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Invited review

Calcium and endocannabinoids in the modulation of inhibitory synaptic transmission

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Abstract

Synapses in the central nervous system can be highly plastic devices, being able to modify their efficacy in relaying information in response to several factors. Calcium ions are often fundamental in triggering synaptic plasticity. Here, we will shortly review the effects induced by postsynaptic increases of calcium concentration at GABAergic and glycinergic synapses. Both postsynaptic and presynaptic mechanisms mediating changes in synaptic strength will be examined. Particular attention will be devoted to phenomena of retrograde signaling and, specifically, to the recently discovered role, played by the endocannabinoid system in retrograde synaptic modulation.

Keywords: Synaptic plasticity; GABA receptors; Glycine receptors; Retrograde signalling; Patch-clamp

1. Introduction

In the adult mammalian nervous system the main inhibitory drive is provided by the neurotransmitters GABA and glycine. Both molecules are released from specialized presynaptic terminals and activate anion-selective receptor-operated channels, thus stimulating rapid changes of chloride fluxes through postsynaptic membranes (see references in [1]). GABA controls the inhibitory neuronal network in the majority of brain regions, whereas glycinergic synapses are predominant in the spinal cord, in the brainstem, and in some areas involved in the processing of sensory signals, like central auditory pathways and the retina. Moreover, presynaptic terminals can co-release glycine and GABA, therefore providing neurotransmission via activation of mixed populations of postsynaptic receptors [2]. The efficacy of neurotransmission at inhibitory synapses is highly plastic: it can be modulated either by phosphorylating/dephosphorylating cycles, receptor turnover and receptor clustering on the postsynaptic side [3–5], or through modulation of neurotransmitter release at presynaptic terminals [6,7].

Our review will aim at discussing relatively recent observations concerning new roles of Ca^{2+} ions in the regulation of inhibitory transmission. In particular, we will focus on the following mechanisms of Ca^{2+} action: (i) modulation of GABAergic and glycinergic receptor function; (ii) presynaptic inhibition of neurotransmitter release due to retrograde signaling.

2. Calcium-induced modulation of $GABA_A$ and glycine-receptor channels: postsynaptic mechanisms

Increases of postsynaptic Ca^{2+} concentration constitute the initial triggering signal for several forms of short- and long-term plasticity at both excitatory and inhibitory synapses [8–10]. At excitatory synapses, augmentations of postsynaptic Ca^{2+} provide a direct hebbian mechanism of detection of coincident post- and presynaptic activity. At inhibitory synapses, the requirement for Ca^{2+} augmentations implies that, during plasticity induction, stimulation protocols must also involve excitatory systems (for example,

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glutamatergic afferents) able to activate postsynaptic Ca^{2+} sources [8].

Increases of Ca^{2+} can be obtained via depolarization-dependent mechanisms (leading to voltage-gated Ca^{2+} channel opening), concomitantly or not with influx through Ca^{2+}-permeable ligand-gated ionotropic receptors (like NMDA or some combinations of AMPA receptor channels), via activation of metabotropic receptors, or via events leading to Ca^{2+} release from intracellular stores [11]. There are, nevertheless, some exceptions. First, GABAergic and glycinergic transmission itself can act in a depolarizing way in particular conditions of high intracellular chloride concentration. For example, at early stages of postnatal development the release of inhibitory neurotransmitter can cause neuronal depolarizations and, thus, Ca^{2+} elevations [12]. Second, astrocytes possess a Ca^{2+}-dependent machinery, which can induce changes in synaptic efficacy at GABAergic synapses via release of an effector molecule, probably glutamate [13]. Finally, G protein-coupled metabotropic receptors can trigger the release of plasticity-inducing messengers in a Ca^{2+}-independent way [14–16].

Although it has been known for many years that Ca^{2+} increases can modify the responsiveness of $GABA_A$ receptors to their agonist (sensory neurons in the bullfrog: [17]), and to plastic changes of central GABAergic synapses (LTD in the hippocampus: [18]), little information is available on the mechanisms at the base of these modifications. This appears particularly surprising if compared with the wealth of available data concerning LTP and LTD phenomena at glutamatergic synapses [9,10].

