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REVIEW

Noncanonical Activity of Endocannabinoids and Their Receptors in Central and Peripheral Synapses

Olga P. Balezina[1], Ekaterina O. Tarasova[1], and Alexander E. Gaydukov[1,a]*

[1]*Faculty of Biology, Lomonosov Moscow State University, 119234 Moscow, Russia*
[a]*e-mail: gaydukov@gmail.com*

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Abstract—This review focuses on new aspects of endocannabinoid functions and mechanisms of activity in central and peripheral synapses, different from the general viewpoint that endocannabinoids are retrograde signaling molecules, which inhibit neurotransmitter release by activating specific presynaptic endocannabinoid receptors CB1 and CB2. Biased agonism of the endogenous and synthetic cannabinoids as well as ability of the CB-receptors to couple not only with classical G_i-proteins, but also with G_s- and G_q-proteins and, moreover, with β-arrestins (thereby triggering additional signaling pathways in synapses) are described here in detail. Examples of noncanonical tonic activity of endocannabinoids and their receptors and their role in synaptic function are also presented. The role of endocannabinoids in short-term and long-term potentiation of neurotransmitter release in central synapses and their facilitating effect on quantal size and other parameters of acetylcholine release in mammalian neuromuscular junctions are highlighted in this review. In conclusion, it is stated that the endocannabinoid system has a wider range of various multidirectional modulating effects (both potentiating and inhibiting) on neurotransmitter release than initially recognized. Re-evaluation of the functions of endocannabinoid system with consideration of its noncanonical features will lead to better understanding of its role in the normal and pathological functioning of the nervous system and other systems of the body, which has an enormous practical value.

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INTRODUCTION

Endocannabinoids are a specific family of signaling molecules produced from lipids (arachidonic acid) in neurons and other cells due to activity of specific enzymes synthesizing endocannabinoids from phospholipids of the cell membrane. According to the initial paradigm (and this paradigm is still generally recognized), which was formulated right after endocannabinoids were discovered in animals in the late 1980s, the main role of endocannabinoids synthesized in postsynaptic structures and released into the synaptic cleft in response to activity of synapses of the central nervous system, consists in their following retrograde action on presynaptic cannabinoid receptors (CB) leading to inhibition of the release of neurotransmitters. The most studied cannabinoids from the whole variety synthesized in animal cells are arachidonoylethanolamine, also called anandamide (AEA), and 2-arachidonoylglycerol (2-AG), although other endocannabinoids have also been described. The latter are present only in low quantities in the CNS and are significantly less explored. An important feature of the two main endocannabinoids (AEA and 2-AG) is that their precursors are always integrated directly in the cell membrane. This makes producing endocannabinoids from certain phospholipids of the membrane by specific enzymes easier: AEA is synthesized by N-acyl phosphatidylethanolamine phospholipase D, while 2-AG is synthesized by diacylglycerol lipase α (DAGLα). Specific membrane cannabinoid receptors of

Abbreviations: 2-AG, 2-arachidonoylglycerol; AC, adenylate cyclase; ACh, acetylcholine; AEA, N-arachidonoylethanolamine (anandamide); CB, cannabinoid receptors; DAG, diacylglycerol; DAGLα, diacylglycerol lipase α; DSE, depolarization-induced suppression of excitation; DSI, depolarization-induced suppression of inhibition; GABA, gamma-aminobutyric acid; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; PKA, proteinkinase A; PLC, phospholipase C; THC, tetrahydrocannabinol; WIN, (R)-(+)-[2,3-Dihydro-5-methyl-3[(4-morpholinyl)methyl]pyrrolo[1,2,3-de]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate salt.

* To whom correspondence should be addressed.

two types – CB1 and CB2 – have been found and identified in nervous and other types of tissues. Both types belong to the family of G-protein coupled 7-transmembrane domain receptors. 2-AG is the full agonist of both types of receptors, whereas AEA acts as their partial agonist. Lately, new potential G protein-coupled endocannabinoid receptors have been discovered but their role is still under investigation [1]. The CB1-receptor is generally located in the CNS, while the CB2-receptor is predominantly present in peripheral tissues, although its presence and functional role has also been described in the CNS [2, 3]. In addition to activation of the classical CB-receptors, endocannabinoids can also modulate the activity of some subtypes of transient receptor potential (TRP) channels and act on nuclear peroxisome proliferator-activated receptors (PPAR) [4, 5]. According to the classical concept of endocannabinoid signaling, CB1 and CB2-receptors are coupled to peripheral G-proteins containing the $G\alpha_{i/o}$-type subunit [6, 7]. Therefore, binding of endocannabinoids to CB-receptors leads to activation of the G_i-protein-mediated signaling pathways with subsequent adenylate cyclase (AC) inhibition and downregulation of cAMP levels and protein kinase A (PKA) activity [8]. At the same time activation of CB-receptors can lead to activation of mitogen-activated protein kinases (MAPKs) in the nerve terminal [9, 10]. The final targets of the cascades starting from the CB-receptors are supposedly suppression of the activity of voltage-gated Ca^{2+}-channels $Ca_V2.1$ and $Ca_V2.2$, also called P/Q-type and N-type Ca^{2+}-channels respectively, or enhancement of the presynaptic potassium conduction through upregulation of the activity of some types of potassium channels, for example K_A-type channels or G protein-coupled inward-rectifying potassium channels [7, 11]. Both effects are due to the interaction of the G_i-protein $\beta\gamma$-subunits with ion channels. This is accompanied by a decrease in AC activity mediated through the α-subunit of the G_i-protein [12]. It is assumed that inhibition of the voltage-gated Ca^{2+}-channels and/or activation of the potassium channels results in depression of the neurotransmitter release in nerve terminals in response to endocannabinoid action in synapses.

Figure 1 summarizes all the steps of endocannabinoid synthesis and canonical signaling pathways, through which the inhibitory effects of endocannabinoids on the release of various neurotransmitters [glutamate, dopamine, gamma-aminobutyric acid (GABA), acetylcholine (ACh)] are realized during activation of CB-receptors in different types of synapses.

INHIBITORY EFFECTS OF ENDOCANABINOIDS IN THE SYNAPSES OF CENTRAL NERVOUS SYSTEM

The ability of 2-AG to suppress the GABAergic inhibition of synaptic activity in the cerebellum and hippocampus is still considered as the cornerstone of the effects of endocannabinoids in the CNS. This effect was named "depolarization-induced suppression of inhibition" (DSI). It was shown that depolarization of the postsynaptic neurons in the CNS caused by neurotransmitter binding leads to the release of endocannabinoids and endocannabinoid-mediated short-term suppression of GABA secretion from the presynaptic inhibitory terminals in the cerebellum and hippocampus [6, 8]. Later, the retrograde endocannabinoid-mediated suppression of the release of the excitatory neurotransmitter glutamate has also been detected in the excitatory synapses and this effect was defined as depolarization-induced suppression of excitation (DSE) [13]. Thus, the role of endocannabinoids in the CNS consists in retrograde suppression of neurotransmitter release regardless of whether the synapse is excitatory or inhibitory.

