SA23

NCS proteins: calcium sensors and regulators of calcium signals

R.D. Burgoyne

The Physiological Laboratory, University of Liverpool, Liverpool, UK

Many aspects of neuronal activity are regulated by calcium signals. The transduction of temporally and spatially distinct calcium signals requires the action of calcium sensor proteins including various EF-hand containing calcium-binding proteins. The Neuronal Calcium Sensor (NCS) family proteins have begun to emerge as key players in neuronal function. Many of these proteins are expressed predominantly or only in neurons sometimes with cell-specific patterns of expression. The NCS protein family is encoded by 14 genes in the human genome that possess four EF hand domains of which 2 or 3 bind calcium. The NCS proteins are high-affinity calcium-binding proteins that act as calcium sensors rather than calcium buffers as they undergo conformational changes on calcium-binding and regulate target proteins. Their ability to associate with membranes either constitutively or in response to calcium elevation allows the NCS proteins to discriminate between different spatial and temporal patterns of calcium signals. Several of the NCS proteins show changes in expression levels in specific disease states. Recent work has established several physiological roles of these proteins including diverse actions on gene expression, ion channel function, membrane traffic of ion channels and receptors and in the control of apoptosis. The NCS proteins act as calcium sensors for diverse functions and also as regulators of calcium signals through their effects on ion channels.

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SA24

How novel Ca^{2+} sensors regulate Ras signalling

P.J. Lockyer

Laboratory of Molecular Signalling, The Babraham Institute, Cambridge, UK

Cells maintain a steep Ca^{2+} ion concentration gradient between the cytosol and the extracellular space, allowing Ca^{2+} to operate as a universal second messenger mobilized from internal stores and the extracellular medium. In the early 1970s it was found that normal cells require extracellular Ca^{2+} to undergo cell proliferation, as least *in vitro*. Other studies demonstrated that *in vivo* extracellular Ca^{2+} gradients regulated cell proliferation and differentiation in tissues e.g. the epidermis and colonic epithelium. Ca^{2+} starvation of non-tumourigenic cells in culture induced cell cycle withdrawal, yet transformation by just a single oncogene - such as the small GTPase Ras - bypassed the need for a Ca^{2+} source to drive cell proliferation.Oncogenic Ras GTPases are found in approximately 15% of human tumours and are known to be necessary and sufficient for tumour induction and maintenance in cancer models e.g. lung adenocarcinoma. These mutant proteins have defective GTPase activity, normally controlled by the binding of GTPase-activating proteins (GAPs), to which oncogenic Ras is insensitive. The canonical view of Ras signalling focuses on receptor/non-receptor tyrosine kinase signalling cascades where a Ras GEF (SOS) is recruited via adapters to the receptor in order to activate Ras at the plasma membrane. Ras is deactivated by the recruitment of p120 Ras GAP via SH2 domains to phosphotyrosine residues on the activated receptor. The delineation of this pathway a decade ago was a major advance in signal transduction research. More recently, multiple families of Ras GEFs (GRF and GRP/CalDAG-GEF) and Ras GAPs (SynGAP and GAP1) have been identified and several of these proteins appear to be regulated dynamically by diacylglycerol (DAG) and/or Ca^{2+} - each a 'product' of phospholipase C (PLC) signalling. Concurrently a novel class of PLC, PLCε, was discovered which operates as a Ras effector in some contexts. Thus, a battery of potential Ras signalling modulators converges with PLC-dependent second messenger pathways.Recently, Ca^{2+} has been directly implicated in the control of Ras cycling with the discovery of twin Ca^{2+} triggered Ras GAPs: RASAL (Ras GTPase-activating-like) and CAPRI (Ca^{2+}-promoted Ras inactivator). I will present work from our lab that demonstrates their intrinsic Ras GAP function using cellular reporter assays of the Ras activation state, combined with spatio-temporal analysis of Ca^{2+}-triggered CAPRI/RASAL translocation. CAPRI and RASAL are dynamic C2 domain-dependent Ca^{2+} sensors, like conventional protein kinase C (PKC) and cytosolic phospholipase A2. We have discovered that these GAPs sense Ca^{2+} signals very differently. Thus, it seems likely that CAPRI and RASAL play novel roles in the information processing of Ca^{2+} signals at the level of the Ras GTPase.