In this part of the review we shortly consider three mechanisms of modulation which could lead to postsynaptic Ca^{2+}-dependent plasticity at GABAergic and glycinergic synapses (Fig. 1): (i) receptor phosphorylation, (ii) rapid modulation of receptor function by Ca^{2+} and (iii) changes in the number of functional receptors. Finally, we will discuss the phenomenon of rebound potentiation (RP), one form of postsynaptic plasticity at GABAergic synapses in the cerebellum.

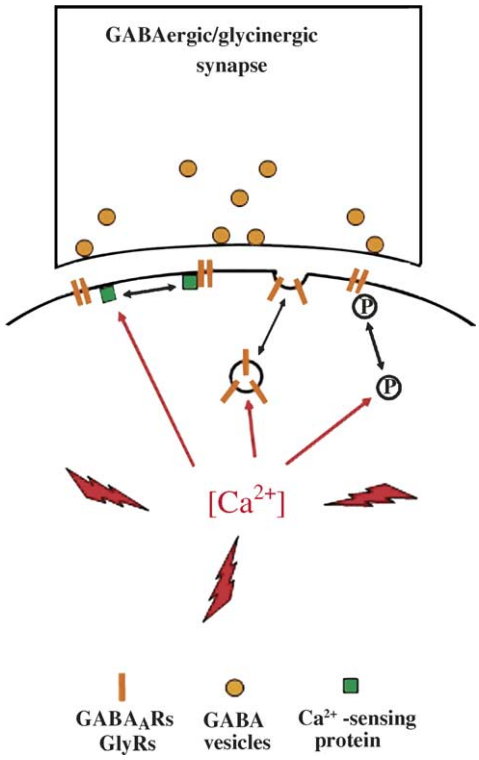

Fig. 1. Three main postsynaptic mechanisms of GABAergic and glycinergic receptor modulation by Ca^{2+}. First, Ca^{2+} elevations can trigger enzymatic pathways leading to receptor phosphorylation/desphophorylation, thereby modifying the synaptic response (right red arrow). Second, receptor-shuffling vesicles can be induced to fuse with the postsynaptic membrane, thus permitting the insertion of new functional receptors and increasing synaptic strength (middle red arrow). On the other hand, increased withdrawal and recycling may lead to inhibition of synaptic strength. Third, Ca^{2+}-sensing proteins (green squares) can be activated by Ca^{2+}, and unbind from (or bind to) synaptic receptors changing their functionality (left red arrow). A mechanism not visualized here, but discussed in the text, consists in the lateral traslocation of receptors from extrasynaptic to synaptic zones (or vice versa), where they can be immobilized and stabilized by scaffolding protein complexes.

2.1. Receptor phosphorylation

Modulation of $GABA_A$Rs and GlyRs by phosphorylation was demonstrated both in heterologous expression systems, and in neurons (review [4,5,19]). The intracellular regions of some $GABA_A$R and GlyR subunits, particularly a long cytoplasmic loop comprised between the third and fourth transmembrane domain, contain consensus phosphorylation sites for both Ser/Thr and Tyr protein kinases. Moreover, several kinases and phosphatases are activated through Ca^{2+}-dependent pathways that link Ca^{2+} signaling cascades to regulation of protein phosphorylation. Thus, phosphorylation and dephosphorylation of $GABA_A$Rs and GlyRs appear to be possible important postsynaptic processes transducing Ca^{2+} increases into modulation of synaptic transmission.

2.1.1. $GABA_A$ receptors

The literature on $GABA_A$R phosphorylation is extremely wide and complex, and it has been thouroughly discussed in reviews specifically devoted to the subject [4,5,19]. Here, we will try to give only a general picture, inviting the interested reader to address the quoted references for more details.

The intracellular domains of $GABA_A$Rs are known to contain phosphorylation sites for several kinases. In heterologous expression systems, protein kinase A (PKA), protein kinase C (PKC), protein kinase G (PKG) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) can phosphorylate the β1, β2, and β3 subunits at a common serine residue located in the cytoplasmic loop (Ser409 in β1 and β3, Ser410 in β2). The two so far characterized γ2 subunits (short and long, for an 8 amino acid difference in composition) have instead been found to be phosphorylated by PKC and CaMKII on Ser327; moreover, the γ2 long subunit has an extra phosphorylation

site in a serine located in the region which differentiates it from the short subunit (reviews in [5,19]).