Traditionally, the endocannabinoid effects in the synapses of the CNS are considered to be a result of their

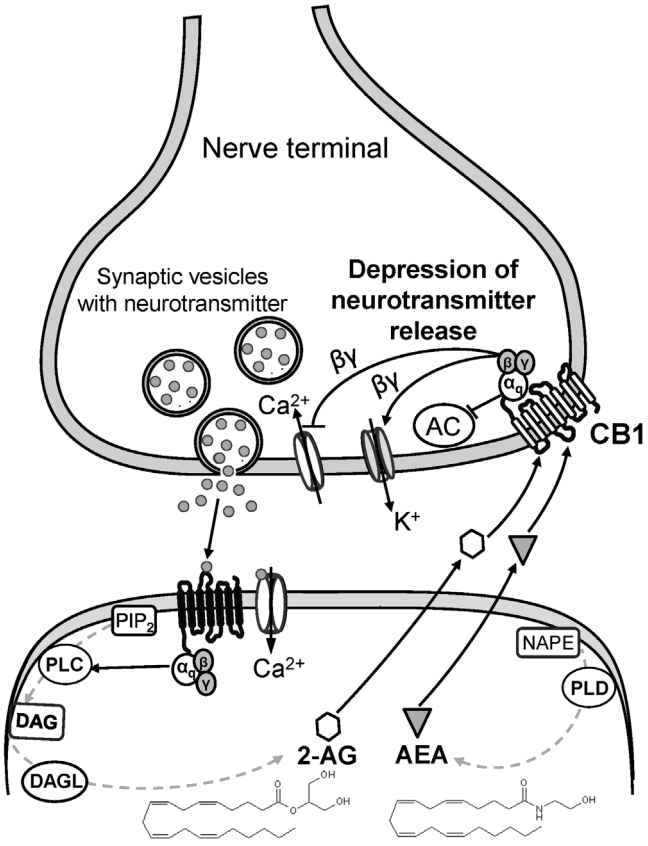

Fig. 1. Schematic representation of canonical inhibitory effects of endocannabinoids in synapses of the central nervous system. Pathways of AEA and 2-AG synthesis in postsynaptic structures, their release into the synaptic cleft and their action on presynaptic CB1-receptors are shown here, as well as signaling pathways triggered by CB1-receptor activation, leading to depression of the neurotransmitter release through modulation of presynaptic Ca^{2+}-channels and K^+-channels activity and downregulation of the activity of AC. Designations: ←, potentiation; ⊥, suppression; α, β, γ, G protein subunits; PIP_2, phosphatidylinositol 4,5-bisphosphate.

"on-demand" synthesis caused by synaptic transmission and neurotransmitter action on the postsynaptic site. The latter may lead, firstly, to depolarization of the postsynaptic membrane, increase in intracellular calcium concentration, and activation of the enzymes of endocannabinoid synthesis, which eventually causes development of DSI or DSE. Secondly, synthesis of diacylglycerol (DAG) occurs due to activation of metabotropic receptors, coupled to $G_{q/11}$-proteins (metabotropic glutamate receptors of type I or muscarinic acetylcholine receptors) followed by activation of phospholipase Cβ. DAG is next deacetylated by DAGL producing 2-AG, which diffuses through the synaptic cleft and inhibits the synaptic transmission by acting on presynaptic CB-receptors. Anandamide production could also be stimulated due to activation of metabotropic glutamate receptors [14, 15]. Such mechanism of endocannabinoid-mediated short-term synaptic plasticity is called "metabotropic-induced suppression of inhibition (or excitation)" – MSI/MSE [16-19]. Both types of endocannabinoid synthesis induction –calcium-dependent depolarization or calcium-independent metabotropic – usually coexist in synapses and mediate manifestation of the retrograde inhibitory effects of endocannabinoids [20-24].

Duration of the endocannabinoid-mediated inhibition of neurotransmitter release or, in other words, depression of synaptic transmission, may be short-term (STD) lasting for several tens of seconds, or may be long-term (LTD), lasting from tens of minutes to even hours. In the latter case, it is assumed that the LTD induction by endocannabinoids requires long-lasting (for at least a few minutes) activation of CB-receptors accompanied by triggering of the long-term mechanisms of neurotransmitter release suppression in synapses [6, 25, 26].

Classical roles of endocannabinoids in the CNS involve regulation of memory formation, learning, pain and other kinds of sensory reception, neuromotor control, food intake behavior, and others [27].

The idea of endocannabinoids acting as specific inhibitory signaling molecules comprising an unique negative feedback system in the CNS synapses is now commonly recognized. Such negative feedback circuit is thought to be able to dynamically regulate reliability of synaptic transmission, allowing postsynaptic neurons to tune sensitivity of their synaptic inputs according to the intensity of the afferentation pattern [6, 28]. Considering the critical role of the endocannabinoid system in various mental and neuropathological conditions [2, 29], this system is also often characterized as a homeostasis-regulating system [3, 27].

NONCANONICAL EFFECTS OF ENDOCANNABINOIDS IN SYNAPSES

The paradigm of endocannabinoids and their CB-receptors as a unique retrograde inhibitory system in the CNS synapses is now under revision due to accumulated data on atypical, noncanonical side effects of endocannabinoids. In particular, just lately the term *endocannabinoidom* was introduced to describe the broadening system of cannabinoids functioning in the human organism that includes, in addition to cannabinoids, some signaling molecules from the N-acylamide family (N-acyltaurine, N-acylserotonin, N-acyldopamine, etc.) [29].

In this review we will be focusing on only one part of these noncanonical effects, specifically on the data regarding the noncanonical tonic action of endocannabinoids in synapses, functional ambiguity of endocannabinoids and various agonists of their receptors, and, finally, we will discuss the ability of endocannabinoids to enhance synaptic transmission by stimulating neurotransmitter release in peripheral and central synapses, instead of inhibiting it. A more detailed description of the broadening spectrum of various noncanonical endocannabinoid-mediated effects can be found in some recent reviews [3, 13, 27].

