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Introduction: tuning the signal: regulation of postsynaptic receptor properties

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Introduction

The control of synaptic strength is a key component of the ability of neurons to adapt to changing environmental conditions. Largely for this reason, the mechanisms which neurons use to modify their responses to altered input activity have attracted considerable attention. Regulation of postsynaptic receptor properties is a common theme in studies of various neuroplastic phenomena, including experience-dependent plasticity during development (e.g. ocular dominance plasticity), and long-term potentiation and depression (LTP/LTD) of synaptic strength. Our goal in this review is to give readers an appreciation of the dynamic nature of ionotropic receptors and their regulation. This review will illustrate the diverse mechanisms of receptor regulation using specific examples, many of which focus on ionotropic glutamate receptors. The principles outlined may apply equally to other ionotropic receptors as well as to some G-protein-coupled receptors, although the response of a given receptor population to a particular stimulus (e.g. chronic depolarization) cannot be predicted absolutely and is dependent on many factors, including receptor type, developmental stage, acute versus chronic and global versus focal patterns of stimulation, etc. (see [1]). By this we hope to provide a brief survey of some of the mechanisms of regulation of ionotropic receptors and the relevance of receptor regulation to neural function and dysfunction.

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Multiple stages of receptor regulation

The composition and functional characteristics of postsynaptic receptors at a given synapse is the product of various regulatory processes. This review has been organized to reflect the multiple levels of receptor regulation that will be discussed in the following sections and in greater detail by the contributing authors.

Genomic control: differential subunit expression and posttranscriptional editing

Receptors are heteromeric protein complexes composed of multiple subunits. The functional properties of receptors are dependent on their subunit composition. Some subunits are obligatory to form functional channels. For example, the NR1 subunit of *N*-methyl-D-aspartate (NMDA) receptors houses the glycine-coagonist binding site which is necessary to form operational NMDA receptors (see [2] for review). For the α-amino-3-hydroxy-5-methyl-isoxazoleproprionate (AMPA) subtype of glutamate receptors, expression of the GluR2 subunit strongly influences the Ca^{2+} permeability of the receptors [3]. Differential subunit expression during receptor biosynthesis is the first level of receptor regulation and is an important determinant of mature receptor functionality. Several receptor subunits exist in alternatively spliced forms such as the flip/flop and long/short splice variants of glutamate receptors. A further level of complexity arises due to posttranscriptional editing (e.g., the Q/R site of GluR2). Differential subunit expression, alternative splicing and posttranscriptional editing of messenger RNA (mRNA) transcripts encoding receptor subunit peptides represent genomic mechanisms of receptor regulation, and this topic is reviewed in the article by Burnashev and Rozov.

Posttranslational control

Receptor protein maturation. During maturation of nascent receptor subunit polypeptides in the endoplasmic reticulum (ER)-Golgi complex, posttranslational modifications occur which can influence the functional properties of the mature proteins. One such modification is the glycosylation of subunit proteins. Early radioligand binding studies using the glutamate analog [^{3}H]AMPA identified the presence of high- and low-affinity AMPA binding sites. The significance of these multiple binding sites for AMPA has been an issue of some debate (see [4] for recent review). Standley and Baudry have provided evidence that the high-affinity AMPA binding site represents intracellular, incompletely glycosylated AMPA receptors, whereas the low-affinity binding site represents mature cell surface receptors. In addition, these authors review evidence suggesting that ionotropic receptor glycosylation can also influence receptor affinity for ligands, current responses in vitro, and the effects of lectins such as concanavalin A on AMPA receptor desensitization.

Synaptic targeting and stabilization. Synaptic transmission requires the establishment of a highly ordered arrangement of specialized membrane domains. Presynaptic neurotransmitter release machinery must be aligned with specialized membrane domains of the postsynaptic cell (i.e. dendritic spines) which house appropriate populations of receptors. The processes involved in targeting nascent receptors to appropriate dendritic structures and the stabilization of newly inserted receptors within postsynaptic membrane elements are critical to our understanding of neurogenesis and of the development of functional synapses and neural circuits. Activity plays an important role in governing the formation and maintenance of synapses during development [5–7] and in determining the functional characteristics of established synapses [8, 9]. Such issues are addressed by Bolton et al., who review the mechanisms behind activity-dependent targeting and stabilization of glutamate receptor subtypes.

Trafficking. The number of synapses and receptor subtypes of an average neuron, the interplay between receptor populations, indirectly by shared second-messenger pathways, via direct receptor-receptor modulation, or by other signaling molecules (e.g. insulin; see Man et al.) promises to add many future surprises and levels of complexity to the story of receptor regulation. The control of cell-surface-receptor expression by membrane insertion and endocytotic internalization is the focus of the article by Man et al. We note in regard to the latter that insulin appears to have opposing action on AMPA and γ-amino butyric acid ($GABA_A$) receptors, decreasing the former while increasing the latter. Such actions may provide a partial basis for the types of 'synaptic scaling' described by Turrigiano et al. [9] and/or the sliding scale for synaptic modification described by Bear [8] in relation to homeostasis and synaptic plasticity, respectively.