In neurons, the situation appears more complicated. First, in contrast with expression systems, the native subunit composition of $GABA_A$ receptors, and consequently the residues accessible to phosphorylation, are less evident. Second, in neuronal systems an important point adding ambiguity to data interpretation is that direct receptor phosphorylation is not the only possible mechanism by which protein kinases can modulate transmission. In such complex systems in fact, kinases and phosphatases are able to affect also distinct enzymatic pathways that, finally converging onto the receptors, can regulate their functionality. An example is given by RP of GABAergic transmission in the cerebellum, which we will review later.

The sign of the modulation exerted by phosphorylation on native $GABA_A$Rs appears at present not uniform. For example, PKA can both increase or decrease currents from the receptors, according to the system analyzed and to subunit composition (references in [19]). The functional consequences of PKC activity seem at first view simpler, because $GABA_A$Rs are in most cases inhibited by this protein kinase. Nevertheless, contrasting data have been reported also in this case. Furthermore, PKC may have complex functions in regulating the receptor life cycle on the postsynaptic membrane [5,19]. At the moment, no obvious rule emerges to predict whether phosphorylation increases or decreases $GABA_A$R activity; each neuronal system might have its own specificity given by subunit composition, protein kinases and phosphatases present, and machinery for receptor membrane trafficking.

Phosphorylation has been suggested to be involved in the LTD of hippocampal GABAergic transmission, particularly through activation of calcineurin [8]. Consistently, this Ca^{2+}-dependent protein phosphatase was shown to reduce $GABA_A$R responses by lowering the affinity for the agonist. Furthermore, CaMKII is an obvious protein kinase candidate for transducing intracellular Ca^{2+} transients into channel function modifications during plasticity phenomena. Known to phosphorylate $GABA_A$Rs in vitro and to modulate responsiveness to GABA in neurons (review [19]), it might play a fundamental role in RP [20,21].

2.1.2. Glycine receptors

Similarly to $GABA_A$Rs, several protein kinases (PKC, PKA and CaMKII) have been implicated in the modulation of GlyR activity. Observations on specific effects of protein kinases on GlyRs are highly variable and even contradictory. In different reports, activators of the same kinase exhibited opposite effects, i.e. either caused decrease or increase of glycine-mediated currents (see references in [1,22,23]). In rat spinal neurons GlyRs can be phosphorylated with opposite functional consequences in response to activation of either PKA or PKC. On the other side, cross-potentiation of GlyR currents by PKC and PKA was reported in acutely dissociated trigeminal neurons [24].

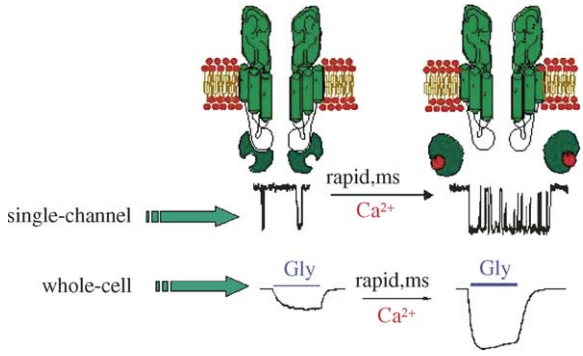

Fig. 2. Hypothesis on the mechanisms mediating rapid Ca^{2+}-induced potentiation of GlyRs. At low intracellular Ca^{2+} concentrations, Ca^{2+}-sensing proteins bind to the cytoplasmic domain of GlyR channels and keep them in a state of low activity. Ca^{2+} elevations trigger the dissociation of the Ca^{2+}-binding proteins from the receptor cytoplasmic domain, thus leading to a prolongation of single channel openings.

In rat spinal sensory neurons, Ca^{2+}-induced increases of evoked glycine currents via CaMKII phosphorylation pathways were reported [25]. However, these results still need clarification. First, so far CaMKII consensus sequences have not been identified in GlyR subunits [23]. Moreover, another study showed that pharmacological block of phosphorylation or dephosphorylation pathways did not prevent Ca^{2+}-induced potentiation of GlyR currents, suggesting possible modulations via other Ca^{2+}-dependent mechanisms [22].

2.2. Rapid modulation by calcium

Besides phosphorylation and dephosphorylation events, faster Ca^{2+}-triggered signaling processes can regulate the activity of ionic channels (Figs. 1 and 2). For example, NMDA receptors, potassium Kv conductances, voltage-dependent Ca^{2+} channels, Ca^{2+}-activated potassium and cyclic nucleotide-gated ion channels can be modulated via interaction with calmodulin or other Ca^{2+}-binding proteins (see review in [26]). The multiplicity of ion channels that are regulated by Ca^{2+}-binding proteins indicates that this mechanism may be a universal modality allowing neurons to respond to incoming stimuli on a fast time scale.