TONIC ACTIVITY OF ENDOCANNABINOIDS AND THEIR CB1- AND CB2-TYPE RECEPTORS IN SYNAPSES OF THE CENTRAL NERVOUS SYSTEM

According to the classical paradigm, the basis of specific endocannabinoid effects in synapses is their on-demand action, which happens only during impulse synaptic activity accompanied either by depolarization of the postsynaptic membrane and subsequent influx of calcium ions or by transmitter action on certain types of $G_{q/11}$-protein coupled metabotropic receptors, or by combination of both ways. This leads to a rapid synthesis and fast release of endocannabinoids, followed by activation of their presynaptic CB-receptors, which causes suppression of transmitter release [7, 30]. Taking into account the pulsed phasic character of synaptic signals, significant efforts were made to prove phasic character of the endocannabinoid-mediated signals. It was shown that not only the release of endocannabinoids, but also their prior synthesis may be phasic. A typical example was provided in the work on hippocampal slices [31], where in response to impulse neuronal activity and glutamate action a fast (phasic) increase in 2-AG synthesis took place via activation of DAGLα by influx of calcium ions. Short (for a few minutes) preapplication of DAGLα inhibitor OMDM-188 prevented the development of DSI – the depolarization-dependent suppression of inhibition of neurotransmitter release – caused by 2-AG. This and similar works prove the fact of on-demand (in response to synaptic activity) synthesis of endocannabinoids with the help of rapidly-activated enzymes, their subsequent release, and fast presynaptic action.

For the last 20 years a huge amount of data has accumulated proving that both endocannabinoids and their

CB1 and CB2 receptors exert tonic or constitutive activity in the CNS even in the absence of impulse neuronal activity [32-34]. One of the first examples was the revealed ability of CB-receptor antagonists to enhance synaptic transmission in sensory terminals and synapses in the CNS by increasing spontaneous release of neurotransmitter and spike activity, respectively, when applied under resting conditions. Thus, introduction of CB-antagonist under resting conditions facilitated the neurotransmitter/co-transmitter release in the synapses of CNS or in the primary sensory afferent nerve terminals. This was shown for the highly specific CB1-antagonist (SR141716A) and CB2-antagonist (SR144528) [32]. The ability of CB-antagonists which were considered as highly specific and neutral antagonists to initiate either spontaneous or evoked synaptic activity, especially under resting conditions, forced researchers to postulate the existence of a special form of tonic activity of the CB1- and CB2-receptors in the absence of synaptic activity or endocannabinoid action. Also, it was inevitable to admit that a lot of CB-antagonists act as inverse agonists, when they bind to tonically active CB-receptors, as they exhibit in this case their own effects reversing inhibition (inverse effects) that facilitate transmitter release in synapses. Indeed, results have been reported proving that the tonic activity of CB1 and CB2 receptors may be the consequence of the existence of a special constitutively active conformation of these receptors, which exerts self-sustained signaling (supporting the inhibitory signaling pathway) even in the absence of their ligand [35-37]. In this case, binding of the ligands acting as inverse agonists/antagonists to CB-receptors will suppress this tonic inhibitory activity and lead to either attenuation of the neurotransmitter release suppression or activation of the synapses [36]. It has been shown that some of the CB1-receptors in the hippocampal slices could form constitutively active conformations in the absence of endocannabinoids and in this state, they were able to inhibit the closely located voltage-gated Ca^{2+}-channels and GABA release [34]. Furthermore, action of the inverse agonist/antagonist AM 251 on these receptors leads to attenuation of the GABA release suppression. It is important to mention that this type of constitutive receptor activity (in the absence of neuronal impulse activity) has already been described in the CNS for both types of CB-receptors owing to the broad screening and detection of effects caused by the antagonists of CB-receptors, which work as inverse agonists in these cases.

Nevertheless, an alternative point of view is actively discussed in the scientific literature, where the endocannabinoid tonic activity is regarded as a result of a basal activity of the enzymes responsible for their synthesis, storage of endocannabinoids in synaptic structures, followed by their mobilization and tonic leakage with subsequent tonic activation of CB1 and CB2 receptors [38, 39]. In this case canonical forms of CB-receptors are under constant exposure to endocannabinoids, thereby being in a subthreshold tonic activation state.

The best-known example of tonic 2-AG production in the CNS is suppression of the activity of GABAergic interneurons in hippocampal slices [34]. It has also been shown that stimulation of the activity of 2-AG and AEA degrading enzymes (monoacylglycerol lipase and fatty acid amide hydrolase, respectively) leads to attenuation of the tonic endocannabinoid-mediated inhibition of the GABAergic system, which is supported by the fact that these enzymes that control basal production of endocannabinoids, are tonically active in certain brain regions [40]. To choose between the two models of tonic endocannabinoid activity, i.e., between the tonic-mediated endocannabinoid release and constitutive tonic activity of presynaptic CB1 receptors in the absence of endocannabinoids, the lipase knockout animals that have limited capacity of endocannabinoid production are used, which makes tonic endocannabinoid synthesis and release impossible [41], as well as comparative analysis of the effects produced by the inverse agonists/antagonists and by the neutral antagonists of CB-receptors, which have been developed (NESS 0327, O-2654, O-2050) and do not cause the effects typical of inverse agonists [37, 42, 43]. Another experimental approach involves testing of the endocannabinoid leakage from the brain tissue samples, where basal activity of endocannabinoids and possibility of their tonic synthesis and release have been established [44]. Finally, the possibility of tonic endocannabinoid leakage has already been shown in experimental models of neuropathological disorders in animals. It was demonstrated that some brain disorders (autism, Huntington's disease and more) could be related to the long-term dysregulation of tonic endocannabinoid-mediated control of GABA release in the brain [45-47]. In particular, the higher levels of tonic retrograde endocannabinoid signaling in the striatum in Huntington's disease, which cause suppression of GABA release in these structures, is considered as a pathophysiological symptom of this disease, according to some researchers [46]. Others claim that tonic endocannabinoid signaling could be a component of autism pathogenesis [47]. Moreover, realization of the endocannabinoid effects in the basket cell's synapses of the hippocampus strictly requires neuroligin-3 – a postsynaptic cell adhesion protein. The exact role of this protein in tonic endocannabinoid signaling is still unknown. It has been suggested that neuroligin-3 is a part of the tonic retrograde signaling machinery operating in synapses of different brain regions via trans-synaptic interaction of neuroligin-3 with presynaptic neurexins [48-50]. It is possible that neurexin forms a transsynaptic coupling with neuroligin-3 in the synapses of people suffering from autism and thereby regulates in some way the tonic synthesis of 2-AG in the postsynaptic cell and facilitates its leakage [51].

