pathway. Depending on the levels of circulating androgens this mode of regulation may notably impact the perception of innocuous cold on the whole organism level.

Keywords: DRG neurons, Prostate epithelial cells, TRPM8, Androgen receptor, Testosterone, Gi

CALCIUM SIGNALING IN SECRETION OF CATECHOLAMINES IN CHROMAFFIN CELLS

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It is well known that Ca^{2+}-dependent exocytosis of synaptic vesicles is the main processes in synaptic transmission in neurons and neuroendocrinne cells. Ca^{2+} signal is main trigger of secretion, but intracellular level of Ca^{2+} can be increased by several ways in the cell. Thus calcium growth in cytoplasm can be evoked by Ca^{2+} entering through voltahe-operated Ca^{2+}-channels, ionotropic channels of receptors or from internal Ca^{2+} stores after activation of membranne receptors. We studied the role of synaptic

proteins such as, synaptotagmin (STG), Munc-13, Munc-18 and CAPS on Ca^{2+}-dependent kinetic properties of secretory events in the rat chromaffin cells using microfluoresnt and amperometric methods and specific antibodies: anti-STG, anti-Munc-13-1, anti-Munc-13-3, anti-Munc-18 and anti-CAPS. Simultaneously, fluorescent dextran-FITC was injected into a cell for the control of antibody entering into the cell. Exocytosis was induced by activating of the potential-dependent Ca^{2+}-channels by activation of voltage-operated Ca^{2+}-channels with KCl solution or increasing of intracellular Ca^{2+} concentration via activating of acetylcholine receptors. The injection of antibodies resulted in the reduction of frequency of appearance of secretory peaks registered by carbon fiber electrode. Most substantially this was observed under anti-STG treatment. Influence of anti-Munc-18 resulted in deceleration of kinetic characteristics of secretory peaks, possible due to narrowing of fusion pore. Using of anti-Munc-18 revealed two types of cells which differed by the value of reduction of the frequency of secretory events. In the first group of tested cells, there was greater reduction of probability of appearance of secretory peaks (by ~71%) after injection of anti-Munc-18.While in the second group, there was the far less this value (~12%). We observed enough substantial decline of frequency of secretory appearances during introduction of specific antibodies to Munc-13, and the value of the reduction depended on the kind of stimulus. Anti-Munc-13-1 was more effective in suppression of secretory peaks than anti-Munc-13-3. Anti-CAPS antibodies induced also reduced appearance of secretory spikes. We concluded that explored synaptic proteins participate in the secretory processes of chromaffin cells, but play their specific role. We also established that secretory responces induced by different ways of Ca^{2+} elevation (ionnic channels or receptors) can be quite different in chromaffin cells.
Keywords: Calcium, synaptic proteins, exocytosis, chromaffin cells

POSSIBLE ROLE OF CALCIUM HOMEOSTASIS IN ALZHEIMER DISEASE

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The main aim of our work was to investigate calcium homeostasis changes in the case of Alzheimer disease. At the first steps of our work with hippocampus cells cultured with в – amyloid (a model of Alzheimer disease) we found a great increase of basal level of calcium in cells cultured in $A\beta_{1-42}$. The cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured using fura-2 based microfluorimetry. We used mitochondrial uncoupler carbonyl cyanide 3-chlorophenylhydrazone (CCCP, 10 nM) in order to compare mitochondrial contribution to Ca^{2+} signals in control and test conditions. It was revealed that after 24-hour incubation of hippocampus cell culture with $A\beta_{1-42}$ Ca^{2+} basal level was 138±12 nM vs. 76±8 nM in control conditions ($P<0.01$, $n=19$). Amplitude of Ca^{2+} transient induced by KCl solution in control conditions was 506±20 nM, and after treatment with $A\beta_{1-42}$ it increased to 622±34 nM ($P<0.05$, $n=15$). In $A\beta_{1-42}$-treated cells Ca^{2+} transients induced by KCl solution with CCCP were higher and broader than that in control neurons. The prolonged returning of calcium signal to the basal level indicates either congestion of calcium by mitochondria or changes in the function of mitochondrial exchangers. If in the control condition the half-time of calcium transients cultured of β-amyloid $t_{0,5} = t_{(max/2)}$ S was 11±3 sec., after application β-amyloid it increased to 32±6 sec. ($P<0.05$). After incubation with в-amyloid and CCCP the corresponding values were increased. It is interesting that during induction of diabetic mellitus similar changes in the function of mitochondria were observed in nociceptive neurons. After application of CCCP in comparison in control conditions a decrease in amplitude and prolongation of the decay phase of depolarization-induced calcium transients were observed. The decay phase could be also described by summation of 2 exponents. Investigating calcium currents in the cultured neurons of hippocampus we also found changes in their voltage-dependence. Current density curves shifted to hyper-polarization by 70% (P< 0, 05, n=12). Steady-state activation characteristics also shifted to more negative potentials. Steady-state inactivation curve did not change. So, we found that almost all characteristics of calcium intracellular homeostasis are changed. We can say that such changes of calcium regulation are one of the important factors in pathogenesis of Alzheimer‘s disease, but whether it is main factor still remain the question. At the same time in peripheral nervous system (small nociceptive dorsal root ganglion neurons) in the case of diabetes mellitus the changes of calcium homeostasis by mitochondria resemble. Obviously if mitochondria are not able to rapid Ca^{2+} uptake during cell excitation, these may lead irreversible pathological changes and neuronal death. These data indicate that changes in calcium exchange function of mitochondria during diabetes and Alzheimer disease are quite similar, indication that the function of mitochondria and related structures (endoplasmic reticulum) are changing similarly in peripheral and central neuronal systems during degenerative processes.
Keywords: Alzheimer disease, calcium mitochondria, в-amyloid, currents

VOLTAGE DEPENDENCE OF NUCLEAR ION CHANNELS IN CENTRAL NEURONS AND ITS POSSIBLE ROLE IN SHAPING CA^{2+} SIGNALS

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Ca^{2+} is a ubiquitous second messenger involved in regulation of numerous cellular functions. In neurons Ca^{2+} enters the cytoplasm through Ca^{2+} channels of the plasma membrane and spreads throughout the cell along the network of the endoplasmic reticulum (ER). Ca^{2+} release from intracellular Ca^{2+} stores is restricted in space and time. The mechanism of spatiotemporal organization of Ca^{2+} signals remains controversial. It is well known that Ca^{2+} release is activated by the agonists of intracellular Ca^{2+} channels, but the mechanism of termination of Ca^{2+} signals is poorly understood. A number of different mechanisms have been proposed including depletion of Ca^{2+} stores and inhibition of intracellular Ca^{2+} channels by high Ca^{2+} concentrations. Ca^{2+} release from intracellular stores leads to transfer of a large positive electric charge from the lumen of the ER into the cytoplasm. This charge transfer can be compensated by counterflow of K^+ into the ER through K^+ and Ca^{2+} channels. However Yamashita and his colleagues have recently reported that Ca^{2+} oscillations in neurons were accompanied by large synchronous changes in electric potential of the lumen of the ER and therefore Ca^{2+} release can generate negative shifts in the lumen electric potential. Authors hypothesized that negative luminal potential attenuates Ca^{2+} release due to decrease in the electrochemical potential for Ca^{2+}. The exact place if any of this mechanism in shaping transient Ca^{2+} signals, such as Ca^{2+} puffs and waves, is not clear. In particular it depends on kinetics of voltage-dependence of ion channels of the ER. Here we studied kinetics of voltage-dependence of ion channels in nuclei isolated from rat cerebellar Purkinje and hippocampal CA1 pyramidal neurons. We have found that all major types of nuclear ion channels, inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$), large-conductance cation and Cl^- channels, were inhibited by negative luminal potential with varied kinetics. Strong and fast inhibition of $InsP_3Rs$ by negative luminal potentials may be one of the major mechanisms of termination of Ca^{2+} signals. The large-conductance cation channels were predominant type of ion channels in the nuclear membrane of both types of neurons and were responsible for the most part of K^+ permeability of their nuclear membrane. Kinetics of voltage-dependence of these channels considerably differed in Purkinje and pyramidal neurons, which may account for particular patterns of Ca^{2+} signalling in these neurons.

Keywords: Ca^{2+} signalling, Inositol 1,4,5-trisphosphate receptors, Cation channels, Cl^- channels, Cell nucleus

NEURONAL-GLIAL NETWORKING

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Our understanding of glial function has changed dramatically over last two decades. This change concerns the whole concept of how the brain is organized, and how the development, life and death of neural circuits are controlled. There is compelling evidence demonstrating that these are the astrocytes that are creating the compartmentalisation in the CNS, and these are the astrocytes that are able to integrate neurones, synapses, and brain capillaries into individual and relatively independent units. Astroglial syncytium allows intercellular communication route, which permits translocation of ions, metabolic factors and second messengers. The resulting potential for parallel processing and integration is significant and might complement the binary coded electrical communication within the neuronal networks.

Keywords: Glia; astrocyte; neuronal-glial circuitry; signalling; receptors

CYS-LOOP RECEPTOR IONIC CHANNELS: STRUCTURE, FUNCTION AND REGULATION BY CALCIUM

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Cys-loop superfamily constitutes a major class of receptor-operated ion channels. Functional channel contains five protein subunits, with a signature sequence of 13 residues flanked by covalently bond

cysteins, situated between binding and channel domains. In vertebrates, Cys-loop receptors are subdivided into cation- and anion-selective channels, corresponding to excitatory receptors activated by acetylcholine (ACh) or serotonin, and inhibitory receptors activated by GABA or glycine. Three main breakthroughs recently accelerated our understanding of the molecular structure of the Cys-loop superfamily. First, the discovery of acetylcholine-binding protein (AChBP) from the freshwater snail *Lymnaea stagnalis*, and the determination of its structure at a resolution of 2.7A, which stimulated homology modeling of ligand-binding domains using AChBP as the template. Second, electron microscopy of ACh receptors from *Torpedo* at a resolution of 4A, which revealed the protein backbone, locations of б- carbon atoms and bulky side chains of compete receptor protein. Third, the determination structure of prokaryotic cys-loop like channels in open and closed conformations. Cys-loop receptors are involved in a rapid synaptic neuro-muscular and neuro-neuronal communication. Function of these channels is regulated via external, intracellular and transmembrane domains. We have shown that cytoplasmic Ca^{2+} leads to rapid potentiation of responses to glycine via Ca^{2+}-binding protein (CaBP). Using the yeast two-hybrid system, screening of a human brain cDNA library against the cytoplasmic loop of human alpha 1 subunit (GlyRh1) we identified this protein and analyzed its functional properties in cell lines and neurons. This mechanism may play an important role in Ca^{2+}-dependent modulation of glycinergic and, presumably, other rapid synapses in the nervous system of vertebrate.

Keywords: calcium, cys-loop receptor, Ca^{2+}-binding protein, human alpha 1 subunit

INTERPLAY BETWEEN CALCIUM AND CAMP IN HEALTH AND DISEASE: IN VIVO IMAGING WITH GENETICALLY ENCODED NEURON-TARGETED PROBES

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cAMP is a ubiquitous second messenger which mediates various neuronal functions that are often coordinated with changes in intracellular calcium. Aiming to visualize calcium and cAMP fluctuations in vivo, we designed neuron-specific FRET-sensors which are based on D3cpv and Epac proteins, respectively. The sensors were introduced into the respiratory neurons in the organotypic brainstem slices via viral transduction and expressed both in neurons and their processes. This allowed us to map the topology of the respiratory network (pre-Bцtzinger complex in the brainstem, pBC). The network appears to consist of interconnected neuronal clusters (modules) presenting the groups of pacemakers which intrinsic rhythmic activity is synchronized to produce a stable respiratory output. The population activity of respiratory network was monitored by recording fluorescence of calcium sensor D3cpv.
The resting cAMP level in pBC neurons was 0.6 µM. After depolarisation-induced calcium influx and calcium release from internal stores cAMP slowly increased to 1 to 2 µM and then returned within 1 min to the basal value. cAMP transients showed facilitation during paired stimulations such as second depolarisation or calcium release followed within <1 min after the first one evoked bigger cAMP responses. The effects were abolished after inhibition of proteinkinase A, indicating the role of phosphorylation of calcium channels which mediate calcium influx or calcium release.
The interplay between calcium and cAMP in neuronal signalling was further examined in the mouse model of Rett Syndrome (RTT). It is a devastating neurodevelopmental disorder caused by mutations in MeCP2 protein which decreased activity leads to diminished production of brain-derived nerve factor (BDNF), a key factor in the differentiation and maturation of neurons during embryogenesis and early postnatal period. We observed distinct disturbances in calcium and cAMP homeostasis in pPBC neurons of model RTT mice (MECP2 -/y). Resting calcium was higher and calcium transients decayed slowed than in wild-type that was due to less efficient calcium uptake into the endoplasmic reticulum by SERCA. cAMP levels in RTT were lower and its changes were smaller due to enhanced activity of phosphodiesterase PDE4 in the mutant. Treatment of slices with BDNF restored both calcium and cAMP homeostasis to that of the wild-type. Global increases in calcium led to retraction of neuronal processes, whereas cAMP increases stimulated their outgrowth. The retractions were bigger and elongations were shorter in the mutant, and they come to those of the wild-type after pretreatment with BDNF. We propose that an orchestrated interplay between calcium and cAMP leads to long-term changes in the wiring within respiratory network that influences its function. A remodelling of respiratory circuitry during development and maturation therefore can set up the conditions for appearance of breathing irregularities in RTT.

Key words: Calcium: cyclic AMP; Respiratory network; Rett Syndrome (RTT)

VARIETY OF MECHANISMS OF CA-PERMEABLE GLUTAMATE RECEPTORS BLOCKADE AS A GATEWAY TO DESIGN OF NEW DRUGS

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Fast excitatory synaptic transmission in the mammalian brain is mediated by the neurotransmitter, glutamate. Most of its receptors are glutamate gated cation-selective ionic channels (GluRs). The channels of two GluR classes, NMDAR and AMPAR lacking of GluR2 subunit, are Ca-permeable. Ca transients in the neurons induced by their activation are widely involved in normal functions and in the pathogenesis of neurological diseases. It motivates the detailed study of receptor structure and pharmacological properties. We considered the blockade of open Ca permeable GluRS as a promising approach to the search of clinically acceptable and tolerant drugs: (1) the noncompetitive antagonism should be more effective than the competitive one; (2) the analysis of structure-activity relationships of homologous series of channel blockers bear information about the molecular structure of the channels that extremely useful for design of new drugs; (3) the differences in the mechanism of drug-channel interactions open possibility to search pharmacological tools addressed to certain functional situations.

The systematic study of a number newly synthesized blockers affecting open channels of NMDA and AMPA receptors was performed by the experiments on isolated brain neurons. We created the first series of AMPAR channel blockers and compared mechanisms of pharmacological blockade of Ca-permeable AMPA and NMDA receptor channels. It was found that: (1) the dicationic compounds with the distance of approximately 10 A between positively charged groups but not the monocationic ones are potent AMPAR channel blockers; (2) the overall blocking effect depends not only on the affinity of drug to the binding site in the channel but on the mechanism of blockade (trapping, penetration through the channel, kinetics of opening-closing, e.g.); (3) the modeling of drug-channel interaction gives the plausible prediction of new active uncompetitive glutamate antagonists; (4) the experiments on animal models of motor disorders showed that elaboration of the clinically used drugs required testing a family of new glutamate channel blockers differing in the selectivity and mode of action.

The main problem of the development of clinically tolerant antagonists of glutamate receptors is that the same receptors participate in both normal physiological and pathological processes. The suggested approaches may help solving the problem.

Keywords: Calcium, NMDA receptors, AMPA receptors, glutamate receptors

THE ROLE OF NON CONDUCTIVE MEMBRANE MECHANISMS IN NEURONAL SIGNAL TRANSDUCTION

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Membrane theory the signal transduction in excitable cell is considered as a membrane conductance change and rejects the electrogenic character of metabolic Na/K pump. However, at present the existence of metabolic component of membrane potential is a proven fact. The present chapter briefly sketches the primary role of electrogenic Na/K pump in regulation of number and activity of cell membrane protein molecules having enzymatic, receptive, and ionic channel forming properties realized by pump-induced modulation of cell volume, membrane water fluxes and intracellular signaling systems. The cell hydration serves as an extrasensitive and universal messenger, through which the under threshold signal transduction in neurons is realized. The negative correlation between brain cortex cell hydration (number of ouabian receptors in membrane) and pain thresholds in rats were shown. The factors having dehydrating effect on brain cortex cells have an analgesic effect on animals, while hydrating effect causes algesic effect. The electromagnetic fields and infrasound, having intensity much less than "thermal thresholds" have a modulation effect on brain cortex cell hydration. It is supposed that nerve disorders and aging induced brain cortex cell dehydration is due by Na/K pump dysfunction-induced activation of cAMP –dependent Na/Ca exchange in regime of 3Na efflux and 1Ca influx which initiate sthe apoptosis cascade in neurons.

