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Signalling mechanisms

Editorial Overview

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Morgan Sheng's research focuses on the molecular structure of synaptic junctions and on the mechanisms that regulate the formation, function and plasticity of excitatory synapses. Using biochemical and molecular genetic approaches, his lab has identified and characterized many of the specific proteins and protein–protein interactions that underlie the functional architecture of the postsynaptic membrane and dendritic spine.

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Terry Snutch's research focuses on the molecular, genetic and physiological analyses of invertebrate and vertebrate voltage-gated calcium channels. In addition to describing the molecular basis for calcium channel heterogeneity, the work has contributed to our understanding of channel regulation by second messengers such as G proteins and protein kinases.

Introduction

In neurobiology, the term 'signaling mechanisms' covers wide ground, ranging from the three-dimensional structure of a neuronal receptor protein, to integration of electrical signals by a segment of dendrite, to the control of neuronal morphogenesis by complex signaling networks. The rapid advances seen in this area in the past few years have been propelled by the increasing sophistication of molecular genetic manipulation, imaging technologies and electrophysiological approaches. This issue of Current Opinion in Neurobiology reflects the breadth of research in neuronal signaling mechanisms and highlights the exciting progress made in many specific areas.

The ion channels and neurotransmitter receptors that control membrane excitability and synaptic transmission lie at the heart of neuronal signaling. Our understanding of ion channels has been catalyzed by technical advances, often borrowed from other disciplines. As reviewed by Beene and co-workers, the conventional site-directed mutagenesis approach for studying ion channel structure and function has been taken to the next level through a nonsense suppression strategy that results in the incorporation of unnatural amino acids. At least with respect to ion channels, this approach has proven to be a powerful tool and has surpassed conventional site-directed mutagenesis in the breadth of functional modifications possible.

Although the incorporation of unnatural amino acids provides a wealth of new information, obtaining the 3D crystal structure of signalling molecules still remains the pot of gold at the end of the rainbow for resolving structure and function issues. Recently, there has been outstanding progress in solving the 3D structure of several ion channels, but a similar degree of success has been largely elusive for the large and diverse superfamily of G protein coupled receptors. Getting us at least part way there, Jingami and co-workers review recent developments in defining the atomic structure of the ligand-binding region of the metabotropic glutamate receptor. The crystal structure provides important clues to the unique mechanism of glutamate binding and receptor activation, and presages many future surprises as the structure of other G protein coupled receptors are revealed.

Despite the vast amount of information gained from current genome sequencing efforts, it is important to realize that the heterogeneity of ion channels and neurotransmitter receptors is not only generated by multiple genes and alternative splicing. A further level of diversity stems from RNA editing, in which a subset of adenosines in mRNAs are post-transcriptionally modified to inosine residues (A-to-I editing), thus changing the amino acid sequence. RNA editing was first described by Seeburg and co-workers [1] for ionotropic glutamate receptors, but it is now known to affect certain serotonin receptors and potassium channels. In their review, Seeburg and

Hartner emphasize that the functional consequences of RNA editing continue to expand, and now include the regulation of channel/receptor gating, conductance, permeability, receptor maturation and assembly. Importantly, the changes at the biophysical level appear to have significant consequences for the organism, influencing synaptic plasticity and susceptibility to seizures.

Although they are not as structurally diverse as potassium channels, or as functionally diverse as calcium channels, sodium channels remain viewed as the workhorses of the nervous system. In the present issue, Goldin reviews recent important developments concerning the molecular and biophysical distinctions between fast and slow inactivation of sodium channels. Alterations in the sodium channel α and β subunits that affect inactivation have been shown to be responsible for certain human cardiovascular and central nervous system disorders. Another clinically important process involving sodium channels is the pathogenesis of a variety of chronic pain conditions. In recent years there has been a concerted effort to define specific sodium channel subtypes that mediate the pathophysiology of neuropathic pain and to exploit these targets for therapeutic intervention. Lai and co-workers describe recent developments in this field and find evidence that strongly points towards the $Na_V1.8$ subtype as being essential for establishment of the hyper-excitability that is associated with persistent pain states. Given the close evolutionary relationship of sodium channel subtypes it will be no easy feat to define selective $Na_V1.8$ antagonists.

Sodium channels are largely restricted to mediating electrical activity directly, whereas voltage-gated calcium channels are charged with contributing not only to membrane excitability but also to the regulation of a plethora of intracellular signalling functions, from neurotransmitter release to gene transcription. The functional diversity of calcium channels arises from a multigene family of pore-forming $\alpha 1$ subunits, which are further regulated by a set of auxiliary subunits. Arikkath and Campbell review the ever expanding list of functions in which calcium channel auxiliary subunits are implicated, ranging from biophysical modulation to disease states such as ataxia and epilepsy.

One of the most studied aspects of calcium channels is their direct contribution to triggering neurotransmitter release. Although the presynaptic N-type and P/Q-type calcium channels appear to be the major players in this process, they by no means act in isolation. Indeed, as reviewed by Spafford and Zamponi, the presynaptic terminal is proving to be quite crowded, with biochemical, molecular, genetic and physiological evidence all pointing to complex interactions among calcium channels, SNARE proteins and a variety of second messenger molecules and scaffolding proteins.