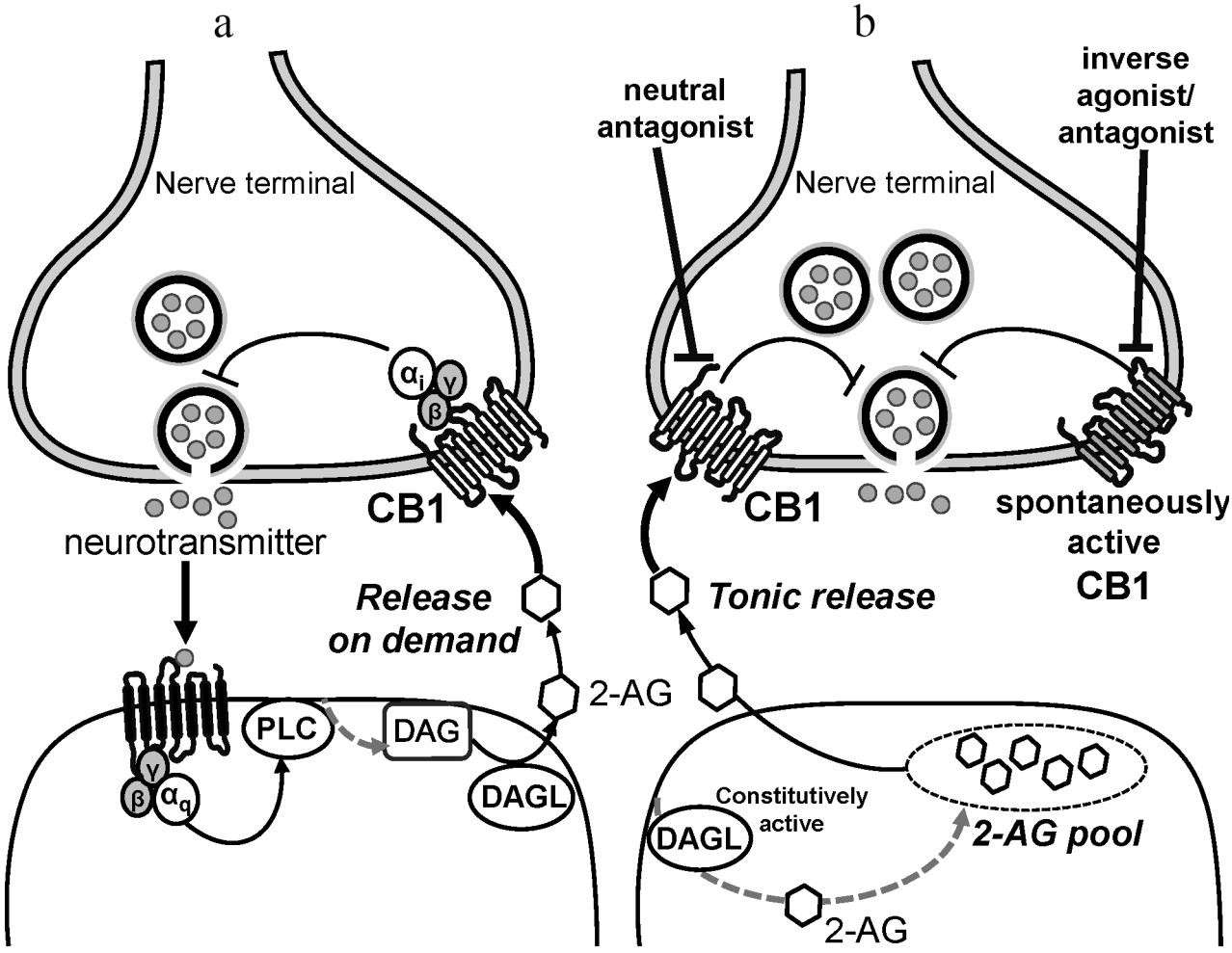

Fig. 2. Possible ways of endocannabinoid action. Canonical activity as the result of strictly on-demand synthesis and action of endocannabinoids in response to neurotransmitter action on the postsynaptic receptors (a); Noncanonical activity, involving endocannabinoid leakage from pre-existing pools and their tonic action on CB-receptors, or constant spontaneous activity of CB-receptors even in the absence of endocannabinoid binding (b). Designations: ←, potentiation; ⊥, suppression; α, β, γ, G protein subunits.

Thus, existence of both models of endocannabinoid signaling – phasic (on-demand) or in form of basal tonic leakage – could be considered proven and equally involved in the endocannabinoid release and function in the CNS (Fig. 2).

Relative contribution and real physiological significance of tonic endocannabinoid leakage in different brain regions is still poorly understood. It has been noted in a recent review that AEA could play a tonic regulatory role in the hippocampus, whereas 2-AG is responsible for solely phasic effects [13]. This, obviously, may be different in other brain regions. In the case of neuronal disorders impairment of the tonic endocannabinoid leakage could change expression of the CB-receptors on presynaptic membranes and therefore alter phasic cannabinoid effects. Furthermore, it has been suggested that basal endocannabinoid leakage and tonic inhibitory activity of their receptors in the primary pain afferents could be the mechanism of tonic control of the pain sensitivity threshold in the peripheral nervous system [32].

NONCANONICAL COUPLING OF CB-RECEPTORS WITH G-PROTEINS, β-ARRESTINS, AND HETERORECEPTORS

Nowadays the possibility of neurotransmitters/co-transmitters and other signaling molecules (hormones, neurotrophins, etc.) to exert oppositely directed effects and broad spectrum of various types of actions on neurotransmitter release is regarded as their specific and distinctive feature. It turned out that the endocannabinoid system, which has been considered as a simple negative feedback system, is not an exclusion.

CB-receptor interaction with different types of G-proteins. The data have been accumulating for a long time

that show that in some synapses of the CNS treated by pertussis toxin (which interrupts canonical interaction of CB-receptors and G_i-proteins) stimulation of the CB1 receptors could lead to the increase in the cAMP level, thus providing evidence (albeit indirect) of the possible coupling of CB-receptors with G_s-proteins and development of noncanonical endocannabinoid effects [52-55]. It was established in the experiments with various endogenous and exogenous CB-receptor agonists in intact animals and in experimental models of neuropathologies that there was significant specificity in the action of these drugs, which was termed dual or biased agonism [9, 56].