Keywords: Na/K pump, neuron, membrane, channels, receptors, cell volume, intracellular signaling

REGULATION OF CARDIAC CALCIUM CHANNEL BY PROTEIN KINASES.

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Classical inactivation of the L-type Cav1.2 (a1C) calcium channel gene is embryonal lethal before day 14.5 pc. We have generated a mouse line in which exon 14 and 15 of Cav1.2 is flanked by two loxP sites. These mice were crossed with various lines expressing tissue-specific Cre recombinase. The off-springs of these mice had severe defects in insulin secretion, blood pressure regulation, intestinal muscle contraction, hippocampal memory acquisition. These mice lines showed that the putative T-type calcium channel blocker mibefradil lowers blood pressure by inhibition of the Cav1.2 channel. Mutation of Ser1928 to Ala has no effect on beta-adrenergic stimulation of the cardiac Cav1.2 channel. We analysed further the coupling between Cav1.2 and the CaM-kinase II. Facilitation of the Cav1.2 channel by CaM-KII involves the phosphorylation of serine 1512 and 1570 in the C-terminus of Cav1.2. Mutation of S1512/1570A results in viable off spring with no obvious phenotype. The mutated cardiac Cav1.2 shows no voltage-dependent and frequency-dependent facilitation. The electrophysiological consequences of these mutations will be discussed.
Keywords: Calcium, calcium channels, CaM-KII, L-type channels, Cav1.2 channels

ROLE OF RYANODINE RECEPTORS IN CA^{2+} SIGNALING AND FUNCTIONS OF HUMAN T LYMPHOCYTES

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$CD4^+$ helper T lymphocytes play a pivotal role in directing the immune responses against foreign pathogens, damaged or transformed cells. Antigenic stimulation of T cell receptors (TCR) triggers spatio/temporally heterogeneous cytosolic Ca^{2+} signals that consequently induce a series of functional transformations in naпve T cells, a process commonly referred to as activation. We have previously shown that in Jurkat T cells, a human lyphoblastic leukemia T cell line, intracellular Ca^{2+}-release channels, inositol trisphosphate and ryanodine receptors, are activated by extracellular Ca^{2+} entry and significantly affect cytosolic Ca^{2+} dynamics during T cell activation by means of regulating the accumulation of Ca^{2+} inside the store (*Dadsetan et al., 2008, J. Biol. Chem., 283:12512-9*). Because normal human lymphocytes exist in multiple functional states, we have further researched the role of ryanodine receptors (RyR) in resting and activated T cells isolated from the peripheral blood of healthy humans. Naпve T cells were purified by negative selection from the peripheral blood mononuclear cells and activated *in vitro* using anti-CD3/CD28 antibodies. To investigate intracellular Ca^{2+} dynamics, resting and activated T cells were loaded with Ca^{2+} indicator Fura-2 and challenged with either RyR agonist caffeine, or reversible sarco-endoplasmic reticulum Ca^{2+}-ATPase inhibitor cyclopiazonic acid in the presence or absence of RyR blockers ryanodine or dantrolene. We found that cytosolic Ca^{2+} signaling and functional expression of ryanodine-sensitive store is upregulated in activated T cells compared with that in resting T cells. We also found that in activated T cells RyR inhibitors significantly reduced Ca^{2+} signaling in the cytosol while increased Ca^{2+} retention within the store, which is consistent with our results obtained in Jurkat T cells. Furthermore, we found that both ryanodine and dantrolene suppress activation-induced T cell proliferation underscoring the importance of RyR in regulation of Ca^{2+}-dependent functions of activated T cells. We conclude that upregulation of RyR is essential for maintaining enhanced intracellular Ca^{2+} signaling in activated T cells and that RyR may serve as a potential target for suppression of pathogenic functions of overactivated T cells.
Keywords: Calcium, T lymphocytes, RyR, Ca^{2+}-release channels

STRUCTURAL PLASTICITY OF NEURONAL CELLS OF CA1 HIPPOCAMPAL AREA AFTER LONG-TERM SYNAPTIC POTENTIATION

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It is known that long-term potentiation results in structural remodeling of the postsynaptic spine,

comprising an enlargement of the spine head and reorganization of the postsynaptic density (PSD) at excitatory synapses of CA1 hippocampal neurons. However, synapses represent complex functional units in which interaction between the presynaptic varicosity and the postsynaptic spine is also modulated by surrounding astroglial processes. In order to investigate how activity patterns could affect the morphological interplay between these three partners, we used an electron microscopic (EM) approach and 3D reconstructions of excitatory synapses to study the activity-related morphological changes underlying induction of synaptic potentiation by theta burst stimulation or brief oxygen/glucose deprivation (OGD) episodes in hippocampal organotypic slice cultures. The area of the PSD significantly enlarged 30 minutes and one hour after stimulation, particularly in large synapses with complex PSD, an effect that was associated with a concomitant enlargement of spine and presynaptic terminals volume. Furthermore, synaptic activity induced a pronounced increase of the glial coverage of both pre- and postsynaptic structures. These data reveal dynamic, activity-dependent interactions between glial processes and pre- and postsynaptic partners and suggest that glia can participate in activity-induced structural synapse remodeling.

Key words: electron microscopy, astrocyte, oxygen/glucose deprivation, LTP, organotypic hippocampal culture

A NEW TYPE OF MITOCHONDRIAL CALCIUM-INDUCED PORE AND PARTICIPATION OF THE PORE IN CALCIUM RECYCLIZATION AND GLUTAMATE-INDUCED MITOCHONDRIAL DEPOLARISATION

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In this decade, serious attention has been given to the study of a novel mitochondrial Ca^{2+}-induced lipid pore. The pore is characterized by the following features: (1) it is induced by Ca^{2+}-binding fatty acids (palmitic (Pal) and stearic); (2) it is insensitive to cyclosporin A (CsA); its opening requires accumulation of Ca^{2+} in the mitochondrial matrix; it closes spontaneously; in contrast to the permeability transition pore (PTP), it does not damage mitochondria. The mechanism of Pal/Ca^{2+} pore opening may be explained in view of our recent data that binding of Ca^{2+} to Pal leads to its segregation into distinct membrane domains, indicating that pore opening can be considered in the light of the theory of lipid pore formation upon chemotropic phase transition. The opening of the pore can induce short or prolonged membrane depolarization (MD) depending on Ca^{2+} and fatty acid concentrations. In the case of prolonged MP, Dy does not fall completely, and the addition of Ruthenium Red (RR) or La^{3+} leads to repolarization of the membrane and inhibition of Ca^{2+} release. This suggests the operation of a mitochondrial Ca^{2+} cycle, with Ca^{2+} influx mediated by the uniporter and Ca^{2+} efflux provided by the opening of Pal/Ca^{2+} pore. Such cycle can also be observed in the absence of external Pal – upon activation of phospholipase A_2 by Sr^{2+}. It is known that high Sr^{2+} activates mitochondrial phospholipase A_2 (PLA_2) but does not induce opening of the CsA-sensitive PTP and degradation of mitochondria. The sequential addition of Sr^{2+} portions (200 nmol/mg protein) to mitochondria up to 1.2-1.4 mM opens the pore, leading to the fall of mitochondrial Дш, Sr^{2+} release, mitochondrial swelling and an increase of external pH (before pore opening, the latter decreases upon each Sr^{2+} pulse). As in the case with an external fatty acid, 1µM RR recovers all these parameters. The inhibitors of phospholipase A_2 (25 мM aristolochic acid and 20 мM trifluoperasine), which essentially suppress the accumulation of endogenous free fatty acids in mitochondria, attenuate the Sr^{2+}-induced CsA-insensitive mitochondrial pore opening. Bovine serum albumin (BSA), which can bind free fatty acids, has the same effect. The combination of a PLA_2 inhibitor with BSA almost completely prevents the pore opening. At the same time, PLA_2 inhibitors do not affect mitochondrial respiration and the rate of mitochondrial Sr^{2+} uptake. The data obtained indicate that an increase in cellular Ca^{2+} (Sr^{2+}) leads to PLA_2 activation and elevation of free fatty acids (including Pal) in the inner mitochondrial membrane, which may result in the induction of the Ca^{2+} (Sr^{2+}) cycle in mitochondria. A possible role of the Pal/Ca^{2+} pore in the glutamate-induced deregulation of Ca^{2+} homeostasis and mitochondrial dysfunction in mammalian central neurons is discussed.

Keywords: Palmitic acid, Calcium, Mitochondria, Ca^{2+}-dependent pore, Ca^{2+} cycle, Sr^{2+}-induced membrane depolarization

HUMAN MESENCHIMAL STEM CELLS FIX RADIATION-INDUCED VASCULAR DAMAGE IN RATS

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The therapeutic potential of bone marrow-derived human mesenchymal cells (hMSC) has been evaluated in a whole-body irradiated (6 Gy) rats. Experimental design of the study comprised phenotypic analysis of hMSC, large conductance Ca^{2+}- dependent K^+ channels (BKCa) activity measurements in aortic smooth muscle cells using patch clamp technique in whole-cell modification, non-invasive systolic arterial blood pressure measurement and simultaneous measurement of contractile force and $[Ca^{2+}]i$. Bone marrow was aspirated in heparin from the sternum of healthy volunteers after informed consent. hMSC were separated using negative selection procedure with monoclonal antibodies (Human RosetteSep Mesenchymal Stem Cell Enrichment Cocktail, StemCell Inc.). The isolated MSC after Ficoll-Hypaque centrifugation were resuspended in MesenCult medium (StemCell Inc.) supplemented with appropriate Mesenchymal Stem Cell Stimulatory Supplements and cultivated 20 - 32 days in the same medium with additional recombinant human growth factors: SDF-1a, EGF, and PDGF-AA (CHO-grade). After two passages MSC were transplanted intravenously to irradiated rats on the 7th day of post-irradiation in a single dose of 16-20x106 cells per rat. Phenotypic analysis confirmed that hMSC used for transplantation are free of hematopoetic contamination and strongly positive for the MSC specific surface markers. Whole-body irradiation produced a decrease of BKCa activity in aortic myocytes. This was paralleled by a reduction of the NO-dependent ACh-induced vascular relaxation and arterial hypertension development. Thus, the vasorelaxing force of BKCa was diminished in irradiated myocytes. Simultaneous measurements of contractile force and $[Ca^{2+}]i$ showed that myofilament Ca2+ sensitivity defined as the ratio of force change to $[Ca^{2+}]i$ significantly increased following irradiation. The hMSC effectively restored outward currents (from 13±1 to 24±1 pA/pF, $P<0.05$, n=12) mainly due to paxilline-sensitive BKCa component, and led to an increase in amplitude of maximal ACh-induced endothelium-dependent relaxation in irradiated vascular tissues from 43±3% to 87±6% ($P<0.05$, n=12). The hMSC transplantation normalized myofilament Ca^{2+} sensitivity (from 0.068±0.007 to 0.030±0.004 mN/nM, $P<0.05$, n=12) and arterial blood pressure from 152±4 to 121±2 mm Hg ($P< 0.05$, n=12).These studies have shown that, being transplanted to the irradiated rats, hMSC demonstrate a clearly expressed therapeutic potential to normalize vascular function damaged following ionized irradiation, and this way appear to be worthwhile therapeutic approach in case of vascular malfunction induced by ionizing irradiation impact.

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Keywords: Calcium, human mesenchymal cells, large conductance Ca^{2+} - dependent K^+ channels, radiation

PRE-AUTONOMIC BUT NOT NEUROSECRETORY CELLS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ARE GLUCOSE SENSING

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Some specialized hypothalamic neurons sense extracellular glucose via increase or decrease in their electrical activity (termed as glucose-excitatory and inhibitory cells, respectively). These neurons have been found in the arcuate, ventromedial nuclei and lateral hypothalamus, sites directly involved in regulation of energy homeostasis. The paraventricular nucleus (PVN) is tightly connected with those ones and mediates a major part of hormonal and autonomic output of the hypothalamus, however the glucosensing properties of its constituent cells have not been studied so far. Here we report that the majority of presumed pre-autonomic (PA) but not neurosecretory (NS) parvocellular neurons in the PVN (~86 vs. 12 %) were directly inhibited via large increase in membrane conductance, when extracellular glucose decreased from 10 to 0.2 mM (EC50 ~0.67 mM). Thus, they can be classified as glucose-excited cells. Blockers of ATP-sensitive K^+ (KATP) channels glybenclamide and tolbutamide were ineffective in preventing inhibition, while channels opener diazoxide did not mimic the effects of low glucose. These data suggest that PA neurons in the PVN sense ambient glucose via KATP channels-independent mechanism, in contrast to majority of glucose-excited neurons in other hypothalamic nuclei.

Keywords: Hypothalamus, paraventricular nucleus, glucose sensitivity, currents

Ca^{2+} SENSITIZING TROPONIN T MUTATIONS LINKED TO HYPERTROPHIC CARDIOMYOPATHY INCREASE APPARENT CYTOSOLIC CA^{2+} BINDING

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Troponin T (TnT) mutations that increase myofilament Ca^{2+} sensitivity are associated with familial hypertrophic cardiomyopathy (FHC) and confer a high risk for sudden death. In mice, Ca^{2+} sensitization causes action potential alternans and ventricular arrhythmia susceptibility, but the underlying mechanism remains unclear. Since Ca^{2+} binding to the troponin complex represents a major portion of cytosolic Ca^{2+} buffering, we hypothesized that TnT mutants alter myocyte Ca^{2+} handling by changing Ca^{2+} buffering. Using cardiomyocytes isolated from mice expressing troponin T mutants, we find that increasing myofilament Ca^{2+} sensitivity produced a proportional increase in cytosolic Ca^{2+} binding. The underlying cause was an increase in the cytosolic Ca^{2+} binding affinity (K_d), whereas maximal Ca^{2+} binding capacity was unchanged (B_{max}). The effect was sufficiently large to decrease Ca^{2+} transients expected during a typical contraction. These results directly demonstrate that myofilament Ca^{2+} sensitivity is an important determinant of cytosolic Ca^{2+} buffering capable of modifying cardiac Ca^{2+} homeostasis. Alterations in cytosolic buffering should be considered as one of the underlying mechanism responsible for the arrhythmogenic effects of TnT mutations that cause myofilament Ca^{2+} sensitization.
Keywords: myofilament Ca^{2+} sensitivity, familial hypertrophic cardiomyopathy, Ca^{2+} buffering, Ca^{2+} homeostasis, arrhythmias

THEORETICAL STUDY OF THE DISTRIBUTION AND PROPERTIES OF Ca^{2+} HANDLING SYSTEMS

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Mathematical modelling provides a useful means of investigating the mechanisms that regulate Ca^{2+} dynamics. Stimulus-evoked Ca^{2+} responses are often spatially highly non-uniform. Considerable evidence exists showing spatial heterogeneity in distribution of Ca^{2+} handling systems that are important for defining localized Ca^{2+} gradients. The complexity of Ca^{2+} regulation makes it difficult to address rigorously how individual Ca^{2+} handling systems contribute to calcium responses. It happens in particular because any use of specific inhibitors of Ca^{2+} signals evokes Ca^{2+}-dependent changes in the activity of other systems that regulate intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). We developed an approach that can be applied for study of the distribution of Ca^{2+} handling systems in the conditions of insufficient experimental data. The two main points for this are the method of rejection and the use of the least favourable for the hypothesis data. We developed a multi-compartmental model of Ca^{2+} dynamics that includes Ca^{2+} entry through voltage-dependent and synaptic receptor channels, diffusion, buffering, Ca^{2+} transport across the endoplasmic reticulum, Ca^{2+} extrusion and mitochondrial transport. Using reasonable descriptions of cell morphology, buffers and other Ca^{2+} handling systems, we checked a number of hypotheses about distribution of these systems in cerebellar granule cells (GrCs), the smallest cells in the brain that are subjected to large Ca^{2+} influx. We successively excluded all those hypotheses that did not allow us to reconstruct accurately the spatiotemporal dynamics of experimental fluorescence responses. Results of our simulations support the hypothesis of a highly uneven distribution of immobile endogenous buffer(s) and presumably Ca^{2+} channels in GrCs leading to predictions that can be tested experimentally. The experimental observations are well explained by a substantial concentration of immobile calcium binding sites (most of all membrane-associated calretinin) in the dendritic endings of GrC. 50 mM of this buffer in the cell is sufficient to reproduce experimental measurements in response to synaptic stimulation and give a satisfactory explanation to a large difference between the typically measured calcium currents in response to depolarization and calcium currents obtained in the model with uniformly distributed buffers. This estimate is consistent with our estimate of the mobile calretinin concentration (1.2-1.3 mM) based on the similarity of its effect on BK channels compared with 150 mM BAPTA and with the estimates of the fraction of calretinin in the cerebellum associated with the particulate fraction.
Keywords: Calcium dynamics, Mathematical model, Calretinin, Endogenous buffers

POSTERS

ABNORMAL SHORT-TERM SYNAPTIC PLASTICITY IN AUDITORY CORTEX IN PATIENTS WITH PARKINSON'S DISEASE

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One of forms of a short-term synaptic plasticity is the paired-pulse depression (PPD). A paired-pulse paradigm is usually used to study synaptic mechanisms in neurotransmitters release. The decrement of the second response upon paired stimulation is likely the result of an inhibition of calcium influx through presynaptic receptors which plays a causal role in the release of glutamate from synaptic vesicles upon afferent stimulation. Parkinson's disease (PD) is a neurodegenerative disorder related to the brain dopamine deficit. Calcium channels are potential targets of dopamine. There are few studies that provide evidence of decreasing PPD of the somatosensory evoked potentials in PD patients.