Presynaptic voltage-dependent calcium channels mediate the calcium influx that triggers neurotransmitter release, but which molecules are responsible for coupling this calcium elevation to synaptic vesicle fusion within a couple of hundred microseconds? Yoshihara and co-workers review the mounting biochemical and genetic evidence that synaptotagmin I, a synaptic vesicle-enriched protein, is the crucial calcium sensor in presynaptic terminals. In particular, *in vivo* electrophysiological analysis of specific mutants demonstrates that synaptotagmin I is responsible for the fourth order calcium cooperativity of release, and for the rapid and synchronized synaptic vesicle fusion triggered by calcium.

The intricate molecular machinery for neurotransmitter release provides abundant substrates for effecting synaptic plasticity. Although much recent work has focused on postsynaptic mechanisms for controlling the strength of synaptic transmission (e.g. the trafficking of AMPA receptors to and from synapses), presynaptic mechanisms of synaptic plasticity are important and perhaps underappreciated. The article by Liu reviews at a fundamental level how the size of the postsynaptic response can be influenced by changes in the amount and kinetics of the neurotransmitter released, and summarizes the experimental evidence that presynaptic mechanisms are important for controlling the efficacy of synaptic transmission.

On the postsynaptic side, we now know a great deal about the regulated trafficking of glutamate receptors that underlies the plasticity of excitatory synapses. However, perhaps the mobility of glutamate receptors is only the tip of the iceberg. Inoue and Okabe review recent green fluorescent protein (GFP) time-lapse imaging studies that reveal a surprising mobility of the other components of the postsynaptic density. Postsynaptic scaffolding proteins translocate dynamically not only during development of synapses but also in response to activity in mature synapses. Are similar processes going on in inhibitory synapses? The review by Kittler and Moss summarizes our state-of-the-art understanding of how $GABA_A$ receptor function is controlled by phosphorylation, interactions with cytoplasmic anchoring proteins, and receptor exocytosis and endocytosis. The GABAergic synapse emerges as a dynamic organization of GABA receptors and associated scaffolding proteins and modulatory protein kinases, not unlike the glutamatergic synapse.

Membrane trafficking controls not only the surface expression of classical neurotransmitter receptors but also the G protein-coupled receptors that are involved in responses to neuromodulators. An excellent example is provided by the opioid receptors, which are of great clinical significance as the targets of morphine and related analgesics. Von Zastrow and co-workers review the cell biology of opioid receptor endocytosis and recycling, and discuss recent evidence that these mechanisms might

relate to adverse adaptations to opiate use, such as drug tolerance and dependence.

Upon binding to excitatory neurotransmitters, glutamate receptors initiate a local signal (such as calcium influx) that is eventually propagated to the nucleus, where transcription of specific genes is either induced or repressed. Although several biochemical pathways have been delineated from the synapse to the nucleus, much remains to be learned about the precise cell biology of mechanisms that transmit localized signals initiated by the opening of a membrane ion channel/receptor all the way to the DNA promoter region of the responsive gene. This subject is covered by Tsien and co-workers, who emphasize the neurobiological 'logic' behind the complex signaling mechanisms. A major theme is that where the signal is generated at the neuronal surface carries valuable information; in other words, that different receptors/ion channels trigger distinct signal transduction processes inside the cell. NMDA receptor signaling offers a particularly striking example of this spatial specificity. Vanhoutte and Bading review the recent evidence that activation of synaptic and extrasynaptic NMDA receptors can have opposite effects on phosphorylation of the transcription factor CREB, gene expression, and cell survival.

How are the signals from postsynaptic ion channels/receptors and individual synapses integrated by neurons? Hausser and Mel review this area, which has benefited from advances in electrophysiology, imaging and computational modeling. A wide range of neuroscientists, from molecular to systems, are likely to find this elegant discussion of functional dendritic compartments both stimulating and educational.

The above articles deal largely with signaling mechanisms in the mature nervous system. During development, synapses and circuits are formed by the growth and connection of axons and dendrites. Rapid progress has been made in understanding the molecular mechanisms that control both the pre- and postsynaptic aspects of neuronal morphogenesis (reviewed by Lundquist, and by Miller and Kaplan, respectively). Although they are morphologically distinct, it is clear that the development of axons and dendrites share some common signaling pathways, in particular, small GTPases of the Rac family. As they are also likely to be involved in the activity-dependent remodeling of axons and dendrites, such mechanisms are relevant for plasticity of the mature nervous system.

Conclusions

At times, the seemingly endless number of signaling molecules and the variety of interconnected pathways can be daunting, especially in the nervous system. More quantitative four-dimensional analysis and computational and 'systems biology' approaches will be necessary to place each of these signaling systems in their proper context within the developing and mature nervous systems and various pathophysiological conditions.

Reference

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