SA25

Bcl-2 interacts with inositol 1,4,5-trisphosphate receptors and inhibits inositol 1,4,5-trisphosphate mediated calcium release from the endoplasmic reticulum

C. Distelhorst[1], L. Roderick[2], M. Bootman[2] and M. Berridge[2]

[1]Case Western Reserve University, Cleveland, OH, USA, [2]The Babraham Institute, Cambridge, UK and [3]University of Cambridge, Cambridge, UK

Inositol 1,4,5-trisphosphate (InsP3) induced release of calcium ions from the endoplasmic reticulum (ER) can induce apoptosis directly by activating cell extrinsic and intrinsic death pathways. Here we report that the anti-apoptotic protein Bcl-2, which resides on the ER membrane, inhibits InsP3-mediated calcium release. Cytoplasmic calcium was measured in the WEHI7.2 T cell line with the calcium indicator Fura-2 (AM), both fluorometrically and by single cell imaging. Bcl-2 markedly suppressed cytoplasmic calcium elevation induced by either antibody to the CD3 component of the T cell receptor or D-myo InsP3BM, a cell permeant InsP3 ester. This action of Bcl-2 was not due to a decrease in the thapsigargin-releasable ER calcium pool, nor was it due to a reduction in lumenal free calcium concentration, measured in digitonin-permeabilized cells with the low affinity dye Fura-2FF (AM). Instead, we found that Bcl-2 suppressed the activity of InsP3 receptors without altering their level of expression. The mechanism

involved an interaction of Bcl-2 with InsP3 receptors, demonstrated by co-immunoprecipitation. This interaction decreased both the state of InsP3 receptor phosphorylation, measured by 32P-labeling, and the affinity of InsP3 receptors for InsP3, measured by a competitive binding assay. In summary, these findings indicate that Bcl-2 is a novel InsP3-receptor interacting protein that suppresses InsP3-linked apoptotic signals by reducing the responsiveness of InsP3 receptors to InsP3.

SA26

Calcium signaling and contraction in smooth muscle of airways and arteries of lung slices

M. Sanderson, A. Bergner and J. Perez

University of Massachusetts Medical School, Worcester, MA, USA

Asthma and pulmonary hypertension involve excessive contractile activity of the smooth muscle of the small airways and arteries within the lungs. While isolated cell studies have provided significant advances in smooth muscle cell physiology, an understanding of how this physiology relates to vessel resistance, requires a preparation that retains the architecture of the pulmonary airways and arteries. One such preparation is the "lung slice". We have used mouse lung slices to simultaneously investigate the contractile and underlying intracellular calcium ([Ca2]i) responses of smooth muscle cells (SMCs) in both intrapulmonary airways and arteries with phase-contrast and confocal microscopy. Lung slices (~100 mm thick) were cut from lungs after filling the alveoli and arteries with agarose and gelatin, respectively. In response to ACH, a sustained contraction of airways was accompanied by a transient increase in [Ca2]i followed by Ca2 oscillations. However, the arteries did not respond to ACH. By contrast, 5-HT induced a sustained contraction in both the airways and arteries and a large transient increase in [Ca2]i followed by oscillations. In both airways and arteries, in response to either ACH or 5-HT, the frequency of the Ca2 oscillations increased with agonist dose and correlated with increased sustained contraction. The removal of extracellular Ca2 did not block the initiation of contraction or the [Ca2]i transients, but did attenuate the Ca2 oscillations and this resulted in relaxation. By contrast, high extracellular K induced transient and unsynchronized contractions of both airway and artery SMCs (twitches) although a greater contraction was observed in arteries. While the [Ca2]i responses of both airway and artery SMCs to high K was oscillatory, the frequency of the oscillations were generally slower than those induced by agonists. The removal of extracellular Ca2 or treatment with Ca2 channel blockers prevented contraction and [Ca2]i signaling induced by K . In summary, these results imply that a sustained contraction of pulmonary SMCs relies on fast repetitive Ca2 oscillations mediated by Ca2 release from intracellular stores.

SA27

Polycystin ion channel signaling complex

P. Delmas

CNRS, Integration des Informations Sensorielles (UMR 6150), Marseille, France

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic disorder occurring in approximately 1 in 1000 births in human, causing renal failure in 50 % of affected individuals and even deaths. In most cases, the disease arises as a consequence of mutations in either of two genes PKD1 and PKD2, which encode polycystins 1 and 2, respectively. The PKD2 family of genes has also been implicated in sensory responses through protein localization on primary cilia of epithelia and neurons and may be critical for left-right asymmetry of the body plan (situs inversus)(1) and sperm motility (2). PKD1 is a large plasma membrane protein involved in cell-cell interaction, whereas PKD2 is a Ca2+-permeable channel belonging to the TRP channel superfamily. Although PKD1 and PKD2 interact and are thought to be part a common sensory signaling pathway, little is known about the gating mechanism of these polycystin complexes. Here, we show that PKD1 and PKD2 form functionally associated 'subunits' of a heteromultimeric signaling complex that functions either as a Ca2+-permeable cation channel (3) or as a G-protein-coupled receptor (4). Within these complexes, PKD1 acts as a cell surface receptor that controls the gating of the PKD2 channel via a structural rearrangement. This coupling is disrupted in disease-associated mutant forms of PKD1 that are incapable of heterodimerization with PKD2.Evidence will be also presented that suggest that polycystin proteins co-assemble to form junctional 'plasma-ERosome' complexes in which plasmalemmal PKD1 gates intracellular PKD2 by a mechanism similar to those outlined in the conformational coupling hypotheses of excitation-contraction and store-operated channels. Thus, PKD1 acts as a prototypical membrane receptor that concordantly activates G-proteins and regulates Ca2+ transport via PKD2 channels, a bimodal mechanism that may account for the multifunctional roles of polycystin proteins in fundamental cellular processes of various cell types.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