The aim of this study was to explore PPD of the N1-P2 complex of the auditory cortical evoked potentials upon double-click stimulation in patients with PD in comparison with healthy age-matched subjects and to determine the correlation of the N1-P2 paired-click amplitude ratios (A2/A1) with some indices of motor and cognitive functions determined by using Unified Parkinson's Disease Rating Scale (UPDRS) and Mini Mental State Examination (MMSE). The central (Cz) auditory evoked potentials were recorded in 52 PD patients and 21 age-matched healthy subjects. The pattern for stimulation consisted of paired auditory clicks with 500, 700, 800, 900, 1100 and 2000 ms interstimulus intervals (ISIs). At each intervals 20 responses were averaged. Amplitudes of N1-P2 complex in the first (A1) and the second (A2) responses were measured. The ratio A2/A1 was calculated for each ISI. The nonparametric Spearman test was used to evaluate possible correlation between the ratio of N1-P2 complexes evoked by paired auditory stimuli and indices of motor and cognitive functions.

The main result of this study shows that in PD patients the A2/A1 ratios were significantly increased to 0.65 ± 0.04 ($p<0.01$), 0.74 ± 0.03 ($p<0.01$), 0.84 ± 0.02 ($p<0.001$) for ISIs 500, 700 and 900 ms respectively as compared to these values (0.46 ± 0.04; 0.56 ± 0.03 and 0.62 ± 0.03) in age-matched controls. The averaged value of A2/A1 ratios for different ISIs correlated negatively with the summary MMSE score ($rS = - 0.64$, $p<0.001$) and with the score of attention plus memory ($rS = - 0.55$, $p<0.01$). Only a weak positive association ($rS = 0.30$, $p<0.05$) of the body bradykinesia score (point 31 of UPDRS) with the averaged A2/A1 ratio was observed. The motor scores of the other UPDRS sub-items had no significant correlation with the A2/A1 ratios.

Results testify to significantly reduced PPD of the cortical evoked potentials at paired-click auditory stimulation in PD patients compared to the healthy age-matched subjects. Altered short-term synaptic plasticity in auditory system in PD patients is likely to be the consequence of central dopamine deficiency and presynaptic changes in transmitter releasing caused by dysregulation of calcium channels and augmentation of cytozolic calcium observed in PD. The results show that reduced cortical PPD contributes to cognitive disturbance in PD patients.

Keywords: Paired pulse depression, Parkinson's disease, Auditory evoked potentials, Cognitive indices.

ANTI-OVARIAN ANTIBODIES, CALCIUM UNIPORT AND MALATE-ASPARTATE MITOCHONDRIAL CARRIERS INHIBITORS INFLUENCE ON RESUMPTION OF OOCYTE MEIOTIC MATURATION AT MICE

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The role of serum anti-ovarian antibodies in development of reproductive disorders remains disputable. The research directed at the establishment of ovarian pathology mechanisms on the basis of experimental models using animal and specific heterogeneous antibodies remains topical. Recent discovery of calcium-dependent mitochondrial carriers allows suggest, that such protein transporters take part in the calcium regulated transport and in the signal system of oocytes. The aim of our researches is the study of the influence of antiovarian antibodies (AOAB), calcium uniport inhibitor (Ru) and malate-aspartate NADH-dependent mitochondrial carriers inhibitors (PPT) on oocyte meiotic resumption on mice. Research was carried out on CBA mice oocytes (8 weeks, weight 18-20 g). It has been established, that under the

action of PPT (0,3 мM) a full supression of oocyte meiotic resumption has been observed ($p < 0.01$), while at 0,003 мM - the amount of oocytes renewing meiosis decreases from 24,70±2,65 % in the control to 12,52±3,78 % ($p < 0.01$); the co-action of octanoate (0,5 мM) and PPT (0,3 мM) have not caused any difference from control volumes. It has not been established any difference in average quantity of group data in the AOAB environment - 15,12±1,83 % or Ru - 17,70±2,34 %, and also AOAB and Ru - 12,13±4,27 %. Under the action of AOAB, PPT and Ru on isolated oocytes there takes place a suppression of oocyte meiotic resumption to 9,34±1,42 % ($p < 0.01$) in comparison to 22,70±2,61 % in the control, whereas PPT together with Ru results in reduction to 12,79±3,63 % that is comparable to action of PPT (12,52±3,78 %) itself. The fact, that mitochondrial NADH/NAD ratio and ATP level can be activated in reply to a calcium signal which does not demand a calcium entrance in mitochondria coordinates the data received by us with the data of the literature [Satrъstegui J, Pardo B, Del Arco A. 2007]. It is necessary to underline, that in experimental conditions of an ischemia, malate-asparate-NADH-dependent carriers in cardiomyocytes do not function exactly because of metabolite redistribution, belonging to mitochondria carriers but not in connection with the carriers damage [Lu M, Zhou L, Stanley WC, et al. 2008]. We have received for the first time the data (with the use of pharmacological preparations of calcium uniport and malate-aspartate mitochondrial carriers inhibitors) that in conditions of antiovarian antibodies action on isolated mice oocytes Ca is captured by oocyte mitochondrials and damaged mitochondrial carriers.

Key words: anti-ovarian antibodies, mitochondrial carriers, oocyte meiotic maturation.

MECHAMISMS OF $[Ca^{2+}]_i$ MOBILISATION FOLLOWING P_2X RECEPTOR STIMULATION IN THE GUINEA-PIG SMALL MESENTERIC ARTERY MYOCYTES

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Rise of intracellular concentration of ionised calcium ($[Ca^{2+}]_i$) is a key event triggering contraction of arterial smooth muscle. There are two major pathways leading to an abrupt increase of $[Ca^{2+}]_i$: (1) Ca^{2+} influx into the cell via plasmalemmal Ca^{2+}-permeable channels and (2) Ca^{2+} release from intracellular calcium stores. We tested an involvement of voltage-gated Ca^{2+} channels (VGCCs), ryanodine receptors (RyRs) and inositol 1,4,5 trisphosphate receptors (IP_3Rs) in $[Ca^{2+}]_i$ mobilisation induced by stimulation of P_2X purinoceptors (P_2XRs) in myocytes freshly isolated from the guinea-pig small mesenteric arteries. The myocytes were loaded with the high affinity Ca^{2+} indicator fluo-3 and stimulated with 10 μM б,в-methyleneadenosine 5-triphosphate (б,в-meATP). Fast x-y confocal imaging revealed that activation of P_2XRs evoked an initial abrupt sub-plasmalemmal (sub-PM) $[Ca^{2+}]_i$ upstroke (SPCU), which was followed by propagating Ca^{2+} wave. The peak amplitude of б,в-meATP-induced SPCU was reduced: (1) by 37% (n=6) after inhibition of IP_3Rs (30 μM 2APB); (2) by 45% (n=6) after inhibition of RyRs (100 μM tetracaine), (3) by 87% (n=5) after depletion of intracellular Ca^{2+} stores (with 10 μM cyclopiazonic acid); (4) by 68% (n=6) following block of VGCCs (5 μM nicardipine) and (5) by 97% (n=4) following block of VGCCs in the myocytes with depleted calcium stores. Immunodetection of RyRs and type 1 IP_3Rs in the myocytes with identified sarcoplasmic reticulum (SR) (with Brefeldin A BODIPY) and nucleus (with DAPI) revealed that sub-PM SR elements are enriched with IP_3Rs while RyRs are predominantly located in the deeper and perinuclear SR elements. These results strongly suggest that in myocytes from the guinea-pig small mesenteric arteries: (1) P_2XRs are Ca^{2+} permeable, however the main physiological role of inward cationic current mediated by P_2XRs is to depolarize the cell membrane and trigger Ca^{2+} entry via VGCCs; (2) this Ca^{2+} entry induces an initial Ca^{2+} release from sub-PM intracellular stores which augments further intracellular $[Ca^{2+}]_i$ mobilisation. Predominant expression of IP_3Rs in sub-PM SR elements and sensitivity of SPCU to 2-APB suggests that IP_3R-mediated Ca^{2+} release is not only involved in $[Ca^{2+}]_i$ mobilisation induced by P_2XR stimulation but also plays a key role thus linking Ca^{2+} entry to RyR-mediated Ca^{2+} release. Since activation of P_2XRs does not engage G-protein related signalling pathway our results also provide an evidence for activation of IP_3Rs in arterial myocytes via Ca^{2+}-induced Ca^{2+} release mechanisms. This novel mechanism involved in excitation-contraction coupling could be common for various smooth muscle types and requires further investigation.

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Keywords: Confocal microscopy, Vascular myocytes, Purinergic receptors, Receptors of intracellular Ca^{2+} stores, Voltage-gated Ca^{2+} channels.

MECHANISMS OF PURINERGIC [Ca^{2+}]i MOBILIZATION IN THE GUINEA-PIG VAS DEFERENS MYOCYTES: PHARMACOLOGICAL STUDY.

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ATP is one of the major neurotransmitters mediating the signals from sympathetic nerves to effector organs and acting upon ionotropic purinoceptors (P2XRs), the signal transduction mechanism of which is confined to fast electrical events on the cell membrane and does not involve G-protein related metabotropic signalling cascades. In this study we evaluated relative contribution of the plasmalemmal and intracellular events to an abrupt increase of cytosolic Ca^{2+} concentration ([Ca^{2+}]i) induced by a brief local application of 10 μM б,в-methyleneadenosine 5-triphosphate (б,в-meATP), an agonist of P2XRs, on smooth muscle cells (SMCs) freshly isolated from the guinea-pig vas deferens and preloaded with the high affinity Ca^{2+} indicator fluo-3. We studied the effect of pharmacological inhibitors of voltage-gated Ca^{2+} channels (VGCCs), ryanodine receptors (RyRs), inositol 1,4,5 trisphosphate receptors (IP3Rs) and sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) on amplitude of the б,в-meATP – induced [Ca^{2+}]i transients visualised using fast x-y confocal imaging. Immunostaining of type 1 IP3Rs and RyRs in SMCs with identified sarcoplasmic reticulum (SR) (with Brefeldin A BODIPY) and nucleus (with DAPI) revealed that sub-plasmalemmal (sub-PM) SR elements express both type 1 IP3Rs and RyRs, while deeper and perinuclear SR elements are enriched with RyRs. The б,в-meATP – induced [Ca^{2+}]i transients were initiated at multiple sub-PM regions (sub-PM [Ca^{2+}]i upstroke, SPCU) and then propagated towards the cell centre. Spatial non-uniformities in SPCU suggest that Ca2+ release from sub-PM sarcoplasmic reticulum (SR) elements contributes to б,в-meATP – induced [Ca^{2+}]i mobilization at early stages. Depletion of intracellular calcium stores (10-min incubation with 50 μM cyclopiasonic acid, a SERCA inhibitor) reduced SPCU by 55±7% (n=8), further supporting the contribution of the SR Ca^{2+} release to б,в-meATP – induced [Ca2+]i mobilization. On the other hand, block of VGCCs (with 5 μM nicardipine) suppressed the SPCU by 71±5% (n=9), thus not only suggesting that P2XR – mediated depolarization activates VGCCs, but also indicating that Ca^{2+} entry via VGCCs induces the SR Ca^{2+} release. Block of VGCCs in the SMCs with depleted SR decreased SPCU by 63±4% (n=8). The remaining [Ca^{2+}]i transient resulted from a direct entry of Ca^{2+} via P2XRs. Block of RyRs with 100 μM tetracaine or IP3Rs with 30 μM 2-APB reduced the SPCU by 38±3% (n=8) and 48±4% (n=10), respectively, suggesting the contribution of the both types of the SR Ca2+ -release channels to genesis of б,в-meATP – induced [Ca^{2+}]i transients. Cumulative inhibition of VGCCs, IP3Rs, and RyRs reduced SPCU by 79±4% (n=8). This inhibition was slightly stronger than that of nicardipine in the cells with depleted calcium stores and suggested certain non-specific component in the inhibitory action of 2-APB and/or tetracaine thus requiring further investigation. Inhibition of phospholipase C-beta (PLC-beta) with 5 μM of U-73122 suppressed the SPCU by 30±4% (n=5), while cumulative inhibition of PLC- beta and IP3Rs reduced the SPCU by 41±5% (n=5). Since activation of P2XRs does not engage G-protein - mediated stimulation of PLC-beta and is confined to the membrane depolarization and voltage-gated Ca^{2+} entry, these results suggest that sensitivity of IP3Rs to Ca^{2+} depends on [IP3]i, which in our case is determined by spontaneous constitutive activity of PLC- beta in SMCs. This study further confirm our previous hypothesis that in SMCs IP3Rs can be activated by voltage-gated Ca^{2+} entry and represent an important mechanism involved in excitation-contraction coupling.

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Keywords: P2X receptors; IP3 receptors; Ryanodine receptors; voltage-gated Ca2+ channels, confocal microscopy

MMUNOCYTOCHEMICAL STUDY OF THE DISTRIBUTION OF N- AND P/Q-TYPES CALCIUM CHANNELS AT FROG MOTOR NERVE TERMINALS

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It is well-known that one of morphological properties of amphibian neuromuscular junction is its comparatively big length. There are many data showing the presence of proximo-distal gradient in the wide spectrum of evoked mediator secretion parameters. In particular, it was shown that in proximal

part of terminal the quantal content, the minimal synaptic delay and the asynchrony of quantal mediator release are higher in comparison with distal part. In this connection the hypothesis that these differences can be due to the distribution and/or ratio of various types of potential-sensitive Ca^{2+}-channels participating in neuromediator secretion has been put forward. To check this hypothesis was the aim of the present research. Work has been carried out on m. cutaneus pectoris preparation of the frogs Rana ridibunda with the use of fluorescent immunocytochemistry and laser confocal microscopy methods. It has been shown that fluorescence intensity of terminals, labeled with antibodies to alpha-1B subunit of N-type Ca^{2+}-channels (the main Ca^{2+}-channels type in the nerve terminals of amphibian responsible for evoked quantal release), varies along whole terminal. However, reliable differences in distribution of this type Ca-channels in proximal and distal parts of terminal have not been revealed. Labeling of other type potential-sensitive Ca^{2+}-channels, namely P/Q-type, with the use of antibodies to alpha-1A subunit, also has not revealed the presence of proximo-distal gradient in distribution of this type of channels on the membrane of nerve terminal. Thus, differences in a number parameters of neuromedaitor secretion of the motor nerve terminal of the amphibian having proximo-distal gradient, cannot be explained by distribution pattern or a ratio between N - and P/Q-types Ca-channels which are distributed along motor nerve terminal rather uniformly.

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Keywords: amphibian neuromuscular junction, N- and P/Q calcium channels.