Biased agonism was first described in the G-protein-coupled catecholamine receptors, but later it was shown in practically all chemical signaling systems, including the ones involving neurotransmitters and hormones [57]. Endocannabinoids are no exception. In particular, certain agonists of the CB1 receptor have been found that produce effects implying that CB1 receptors could be coupled not to G_i-proteins (or not only to G_i-proteins), but also to other types of G-proteins [58]. The synthetic agonist of CB-receptors WIN 55,212-2 (WIN), which is widely used in experiments, leads to activation of not the G_i-proteins while binding to CB1 receptors, but of G_z-, $G_{q/11}$-, $G_{12/13}$-proteins, which is accompanied by activation of phospholipase C (PLC) isoforms in the cortical synapses [59]. Similar effects of WIN have been shown in mice motor synapses not so long ago, where WIN caused increase in the frequency of spontaneous transmitter release [60]. Finally, it was shown in the recent work where six different cannabinoids –two endogenous (2-AG and AEA) and four exogenous, including WIN and CP55,940 – have been tested in detail in cell cultures, modeling neuronal activity in the striatum and consisting of cells containing CB-receptors, that different agonists acting through the same CB1 receptors were able to trigger different signaling cascades via activation of different types of G-proteins, including G_s-proteins [61]. This all compels one to postulate functional ambiguity of the CB-receptor agonists, as well as of the CB-receptors themselves, from the viewpoint of functional implications for the neuron. This functional diversity of effects, which is termed biased agonism, is explained by the existence of different conformational forms of these receptor proteins, which have potentially different affinity to different G-protein types. This also means that depending on the chemical structure of the agonist the CB-receptor molecule is stabilized in a certain conformational state with selective affinity to the pool of G-proteins containing specific subunits. Interaction with the specific G-proteins triggers molecular signaling pathways, which do not necessarily lead to the inhibition of neurotransmitter release. It is believed nowadays that activation of a certain G-protein and triggering of a certain signaling pathway strongly depends on the agonist type, which stabilizes the CB-receptor in a specific state that interacts preferably with one of the G-protein types [62-64] (Fig. 3a). It is likely that the regulators of G-protein signaling (RGS) contribute to this complex manifestation of the biased agonism [65, 66].

Currently, the ligands of CB-receptors are divided into 4 chemically distinct classes. Those are: (i) eicosanoids [including 2-AG and AEA], (ii) classical phytocannabinoid from plants of the *Sativa* genus – Δ^9-tetrahydrocannabinol (THC) and its derivatives, (iii) nonclassical synthetic agonist CP 55,940 and (iv) aminoalkylindole WIN. Due to the discovered ability of CB-receptor ligands to exert biased (functionally diverse) agonism, many researchers believe that it is worthwhile to search for specific CB-receptor ligands (agonists) among the set of already known synthetic cannabinoids. These ligands should lead to the development of only one desired effect by binding to the CB-receptors in the CNS, so that they could be used in clinical practice for various goals [67-70]. Development of new synthetic CB-receptor agonists is an on-going process. Lately it has been shown that in the presence of pertussis toxin, when interaction of $G_{i/0}$-proteins with the cell receptors is blocked, high concentrations of synthetic cannabinoids such like WIN, CP55,940, JWH-018, and AB FUBINACA cause increase in the cAMP levels above the levels observed during AC activation by forskolin [71, 72]. On the other hand, THC in the same concentration range failed to increase cAMP levels while binding to the same CB1 receptors. Thus, the notion of biased agonism and functional diversity of the ligands, which are able to induce various signaling pathways, including stimulation of the G_s-protein-mediated cascades, while binding to the same type of CB-receptors, gets more and more support.

CB-receptor interaction with β-arrestins. Analysis of the diverse functional implications caused by binding of various ligands to CB-receptors in the CNS led to another surprising discovery – when activated by certain ligands, CB-receptors are able to interact not only with G-proteins but also with β-arrestins. β-Arrestins are a small family of cytoplasmic proteins with molecular weight of about 50 kDa, which are able to bind to cytoplasmic domains of G-protein-coupled receptors and cause termination of the G-protein activation, firstly, via receptor inactivation and, secondly, via its following internalization. As known, activation of the CB-receptors is followed by their phosphorylation by G-protein-coupled receptor kinases, which allows β-arrestins – β-arrestin 1 and β-arrestin 2 – to translocate to the cell membrane and to bind to the cytoplasmic parts of CB-receptors. It is currently established that both types of arrestins can bind to CB1 and CB2 receptors. Binding of β-arrestin 2 leads to G-protein coupled CB-receptor desensitization and probably internalization, i.e., their removal from the cell membrane, as was demonstrated for the case of activated CB1-receptor and β-arrestin interaction [73] (Fig. 3b). But it becomes apparent that participation of β-arrestins