Receptor modification. Several articles in the review touch on emerging notions of mechanisms of posttranslational receptor modification, notably by glycosylation (Standley and Baudry), phospholipase A_2 modulation (Massicotte), and by oxidation-reduction reactions (Choi and Lipton). These descriptions serve as a welcome balance to a field that has long been dominated by the notion that most receptor regulation is governed solely by phosphorylation/dephosphorylation reactions. Evidence for phosphorylation as a key mechanism governing ionotropic regulation has been the subject of numerous reviews [10–13] and will not be discussed at length here. In many of the following articles, however, phosphorylation reactions are implicitly recognized to be involved at each of the stages cited above: Direct phosphorylation of consensus sequences on receptor subunit proteins by particular kinases (e.g. PKA, CamKII, PKC) appears to be an early stage of the regulatory process in response to agonist stimulation or neural activity and can be expected to have major roles controlling the various proteins involved in receptor trafficking (see Bolton et al.) and in the expression of receptor subunits (see Burnashev and Rozov). The additional regulatory mechanisms discussed in the following articles appear to act in concert with phosphorylation to provide an exquisite level of control of receptor expression and function.

In regard to redox modulation it is perhaps worthwhile to emphasize that it is becoming clear that endogenous redox agents may be extremely important modulators of receptor function. In their article, Choi and Lipton provide important evidence to suggest a fundamental role of nitric oxide (NO) in maintaining NMDA receptor activity within a normal range of function via oxidation. However, an equally important set of functions can be attributed to an endogenous reducing agent, the tripeptide glutathione (GSH), which may act on ionotropic receptors containing cysteine residues, e.g., NMDA subtypes, AMPA, glycine and $GABA_A$ receptors (for review, see [14]). Janaky et al. [14] propose that GSH may have multiple roles at ionotropic receptors: At low concentrations, GSH may act as a γ-glutamyl compound displacing glutamate from the NMDA binding site [15]. Extracellular and intracellular GSH action may also lead to *S*-nitrosoglutathione formation [16, 17], the latter serving as a buffer for NO. At higher concentrations, GSH may act like any other thiol redox compound: Notably, at the NMDA receptor it may serve to oppose the oxidizing action of NO and also

form complexes with Zn^{2+} [18], both actions impacting NMDA receptor function. The foregoing makes clear that redox modulation of ionotropic receptors by naturally occurring molecules offers yet another fundamental level of control whose ultimate impact on neural activity remains to be determined.

Physiological and pathophysiological consequences. Several articles in this review deal extensively with the role played by aspects of receptor regulation on neuronal plasticity (Massicotte; Kullmann et al.) and pathology; all touch upon it to varying degrees. Plasticity in these instances is represented by the various phenomena termed LTP and LTD, considered by many to represent the physiological basis of learning and memory (for contrasting views, see [19]). Although not definitive, current data tend to support the view that such changes in synaptic gain are likely postsynaptic in nature, probably involving alterations in receptor characteristics. Massicotte and Kullmann et al. attempt to place the diverse and often contradictory results into context in order to better understand the relationship of receptor regulation to both plastic and pathological events. What emerges is the view that the role played by receptors in such events is complex and variable. How such receptor modifications come about, how, or if, they are maintained, and the role of the various stages in regulation (e.g. trafficking vs. in situ surface regulation of function) are key questions that must be resolved.

Future directions

Various issues in receptor regulation seem likely to be the focus of future investigations. First, and most obviously, much more remains to be discovered about the 'machinery' of receptors and regulation, the various scaffolding and trafficking proteins, their interactions, the different types of cells in which they are expressed, etc.

More fundamental, however, are questions about receptor function and regulation in relation to neural function. For example, what is the role of age and/or development in receptor expression, function and regulation? As many of the characteristics of neurons and synapses appear to be highly age dependent, it is not unreasonable to suspect that the basis for much of this will reside in age dependent variations in receptor expression, trafficking, and regulation. This subject is still largely unexplored but clearly may be crucial for understanding normal synaptic function, mechanisms of neuroplasticity, and the intersection of plastic processes with pathological outcomes (see [19]).

Another fundamental topic will be that of the role of receptors in pathological conditions and the genetic or environmental factors which affect receptor expression and function. Alterations in receptor characteristics are observed in various neurological disease states, and it will be crucial to determine if these are causal or incidental to the mechanisms underlying neurodegeneration [20, 21].

These and other topics seem formidably complex. However, the current pace of research, as typified by the articles in this review, suggests that they, too, will soon be within reach.

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