ALTERATIONS IN VOLTAGE-GATED CALCIUM CHANNELS IN AMYLOID-β_{1-42}-TREATED HIPPOCAMPAL CELL CULTURE

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β-Amyloid peptide of 42 amino acids ($A\beta_{1-42}$) is constituent of senile plaques in Alzheimer's disease (AD). It produces memory deficits characteristic in AD. Dysfunction in calcium homeostasis is one of the events in pathogenesis of AD. The aim of the study was to discover changes in properties of voltage-gated calcium channels (VGCC) in hippocampal cell culture with experimental induced AD.
Voltage-dependent characteristics of calcium channels were studied on rat hippocampal cell culture in control condition and after 24-hour incubation of the cell culture with $A\beta_{1-42}$. Current-voltage relationship (CVR), steady-state activation and steady-state inactivation curves of VGCC were obtained using whole-cell modification of patch clamp technique. Current density in Aβ-treated cells was 70 % higher ($P<0.05$, n=12) than in control ones. CVR of VGCC after incubation of hippocampal cell culture with $A\beta_{1-42}$ had 10-mV shift to the hyperpolarization direction in comparison with control neurons. Steady-state activation curve of VGCC in hippocampal cells modified by $A\beta_{1-42}$ had 12-mV shift ($P<0.001$, n=10) to more negative values of membrane potential as compared to control curve. In contrast with first two voltage dependencies steady-state inactivation curve of VGCC was not changed after action of $A\beta_{1-42}$.
It is concluded that $A\beta_{1-42}$ affects VGCC of hippocampal cell culture by means of some intermediate mechanisms or directly that leads to disturbance in cell calcium homeostasis.

Keywords: β-amyloid peptide, Alzheimer's disease, hippocampal cell culture, voltage-gated calcium channels, voltage-dependent characteristics

CALCIUM TRANSIENT IN DIFFERENT PARTS OF THE FROG NEUROMUSCULAR JUNCTION IN THE RESPONSE TO NERVE PULSE

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Calcium ions play the primary role in promoting the stimulus-induced secretion of acetylcholine from nerve terminals. It was established that an increase in concentration of calcium concentration leads to rise of quanta mediator secretion. In previous research it was established that an increase of calcium concentration leads to synchronization of mediator secretion. Thus, change of concentration of calcium in the field of an active zone of secretion defines not only quantity, but also the time parameters of

release of quanta mediator after arrival of action potential. But these data was received by electrophysiological methods which do not allow estimating the change in concentration of calcium ions in the active zone. At the same time for large synapse of central nervous system and lobster neuromuscular junction the way of the estimation of fast calcium transient with the fluorescent dyes is known. In this connection there was a question about the possibility of realization of this technique and its application on small neuromuscular junction for an estimation of change of the intracellular calcium concentration changes and comparison of these changes with the data received under simultaneous electrophysiological registration of synapse activity. The method of registration of the fast changes of calcium ions was based on application of a fluorescent marker with high affinity to calcium – Oregon Green BAPTA 1. Marker was loaded into the terminal of the cutaneous pectoris preparation of a frog from the cut end of the nerve bundle. Registration of the fast changes of calcium concentration was made by high sensitive photodiode. Nerve action potentials and extracellular endplate currents were recorded by using standard electrophysiological techniques. In our experiments we registered fast calcium transient as from the long nerve terminal, and separately from proximal and distal parts in reply to single stimulus and at frequency stimulation of a nerve. Thus, the realized technique allowed us to estimate fast changes of intracellular calcium concentration and simultaneously extracellular currents from the same nerve terminal in the replay both on individual, and on frequency stimulation of the motor nerve. In this experiments we show that in proximal part calcium transient was bigger than in distal one. This difference in calcium transient along presynaptic nerve ending can explain obtained early gradient in quantum secretion of mediator in different part of long frog terminal.
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Keywords: Calcium, Neuromuscular junction, Calcium transient

EFFECT OF NEURAMINIDASE BLOCKER ON NEURONAL ACTIVITY AND FORMATION OF HIPPOCAMPAL SYNAPSES

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Neuraminidase (NEU) effects on neuronal and network activity by controlling of sialilation of the extracellular membrane surface. Treatment with NEU reduces sensitivity of sodium channel gating and action potential threshold to extracellular calcium by desialilation. At the network level exogenous NEU exerts powerful anticonvulsive action in vitro and in vivo. NEU blocker N-acetyl-2,3-dehydro-2-deoxyneuraminic acid (NADNA) significantly reduces seizure threshold and aggravates hippocampal seizures in vivo. In present work we show that endogenous NEU limits a synaptogenic process and alter seizure like activity. Extracellular field potential recordings were made from pyramidal CA1 layer from cultured hippocampal slices. The level of sialylation and morphological characteristics of synapses were estimated using electron microscopy. We examined the effect of bath application of NADNA on field potentials recorded from the hippocampal CA1 pyramidal layer. In extracellular recordings from CA1 pyramidal layer spontaneous recurrent network activity were observed in 5 from 15 cultured slices at DIV12-16. About 3-5 min after application of NADNA (2 mM), we observed an increase in the firing frequency from 0.35± 0.01 Hz to 0.68 ± 0.08 Hz (n=5, $p<0.05$). The amplitude of synchronous oscillations also significantly increased from 0.46 ± 0.01 pA (pooled from 234 events out of 5 recordings) to 0.56 ± 0.01 (pooled from 320 events out of 5 recordings). In the next set of experiments we examine the effect of blockade of endogenous NEU on seizure like activity in low-magnesium model of epilepsy. Cultured hippocampal slices were incubated with NADNA (2mM) during 2 hours. The effect of low magnesium on extracellular neuronal activity was examine and compared on NADNA treated and untreated slices. Using low magnesium model of epilepsy we show that seizures recorded from hippocampal slices pretreated with NADNA have recurrent cycles of activity which contained in sequence: initial burst, tonic activity and clonic activity, with tonic/clonic ratio about 30%. In the untreated slices we did not find any regular sequences in seizure manifestation and tonic/clonic ratio was about 10%. Electron microscopy shows significant increase of perforated synapses which associated with NMDA receptor-dependent LTP after NADNA treatment. However when concentration of calcium in incubation ACSF was increased to 5mM we did not find any effect of NADNA on amount of perforated synapses. Based on our data we suggest that endogenous NEU which control level of surface near the voltage gated sodium channels influence on neuronal net activity and through that at least partially control synapses formation.
Keywords: neuraminidase; sialic acid; seizure; hippocampus

OPIOIDS FACILITATE P-TYPE CALCIUM CURRENT IN PURKINJE NEURONS OF RAT IN G-PROTEIN-INDEPENDENT MANNER

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P-type calcium channels play a key role in the synaptic transmission between mammalian central neurons: major part of calcium entering pre-synaptic terminals is delivered via these channels. Using conventional whole-cell patch clamp techniques we have studied the effect of mu-opioids on P-type calcium channels in acutely isolated Purkinje neurons from rat cerebellum. In 75% of tested neurons selective mu-opioid agonist DAMGO (10 nM) produced a small, but consistent facilitation of current through P-type calcium channels (11±1%, n=24, p<0.01). Endomorphin-1 demonstrated similar action. This effect of mu-opioids was rapid and fully reversible. In the rest of tested neurons application of DAMGO was of no effect. In the sensitive neurons the effect of this drug was already well noticeable in concentration of 1 nM and fully saturated at 100 nM. Facilitatory action of mu-opioids was totally preserved in the presence of 0.5 mM GDP inside the cell indicating that the interaction of drugs with the channels is independent on the activity of G-proteins. This is in contrast to the properties and behavior of "classical" opioid receptors. Furthermore, mu-opioid antagonist naloxone (100 nM) mimicked facilitatory effect of mu-opioids abolishing further facilitation of the current when DAMGO was added on top of naloxone. In conclusion, mu-opioids modulate P-type calcium channels via "non-classical" site which demonstrates high affinity to mu-opioids.
Keywords: Calcium, opioids, P-type calcium channels

NONSPECIFIC INWARD POTASSIUM CURRENT IN RAT DRG NEURONS

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Voltage-gated calcium channels (VGCC) represent the main calcium entry pathways in many cell types and regulate a number of intracellular processes. It is known that VGCC are highly selective structures for Ca^{2+} ions at physiological conditions. However, at low concentrations of divalent cations VGCC lose their selectivity. In this aspect K^+ permeability of neuronal VGCC was not enough elucidated. The aim of investigation was to discover nonspecific K^+ current through VGCC of rat dorsal root ganglion (DRG) neurons in Ca^{2+}-free solution (Ca0). Currents through VGCC have been registered in control solution and in Ca0 contained K^+ ions using whole cell configuration of patch clamp technique. Voltage-dependent relationships of VGCC have been studied applying for two mentioned solutions. Current-voltage relation (CVR) of VGCC in Ca0 had 10-mV shift ($p < 0.05$, $n = 9$) to more negative membrane potentials in comparison with CVR in control solution. Steady-state activation characteristic of VGCC in Ca0 was shifted for 10 mV ($p < 0.02$, $n = 15$) to hyperpolarization direction rela-tively to steady-state activation curve obtained in control solution. Unlike previous two voltage-dependent relations steady-state inactivation curve of VGCC has not been changed after applying Ca0. Nickel in 300-mkM concentration inhibited K^+ current predominantly through T-type VGCC for 69 ± 4 % ($p < 0.001$, $n = 5$) in Ca0. Additionally, nifedipine (50 mkM) reduced K^+ current mainly via L-type Ca^{2+} channels to 6 ± 2 % ($p < 0.001$, $n = 6$) in Ca0. Cobalt in concentration 4 mM depressed K^+ current almost completely over all voltage range of activation VGCC in Ca0. It was concluded that low- as well as high-threshold VGCC of rat DRG neurons are permeable for K^+ ions at the absence of Ca^{2+} in the external solution.
Keywords: voltage-gated calcium channels, DRG neuron, voltage-dependent relations, calcium-free solution

EFFECT OF OMEGA – CONOTOXIN – GVIA ON CALCIUM – DEPENDENT PAIRED PULSE DEPRESSION IN CULTURED HIPPOCAMPAL NEURONS

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Multiple types of high-voltage-activated Ca^{2+} channels trigger neurotransmitter release at the mammalian central synapse. Now it is clear that N-type Ca^{2+} channels contribute to synaptic transmission at many

of CNS synapses. However, it is not known whether presynaptic N-type Ca^{2+} channels contribute to calcium-dependent paired pulse depression (PPD) mediated by GABA release in inhibitory synapses of cultured hippocampal neurons. We studied the sensitivity of GABAergic PPD to selective N-type high-voltage-activated Ca^{2+} channels blocker omega-conotoxin GVIA (ω-CgTx). Patch-clamp technique in whole-cell configuration was used in postsynaptic neuron and local exteracellular paired - pulse stimulation of single presynaptic axon by rectangular pulse with 0.4 ms duration, the interpulse interval in pair was 150 ms. ω-CgTx (200 nM; 1 μM) in a dose-dependent manner irreversibly reduced the amplitude of paired eIPSCs by 25 - 49% and decreased PPD by 11 – 22 % compared with control.
The fraction of N-type Ca^{2+} channel in calcium-dependent PPD was estimated in presence of ω-CgTx (200 nM) under different calcium concentrations. The present data show that the relationship between eIPSC and extracellular Ca^{2+} concentration are highly nonlinear. The application of ω-CgTx did not change nonlinear relationship. It was shown that PPD is qualitatively affected by the extracellular Ca^{2+} concentration. Lowering external Ca^{2+} concentration reduced the depression powerfully in presence of ω-CgTx than in absence. In addition we found difference between the degree of cooperativity for transmitter release in the presence and absence of the N-type Ca^{2+} channel blocker. So, at inhibitory synapses in the hippocampus more than one Ca^{2+} ions act in a cooperative manner to cause release of GABA. After ω-CgTx application the degree of cooperativity was increased.
These results confirm that N-type Ca^{2+} channels are highly involved in calcium-dependent paired pulse depression at inhibitory synapse in cultured hippocampal neurons.
Keywords: N-type calcium channels, omega-conotoxin GVIA, GABAergic synaptic transmission, paired pulse depression

THE MITOCHONDRIAL PALMITATE/Ca^{2+}-INDUCED PORE: A ROLE IN APOPTOSIS AND ANIMAL'S ADAPTATION TO HYPOXIA

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Earlier we found that being added to rat liver mitochondria, Ca^{2+} plus palmitic acid (Pal) opened a cyclosporin A-insensitive pore, which remained open for a short time. Apparently, this pore is involved in the Pal-induced apoptosis and may also take part in the mitochondrial Ca^{2+} recycling as a Ca^{2+} efflux system (Belosludtsev et al. J Bioenerg Biomembr 38:113–120, 2006; Mironova et al. J. Bioenerg. Biomembr. 39:167–174, 2007). It was shown that the Pal/Ca^{2+}- induced pore (PalCaP) is different from the mitochondrial permeability transition pore (MPT pore), concerning both regulation and, apparently, the mechanism of formation. According to our data, formation of PalCaP results from complexation of Ca^{2+} with Pal anions on the matrix side of the inner mitochondrial membrane. In the present work, we continue studying physiological and regulatory aspects of the pore. The following observations have been made. (1) The opening of Pal/Ca^{2+}-induced pore is accompanied by the release of apoptosis-induced factor (AIF) from mitochondria. (2) Cardiolipin has been found to facilitate the Ca^{2+}-induced formation of pores in the Pal-containing liposomal membranes. (3) The rate of Pal/Ca^{2+}-induced swelling of rat liver mitochondria increases substantially with the age of animals. (4) The pore opening depends on the resistance of animals to hypoxia: in the highly resistant to hypoxia rats, the mitochondrial Pal/Ca^{2+}-induced pore opens easier than in the low resistant animals. At the same time, the classical, cyclosporin A-sensitive MPT pore opens easier in the low resistant to hypoxia rats. The adaptation of the low resistant rats to oxygen deficiency increases the sensitivity of their mitochondria to PalCaP inductors. It seems that the physiological activation of PalCaP in mitochondria of the highly resistant and hypoxia-adapted rats is a compensatory mechanism, which protects tissues against ischemic injury. We suppose that a possible mechanism of animal's adaptation to hypoxia is the activation of a Ca^{2+} cycle, which is mediated by Ca^{2+} uniporter and PalCaP and leads to the mild uncoupling and decreased ROS production in mitochondria.
The work was supported by grants from ISTC (3301) and RFBR (09-01-01024-a).
Keywords: Palmitic acid, Calcium, Mitochondrial Ca^{2+}-dependent pore

TIME COURSE OF THE EVOKED QUANTAL SECRETION UNDER CHANGE OF THE INTRACELLULAR CALCIUM AT THE MOTOR NERVE TERMINAL

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Calcium ions initiate neurotransmitter release and thereby play an important role in the transmission of excitation. In addition to the amount of released neurotransmitter quanta (the quantal content of the endplate potential), another important characteristic of exocytosis is the kinetics of release of individual quanta that form total multiquantal response. Previously, we showed the dependence of the temporal parameters of neurotransmitter quantum release on the extracellular calcium ion concentration. To reveal the mechanisms of calcium regulation of the kinetic of evoked quantum neurotransmitter release we have investigated the temporal parameters of acetylcholine secretion in the experiments made on neuromuscular junction of mouse by frequency stimulation of motor nerve, which did produce the release facilitation, under various extracellular calcium concentration or in the presence of calcium channels blockers or with intracellular calcium buffers. Analysis of histograms of synaptic delays of uniquantal endplate currents recorded during 50 ms interval after presynaptic action potential arrival revealed three components of secretion process: early and later period of synchronous release and delayed asynchronous release. At reduced level of extracellular calcium the relative number of quanta released during the asynchronous phase of secretion was enhanced, with a concomitant decrease of quantum release rate during the early synchronous period of secretion. Blockade of calcium channels by cadmium ions, non-specific calcium channel blocker, led to decrease in quantum content and increase in number of signals in the delayed asynchronous phase of release. Since on motor nerve ending was shown presence of different types of calcium channels we used specific blockers of L-type calcium channels (nitrendipine) and P/Q-type calcium channels (w-Agatoxin IVA) and non-specific blocker of potassium channels aminopyridine. Specific blocker of P/Q-type calcium channels abolished the facilitation of synchronous component while L-type blocker decreased the facilitation of asynchronous component of secretion. Data obtained support the hypothesis that both low- and high-affinity calcium sensors with different calcium binding kinetics participate in the regulation of respective synchronous and asynchronous release of mediator quanta.

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Key words: uniquantal release, time course of secretion, neuromuscular junction, calcium channels.