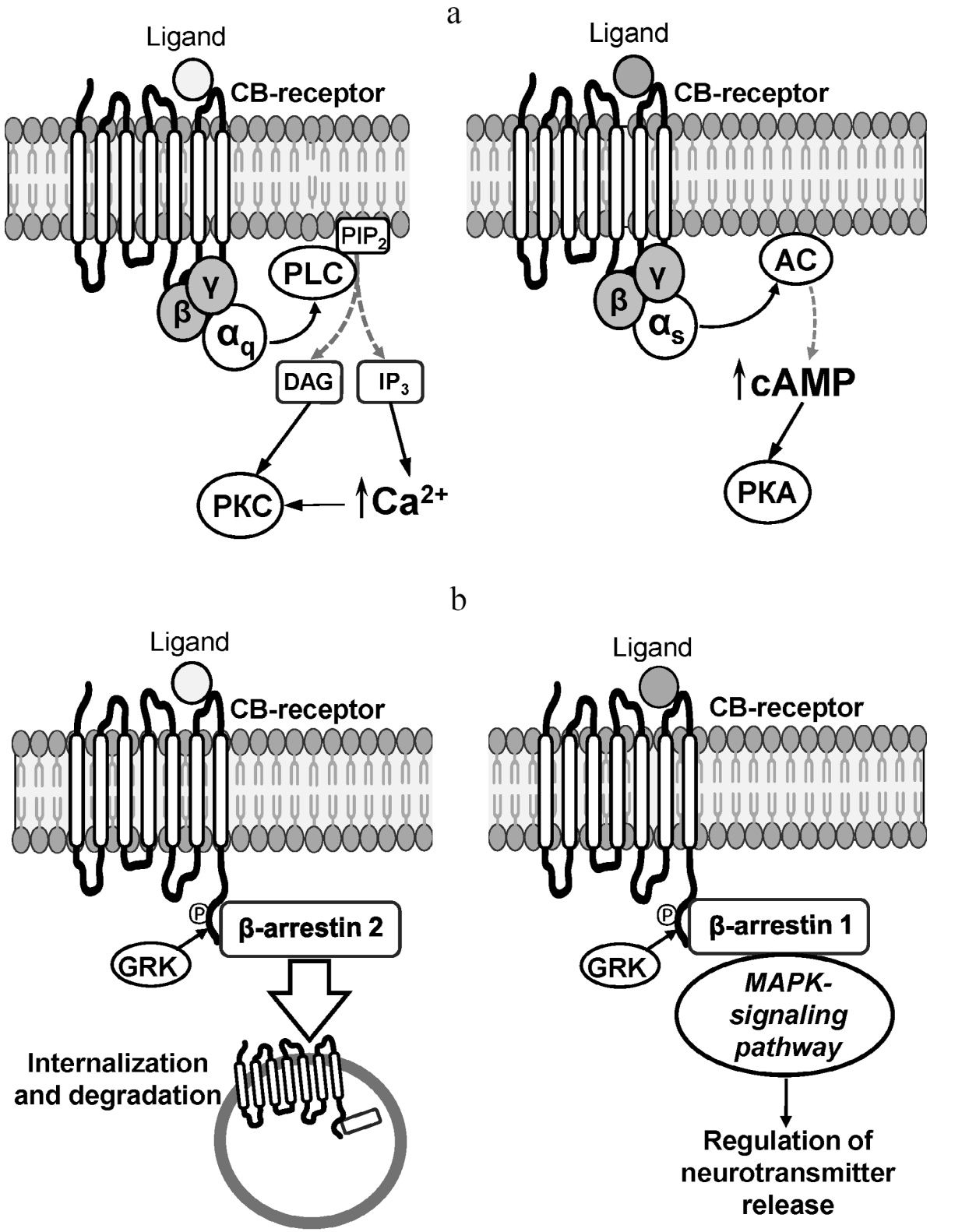

Fig. 3. Examples of possible noncanonical interactions of CB-receptors with G_q or G_s-proteins and triggering of intracellular signaling pathways leading to atypical cell responses to endocannabinoid action (a); with β-arrestins 1 and 2 (b). On the right the possibility of interaction of CB-receptors with β-arrestin 1 is shown, which could lead to initiation of noncanonical signaling pathways. Designations: ←, potentiation; ⊥, suppression; α, β, γ, G-protein subunits; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol-1,4,5-trisphosphate; GRK, G-protein coupled receptor kinase; P, phosphate.

in CB-receptor desensitization and internalization is only one of their multiple roles in the cells [12, 70]. It was discovered that β-arrestin 1 was able to bind CB1 receptors and to mediate subsequent effects of their activation, which demonstrated that β-arrestins could act as signal transducing agents independent on G-proteins. In neurons and synapses of the CNS β-arrestin 1 plays a role of the scaffold protein, which forms multiprotein submembrane complexes initiating cell signaling. Therefore, direct interaction of β-arrestin 1 with CB1-receptor molecules can initiate signaling cascades involving activation of MAPKs, including extracellular signal-regulated kinase Erk1/2, p38, etc., in particular [74-77] (Fig. 3b).

Moreover, it has been reported that cannabinoid ligands acting at concentrations many orders of magnitude lower than during triggering of G_i-protein-coupled cascades from CB1 receptors, are able to prompt recruitment of β-arrestin 1 to CB1 receptors and therefore cause stimulation of the G_s-protein-mediated signaling pathway (that happens in the presence of pertussis toxin) and increase in the cAMP level [63]. Therefore, to provide a complete picture of the implications of CB1 receptors interactions with their ligands in the CNS, we need to consider another manifestation of biased agonism –triggering of the G-protein-independent signaling pathways, which include β-arrestin 1 and subsequent MAPK (Erk1/2, p38) or AC/cAMP-system activation, which leads to variable functional effects [63, 73, 77]. This undoubtedly important but yet not very thoroughly conducted investigation of this noncanonical activity of the endocannabinoid system is hindered by the absence of specific blockers of β-arrestin 1 binding to CB1 receptors. Although,there is already a candidate for this role in the form of peptide fragment of one of the loops of β-arrestin 1, for which specific binding to the cytoplasmic loop motif on the C-end of the CB-receptor and ability to disturb β-arrestin 1 binding to CB1 receptors has been demonstrated [73].

Heteroreceptor interaction of CB-receptors. In addition to the recently becoming popular idea of ambiguous biased agonism of endocannabinoid receptors and their agonists, another distinctive feature of the CB-receptors has become obvious in the recent years. That is their tendency to form heteroreceptor complexes with other membrane receptors, which are either also G-protein coupled receptors (for opioids, adenosine, dopamine, serotonin, angiotensin receptor type 2) or possess intrinsic kinase activity (Trk-receptors of neurotrophins, epidermal growth factor receptors and others) [3, 9].

The data on co-expression and interaction of CB1 receptors with other membrane G-protein-coupled receptors in neurons and synaptic terminals indicate ambiguous functional implications. For example, in the case of co-expression of the CB1-receptors with the μ-opioid receptors selective activation of one of the two receptor types suppressed manifestation of the effects of selective activation of the other receptor type [78]. Reciprocal interaction between the $GABA_B$-and CB1 receptors in hippocampal synapses has been described, where neurotransmitter release inhibition due to activation of one of these receptor types was suppressed by activation of the other type of receptors [79]. The expression of CB1 receptors and D2-autoreceptors for dopamine was shown in dopaminergic terminals. Selective activation of only CB-receptors led to suppression of the [^{3}H]-dopamine release via G_i-protein activation and inhibition of AC activity. But when the CB-receptors and D2-autoreceptors were simultaneously activated, CB-receptors demonstrated a shift from activation of G_i-protein followed by dopamine release suppression to domination of the G_s-protein-mediated signaling: increase in the AC activity was observed together with accumulation of cAMP, activation of PKA, and potentiation of the [^{3}H]-dopamine release in brain slices [55]. Possible involvement of the regulators of G-protein signaling in realization of such effects is a matter of discussion [66]. It is important to note in regards to heteroreceptor interactions, that there are numerous examples of endocannabinoid and CB-receptor transactivation, when during stimulation of postsynaptic receptors by selective agonists modulation of the G_q-protein-mediated signaling pathways in the postsynaptic neuron occurs with increase in postsynaptic calcium levels and parallel increase in endocannabinoid production and CB-receptor activity in these structures [80]. For example, it is known that the brain-derived neurotrophic factor is able to induce release of endocannabinoids and their presynaptic action due to the binding to postsynaptic TrkB-receptors [81]. Therefore, pharmacological analysis of the noncanonical effects of CB-receptor agonists in the CNS synapses is getting even more complicated considering the possible indirect activation of these receptors and possible parallel involvement of other signaling molecules in the realization of endocannabinoid effects [12].