SA28

Imaging IP_3-mediated Ca^{2+} signaling in cortical neurons: Physiology and implications for Alzheimer's disease

G. Stutzmann, F. LaFerla and I. Parker

University of California, Irvine, Irvine, CA, USA

Ca^{2+} signals regulate numerous cellular functions, and are generated by Ca^{2+} ions entering the cytosol from extracellular sources (e.g. entry through voltage gated channels), and by Ca^{2+} ions liberated from intracellular stores through inositol triphosphate (IP_3) receptors and/or ryanodine (RyR) receptors. Ca^{2+} ions subserve particularly complex signaling roles in neurons, regulating functions ranging from gene transcription to modulation of membrane excitability. Although the roles of extracellular Ca^{2+} entry are well characterized, the roles of intracellular Ca^{2+} liberation are less well understood. We therefore used a combination of techniques including whole cell electrophysiological recordings, photolysis of caged IP_3 and 2-photon microscopy to image IP_3-evoked Ca^{2+} signals in pyramidal cortical neurons in mouse brain slices. We describe here both the physiological functioning of the IP_3-signaling pathway in neurons, and the involvement of disruptions of this pathway in the pathogenesis of Alzheimer's disease (AD). Physiological Ca^{2+} responses to photoreleased IP_3 in wild-type mice varied greatly between different neurons; however, within IP_3-responsive neurons, the soma invariably showed the highest sensitivity, and Ca^{2+} signals increased nonlinearly with [IP_3]. IP_3-evoked Ca^{2+} release was potentiated by Ca^{2+} entry during action potentials and vice versa, indicating bidirectional facilitation between intra- and extracellular Ca^{2+} sources. In particular, IP_3-evoked Ca^{2+} signals strongly inhibited spike firing through activation of an outward hyperpolarizing membrane conductance. Thus, the IP_3/Ca^{2+} signaling pathway serves as a powerful and sustained modulator of excitability in cortical neurons and may mediate complex reciprocal interactions between electrical signals and chemical signals arising through metabotropic synaptic inputs (Stutzmann et al., 2003). The dark side of the IP_3/Ca^{2+} signaling pathway, however, is that perturbations outside its normal operating range can lead to pathological changes, including necrotic and apoptotic cell death. For example, disruptions in intracellular Ca^{2+} signaling have been proposed to underlie the pathophysiology of Alzheimer's disease (AD), and it has recently been shown that AD-linked mutations in the *presenilin 1* gene (*PS1*) enhance IP_3-mediated Ca^{2+} liberation in nonexcitable cells (Leissring et al., 2001). However, little is known of these actions in neurons, which are the principal locus of AD pathology. We therefore examined how *PS1* mutations affect Ca^{2+} signals and their subsequent downstream effector functions in cortical neurons. We found that IP_3-evoked Ca^{2+} responses are more than threefold greater in *PS1* knock-in mice relative to age-matched non transgenic controls, and electrical excitability was concomitantly reduced via enhanced Ca^{2+} activation of outward hyperpolarizing K^+ conductances. Moreover, IP_3 receptor levels in cortical homogenates were not different between knock-in and control mice, suggesting that the exaggerated cytosolic Ca^{2+} signals likely result from increased store filling and not from increased flux through additional IP_3-gated channels. Notably, action potential-evoked Ca^{2+} signals were unchanged, indicating that *PS1* mutations specifically disrupt intracellular Ca^{2+} liberation rather than impairing cytosolic Ca^{2+} buffering or clearance. A limitation of the *PS1* knock-in model is that these mice do not go on to develop the plaques and tangles characteristic of AD. We have thus begun to investigate Ca^{2+} signaling in a novel triple transgenic model of AD, where mice expresses mutant *PS1, APP* and *tau* genes simultaneously, and display the histopathological markers of AD at later ages (Oddo et al., 2003). These studies in transgenic AD mouse models suggest that *PS1* mutations are predominantly responsible for the IP_3-mediated Ca^{2+} dysregulation in neurons, and that Ca^{2+} dysregulation precedes the histological and cognitive impairments observed in AD. The profound effects of these mutations on neuronal Ca^{2+} and electrical signaling patterns may contribute to the long-term pathophysiology of AD.

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