INCREASED SENSITIVITY OF THE MITOCHONDRIAL PERMEABILITY TRANSITION PORE TO CALCIUM IN RAT HEART WITH CHRONIC DEFICIENCY OF NIGROSTRIATAL DOPAMINE

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Parkinson's disease is caused by the mass death of dopaminergic neurons of the substantia nigra and as a result by the deficiency of nigrostriatal dopamine (NSDA). Previously we have demonstrated the functional deterioration of the myocardium of rats with the chronic deficiency of NSDA, which reflected the dysregulation in mitochondria of the cardiac myocytes. We studied the sensitivity of mitochondrial permeability transition pore (MPTP) opening to natural inductors – Ca^{2+} ions in the heart mitochondria of the rat with chronic deficiency of nigrostriatal dopamine caused by an injection of selective neurotoxin 6-hydroxidopamine in an ascending lateral bundle of the forebrain. MPTP-opening was determined specrophotometrically (λ=520 nm) by a decrease in an optical density resulting from mitochondrial swelling. It has been shown that the rat heart mitochondria with chronic deficiency of nigrostriatal dopamine are more sensitive to Ca^{2+} in its physiological concentration (10^{-7} mol/l) and overload (10^{-6} - 10^{-4} mol / l) in comparison to control animals. Besides, the melatonin administration one hour before the decapitation significantly reduced the amplitude and the speed of swelling of the heart mitochondria. Obtained results lead to a conclusion that an increased sensitivity of the MPTP to calcium and mitochondrial membrane permeability may be one of the causes previously reported of disturbance in contractile function of the rat heart with chronic deficiency of nigrostriatal dopamine. Melatonin due to its antioxidant properties can reduce the sensitivity of MPTP and thus make the protective effect on the heart.

Keywords: dopamine deficiency, myocardium, mitochondrial permeability transition pore, melatonin

REACTIVE OXYGEN SPECIES MODULATE NEURONAL NETWORKS PROPERTIES WITHIN CNS IN INVERTEBRATES

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"Reactive oxygen species" (ROS) refers to a group of oxygen containing compounds with the ability to react with reducible compounds: superoxide, hydrogen peroxide and hydroxyl radical. Growing evidence suggests that ROS, initially assumed to be harmful compounds, could serve as important signaling molecules. Here we used the combination of electrophysiological (microelectrode technique) and biochemical methods to analyzed the action of ROS within central nervous system of fresh-water pond snail *Lymnaea stagnalis*. Observation of antioxidant defence in *Lymnaea*'s CNS at different functional states established that food consumption associated with free radical production in the nervous system of mollusks: increase of thiobarbituric acid reactive substances amount, reduce of glutathione level and Se-glutathione peroxidase activity. It was determined that changes in environmental temperatures can also modulate ROS level in the nervous tissue of molluscs. Spontaneous electrical activity of identified neurons within respiratory (RPeD1, VD4, VD1/RPaD2, Vi-cells), feeding (L/R CGC, L/R B1–4) networks, cells involved in locomotion (LPeD1, PeA-cluster) and defense reactions (RPaD1, LPaV1, VD2/3) was partially modified by hydrogen peroxide (10^{-4} M bath application, final). Hydrogen peroxide in low concentrations, i.e. 10^{-5} M or less was ineffective. Respiratory central pattern generator (CPG) activity was more sensitive to H_2O_2 impact. On the other hand, we were unable to determine significant differences in feeding CPG operation as well as in electrical properties of "defense" neurons and cells involved in locomotion of mollusks. We also describe the responses of identified synapses, both electrical and chemical, on ROS level growth. In these conditions electrotonical coupling between VD1/RPaD2 was reduced above twice. As for chemical synapses formed by dopamine-containing neuron RPeD1, bath application of H_2O_2 (10^{-4} M, final) was associated with the postsynaptic potentials amplitude decrease and synaptic delay raise. The conductance of peptidergic synapses formed by FMRF-containing cell VD4 and its followers didn't vary at the presence of hydrogen peroxide. Washing of preparations by fresh Ringer's solution partially restored the initial pattern of neuronal and synaptic activity. Thus, ROS can be critical for neuronal network operation within CNS of mollusks realizing their action on synaptic level.

Supported by BRFFI (grant B08R–075).

Keywords: free radicals, mollusc, synapse, neuron

COMPARATIVE ANALYSIS OF CALCIUM EXCHANGE BETWEEN CYTOSOLE AND ENDOPLASMIC RETICULUM OR MITOCHONDRIA AS THE STORES

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The dependence of intracellular Ca^{2+} dynamics on geometrical size relations between Ca^{2+}-exchanging parts of the intracellular space was studied in single-compartment mathematical models corresponding to a thin fragment (compartment) of Purkinje neuron spiny dendrite. The plasma membrane contained ion pumps and channels characteristic of this cell type, including those conducting excitatory synaptic current. The model equations took into account Ca^{2+} exchange between cytosole, extracellular medium, intracellular store (mitochondria, Mit, or endoplasmic reticulum, ER), endogenous Ca^{2+} buffers and a fluorescent dye and Ca^{2+} diffusion in the bulk of the cytosole. The ER membrane contained a Ca^{2+} pump and channels of Ca^{2+}-induced and inositiol-3-phosphate-induced Ca^{2+} release as well as leakage channels. Mit exchanged Ca^{2+} with the cytosole via uniporter and Na^{+}-Ca^{2+} exchanger. The mitochondrial metabolic reactions were also included. The stores occupied from 1 to 36 % of the total compartment volume. Identical synaptic excitation caused similar electrical reactions (calcium spikes) but different concentration responses. Equal increments of the space occupancy by the stores led to unequal increments of the peak cytosolic concentrations $[Ca^{2+}]_i$ and Ca^{2+}–fluorescent dye complex [CaD], as well as those of Ca^{2+} concentration in the store. The changes in $[Ca^{2+}]_i$ and [CaD] followed more accurately those in the volume of the organelle-free cytosole, whereas changes in the stored Ca^{2+} were more similar to those in the store membrane area. Ca^{2+} exchanges with the cytosole were compared for the ER and Mit responding to the same bell-shaped $[Ca^{2+}]_i$ pulses obtained when the diffusion form the operating pool to the bulk was set to prevail significantly over other fluxes. In this example, ER captured from the cytosole approximately four times greater amount of Ca^{2+} with two times greater rate than Mit did at the same space occupancy (36 %). Hence, in a dendritic compartment the relative occupancy of the

intracellular volume by organellar Ca^{2+} stores and the size of the latter are important structural factors essentially modulating calcium dynamics, and this structural dependence can be adequately reflected in experiments using Ca^{2+} probes.
Keywords: Spiny dendrite, Sub-cellular geometry, Endoplasmic reticulum, Mitochondria

THE REGULATION OF ATP-INDUCED POTENTIATING OF SYNAPTIC TRANSMISSION BY ENDOCANABINOID SYSTEM IN RAT HIPPOCAMPUS

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The signaling transmission in nervous system between numerous of neurons is described as specific connections named synapses. Various mechanisms are involved in a regulation and modulation of processes within excitatory synapses. One of them, synaptic plasticity, is the ability of neurons to modify the efficacy of synaptic transmission, that thought to provide the cellular basis for the profound influence of experience over information processing and storage in the brain. Long-term potentiation (LTP), a long-lasting increase in excitatory synaptic strength after heightened synaptic activity depends on the pattern of synaptic inputs impinging on the postsynaptic CA1 neurons shortly afterward, involves Ca^{2+} influx through NMDA receptors (NMDARs), leading to a high level of intracellular Ca^{2+} concentration, and activation of calcium/calmodulin- dependent kinase II (CaMKII). Exogenous application of ATP causes the potentiation of synaptic transmission. This effect, which was observed after drug removal, was described to persist in in vitro preparations for a relatively prolonged period. A major physiological role of the endocannabinoid system is a regulation of neurotransmitter release at various types of synapses throughout the brain. The production of endocannabinoids is evoked by specific physiological stimuli proposed to act presynaptically on cannabinoid receptors. This reverse mode of action makes them ideal candidates as retrograde signals in several paradigms of short- and long-term synaptic plasticity. The current study was devoted to understand how the endocannabinoid system contributes to the phenomenon of ATP-induced long-lasting synaptic plasticity in CA1 region of hippocampus, since cannabinoids CB1 receptors are widely expressed both presynaptically and postsynaptically in hippocampus. The long-lasting potentiation of population spike evoked by electrical stimulation of the Schaffer collaterals in rat hippocampal slices (125 %) was completely eliminated by exogenously applied CB1 receptor agonist WIN-2 (2 μM) (PS/PS0 = 90 %) and turned to depression of PS when WIN-2 was used in higher concentration (4 μM). However, the selective CB1 antagonist AM251 (500 nM) was found to prevent the inhibitory effect of WIN-2 on PS with the LTP generation (110 %) when WIN-induced LTD was averted to the control level. These data suggests that the blockade of LTP by cannabinoid agonists is resulted in decrease in the probability of glutamate release via presynaptic receptors and thus draws up the complementary role of CB1 receptors into metaplasticity process in hippocampus
Keywords: Long-term potentiation, cannabinoid receptors, EPSP, hippocampus

NON-QUANTAL ACETYLCHOLINE RELEASE: CALCIUM INDEPENDENT MECHANISM BUT CALCIUM DEPENDENT MODULATION

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At present time the fact of existence of another mode of neurotransmitter release (non-quantal secretion) besides well known quantal release is recognized. It is supposed that there are several molecular mechanisms of non-quantal neurosecretion and to this time the process of non-vesicular release in the mammalian neuromuscular junction is the most extensively studied. However, many features of this type of secretion remain unclear. In particular, there was open question concerning the influence/participation of extracellular calcium on/in the mechanisms of non-quantal acetylcholine release and its regulation. In the experiments carried out on neuromuscular preparations of rat and mouse diaphragms, using electrophysiological methods we found that the dependence of the intensity of non-quantal release on calcium concentration in the medium is bell-like with the mode at the 2 mM level (normal physiological concentration). Further, when Ca^{2+} was removed from extracellular solution a delayed slow decrease of intensity was observed. On the contrary, the quantal release responded to the removal of Ca^{2+} much more rapidly. Intriguing results were obtained when the blockers of calcium channels were used. 4 mM

Mg^{2+} completely and rapidly eliminated the non-quantal acetylcholine release. However Cd^{2+} being more effective Ca^{2+} channel blocker did not affect this type of secretion as well as L-type calcium channel blocker nitrendepine. When we studied the mechanisms of regulation of non-quantal release intensity we found that the activation of glutamate NMDA receptors and muscarinic M_1 receptors led to the decrease of intensity of this type of mediator release. It was shown that activation of both types of receptors was accompanied by increase of calcium-dependent nitric oxide production and none of agonists of these receptors did not affect the non-quantal release when Ca^{2+} has been removed from extracellular medium. These data suggest that the molecular mechanism of non-quantal release of acetylcholine from motor nerve ending is Mg^{2+}-sensitive and Ca^{2+}-independent and but some pathways of intensity regulation are Ca^{2+}-mediated.
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Keywords: non-quantal release, calcium, neuromuscular junction.

P-AND R-TYPE Ca CHANNELS IN GLYCINERGIC NERVE TERMINALS OF RAT SPINAL NEURON

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The functional contribution of high- and low–voltage activated (HVA and LVA) Ca^{2+} channel subtypes on glycinergic nerve endings (boutons) projecting to the rat spinal sacral commissural nucleus (SDCN) neurons were investigated by measuring the inhibitory postsynaptic currents (eIPSCs) evoked from individual boutons by focal electrical stimulation. Glycine release from most boutons required activation of both P–type (antagonized by 2 nM щ–Aga IVA) and R–type (antagonized by SNX–482, Ni^{2+} and Cd^{2+} at low concentration) as indicated by both amplitude and success rate (Rsuc) of glycinergic eIPSCs. Low (P–type selective) and high (Q–type selective) concentrations of щ–Aga IVA almost equally suppressed eIPSCs indicating a little and/or no contribution of Q–type channels on eIPSCs. N–type antagonist (щ–con GVIA) and L–type antagonist (nifedipine) reduced the eIPSCs in about half of boutons tested, although the inhibition was much less suggesting a smaller contribution than P– and R–type channels. A T–type antagonist, R(-) efonidipine, had little effect on eIPSCs. These results indicate that HVA Ca^{2+} channels nonuniformly regulate glycine release from single boutons and might have different spatial distributions. The P– and R–type Ca^{2+} channels almost ubiquitously populate glycine release sites while L– and N–type channels are localized either close to or far from the release site. Neither Q– nor T–type channels appear to importantly influence glycine release in these nerve endings.
Keywords: Calcium, N–type calcium channels, P-type calcium channels, R-type calcium channels, spinal neurons

CALCIUM SIGNALLING DURING HYPOXIA IN FISH *Carasius gibelio*

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It is well known that mammalian neurons are exclusively sensitive to oxygen deprivation, undergoing irreversible damage after short periods of anoxia. In contrast, some vertebral animals are unusually tolerant of anoxia, surviving several hours of anoxia. Fish *Carasius gibelio* is one of such hypoxia-tolerant animals. It was established that hypoxic conditions induce membrane depolarization of neurons which increases intracellular $[Ca^{2+}]_i$ via the voltage-dependent activation of several types of voltage-gated Ca^{2+} channels. Besides Ca^{2+} can also enter as a result of other events e.g. as a result of the reversal of the Na^+/Ca^{2+} exchanger). Finally, the $[Ca^{2+}]_i$ increase in the cytosol can occure from the release of Ca^{2+} from intracellular stores – mitochondria or Ca^{2+}-induced release from the endoplasmic reticulum. Elevated intracellular free $[Ca^{2+}]_i$ causes disorders numerous enzymes such as protein kinases, phosphatases, lipases and proteases that can lead to damage cellular structures which following apoptosis and cell death. Therefore, intracellular components involved in calcium clearing process can play a great role in adaptation to hypoxia. Several cellular components effectively compensate calcium overload induced by external calcium ion entry through plasmalemmal channels. Ca^{2+}-ATPases (PMCA and SERCA-pump) as well as NCX and intracellular organells – mitochondria and endoplasmic reticulum are among them. These components also effectively control the influx of calcium into the cell by modulation of

store-operated plasmalemmal channels which is the most general mechanism affecting calcium homeostasis. The participation of all these mechanisms in cell adaptation to changes in external medium can be demonstrated in particular during hypoxic conditions. Therefore in our studies we estimated the role of these organelles in hypoxic conditions by using microfluorescent technique in hypoxia-tolerant fish *Carasius gibelio* by measuring the changes in intracellular calcium concentration using Fura-2 flurescent dye downloaded in isolated neurons. Also we used a polarographic technique to measure partial oxygen pressure in bath solution. In our experiments we found that hypoxia induces elevation of cytosolic calcium in cerebellar neurons of fishes. The sources of such elevation is the massive influx of ions through activation of calcium-permeable plasmalemmal channels. An important further step is a substantial increase in calcium accumulation by mitochondria and endoplasmic reticulum. In comparison to mammal neurons in neurons of hypoxia-tolerant animals the level of intracellular calcium easily returned back to its initial level after reoxygenation. We concluded that cerebellar neurons of hypoxia-tolerant carasius have more powerful or more resistant Ca^{2+}-clearing systems to hypoxia than rats ones.
Keywords: Ca^{2+}-ATPase, SERCA, PMCA, NCX, hypoxia, carasius, calcium, neurons, cerebellum