As far as inhibitory receptors are concerned, no such Ca^{2+}-dependent mechanisms are at present known for $GABA_A$Rs, although these channels can be complexed with other proteins able to modulate their function (reviews in [4,19]). On the other hand, emerging evidence indicates that neurons and cells expressing GlyRs possess the machinery required for a rapid Ca^{2+}-dependent regulation.

In cultured spinal cord neurons and in transfected human cell lines, Ca^{2+} elevations result in the fast augmentation of glycine-induced currents [22]. This form of modulation develops in less than 100 ms. It is characterized by an increase in the apparent affinity of GlyRs for glycine, and it presumably involves an unknown Ca^{2+}-binding protein. It was suggested

that, in the absence of Ca^{2+}, this protein might bind to the cytoplasmic domain of GlyRs, thus keeping them in a state of lower gating activity. Upon Ca^{2+} entry, the protein would dissociate from its binding site and cause prolongation of channel openings (Fig. 2).

This phenomenon of Ca^{2+}-induced potentiation was demonstrated in various praparations: spinal cord neurons [22], ventral tegmental neurons [27], hypoglossal motoneurons (Ragozzino et al., submitted), retinal bipolar and ganglion cells (Bertollini et al., data in preparation), trigeminal neurons (Huang, personal communication), and it may be important for the rapid modulation of glycinergic networks in vertebrates.

2.3. Modulation of receptor membrane expression

In recent years, growing attention has been devoted to the molecular mechanisms regulating the trafficking and anchoring of ionic channels to synaptic sites. Receptor-operated channels are now regarded as highly dynamic variables of the synapse architecture and, indeed, their expression at the postsynaptic membrane can be modified by extracellular factors and by activity-dependent increases in Ca^{2+}. NMDA receptor-dependent LTP and LTD at glutamatergic synapses [9] are important examples, where the study of receptor life cycles has gained particular relevance. Recent data strongly support the idea that the regulation of the functional receptor number is one of the major factors determining these forms of plasticity [9,10]. The postsynaptic membrane of inhibitory synapses is also dynamically regulated. Several recent reviews are devoted to this analysis [5,4,19,28], and we only highlight a few points here.

$GABA_A$Rs can be translocated to the postsynaptic area of inhibitory synapses on fast time scales (in the order of minutes). In a pioneer study, Otis et al. [29] provided evidence that, following experimental temporal-lobe epilepsy (kindling), the number of functional postsynaptic $GABA_A$Rs strongly increased. These observations were later confirmed by combining quantal analysis of GABAergic currents with quantitative immunogold staining of $GABA_A$Rs [30].

Fast receptor translocation to synaptic sites can, in principle, be performed via two distinct mechanisms: either by lateral diffusion from non synaptic areas, or by direct receptor insertion from cytoplasmic compartments (Fig. 1). Lateral diffusion of GlyRs between synaptic and extrasynaptic areas was demonstrated using video tracking of antibody-coated fluorescent particles and quantum dot labeling of single GlyRs [28,31]. Similar behaviour for $GABA_A$Rs was suggested by early, low-resolution experiments using photobleach recovery monitoring [32]. These and other observations [5] indicate that the dynamic movement of receptors from extrasynaptic to synaptic areas, and vice versa, may represent a powerful mechanism for a rapid regulation of the efficacy of inhibitory synapses.

Similarly to AMPARs, inhibitory receptors undergo a continuous cycle of endo/exocytosis between intracellular compartments and cell surface, and external factors can modulate receptor surface expression in both directions: for example, insulin increases $GABA_A$ receptor number [33], whereas BDNF exerts the opposite action [34]. Furthermore, Ca^{2+}-dependent phosphorylation (via PKC and CaMKII) may be important for functional regulation of surface expression and stabilization of $GABA_A$ receptors (review [3,4,19]). Finally, delivery of receptors to developing glycinergic synapses is stimulated by elevation of Ca^{2+} [35].