In the case of formation of heterodimeric complexes of CB1 and CB2 receptors, mutual reciprocal antagonistic interaction between them exists. Testing of agonist action on one of the receptor types in the CB1-CB2 complex showed a weaker binding of the agonist to its receptor, which is probably caused by the other receptor in the pair. In the same way, binding of the agonists to the second type of receptors in the pair was impaired by the first type of the receptors. Such bidirectional mutual antagonism (mutual suppression) of the receptor activity in the CB1-CB2 heteroreceptor complexes reflects that additional patterns of molecular interactions of CB-receptors exist, as well as yet unexplored ways of their activity regulation [82, 83].

Intracellular CB-receptors and CB-receptors of astrocytes. Without any doubt, the ability of cannabinoids to act on intracellular CB1 receptors located on mitochondrial membranes [84-86], endosomes and lysosomes [87] of neuronal cells can account for their noncanonical

effects. Mitochondrial CB1-receptors are coupled to G_i-proteins, but since this pathway starts in the mitochondrial matrix instead of the cytoplasm, it plays only an indirect role in the neurotransmitter release regulation via decrease in ATP production. In this case activation of the mitochondrial CB1 receptors could underlie the development of short-term depression of synaptic transmission in the hippocampus and the failure of memory formation during long-term synaptic activity [88, 89]. Only CB2 receptors were found so far in the endosomes and lysosomes of cortical and hippocampal neurons. The IP_3-receptor-mediated increase in intracellular calcium concentration takes place upon their activation, which implies the noncanonical coupling of these receptors to G_q-proteins [90, 91]. Increase in the intracellular calcium concentration, in turn, leads to the opening of the Ca^{2+}-activated chloride channels, which decreases neuron excitability [92]. Hence, the possibility of atypical intracellular localization of CB-receptors has been already recognized, as well as their noncanonical activity that specifically affects synaptic transmission and neuronal activity in the CNS.

Finally, possible implications of the CB1-receptors activation on astrocytes should be mentioned, where CB1 receptors were found on the cell membrane [93, 94] as well as on mitochondrial membranes [95-97]. While activation of the CB1 receptors in nerve terminals triggers the G_i-protein-coupled signaling cascades leading to depression of neurotransmitter release, activation of the astrocytic CB1 receptors by endocannabinoids released from the neurons causes increase in the cytosolic calcium concentration in astrocytes, which enhances the release gliotransmitters (glutamate, D-serine, adenosine) from astrocytes, including the endocannabinoids themselves [98, 99]. It is assumed that on the contrary to the relatively localized retrograde action of endocannabinoids in synapses, the spatiotemporal character of the CB1-induced calcium signals in astrocytes followed by the gliotransmitter release allows regulating synaptic transmission in a large number of synapses, located at a distance from the initial spot of endocannabinoid release [100]. Moreover, the astrocytes under the effect of endocannabinoids could be indirectly involved in realization of different forms of synaptic plasticity depending on the gliotransmitters they release and their receptors on the pre- and/or postsynaptic membranes, moreover, they could be involved in either inhibition of neurotransmitter release or its potentiation [99-103].

ENDOCANNABINOID-MEDIATED POTENTIATION OF SYNAPTIC TRANSMISSION IN CENTRAL AND PERIPHERAL SYNAPSES

Central synapses. The facts illustrating potentiating rather than inhibiting effects of endocannabinoids and their receptors on synaptic transmission in the CNS have been known for quite a while. But they are still few in numbers and for realization of these effects special conditions are required [104]. One example of such conditions is the case of heteroreceptor interaction of CB-receptors and dopamine D2-receptors in striatal synapses, where along with the well-known effects of G_i-protein activation in the nerve terminals upon exposure to exogenous agonists of CB-receptors leading to dopamine release inhibition, the simultaneous application of both CB-agonists and D2-agonists causes shift of intracellular signaling mechanisms (triggered by CB-agonists) from the G_i-protein-mediated pathways to the activation of G_s-protein followed by upregulation of the AC-cascade and potentiation of dopamine release [105, 106]. It is also often mentioned in the scientific papers that involvement of endocannabinoids in the long-term potentiation (LTP) in the hippocampus and the striatum is simply due to disinhibition and facilitation of the synaptic transmission in glutamatergic synapses. It occurs due to suppression of the GABA-mediated inhibition of the glutamate release by endocannabinoids via interaction between the heteroreceptor parts, i.e., it is an example of DSI development [107].

However, the real potentiating effects of endocannabinoids in the CNS synapses are also described, where the retrograde action of endocannabinoids in homosynaptically active synapses leads to facilitation of the neurotransmitter release. In particular, paired-pulse stimulation of post- and presynaptic structures in synapses with parallel registration of excitatory postsynaptic potentials was used in the study of activity of glutamatergic corticostrial synapses in the mice brain slices. Varying the number, frequency, and duration of the paired-pulse stimuli allowed finding conditions, which led to the development of synaptic plasticity in the form of either LTP or LTD, depending on the time interval between the pre- and postsynaptic stimuli. It turned out, that manifestation of the different forms of synaptic plasticity depended also on the level and pattern of endocannabinoid production in these synapses. The authors showed that formation of the short-term but highly concentrated endocannabinoids transients developed under specific conditions of synaptic stimulation caused LTP development, whereas long-term but moderate increase in endocannabinoid concentration induced LTD in these synapses. The observed LTP indeed was developed due to endocannabinoid release, since its induction was prevented by blocking CB1-receptors and was not observed in the CB-receptor knockout mice [108]. It was also shown that the diverse effects of endocannabinoids (LTP or LTD induction) could be associated with the balance between the activities of presynaptic PKA and calcineurin regulated by the presynaptic action of corresponding concentrations of endocannabinoids in the nerve terminals, and could also depend on the pattern of DAGLα activity in the postsynaptic structures [108, 109].

The ability of endogenous cannabinoids to cause LTP development was also shown in hippocampal synapses [110]. High frequency stimulation was used to induce weak and strong forms of LTP in the hippocampal slices. It turned out that under these conditions of synaptic activity endocannabinoid release could facilitate strong forms of LTP and inhibit the weak ones. The authors believe that the release of endocannabinoids and their action play a role of a high-pass filter, which controls the signal-to-noise ratio during intensive synaptic activity in the noisy synaptic circuits of the CNS [111].