BK-CHANNELS AS A TARGET OF THE THERAPY OF URINARY BLADDER DYSFUNCTION

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More than a quarter of diabetic patients suffer from overactive bladder syndrome (OAB). Contractility of the urinary bladder smooth muscle (detrusor) is primarily regulated by muscarinic M3 cholinoreceptors, therefore, muscarinic antagonists along with botulinum toxin A, which decreases the release of acetylcholine from cholinergic nerve terminals, have long been the agents of choice for OAB treatment (Roxburgh C, Cook J, Dublin N. Anticholinergic drugs versus other medications for overactive bladder syndrome in adults (Review), Copyright © 2007 The Cochrane Collaboration. Published by JohnWiley & Sons, Ltd). However, these agents have strong side effects prompting for the search of additional mechanisms modulating detrusor contraction.
Large conductance Ca^{2+}-activated K^+ channels (BK channels) are key elements in the negative feedback mechanism regulating detrusor myocyte excitability and detrusor tone. Ca^{2+} influx during the action potential facilitates repolarization due to BK-channel activation, whereas localized Ca^{2+} transients (Ca^{2+} sparks) caused by spontaneous opening of a cluster of ryanodine receptors (RyRs) on the sarcoplasmic reticulum (SR) membrane give rise to the BK channels-mediated spontaneous transient outward currents (STOCs) promoting hyperpolarization (G.J. Christ & S. Hodges. Molecular mechanisms of detrusor and corporal myocyte contraction: identifying targets for pharmacotherapy of bladder and erectile dysfunction. British Journal of Pharmacology (2006) 147, S41–S55.). Therefore, in our work we have compared currents through BK channels in detrusor myocytes from control rats and rats with diabetic model of OAB. Experiments were carried out on enzymatically dispersed smooth muscle cells (SMC) from the detrusor muscle of normal rats and rats subjected to the streptozotocin-induced diabetes using whole-cell patch-clamp technique. BK component (I_{BK}) of the net membrane current was isolated based on the sensitivity to the specific BK-channel blocker paxilline (300 мМ). Current amplitudes were normalized to the SMC membrane capacitance to yield current density (pA/pF) and expressed as mean±s.e.m. Depolarization-evoked I_{BK} in the detrusor SMC of diabetic rats appeared significantly suppressed compared to normal rats: at 0 mV its density constituted only 0.3±0,05 pA/pF vs. 5.4±0.04 pA/pF under normal conditions. Mean frequency and mean amplitude of STOCs recorded from detrusor SMC of diabetic animals at holding potential -20 mV were also markedly inhibided from control values of 12±1.5 Hz and 0.87±0.05 pA/pF to 2.4±0,6 Hz and 0.47±0.06 pA/pF, respectively, during diabetes. Thus, in the present study, we have demonstrated substantial downregulation of BK-channel-mediated currents in detrusor SMC under diabetic model of OAB. Such downregulation may result from altered expression of these channels, impairment of their function and/or disturbances in the RyR-mediated Ca^{2+} release.
Keywords: BK channels, streptozotocin-induced diabetic rats, overactive bladder syndrome

QUANTAL ATP RELEASE FROM ACUTELY ISOLATED CORTEX CELLS ON SINGLE BOUTON LEVEL

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ATP-mediated neurotransmission functionally important mechanism and might significantly influence neuronal Ca signaling synaptical plasticity and development in CNS. Postsynaptic action of ATP through purenergic modulation of synaptic strength engages many processes in brain like neurotransmitter receptors regulation or glia-neuron interactions. In current work we focus on quantal release of ATP mediated by stimulation of the single synaptic terminal isolated from whole synaptic network. Acutely isolated cortex cells were prepared with non enzyme vibrodissociated technique. The ATP postsynaptic currents were measured during local extracellular electrical stimulation of one single bouton. The successful mediator fusion events were visually controlled by stimulus activating staining by Synapto-Green. The ATP was released from distinct vesicle population as evidence by existence of two separate populations of synaptic currents. As membrane potential was set above the reversal potential for Cl-, we observed fast inward currents mediated by P2X receptors and slow outward currents mediated by GABA. Furthermore, these two populations of synaptic currents we separated by pharmacological tools. When slow outward postsynaptic currents we inhibited by picrotoxin, the residual fast inward currents were blocked by 30 μM PPADS. The quantal parameters of postsynaptic currents in the cortical cells were obtained and verified using maximum likelihood and autocorrelation techniques. The amplitude histograms for both mediators have a binominal distribution character with quantal size near 6.5 pA for ATP and 10 pA for GABA. We measured spontaneous postsynaptic events in parallel with single bouton stimulation. The quantal and kinetic parameters of spontaneous ATP and GABA receptor-mediated currents correlated to the parameters of corresponding evoked synaptic currents. In a conclusion, our results demonstrate that release of ATP in cortical inhibitory terminals occurs from separate pool of synaptic vesicles. Upon excitation of postsynaptic site release of ATP and GABA can be synchronized when both transmitters are released in multivesicular package.
Keywords: ATP, postsynaptic currents, quantal release, cortex

BETA-ADRENERGIC MODULATION OF GABA RECEPTOR-MEDIATED INHIBITION IN THE RAT HIPPOCAMPUS *IN VITRO*

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Noradrenaline (NA) from the locus coeruleus and GABA from hippocampal nonpyramidal cells exert strong influences on neuronal activity in hippocampus. An interaction between GABAergic and noradrenergic systems is often observed in the CNS, but there is not studied question concerning influence noradrenaline via beta-adrenoceptor subtypes on GABA-ergic inhibition in the hippocampus. To demonstrate the noradrenergic modulation of GABA-induced inhibition the effect of selective beta2-adrenoceptor agonist metaproterenol and GABA on population spikes (PS) in CA1 in rat hippocampal slices were studied *in vitro*. We found that metaproterenol did not significantly change PS. Metaproterenol alone slightly increased the amplitude, latency and duration of PS, but its effect was not statistically significant. The local application of GABA inhibited PS decreasing the amplitude, latency and duration right up to a total disappearance of PS in reply to stimulation. During simultaneous application of both compounds metataproterenol decreased GABA-induced inhibition in most cases, therefore the amplitude, latency and duration of PS decreased less than during isolated application of GABA. In few recordings the GABA-induced inhibition was enhanced by metaproterenol.
Our results indicated that beta2-adrenoceptor agonist metaproterenol mainly relieving GABA-induced inhibition may also cause an opposite effect. These dual effects of metaproterenol on GABA-induced inhibition of PS could be explained by different modulation of GABAA and GABAB receptors during beta2-adrenoceptor activation. The suggestion is made that NA may modulate GABA-induced electrophysiological responses in hippocampus via beta-noradrenergic receptors activation and may play an important role in the regulation of the excitability of neurons and the fine-tuning of information flow in hippocampus.
Keywords: hippocampus, noradrenaline, beta-adrenoceptors, GABA.

INITIATION OF LONG TERM-POTENTIATION IN RAT HIPPOCAMPUS REQUIRES HYDROLIZIBLE PHOSPHATE GROUPS AND ACTIVATION OF VARIOUS P2X RECEPTORS

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Purinergic receptors, activated by purines and pirimidines, play a major role in different cellular interactions, such as smooth muscle contraction, neurotransmission, exocrine and endocrine secretion, nociception, control of cardiac function and other. The activation of purinergic receptors in hippocampus is known to modulate synaptic plasticity considered to be the molecular basic of learning and memory. In the present study standard electrophysiological recordings of EPSP in rat hippocampal slices were applied to investigate the effect of ATP analogues on ATP-induced long-term potentiation (LTP). Extracellular application of non-hydrolizible analogue of ATP, б,в-Methylene-ATP (б,в-MeATP; 50 мM), prevented initiation of LTP (87%±5% vs. control). However, application of ATP-г-S (10 мM) showed the dramatic facilitation of synaptic plasticity (148%±13% vs. control). These data indicate on the option of importance for phosphate groups of ATP within LTP initiation. The novel antagonist of P2X7 receptors A740003 was also used in order to examine the possible contribution of this type of purinergic receptors to ATP-induced LTP. Co-application of A740003 (10 мM) and ATP (10 мM) prevented the induction of ATP-induced LTP (102%±4%). However, effect of A740003 used in lower concentration (1 мM) was not significantly different from previous experiment (94%±5%). These data demonstrate that various types of P2X receptors might be involved in ATP-induced LTP in hippocampus.

Keywords: LTP, purinergic receptors, P2X receptors

RAPID MODULATION OF GABA-ERGIC SYNAPTIC TRANSMISSION BY THYROXINE

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Thyroid hormones (THs) are well known for their genomic effects but recently several studies revealed their actions as rapid modulators of membrane receptors. In particular, fast effect of THs (in the micromolar range) on glutamatergic synaptic transmission has been reported (Losi et al., 2008). We studied whether thyroxine affects GABAergic synaptic transmission in the rat hippocampal cell cultures Using patch-clamp technique we examined effects of thyroxine (0,5 –2 uM) on evoked GABAergic inhibitory post-synaptic currents (IPSCs). We found that IPSC amplitude was decreased in the presence of thyroxine by 30%. The decrease was accompanied by changes of paired-pulse ratio and IPSC coefficient of variation (CV), which is suggestive of a presynaptic mechanism.

Keywords: GABAergic, hippocampal neurons, thyroxine, synaptic plasticity.

KV3 CHANNELS MODULATE CALCIUM INFLUX EVOKED BY HIGH FREQUENCY ACTION POTENTIAL FIRING IN THE RAT RETINAL GANGLION CELLS

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Retinal ganglion cells (RGCs) are final output neurons transmitting impulses from visual receptors to the brain via optic nerve. RGCs demonstrate depolarization-evoked high-frequency firing patterns. It is known that voltage-gated potassium Kv3 channels maintain high-frequency action potential (AP) firing in central neurons. Earlier, using potassium channel blockers 4-aminopyridine and tetraethylammonium (TEA) we have shown that Kv3 channels are essential for fast firing pattern generation in the rat RGCs. In particular, activation of Kv3 channels provides high AP repolarization rate and conditions generation of short duration AP. Main calcium influx in RGCs is provided by activation of voltage-gated calcium channels during action potential generation. In present work we studied how activation of Kv3 cannels during action potential generation affects calcium influx in the rat RGCs. Simultaneous whole-cell patch-clamp recordings and microfluorometric calcium measurements were made on mature rat RGCs (4-5 weeks old rat). Calcium-sensitive dye Indo-1 (100 μM) was added to intracellular solution. The cells were activated by applying 500-ms depolarizing current steps with incremental amplitude of 20 pA (up

to 150 pA). Changes of maximal steady firing frequency, single AP width (at half-height) and (as a consequence) intracellular calcium concentration caused by bath application of potassium channels blocker TEA (500 μM) were analysed. We observed linear increasing of the calcium signal during depolarization-evoked AP firing. Intracellular calcium concentration exponentially reduced after AP generation ending. Time constant of the calcium signal decay was 5.1±0.3 s (n=5) and not affected by the blocker application. Amplitude of the calcium signals depended linearly on RGCs firing frequency. TEA application reduced maximal steady firing frequency from 54.4±5.7 s-1 to 25.5±4.8 s-1 (n=11); increased single AP half-width from 1.2±0.1 ms to 1.8±0.1 ms (n=11) that resulted in increasing of calcium influx from 1.2±0.2 nM•s to 1.9±0.3 nM•s (n=5). Thus, Kv3 channels play key role in maintenance of high frequency action potential firing in the rat RGCs and, at the same time, they modulate calcium influx via modulation of single AP duration. Probably, it prevents cell form calcium overload during high frequency action potential firing leading to calcium-mediated cytotoxic death.
Keywords: Retinal ganglion cells, Kv3 channels, action potential

HOPPING PROCESS CAUSES ONE-DIMENSIONAL BROWNIAN MOTION OF MOTOR PROTEINS ALONG MICROTUBULES

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The motor proteins are known to move along the microtubule (MT) via cyclic alternation of strong and weak binding states. During strong binding state the motor protein at a specific binding site is anchored to MT. During the weak binding state, the motor protein is loosely and non-specifically coupled to the MT surface and accomplishes one-dimensional (1D) Brownian motion along its long axis, until it finds the next specific binding site. While in strong binding state the motor protein is anchored to MT, the actual motility depends on the motion in weak binding state. Thus 1D Brownian motion is important for motility of motor proteins but its mechanism is still poorly understood. Two models have been proposed for protein 1D Brownian diffusion for case of movement along DNA: sliding and hoping. Here we discuss relevance of these two modes for movement of motor proteins along MT. The sliding mode involves a constant motor protein-MT contact, and the hopping implies repeated rounds of dissociation and reassociation at a nearby location. The association/dissociation during weak binding state is determined primarily by electrostatic interactions. If hopping process causes one-dimensional diffusion, the increase in ionic strength will increase the time period the motor protein spends in solution via screening of the electrostatic interactions raising the measured diffusion constant. The diffusion constant of both dyneins and kinesins (kinesin-1, Eg5, Ncd) as well as of the positively charged polyacrilamide nanoparticles has been reported to be sensitive to the ionic strength of solution. Here we explain this dependence by the hopping mechanism. In case with charged nanoparticles as well as with actual motor Ncd, the diffusion constant changes nonlinearly with the increasing ionic strength. The diffusion constant for nanoparticle has the minimum at the ionic strength of 70 mM and increases with either the increase or decrease of ionic strength. Here we propose a mathematical model explaining this phenomenon. Our model is based on the assumption that microtubules as polyampholytes have both negatively and positively charged sites on their surface and that these sites are unequally distributed. We supposed that an average negative binding site on microtubule has bigger radius and is located closer to the positively charged motor protein than a positive one. At low values, the increase in the ionic strength screens the repulsion between the positive site on microtubule and the positive motor protein, which causes increase in attractive electrostatic interactions. When ionic strength reaches the critical value (50 mM) the repulsion becomes minimal, but attraction at the negative site remains the same. This is the point of strongest electrostatic bond and respectively minimal diffusion constant. According to hopping model, an additional increase in ionic strength will lead to screening of attraction and respectively to increase in the diffusion constant which well fits the experimental data.
Keywords: microtubule, motor proteins, hopping model

RESTRICTION OF HOPPING DURING THE WEAK BINDING STATE INCREASES THE PROCESSIVITY OF MOTOR PROTEINS

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The motor proteins are known to move along the microtubule (MT) by cyclic alternation of two strong and weak binding. During strong binding state the motor protein is anchored to MT. In the weak binding state it accomplishes one-dimensional (1D) Brownian motion along the long axis of the microtubule. Classically, the two-headed coordination in a hand-over-hand mechanism is required for processivity of the conventional two-headed kinesin, i.e. one head is strongly bound to the MTs and restricts 1D Brownian motion accomplished by the other weakly bound head. However, the mechanism of one-headed motor protein motion remains unclear. The electrostatic tethering of single head to the MT during the weak binding state is believed to be responsible for the processive movement for both kinesins and dyneins. Within the motor protein, the number of positive charges available for contact with MTs is believed to be the key factor regulating its processivity. An additional electrostatic interaction between the positively charged K-loop of a monomeric kinesin KIF1A and the negatively charged E-hook of tubulin is considered to be a tether which enables processivity in the absence of second head. It has been reported that fusion of BDTC (a subunit of transcarboxylase, which weakly binds to MTs) to the single-headed conventional kinesin reduces its diffusion constant, stabilizes the motility, and enables successive steps, which are otherwise impossible because of the absence of the second head. For single-headed conventional kinesin, processivity can be analogously enabled by the restriction of the diffusion with optical tweezers. Similarly, additional positive charges, derived from the dynactin when it acts in complex with the dynein, restrict dissociation of the dynein and increase complex processivity. Here we try to find the model that more precisely explains the forces controlling the one-dimensional diffusion over the MTs and to identify roles of sliding and hopping in this process. Two hypothetical causes of the one-dimensional diffusion are discussed: sliding and hopping, and the resulting scenarios for the electrostatic tethering of the motor protein to the MT. If a hopping process causes one-dimensional diffusion, the electrostatic tethering will increase the nonspecific binding affinity, decreasing the time period the protein spends in solution and effectively decreasing the measured diffusion constant. The opposite situation is expected if such one-dimensional diffusion is caused by sliding. The tighter electrostatic interactions should lower the activation barrier for protein translocation along the MT in a manner similar to the reduction of activation barriers for chemical reactions by enzymes. The reduction of the activation energy for sliding will increase the measured diffusion constant. Based on the observation that additional positive charges on motor protein reduce its diffusion constant, we conclude that the hopping process contributes most to one-dimensional diffusion and the restriction of hopping is responsible for increased processivity.