Thus, it is tempting to speculate that postsynaptic forms of plasticity at excitatory and inhibitory systems may consist in modifications of the receptor life-cycle performed by similar mechanisms. There are indeed analogies between the two synaptic systems in this sense. For example, AMPA receptor endocytosis, which appears to promote NMDARs-dependent LTD at glutamatergic synapses, requires calcineurin [9]. A similar mechanism is suggested for some NMDAR-dependent forms of GABAergic LTD [36]. These analogies, however, have to be considered with precautions because, for example, in neocortical pyramidal cells GABAergic LTP was not affected by the antagonists of vesicular exocytosis, botulinum toxin D and GDP-β-S [37].

2.4. Rebound potentiation

RP is a powerful form of plasticity of the GABAergic transmission from molecular layer stellate/basket cells onto cerebellar Purkinje cells. The elevations of Ca^{2+} in Purkinje cells, which follow either depolarization pulses [38], or stimulation trains to the climbing fibers [39], produce an increase in synaptic efficacy lasting several tens of minutes. Firstly described by Llano et al. in 1991 [38], and by Kano et al. [39], who gave the phenomenon its present name, RP was early attributed to increases in $GABA_A$R responsiveness to the neurotransmitter, and thus associated with a postsynaptic site of expression.

The most detailed descriptive model of this phenomenon is based on pharmacological studies of RP in cultured Purkinje cells [21,40]. The proposed model explains previous observations reporting the dependence of RP on both CaMKII [20] and PKA [40] activity. Moreover, it also involves several other players which, besides PKA and CAMKII, are often found to play important roles in phenomena of synaptic plasticity [9,10]. These are phosphatases (PP-1 and calcineurin (PP-2)), and DARPP-32 (the dopamine- and cAMP-regulated phosphoprotein), which is an inhibitor of PP-1. Interestingly, PKA is here proposed to play only a “gating”, or modulatory, role, whereas activation of CAMKII would be a necessary and sufficient condition to induce the potentiation of synaptic responsiveness. These actions of PKA and CAMKII bear a strong resemblance with glutamatergic LTP [9]. For the detailed description of the model, we invite the interested reader to the corresponding publication [21].

3. Calcium-induced modulation of $GABA_A$ and glycine-receptor channels: presynaptic mechanisms and endocannabinoids

In synaptic physiology the expression *retrograde signaling* generically defines several known phenomena of synaptic modulation, which are triggered by stimulation of postsynaptic cells, but are mediated by a presynaptic regulation of transmitter release [6]. Retrograde signaling mechanisms provide postsynaptic neurons with efficient tools for controlling presynaptic afferents in an activity-dependent way, and are now recognized as an important modality of central synapses.

3.1. Depolarization-induced suppression of inhibition (DSI)

The importance of Ca^{2+} ions in triggering forms of retrograde modulation was established in the early 1990s with the discovery of the phenomenon called "Depolarization-induced Suppression of Inhibition" [38,41], and was later confirmed by numerous studies (review [6,7]). Postsynaptic depolarizations in Purkinje and CA1 pyramidal cells were found to induce a reversible depression of afferent GABAergic inputs, which lasted for tens of seconds. These and later studies provided a complete characterization of DSI. Its main properties are the following (see references in [6,7]): (i) its induction is postsynaptic, (ii) it is triggered by Ca^{2+} elevations in the postsynaptic neuron and (iii) its mechanisms of expression are presynaptic (Fig. 3A).

The existence of a retrograde Ca^{2+}-dependent messenger transducing the postsynaptic Ca^{2+} signal into a presynaptic down-modulation of neurotransmitter release was proposed in the very first study [38]. For around 10 years research on DSI was restricted to a small scientific niche, which essentially consisted of the original laboratories where the phenomenon was first described. Investigations mainly revolved around the possible identity of the retrograde messenger.

The field was given a new acceleration when the first reports about the involvement of endocannabinoids in DSI (Fig. 3A) and in a similar phenomenon concerning glutamatergic synapses (Depolarization-induced Suppression of Excitation, or DSE) appeared (hippocampal DSI [42]; cerebellar DSE: [43]). The unequivocal identification of endocannabinoids as retrograde messengers in DSI/DSE is now further supported by the multiplication of reports appearing in several brain areas (review in [7]). DSI/DSE has now become a widely recognized mechanism of modulation for many central synapses, both glutamatergic and GABAergic ones. Moreover, this phenomenon has been recently found for glycinergic synaptic transmission (glycinergic DSI) in hypoglossal motoneurons from rat and mouse brainstem slices (Ragozzino et al., submitted).