Thus, it has been shown in the recent studies that noncanonical potentiating effects of endocannabinoids in the CNS do actually exist and can be observed experimentally. But manifestation of such effects requires certain time and frequency parameters of synaptic activity, close to the physiological ones, providing the right patterns of synthesis and concentration profiles of endocannabinoids in synapses [108, 111]. Anyway, the idea of noncanonical endocannabinoid activity underlying potentiation of synaptic transmission and transmitter release in the synapses of the CNS gets lately more recognition and support [13, 107, 108, 111].

Peripheral synapses. One of the peripheral structures possessing the endocannabinoid system is the skeletal muscle. The expression of not only CB1 and CB2 receptors, but also the enzymes of endocannabinoid synthesis and degradation were shown in skeletal muscles [112-114]. It was found that endocannabinoid release from the contracting muscle could lead to the development of inherent endocannabinoid effects in the muscle fibers themselves [115-117], and likely in the motor synapses as well [118, 119].

The first attempts to study the role of endocannabinoid system in the neuromuscular synapses of vertebrates were made in the end of the 20th and beginning of the 21st century [120-122]. The canonical inhibitory action on ACh release of these signaling molecules has been found in the synapses of cold-blooded animals [123, 124]. But even in the earliest work in this field increase in the amplitude of spontaneous miniature endplate potentials (MEPPs) and their frequency occurring without changes of evoked ACh release has been detected in the presence of THC [120].

Recently new evidence of the noncanonical action of cannabinoids underlying potentiation of ACh release in the motor synapses has been reported. In particular, investigation of the WIN effects in the mouse diaphragm motor synapses showed significant increase (by about 50%) in the MEPP frequency [60]. This was associated with the CB1 receptor activation and triggering of the presynaptic signaling pathway involving PLC, PKC and stored Ca^{2+} from the ryanodine-sensitive Ca^{2+}-stores. This facilitation of the spontaneous neurotransmitter release caused by WIN may seem surprising considering that inhibition of the evoked synaptic activity by exogenous cannabinoids in the CNS synapses is usually also accompanied by suppression of their spontaneous activity [125, 126]. The same classical experimental object – neuromuscular synapses of the mouse diaphragm – was used to reveal noncanonical potentiating effects of tonic use of endocannabinoids (2-AG and AEA) on the spontaneous neurotransmitter release. It turned out that although both AEA and 2-AG activated the same presynaptic receptor type – CB1, they caused changes in different parameters of MEPPs related to the spontaneous ACh secretion: AEA caused increase in the MEPP frequency by about 75% preventable by blocking of the L-type Ca^{2+}-channels or inhibition of PKA but not PLC. Whereas 2-AG caused increase in the ACh quantal size and in the MEPP amplitude by 50% preventable by vesamicol – an inhibitor of the vesicular ACh transporter [127]. It seems likely that the difference in AEA- and 2-AG-induced effects, which both develop from the CB1 receptor activation, but affect different parameters of the quantal ACh release, is due to the involvement of different signaling pathways and final targets in the nerve terminal (Fig. 4).

Such duality of the effects of activation of the same receptor type by the different agonists may be manifestation of the biased agonism in the CB1 receptor activation, existence of which is actively discussed nowadays in relation to the endocannabinoid system. Regarding the 2-AG-mediated increase in quantal size, a similar effect was also described in another work, where the long-time

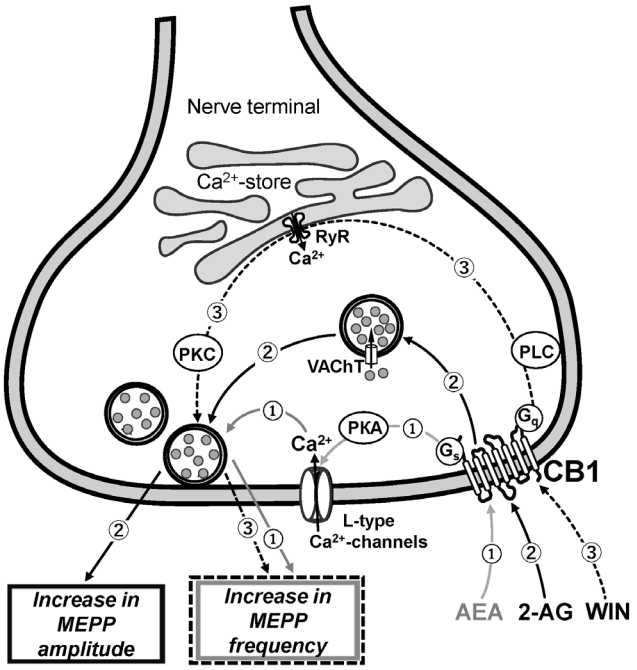

Fig. 4. Scheme of the possible different pathways starting from the CB1 receptors triggered by AEA – 1, 2-AG – 2, and WIN – 3, which lead to potentiation of the different parameters of spontaneous release of ACh quanta. Designations: ←, potentiation; RyR, ryanodine receptors; VAChT, vesicular acetylcholine transporter.

application of CB1 agonists also led to significant increase in the MEPP amplitude in the mouse diaphragm motor synapses, and this increase was prevented by blocking the vesicular ACh transporter. Enlargement of the size of cholinergic vesicles was detected 2-4 h after cannabinoid application to the muscle [118]. Potentiation of the neurotransmitters quantal size on the presynaptic level by enhancing of the neurotransmitter pumping into the vesicle is currently a well-known phenomenon in both central and peripheral synapses [128, 129]. This mechanism is considered as a specific presynaptic way of facilitation of the synaptic transmission mediated by various neuromodulators. Nevertheless, involvement of the endocannabinoid system in this mechanism of synaptic transmission potentiation is a new, noncanonical feature of these signaling molecules, which needs further investigation.

CONCLUSIONS

Analysis of the literature shows a large variety of targets and signaling pathways associated with endocannabinoid action in the central and peripheral synapses. The biased agonism, typical for endocannabinoids and their receptors, together with the functional and structural interaction of this system with other receptors significantly expand the range of endocannabinoid action and allow defining these substances as pleiotropic signaling molecules, which are able to exert diverse effects in form of both inhibition as well as potentiation of neurotransmitter release. The described ability of endocannabinoids to exert diverse and variable modulation of neurotransmitter secretion, demonstrates basic resemblance of this at first glance unique signaling system with other systems of chemical modulation of synapse activity. Re-evaluation of the potential functional implications and mechanisms of endocannabinoid action, with consideration of their noncanonical effects, will provide a deeper and versatile understanding and evaluation of the role of this signaling system under normal conditions and will allow to use the targeted changes of the modulatory potential of endocannabinoids in pathological states of the nervous system and other systems of the organism.

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