Keywords: microtubule, motor proteins, hopping model

DNA IS CLOSE-PACKED

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Helical structures appear in nature, their formation is ubiquitous. E.g. alpha-helices in proteins and DNA double helices. Akin to the problem of obtaining the best packing of hard spheres we ask the question: what is the best helical packing of flexible tubes with hard walls? We solve this mathematical problem by finding the helix pitch angle where the volume is most efficiently used. We call these unique structures close-packed. There are several different helical structures of DNA. We find the A-DNA to be essentially closed packed at a pitch angle of 34.8 deg. with a volume fraction, which is within 2 parts in a thousand from that of the ideal close-packed tube structure. The prevalent structure of B-DNA has a broken helical symmetry with minor and major grooves and a pitch angle of 37.4 deg. When the symmetry breaking is taken to be a priori, the best use of volume for a tube model appears at a pitch angle of 38.3 deg. which we find to be in significant agreement with the before mentioned experimental structure (37.4 deg.). For a detailed discussion and further references, see Ref. 1. In summary, we show that the close-packed structures with pitch angles of 18.1 deg. and 32.5 deg. for single and double helices, respectively, are the essential structures from a best packing perspective, thereby underling the importance

of volume fraction for our understanding of alpha-helix and for DNA.
Keywords: Protein structure, DNA, Helices, Close-packed structures
[1] K. Olsen, J. Bohr, The generic geometry of helices and their close-packed structures. Theoretical Chemistry Accounts, http://dx.doi.org/10.1007/s00214-009-0639-4

CYTOCHROME *C* AS AMPLIFIER OF ROS PRODUCTION IN MITOCHONDRIA

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It is known that calcium signalization in the cell is closely connected with mitochondria ability to accumulate calcium. Impaired Ca^{2+}-signaling, opening of mitochondrial permeability transition pore (MPTP), which is accompanied by the uncoupling and the decreased effectiveness of ATP synthesis, massive release of ROS and release of cytochrome *c* from the intermembrane space into cytosol are often considered as the first steps in the induction of cellular apoptosis. As a rule, cytochrome *c* serves for electron transport between III and IV complexes of respiratory chain and in the normal state could exhibit antioxidant properties as an acceptor of electrons. However, the role of cytochrome *c* in regulation redox reactions outside of the mitochondria is controversial. The aim of our work is to estimate the influence of exogenous cytochrome *c* on ROS formation in mitochondria and its dependence on the opening of permeability transition pore. The data on ROS formation were obtained using dichloro-fluorescein (DCF) fluorescence. It is shown that cytochrome *c* in the incubation medium has weak influence on the ROS formation in mitochondria in the course of Ca^{2+}-uptake, when MPTP opening is blocked by cyclosporine A. On the contrary, MPTP opening is accompanied by a dramatic increase in ROS production in the presence of cytochrome *c*, which could not be explained merely by the increase in the rate of electron transport resulting from MPTP opening and the uncoupling of respiratory chain. It is shown that the rise in DCF signal takes place in the matrix and could not be ascribed to direct oxidation of the probe by cytochrome *c* outside of the mitochondria. To explain observed rise in ROS formation in the matrix induced by cytochrome *c* outside of the mitochondria, we propose that mitochondrial pore might exchange ROS between the matrix and the medium. ROS, generated in the matrix, would be released at first through the pore into medium, where ROS metabolism could be manifold activated by Fe-heme complex of cytochrome *c*. The last one would act as a catalyst in the amplification of ROS production by the way of Fenton-type reactions. In turn, even transient increase in ROS concentration would lead to their flow back into matrix space through the same mitochondrial pore which would be in its open state and increase ROS signal inside the mitochondria. We suppose that, apart of its conventional function, cytochrome *c* could serve in such a way as an amplifier of ROS signaling between mitochondria providing a background for the development of apoptosis induced by MPTP opening.
Keywords: mitochondria, mitochondrial pore, calcium, ROS, cytochrome c.

INTERSPIKE MODEL OF NEURONAL IMPULSE ACTIVITY

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Modelling of neuronal activity is a nontrivial task considering either the intrinsic properties of the generation of neurons and processes of interaction between them. Description of neuronal activity by a sequence of interspike intervals is a nice mean between complex models like Hodgkin–Huxley or Izhikevich models, which compute the transmembrane potential, and excessive loss of information in describing the neuronal activity with the averaged frequency of generation. The presented phenomenological model deals with a set of interspike times on the single-neuron level and has the advantage of being computationally less demanding. The transient function of neuronal generation may be simply obtained in “ramp” protocol of patch-clamp experiment and approximated by rational function with three parameters, and take into consideration the calcium dependence of the generation. Accommodation properties are well described by an exponential function with saturation. We assume that the mechanism of accommodation can mainly be related to calcium intracellular accumulation and balance during long-term generation. The dynamic system of two equations allows calculating the impulse generation, taking into account an inward current and accommodation properties of single neuron. This model can be used for network simulations and may thus help to elucidate the functional role of neural dynamics on the systems level.
Keywords: neuron model, impulse activity, accommodation, interspike intervals

THE SPATIAL DISTRIBUTION OF THE Ca^{2+} SIGNALS INDUCED BY Ca^{2+} ENTRY THROUGH LVA Ca^{2+} CHANNELS IN P12 LD THALAMIC NEURONS

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Entry of Ca^{2+} through LVA Ca^{2+} channels is important event for low-threshold Ca^{2+} spike initiation. How much charge is needed to initiate the spike and how it is distributed in particular neuron is unknown. Here, LVA Ca^{2+} channels were activated in whole-cell configuration. We monitored the amount of charge injected and the results of Ca^{2+} entry using Ca^{2+} sensitive dye, Fluo-3. Wide-field fluorescent light images were accumulated by CCD camera before and during step depolarization. Accumulation of Ca^{2+} in cytosol was associated with changes of Fluo-3 signal. Analysis of the spatial distribution of this signal revealed two characteristic features. It had either a diffusive appearance or a well near-membrane localised patchy distribution in the form of either flashes or elongated sources of light. A 10 step protocol revealed stochastic spatial appearance of flashes and fluctuations in their peak intensity. We also observed places where step depolarization does not produce detectable ДF signal. Direct measurement of the single-channel activity in cell-attached configuration showed that 9 from 10 somatic patches contained no one LVAf Ca^{2+} channel if patched surface area was of ~10 μm^2. The success rate increased 5 times when surface area was only twice larger. Successive patches had from 1 to 7 simultaneously open LVAf Ca^{2+} channels.

Keywords: Calcium, calcium channels, LVA calcium channels

IMPAIRED MITOCHONDRIA HANDLING CONTRIBUTES TO THE ALTERATION OF STORE-OPERATED Ca^{2+} ENTRY UNDER DIABETES-INDUCED XEROSTOMIA

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Xerostomia is a troublesome complication of diabetes mellitus associated with irreversible damage of salivary glands functioning resulted in the decreased salivation. The most common complications of xerostomia are severe oral dryness, loss of taste acuity, increased incidence of dental caries. Salivation is activated by a neurotransmitter acetylcholine (ACh). ACh-induced fluid secretion is mediated by a complex cytosolic calcium ($[Ca^{2+}]_{cyt}$) signal that is required for a synchronized activation of Ca^{2+}-dependent apical Cl^- channels and basolateral K^+ channels driving water flow out of the gland. Previously we showed that diabetes is associated with alterations of ACh-mediated $[Ca^{2+}]_{cyt}$ signaling in submandibular saliva gland which provides a major contribution to secretion of fluid and electrolytes (Fedirko et al., 2006). Since salivation is initiated by an InsP3-mediated Ca^{2+} release from the endoplasmic reticulum (ER) but subsequently depends on the elevated $[Ca^{2+}]_{cyt}$ maintained by a store-operated Ca^{2+} entry (SOCE), we hypothesized that both processes could be altered under the diabetes and therefore contribute to the development of xerostomia. Diabetes was induced by a single i.p. injection of streptozotocin. Measurements of $[Ca^{2+}]_i$ was performed using fluorescent Ca^{2+} dye fura-2/AM. We found a decrease of the amplitude and significant deceleration of ACh-induced $[Ca^{2+}]_i$ under the diabetes. On the other hand, our data demonstrated increased contribution of mitochondria to the cytosolic calcium clearance in the acinar cells from diabetic rats. In particular under the diabetes we found: i) an increase in the amount of Ca^{2+} accumulated in the mitochondria in the resting state (by 46 ± 6 %; $p < 0.05$); ii) significantly smaller effect of mitochondrial Ca^{2+} uptake inhibition on the ACh-induced $[Ca^{2+}]i$ transients in Ca^{2+}-containing extracellular medium (by 69 ± 11 %; $p < 0.0001$ in diabetic cells vs. 29 ± 6 %, $p < 0.05$ in control). Since both SOCE and subsequent ER Ca^{2+} refilling are precisely regulated by mitochondria (Kopach et al., 2009), thus we studied the effectiveness of these processes under the diabetes. We found that SOCE induced by short ACh stimulation was increased in diabetic acinar cells (by 70 ± 15 %). Inhibition of mitochondrial Ca^{2+} accumulation equalized SOCE magnitude in the control and diabetic cells indicating the increased role of mitochondria to provide positive feedback on SOCE under diabetes. In contrast, during the sustained cells stimulation (continuous presence of ACh or TG), SOCE was decreased and decelerated under diabetes (~ by 40%) suggesting inability of acinar cells to maintain SOCE under potent agonist stimulation. Concluding, our data directly showed severe impairment of the intracellular signaling mechanisms responsible for the activation and maintenance of SOCE and highlight the significant contribution of alteration in mitochondria Ca^{2+} handling to the development of xerostomia under the diabetes.

Keywords: calcium, salivary cells, mitochondria, store-operated calcium entry, diabetes mellitus.

CALCIUM-DEPENDENT MECHANISM OF IRRADIATION-INDUCED XEROSTOMIA

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Salivary glands are highly susceptible to acute exposure to ionizing radiation (i.e. obtained within a few days or even hours). Salivary gland damage is observed after relatively low irradiation dose of about 10 Gy, making them 2nd sensitive organ of the human organism after the bone marrow which can be damaged with doses as low as 1 Gy. The most common complications of irradiation are severe oral dryness, loss of taste acuity, increased incidence of dental caries, and oral ulceration, which are some of the symptoms associated with radiation-induced xerostomia. Acquired evidences indicate that radiation does not kill the acinar cells but leads to the unknown damage in the signal transduction system. Regulation of Ca^{2+} in the cytosol and endoplasmic reticulum is central to the regulation of synthesis and secretion of saliva components, thus alteration of intracellular Ca^{2+} handling could be a basis for impaired salivary gland functioning associated with radiation. However, the information about the changes of Ca^{2+} signaling in radiation-induced xerostomia is very limited. We studied the effect of local irradiation (a single dose of 1 and 10 Gy of X rays) on the rate of salivation, protein and calcium concentration in the saliva secreted by submandibular salivary gland. The measurements were performed in 2 h and 24 hours after irradiation procedure. We found that single dose of 1 Gy caused significant decrease in the rate of salivation. In 2 hours after irradiation the rate of salivation decreased by 54±14%; protein and calcium concentration in the saliva - by 52±9% and 34±11 % correspondingly. We suggested that observed changes in the salivation could be due to the changes in the intracellular calcium dependent signaling mechanisms involved in the regulation of synthesis and secretion of proteins and fluid secretion. We also found that after irradiation (10 Gy) the total calcium content in the isolated acinar cells after their incubation with specific inhibitor of SERCA – thapsigargin TG (1 nM) in calcium containing and calcium free extracellular solution decreased by 13±2% ($p<0,01$) and 44±2% ($p<0,001$) compare to corresponding non-irradiated control. Application of ACh in calcium containing extracellular solution to the acinar cells isolated from the irradiated animals induced increase in total cellular calcium content by 32±9 % ($p<0,05$) compare to non-irradiated control. Application of ACh in calcium free solution induced decrease in calcium content by 23±4 % ($p<0,05$). These data suggest that after irradiation the calcium concentration in the endoplasmic reticulum is decreased whereas the sensitivity of muscarinic receptors to ACh is increased. We also showed that irradiation alters activity of plasma membrane and endoplasmic reticulum Ca^{2+}-ATPases. In particular, PMCA activity - did not change significantly whereas SERCA – decreased by 63±6 % ($p<0,01$) correspondingly. Thus these data directly showed the high susceptibility of cellular Ca^{2+}-ATPases to irradiation. Moreover, we showed that after preincubation of the acinar cells with ACh (10 мM) the activity of PMCA – increased by 16 ±7 %; activity of SERCA – increased by 64±26 % ($P<0,05$) compare to the nonstimulated control. After irradiation, effect of preincubation with ACh was completely opposite. In particular, the activity of PMCA – decreased by 42 ± 8% ($P<0,05$); activity of SERCA – decreased by 44±11% ($P<0,05$) compare to the effect of ACh pre-application in non-irradiated control. Concluding, we found the complex changes in the intracellular calcium homeostasis and calcium signaling cascades under the radiation-induced xerostomia. In particular we showed that low doses of irradiation induces: i) decrease of the endoplasmic reticulum calcium content; ii) significant sensetisation of muscarinic receptors; iii) diminishing of the activity of both plasma membrane and endoplasmic reticulum membrane Ca^{2+}-ATPases.

Keywords: radiation, salivary gland, calcium, salivation, Ca^{2+}-ATPases, achetylcholine

ROLE OF ENDOCANNABINOIDS IN THE REGULATION OF CALCIUM HOMEOSTASIS AND SALIVATION

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It is well known that endocannabinoids is an important regulatory system that alters different physiologic parameters in biologic systems. Mammalian tissues contain at least two types of cannabinoid receptors (CBs), CB1 and CB2, mainly located in the nervous system but recently by immunohystochemical methods it was shown their expression in salivary glands. However, the role of cannabinoids in the salivary cells physiology remains unclear. Our experiments were performed on male Wistar rats (6-7 weeks old, 100-150 g). Saliva was collected *in vivo* using variable speed peristaltic pump. We have

found that agonist of CB2 cannabinoid receptors – virodhamine (30 мM) *in vivo* caused decrease of the rate of unstimulated salivation, calcium and protein concentrations in secreted saliva by 31±6 % (P<0,01, n=7), 12±3 % (P<0,05, n=5) and 14±4 % (P<0,05, n=6) correspondingly, compare to control. Intraglandular injection of CB1 cannabinoid receptors agonist – (R)-(+)-Methanandamide (5 мM) caused significant decrease of the rate of unstimulated salivation and Ca^{2+} concentration in saliva by 22±8 % (p<0,05, n=6) and 25±2 % (p<0,05, n=4) accordingly, compare to control.
We also showed that application of virodhamine (30 мM) *in vitro* to the isolated acinar cells of rat submandibular salivary gland in Ca^{2+}-free extracellular solution (1 mM EGTA, 5 min) decreased total calcium content by 28±4 % (p<0,01, n=8) whereas (R)-(+)-Methanandamide (5 мM) – by 21±4 % (p<0,01, n=5). Notable, we found that endocanabinoids significantly potentiated store-operated Ca^{2+} entry (SOCE) into the acinar cells. In particular, following readdition of 2 mM $CaCl_2$ to the extracellular solution in the presence of virodhamine (30 мM) the total cellular calcium content increased by 130±22 % (p<0,001, n=6) whereas in control - by 57±15 % (p=0,01, n=6). Similarly, in the same conditions (R)-(+)-Methanandamide (5 мM) caused significant increase in total acinar calcium content on 166±33 % (p<0,01, n=5) whereas in control activation of SOCE resulted in the increase of calcium content by 93±12 % (p=0,001, n=5). We also demonstrated that *in vitro* virodhamine (30 мM) applied to isolated acini in the presence of SERCA inhibitor - thapsigargin (0,1 мM) in Ca^{2+}-free medium (1 mM EGTA, 10 min) caused decrease in total calcium content by 19±3 % (p<0,01, n=8) compare to the effect of thapsigargin only. The latter suggest the potentiation of the Ca^{2+} release from the ER upon activation of CB receptors. Following the incubation of acinar cells in the solution containing 1 mM EGTA, 1 mM EGTA+thapsigargin (0,1 мM) or 1 mM EGTA + thapsigargin (0,1 мM)+virodhamine (30 мM) 2 mM $CaCl_2$ was added to the mediaum to activate SOCE. The latter induced increase in the total cellular Ca^{2+}-content by 213±46 % (p<0,01) in the presence of virodhamine whereas in the presence of thapsigargin only – by 108±38 % (p<0,01). Thus acinar cells of submandibular salivary gland posse's active cannabinoid receptors (CB1 and CB2 type) which activation leads to the inhibition of salivation and alteration in electrolyte saliva content. Observed changes in the salivation can be caused by endocannabinoids-induced modulation of Ca^{2+} release from the ER and SOCE.
Keywords: cannabinoid receptors, virodhamine, (R)-(+)-Methanandamide, submandibular salivary gland, salivation.

Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II MODULATES SHORT-TERM PLASTICITY AT THE FROG NEUROMUSCULAR JUNCTION

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Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) plays a key role in use-dependent plasticity of central synapses. However, the contribution of CaMKII to the function of peripheral synapses of vertebrates remains unknown. Here we studied the role of CaMKII in the basal neurotransmission and short-term synaptic plasticity at the frog neuromuscular junction. Utilizing conventional electrophysiological technique we found that application of CaMKII inhibitor – KN-93 (5 mkM) does not affect the amplitude-temporal parameters of end-plate potentials (EPP) and currents (EPC), miniature EPC (MEPC) and extracellular responses of nerve terminal. KN-93 also did not influence the level of basal quantal neurotransmitter release. However, CaMKII blockade produced the inhibition of second component of paired-pulse facilitation (interpulse interval from 50 to 500 ms) and enhanced short-term depression of EPCs at high-frequency stimulation, prominently at 50 imp/s frequency. Thus, CaMKII has an important role in function of frog neuromuscular synapse under the high-frequency activity conditions, concerned with enhancement of neurotransmitter release. Probably, the contribution of CaMKII can be explained be its influence on the mobilization of synaptic vesicles to active zones.
Project is supported by Russian Foundation for Basic Research (07-04-01331).
Keywords: Ca2+/calmodulin-dependent protein kinase II, synaptic plasticity, neuromuscular junction

ROLE OF NEURONAL GLUTAMATE TRANSPORTERS IN REGULATION OF SYNAPTIC TRANSMISSION WITHIN HIPPOCAMPAL NETWORK

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Glutamate is the major excitatory transmitter inCNSalthough it causes severe braindamageby pathologic excitotoxicity. Efficient neurotransmission is controlled by powerful protection and support afforded by specific high-affinity glutamate transporters in neurons and glia, clearing synaptic glutamate. While the role of glial cells in glutamate uptake is well defined, the role of neuronal transporters remains poorly understood. The evaluation of impact of neuronal transporters on spontaneous and evoked EPSC in hippocampal CA1 neurons within a model 'single bouton preparation' by pre- and postsynaptic uptake was addressed. In whole-cell patch clamp experiments the influence of blocking, pre- or both pre- and postsynaptic glutamate transporters (GluT) on spontaneous and evoked postsynaptic currents (sEPSC and eEPSC), was examined by manipulating the content of intracellular solution. Suppressing GluT by non-transportable inhibitor TBOA (10μM) led to remarkable alteration of glutamate uptake process and was reflected in measurable changes of general properties of synaptic currents. Elimination of intracellular K^+ concentration required for glutamate transporter operation by using Cs^+-based internal solution (postsynaptic GluTs are non-functional a priori), causes the deficient of presynaptic glutamate transporters. Applied in such conditions glutamate transporter inhibitor TBOA (10μM) affected the occurrence of synaptic event and thus unregulated the transmitter release. eEPSCs were generally suppressed both in amplitude (to 48.73±7.03% vs. control) and in success rate (Rsuc) by TBOA (from 91.1±7.5% in control to 79.57±13.2%). In contrast, with K^+-based solution in patch pipette (preand postsynaptic GluT are intact), amplitude of eEPSC was substantially potentiated by pre-treatment with TBOA (152.1±11%), whereas (Rsuc) was reduced to 79.8±8.3% in average. The identical reduction of event success rate as well as increased pair-pulse ratios (PPF ratio) for eEPSC in both cases indicates the effect of TBOA on presynaptic uptake. sEPSCs simultaneously recorded from neurons, showed the same pattern of regulation but with less potency, indicating the similar processes in most of excitatory synapses. In conclusion, presynaptic transporters are suggested to act mainly as negative feedback signal on presynaptic release and/or referred to vesicle refilling processes.

Keywords: Excitatory postsynaptic currents (eEPSC); Neuronal glutamate transporters; Single bouton stimulation; CA1 hippocampal neuron

KINETIC PARAMETERS OF THE Zn^{2+} -INDUCED MITOCHONDRIA SWELLING AND Zn^{2+}-INDUCED Ca^{2+} RELEASE FROM MITOCHONDRIA

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It is known that Zn^{2+} is able to activate the mitochondrial (Mit) swelling following by opening the permeability transition pore, fast Ca^{2+} release from the Mit matrix as well as the other pathological processes which may result in the cell damage and death. However the kinetics of the Zn^{2+}-induced Mit swelling and the Zn^{2+}-induced Ca^{2+} release from these organelles remain unknown. The aim of the present study was to find out the kinetic parameters of the Zn^{2+}-induced Mit swelling and the Zn^{2+}-induced Ca^{2+} release from these organelles. The experiments were performed on the isolated rat liver Mit obtained by differential centrifugation. Mit swelling was measured using spectrophotometer SF-26 and analyzed by three parameters: i) maximal rate; ii) lag time; iii) peak amplitude. Ca^{2+} release from the Mit was investigated using Ca^{2+}-selective electrode Orion 93-20 and analyzed by three parameters: i) maximal rate; ii) duration of the process; iii) concentration of released Ca^{2+}. The concentration of protein was measured by Lowry method. We showed, that increase of extra-Mit concentration of Zn^{2+} (0,1-7,0 μM) caused significant decrease in the lag time of the Zn^{2+}-induced Mit swelling, but did not induce significant changes in the amplitude of the process. The dependence of the rate of Mit swelling on extra-Mit concentration of Zn^{2+} was bell-shaped with the peak magnitude equal to 3 mcM of extra-Mit Zn^{2+}. Vmax and K0,5 of the ascending branch of the parabola are equal to 0,280 A540/min*mg of protein and the 0,22 mcM accordingly. Also we found that increase of extra-Mit concentration of Zn^{2+} (0,5-10,0 μM) caused significant bell-shaped changes both of the duration of the Ca^{2+} release from the Mit and the concentration of released Ca^{2+} with the peak magnitudes of these parameters in the points of

3 mcM of extra-Mit Zn^{2+} in both cases. The dependence of the rate of Ca^{2+} release from the Mit on extra-Mit concentration of Zn^{2+} was also parabolic. Thus, our results demonstrated the kinetic parameters of the Zn^{2+}-induced Mit swelling and the Zn^{2+}-induced Ca^{2+} release from these organelles.
Keywords: mitochondria, zinc, permeability transition pore

THE ADAPTABILITY OF POTASSIUM CHANNELS ACTIVITIES

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Potassium (K^+) channels are the most diverse class of ion channels, and are important for regulating neuronal excitability and signaling activity in a variety of ways. They are major determinants of membrane excitability, influencing the resting potential of membranes, wave forms and frequencies of action potentials, and thresholds of excitation. Potassium channels fulfill important function in many signal transduction pathways in the nervous system. Complex processing and integration of the signals observed in neurons are facilitated by a diverse range of the gating properties of the ion channels in this cell type, particularly of the voltage-gated K^+ channels (K_v). A distinctive combination the of K^+ channels endows neurons with a broad repertoire of the excitable properties and allows each neuron to respond in a specific manner to a given input at a given time. The properties of many K^+ channels can be modulated by second messenger pathways activated by neurotransmitters and other stimuli. K^+ channels are among the most frequent targets of the actions of several signaling system. Voltage-gated K^+ cannels exist not as independent units merely responding to changes in transmembrane potential but as macromolecular complexes able to integrate a plethora of cellular signals that fine tune channel activities. Specificity of information is generally encoded by the kinetics of action potential frequency, duration, bursting, and summation. A neuron (or specific axon or dendrite), when it is required to change its firing pattern, can rapidly regulate the gating behavior of existing channels. If longer term modifications in firing patterns are required, the cell may alter the transcriptional expression of ion channel genes for diverse functions. The number of K^+ channel genes is relatively large; however, the diversity of endogenous K^+ current phenotypes observed from various excitable cells is much greater. Additional processes such as alternative splicing, posttranslational modification, and heterologus assembly of pore-forming subunits in tetramers contribute to extend the functional diversity of the limited repertoire of K^+ channel gene products. Even greater diversity can be achieved through interactions between K^+ channel proteins and accessory proteins. The number of likely therapeutic indication for K^+ channel modulators will increase as insight into the dynamics of expression of these channels in various diseases grows and the issue of the required selectivity is resolved.
Keywords: potassium channels, neuronal excitability, signaling activity, pharmacology

VOLTAMMETRIC DETECTION OF QUANTAL RELEASE OF CATHECHOLAMINES FROM CHROMAFFIN CELLS

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Adrenal chromaffin cells are ideal models to investigate stimulus-secretion coupling of catecholamines because of their neuro-ectodermal origin and close biochemical and functional similarities to postganglionic sympathetic neurons. In numerous experiments it was shown that this secretion is an exocytotic process. Electrochemical measurements with microelectrodes at isolated adrenal medullary cells provide direct chemical evidence for the quantal nature of exocytosis in real time. Chromaffin cells can release two main types of catecholamines – adrenalin (Adr) and noradrenalin (NA) which play different role in hormonal regulation of organism. In our previous experiments we established that there are two subpopulation of chromaffin cells which predominantly release one of these transmitters. Therefore, in presented data we tested the possibility to determine what kind of substance is released from studied cell by using cyclic voltammetric method. A cyclic voltammetry is used in the measurement of neurotransmitters and related substances in physiological preparations. Voltammetric measurements allow measuring the rapid concentration dynamics of redoxactive biological substances to be followed. Usually procedure involves the use of carbon-fiber microelectrodes with fast-scan cyclic voltammetry with scan rates exceeding 100 V/s. In our experiments, a secretion of catecholamines from individual

rat adrenal medullary cells grown in primary culture has been investigated with a carbon-fiber microelectrode placed adjacent to the cells. Oxidation of catecholamines at the electrode surface results in changes in current, which give a real-time measure of catecholamine secretion. We obtained voltammograms for 20 mM Adr or 20 mM NA, which were measured in bath solution and further were used as standart curves for these mediators. Further we have measured an electrode current induced by secreting chromaffin cells at charasteristic electrode potentials. Comparing obtained values for measured cell and standard curve we could determine what kind of compound (Adr or NA) is secreted by given cell. Thus, we found that particularities of voltammograms for Adr and NA could be used for determination of cathecholamine that is released from chromaffin cell during secretion.
Keywords: Calcium, secretion, exocytosis, cathecholamines, chramaffin cells, voltammetry

EFFECT OF GABAPENTIN IN DIFFERENT RAT DORSAL ROOT GANGLION NEURONS IN NORMA AND UNDER STREPTOZOTOCIN-INDUCED DIABETES

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Pain is the challenging problem in medical practice and one of the most frequent reasons of asking medical help by patient. There is difficult to find a proper method, type of drug and dosage for effective treatment of pain senses. Nowadays a wide range of pain-attenuating chemicals such as opioid analgesics, nonsteroidal anti-inflammatory drugs and local anesthetics have been created. There are a lot of targets which these drugs specifically influence to mediate its analgesic effect. But the problem is these agents are not able to produce sufficient enough analgesic effect or/and have significant side effects. Drug tolerance, mental disorders, kidney failure, liver disorder, dementia, problems in the digestive tract, bleeding problems, numbness est. often appears as side effect under administration of analgetics. According to this problem such drugs as gabapentin with its low level of side effect is very prospective in reliving of pain. Gabapentin (1-(aminomethyl)cyclohexaneacetic acid) was designed as one of the GABA analogues. It shows significant anticonvulsive effect, low side effect level and utility for epilepsy treatment. Moreover, it was revealed that gabapentin has antinociceptive effect in models of mechanical and thermal hyperalgesia. One of the possible ways of gabapentin action is its influence on voltage-operated calcium channels. The action of gabapentin on voltage-operated calcium channel mediating intracellular calcium elevation has been examined in small, medium and large types of primary sensory neurons. The action of gabapentin on large size neurons demonstrated no changes in hyperpotassium evoked Ca transients. In medium sized neurons gabapentin application indicated reduction of Ca influx. Ca transients in small size neurons showed statistical non significant increase relative to control value. To select among examined neurons the nociceptive ones we made an extra test by capsaicin application as selective agonist of vaniloid subtype 1 receptors which are exclusively expressed in small and medium DRG neurons. Gabapentin action on capsaicin-positive medium sized neurons revealed twice more substantial reduction of Ca transient with respect to such reduction in capsaicin-negative neurons. In case of rats with streptozotocin-induced diabetes the changes in calcium elevations in different DRG neurons were different to those in normal conditions. In contrast to normal large DRG the same neurons under pathological conditions demonstrated significant sensitivity to gabapentin. Effect of gabapentin on medium neurons of diabetic rats demonstrated smaller elevations of intracellular free calcium but sensitivity to gabapentin applications on capsaicin-positive and capsaicin-negative neurons was opposite to that in control group. The obtained data show that gabapentin has specific action to medium sized nociceptive neurons and may be useful for treatment of acute pain syndromes.
Keywords: dorsal root ganglion neurones, gabapentine, pain

THE USAGE OF CALCIUM CHANNEL-BLOCKING AGENT IN THE ACUTE PERIOD OF TRAUMATIC DISEASE

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The subject of the investigation is the usage of calcium channel-blocking agent in the acute period of traumatic disease. Research was performed on the rats with traumatic injury. All traumatic influences was performed with anesthesia. The verapamil was injected after traumatic affect. The general and

ionized calcium, parameters of systemic hemodynamics and lethality was study. The direct correlation was reveal between decrease of calcium level, disturbances of systemic hemodynamics and lethality. The decrease of arterial pressure, heart dysfunction and unfavorable outcome was observed in the animals with low calcium level. The usage of calcium channel-blocking agent after traumatic injury enabled to enhance indexes of systemic hemodynamics and survival rate.
Keywords: Calcium channel-blocking agent, Traumatic disease

CALCIUM CHANNELS IN CALCIUM SIGNALLING OF MURINE OOCYTES

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In mammals, the increase in $[Ca^{2+}]_i$ during fertilisation occurs in the form of $[Ca^{2+}]_i$ oscillations, which start when the oocyte is penetrated by spermatozoon and last for several hours until the formation of pronuclei. Thus, calcium ions is known to participate in the early events of fertilization but role of transmembrane calcium flux during maturation of oocyte is not quite known. One reason for this blank in our knowledge lies in the difficulty in studying a single mammal oocyte. The goal of our investigations was to investigate the properties of Ca^{2+} channels of individual murine oocytes and to begin to characterize changes in Ca^{2+} channel activity during its development before fertilization. In our experiments we used isolated oocytes from mature mouses and measurements of their Ca^{2+} channel activity by microfluorescent methods with Ca^{2+} indicator Fura-2AM. We found that mouse oocytes, which were studied during their maturation were able to generate $[Ca^{2+}]_i$ transients evoked by membranne depolarizations. We did not observed $[Ca^{2+}]_i$ oscillations in such experimental conditions, however Ca^{2+} transients evoked by depolarization of oocytes were persistent and observed during long-lasting experiments.
Keywords: Calcium, oocytes, mouse, calcium current

Ni^{2+}-SENSITIVITY OF THE Ca_v3.1 T-TYPE CALCIUM CHANNEL WITH AND WITHOUT HISTIDINE MUTATION IN THE EXTRACELLULAR IS3-IS4 LOOP

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Nickel is long known as a selective blocker of low voltage-activated T-type calcium channels. However the Ni^{2+}-sensitivity of three cloned T-channel subtypes, Cav3.1, Cav3.2 and Cav3.3, is different with Cav3.2 one being around 20- to 60-fold more sensitive than Cav3.1 and Cav3.3. Recently the nickel-interacting site, which principal component is histidine-191 (H191) in the extracellular S3-S4 loop of domain I, has been identified in the Ni^{2+}-sensitive Cav3.2 channel, suggesting a novel role for the S3-S4 loop in the control of T-type Ca-channels permeation and block. However, the dependence of Ni^{2+}-sensitivity on the permeating ion species was not established. In the present study we compared nickel sensitivity of the wild-type recombinant Cav3.1-channel with Cav3.1-mutant, in which glutamine (Q) in the position equivalent to the H191 of Cav3.2 was substituted by histidine (Q172H mutant). Each channel was expressed in *Xenopus* oocytes and the blockade of the respective current by Ni^{2+} was assessed in the presence of different charge carriers: 10 mM Ca^{2+}, Sr^{2+} or Ba^{2+}. Our data show that the introduction of histidine in the Cav3.1 S3-S4 loop of domain I enhanced Ni^{2+}-sensitivity on average 4-fold: the IC50 for Ni^{2+} blockade of Ca^{2+}, Sr^{2+} or Ba^{2+} currents decreased from the control values for the wild-type Cav3.1 of 206 mM, 271 mM and 292 mM to 91 mM, 67 mM and 61 mM, respectively, for the Q172H mutant, consistent with the role of this amino acid in the formation of Ni^{2+} binding site. The dependence of IC50 for the blockade of Q172H mutant by Ni^{2+} on the charge carrier indicates differential competition of Ca^{2+}, Sr^{2+} or Ba^{2+} with Ni^{2+} for this site. Thus, in the absence of Ni^{2+} histidine-containing binding site is occupied by permeating divalent cation, which may influence channel's permeation properties.
Keywords: T-type calcium channels, Xenopus oocytes, Ni^{2+}