3.2. Ca^{2+} and the endocannabinoid system

Several comprehensive reviews have recently described the cannabinoid system in the brain and its possible therapeutic exploitations [6,44–46]. Here we will shortly highlight the main properties of endocannabinoid signaling and of the Ca^{2+}-induced plasticity forms operating via this mechanism.

The endocannabinoid system consists of cannabinoid receptors (CBRs), their endogenous ligands and the cellular machinery for their synthesis and degradation [44,45]. So far, two distinct isoforms of G protein-coupled CB receptors (CB1Rs and CB2Rs) have been cloned. CB1Rs mediate most of the endocannabinoid effects in the CNS, and their prevailing localization in the brain is on presynaptic axons (review [44]). In contrast, CB2Rs are predominantly located on peripheral structures and on cells of the immune system. Furthermore, other CB-sensitive receptors are suggested to be present in the CNS (see references in [45]), although so far they have not been identified.

Endocannabinoids are, by definition, endogenously produced molecules, which are able to activate CBRs. Several substances with such properties have been isolated from the brain (see references in [44,45]). They are typically produced from membraneous precursors and have in common an unsaturated fatty acidic structure. In contrast to classical neurotransmitters, they may be released not via vesicular mechanisms, but rather via passive or facilitated diffusion. Ca^{2+} elevations seem to be essential for their synthesis rather than for their release (see references in [44]). Great attention has been in particular devoted to two of them (Fig. 3A), anandamide (AEA) and 2-arachidonoylglycerol (2-AG), because they can be produced by neural structures in an activity-dependent way [44,45].

Ca^{2+}-induced release of endocannabinoids and consequent activation of presynaptic CB1 receptors causes the decrease of presynaptic Ca^{2+} transients [43,47], a direct inhibition of the release machinery [38], and a reduction in the spike rate of presynaptic interneurons [48]. Among the range of mechanisms by which CB1 receptors inhibit transmission, the downregulation of voltage-dependent Ca^{2+} channels and the upregulation of K^+ conductances on presynaptic axons have been demonstrated in different brain regions [44] (Fig. 3B).

3.3. Postsynaptic Ca^{2+} is not all: the case of endocannabinoid synthesis triggered by G protein-coupled receptors

As mentioned above, endocannabinoids can be produced in neurons following Ca^{2+}-concentration increases. Nevertheless, postsynaptic Ca^{2+} is not the only factor activating the endocannabinoid synthesis machinery. Activation of G protein-coupled metabotropic receptors, either group I metabotropic receptors (group I mGluRs, Fig. 3A) [14,16,49–52], muscarinic receptors [15], or dopamine receptors (D2R; see references in [45]), have been found to trigger production of endocannabinoids.

Endocannabinoid synthesis through G-protein coupled receptors can be either dependent [50–52], or independent [14–16,49] from postsynaptic Ca^{2+}. In some cases, down-

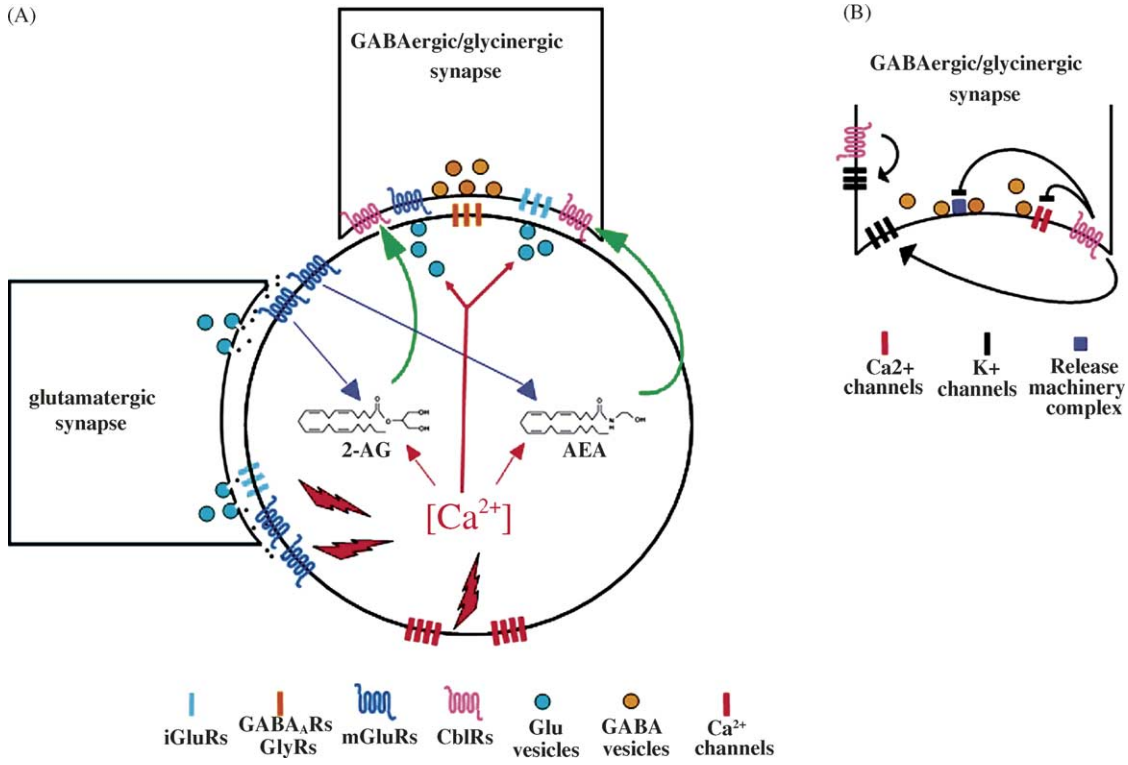

Fig. 3. Presynaptic modulation of transmitter release: endocannabinoids and glutamate as retrograde messengers.(A) Main pathways of retrograde modulation of inhibitory synaptic transmission. Increases of postsynaptic Ca^{2+} concentration can trigger the release of retrograde messengers like the endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), or like glutamate. These substances then activate presynaptic receptors, and regulate synaptic strength.Elevations in postsynaptic Ca^{2+} can be produced by several mechanisms. At glutamatergic synapses, the release of glutamate (light blue circles) can activate postsynaptic ionotropic AMPA and/or NMDA receptors (light blue bars) or group I metabotropic receptors (mGluRs, blue transmembrane protein). Activation of ionotropic receptors can lead to increases of Ca^{2+} levels either directly (due to their permeability to Ca^{2+}), or through depolarizations leading to activation of voltage-gated Ca^{2+} channels (red bars). Group I mGluRs can either cause depolarizations through activation of non-specific cationic conductances, or induce Ca^{2+} release from intracellular stores (not shown). The rise of postsynaptic Ca^{2+} leads to the production of endocannabinoids (like AEA or 2-AG), which can retrogradely activate type 1 cannabinoid receptors (CB1Rs; pink transmembrane protein) on the presynaptic membrane, and inhibit neurotransmitter release. In some cases, group I mGluRs can activate enzymatic pathways leading to release of endocannabinoids in a Ca^{2+}-independent way (blue arrows).Recent evidence has shown that glutamate may be released from postsynaptic dendrites via Ca^{2+}-dependent mechanisms. The retrograde activation of presynaptic glutamatergic receptors (ionotropic or metabotropic) can then change neurotransmitter release probability at GABAergic terminals. The release of glutamate from postsynaptic membrane may be vesicular (see text for a detailed description).(B) The most common mechanisms of control of transmitter release by CB1Rs. Activation of CB1Rs typically causes reduction of the Ca^{2+} influx into presynaptic terminals either via downregulation (black bars) of voltage-gated Ca^{2+} channels, or via upregulation (black arrows) of K^+ channels (black bars). At some synapses, CB1Rs can directly inhibit the release machinery (blue squares). CB1Rs can increase K^+ channel function also on the axonal shaft, or at cellular locations where action potentials are generated. These effects reduce neurotransmitter release by decreasing the presynaptic spike rate. Recent data also suggest possible changes of action potential shape, or possible modulations of the success rate of action potential propagation into axon collaterals.

stream of the G protein-coupled receptors the production of the endocannabinoid 2-AG may require activation of the enzymes phospholipase C and DAG lipase [14,16,52]. Interestingly, a same cell type may be endowed with multiple alternative pathways leading to endocannabinoid synthesis. An example in point is given by the hippocampal CA1 pyramidal cells. Here, 2-AG seems to mediate both DSI [53], and an endocannabinoid-dependent form of GABAergic LTD that is triggered by group I mGluR activation [14]. In CA1 cells, blockers of PLC and DAG lipase inhibit LTD, whereas they leave DSI unmodified, thus suggesting the existence of at least two modes of 2-AG synthesis. A similar situation appears to pertain to Purkinje cells, where DSI is Ca^{2+}-dependent, whereas the group I mGluR-mediated production of endocannabinoids is not [16]. Finally, a same cell may produce different endocannabinoids, according to the pattern of postsynaptic receptors that afferent inputs may activate [54].

The characterization of the endogenous cannabinoid system is still a work in progress. Many points concerning the identity of the endogenous molecules involved, the existence of so far uncharacterized receptor(s), and a true assessment of the physiological relevance of protocols leading to activation of this system, are still open (reviews in [7,44,45]).

3.4. Cannabinoid-independent retrograde modulation of synaptic transmission: the case of glutamate

Endocannabinoids are the most common molecules exerting retrograde functions in the brain. Nevertheless, they

are not the only ones (review in [6]). We would like to devote a small final section on the possible actions of an aminoacid mainly known for its "orthodox" neurotransmitter functions: glutamate. In several cases, this neurotransmitter has received valuable credit for its possible functions as retrograde modulator. Indeed, protocols inducing postsynaptic depolarizations can lead to presynaptic modulation of synaptic transmission not via CB1 receptors, but rather via activation of presynaptic glutamate receptors. Evidence comes from studies concerning cerebellar parallel fiber [55] and GABAergic interneuron synapses [56] onto Purkinje cells, and synapses between neocortical layer 2/3 [57] or layer 5 [58] fast-spiking interneurons and pyramidal cells. Similarly to DSI/DSE, in these systems transmission is modulated via presynaptic mechanisms after augmentations of postsynaptic Ca^{2+} (Fig. 3A). This implies the existence of a retrograde messenger, and glutamate is a convincing candidate.

The required postsynaptic elevations in Ca^{2+} can originate either from activation of group I mGlu receptors [55] and back-propagating action potentials [57], or following depolarizations via voltage-clamp commands [56,58]. Moreover, retrogradely active glutamate can mediate either a depression [55,57,58] or a potentiation [56] of transmitter release. Presynaptic receptors (Fig. 3A) mediating these effects can be metabotropic [57], but also glutamate ionotropic ones [55,56,58].

A particularly interesting case concerns GABAergic afferents onto cerebellar Purkinje cells. Duguid and Smart [56] have shown that Purkinje cell depolarizations either via voltage-clamp commands, or activation of the climbing fiber input may induce activation of NMDA receptors on presynaptic axons. The resulting potentiation of synaptic strength provides a transient modification in transmission for around 8–10 min. This form of plasticity is temporally intermediate between the faster endocannabinoid-mediated depression (tens of seconds) and the longer-lasting RP, which decades in several tens of minutes. The three phenomena can be induced by activation trains to the climbing fiber input [39,56]. It would be of great physiological importance to quantitatively assess the Ca^{2+}-dependence of each phenomenon.

In contrast to endocannabinoids, in the neocortex glutamate is suggested to be released from postsynaptic dendritic compartments via vesicle exocytosis [57]. In the cerebellum, although the simplest hypothesis assumes that glutamate be released directly from Purkinje cell dendrites, either via exocytosis or other mechanisms, this point must still be rigorously demonstrated. In the case of glutamate, it is interesting to speculate that other structures known to release the neurotransmitter in an activity-dependent way, e.g. glial cells, may be involved. Glial cells are indeed known to modulate GABAergic transmission via glutamate-related pathways, both by potentiating [13] and depressing [59] synaptic strength. This possibility would imply the existence of yet unknown mechanisms of communication between Purkinje and Bergmann glial cells.

4. Conclusion

We have tried to give a short, but nonetheless comprehensive, view of our actual knowledge on Ca^{2+}-dependent forms of plasticity at inhibitory synapses. From our discussion it can be inferred that GABAergic and glycinergic synapses are highly modifiable structures, equipped with several tools, which can finely tune their strength under the direct control of postsynaptic activity. In this context, the finding of endocannabinoids as important mediators of inhibitory plasticity has given a formidable boost to a domain that, beforehand, had somehow been lingering in the shadow cast by glutamatergic synapses. Obviously, there are still many open points on the subject; therefore, we believe, new reviews will soon be required to keep up with the advances of the field.

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