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REVIEW

Myogenic Classical Endocannabinoids, Their Targets and Activity

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Abstract—This review focuses on the recently discovered specific action of two classical endocannabinoids (ECs), 2-arachidonoylglycerol (2-AG) and arachidonoyl ethanolamide (AEA), in the case of their synthesis and degradation in skeletal muscles; in other words, this review is dedicated to properties and action of the myoendocannabinoid (myoEC) pool. Influence of this pool is considered at three different levels: at the level of skeletal muscles, motor synapses, and also at the level of the whole organism, including central nervous system. Special attention is paid to the still significantly underestimated and intriguing ability of ECs to have positive effect on energy exchange and contractile activity of muscle fibers, as well as on transmitter secretion in motor synapses. Role of muscle contractions in regulation of activity balance between the enzymes catalyzing synthesis and degradation of myoECs and, therefore, in the release of myoECs and exertion of their specific effects is thoroughly considered. Increasingly popular hypotheses about the prominent role of myoECs (AEA and/or 2-AG) in the rise of the overall level of ECs in the blood during muscle exercise and the development of "runner's high" and about the role of myoECs in the correction of a number of psychophysiological conditions (pain syndrome, stress, etc.) are discussed here. Thus, this review presents information about the myoEC pool from a totally new viewpoint, underlining its possible independent and non-trivial regulatory role in the body, in contrast to the traditional and well-known activity of neurogenic ECs.

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INTRODUCTION

In the late 20th and early 21st centuries, nervous and other animal and human tissues were shown to contain the animal analogs of phytocannabinoids (plant cannabinoids) that were termed endocannabinoids (ECs). They proved to be a specific group of lipophilic signaling molecules formed by enzymatic cleavage of phospholipids in the cell membranes. There are two major ECs best-studied to date: 2-arachidonoylglycerol (2-AG) and arachidonoyl ethanolamide (AEA), or anandamide. Pathways of their enzymatic synthesis and degradation and ability to be released from neurons and other cells have been described, as well as their specific effects on cannabinoid receptors (CB), the best-known of them being CB1 and CB2. Presence of the receptors for ECs has been shown in the nerve terminals and other regions of neurons, as well as in neuroglia and in the cells of some other tissues and organs [1].

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, arachidonoyl ethanolamide (anandamide); ACh, acetylcholine; BDNF, brain-derived neurotrophic factor; CB, cannabinoid receptors; CGRP, calcitonin gene-related peptide; DAGL, diacylglycerol lipase; ECs, endocannabinoids; EMC, electromechanical coupling; FAAH, fatty-acid amide hydrolase; MAGL, monoacylglycerol lipase; MEPP, miniature end-plate potentials; myoECs, myogenic endocannabinoids; NAPE-PLD, N-acylphosphatidylethanolamine-specific phospholipase D; PKA, protein kinase A; PLC, phospholipase C; PPAR, peroxisome proliferator-activated receptors; TRPV1, transient receptor potential vanilloid 1 channels.

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Activity of ECs in the CNS has been investigated in detail. It has been established that AEA and 2-AG are synthesized in the CNS synapses and released from postsynaptic structures "*on demand*", i.e., in response to synaptic activity and postsynaptic effect of the transmitter. Having been released from postsynaptic structures, ECs act on presynaptic CB receptors, causing retrograde inhibition of transmitter secretion [2].

However, the possibility of EC synthesis, release, and (reception?) recognition (with involvement of CB1 and CB2 receptors) has been currently proven not only for the nervous system, but also for other tissues, including adipose tissue, glands, blood cells, immune cells, skeletal muscle cells, where special EC-metabolizing enzymes are also localized [3-5]. Daily fluctuations in the EC levels in human and animal blood have been described, as well as their increase under stress conditions, obesity [6-10], certain types of muscle exercise, muscle and other disorders [11-13]. The problem of cellular sources that form systemic pool of ECs in blood and its mission under different conditions of body function has yet been poorly studied.

Skeletal muscles, which make up to 40% of the total weight of human body, are of particular interest in this respect as a potential source of ECs [11]. The ability of skeletal muscles to perform not only contractile but also a very significant secretory activity, which has been discovered in the past two decades, made it possible to detect a broad range of signaling molecules (myokines) synthesized in muscles and released into the extracellular environment and blood. In recent years, the list of myogenically secreted protein molecules and molecules of different chemical nature has increased to several hundreds of names [14].

Unexpectedly, ECs have been identified among these molecules [14, 15], which are referred to in this review as myoendocannabinoids (myoECs), including 2-AG, AEA, and related compounds [16]. It has been shown that these myoECs could be synthesized and cleaved by special enzymes, expression of which has been demonstrated in muscles along with the expression of CB receptors [17, 18]. At the same time, the level and effects of myoECs on muscle fibers and other targets could vary depending on the regimes of muscle activity [19, 20] and pathology [16, 21]. Despite this discovery and continuing high interest in cannabinoids as tools for correction of various functional conditions [22-24], the role of myoECs as a separate pool in the endocannabinoid system, which is capable of independent and diverse regulatory activity at the regional and systemic levels of the body, has not yet been specially considered in the reviews dedicated to the central [1, 2] or peripheral [11, 24-26] effects of ECs. In view of the above, aim of the present review was to summarize the data on the properties and effects of myoECs obtained in recent years and to describe their specific activities and mechanisms of action at different levels of the organism, taking into account the currently known membrane and intracellular targets of ECs.

ENZYMES FOR SYNTHESIS AND DEGRADATION OF MYOENDOCANNABINOIDS IN SKELETAL MUSCLES

In the recent decades, specific enzymes controlling synthesis and degradation of two best-known endocannabinoids, 2-AG and AEA, have been found in muscle tissue. It is known that ECs can be synthesized in neurons and other cells from the membrane phospholipids with involvement of specific lipid-modifying enzymes. The latter include diacylglycerol lipases (DAGL) and specific phospholipases, primarily phospholipase D (PLD) that cleaves N-acyl-phosphatidylethanolamines (NAPE) in the membrane. ECs are cleaved by monoacylglycerol lipase (MAGL) or fatty acid amide hydrolase (FAAH) (Fig. 1). It was established that the synthetic and metabolic pathways of 2-AG and AEA are fundamentally different [27]. 2-AG is synthesized with involvement of DAGL and metabolized mainly by MAGL. AEA, on the other hand, is synthesized mainly by the N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD) system and cleaved by FAAH [2, 28, 29].

Immunoblotting and reverse transcription polymerase chain reaction (RT-PCR) have revealed presence of the enzymes catalyzing EC synthesis and degradation (DAGLα and -β, NAPE-PLD, MAGL, and FAAH) in human and rodent skeletal muscles [17, 18]. At the same time, MyoEC synthesis in the muscles is not limited to two classical ECs: AEA and 2-AG. Structural analogs of AEA are formed via the pathway analogous to AEA synthesis; among them, the best-known analogs in muscles are the related compounds such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA). They can be released from the muscles simultaneously with AEA and 2-AG [16], though in smaller amounts. They exhibit their physiological effects bypassing CB1 or CB2 receptors, acting mainly as the endogenous agonists of peroxisome proliferator-activated receptors (PPAR), or transient receptor potential vanilloid 1 channel (TRPV1) receptors [11, 30].

The levels of ECs and related compounds in the skeletal muscles of 56 patients were determined by liquid chromatography. There was a significant spread of individual data, and the averaged values in the subjects of different sexes in the daytime were as follows: 1.5 ± 0.8 pmol/g for AEA; 30.4 ± 22.0 pmol/g for OEA; and 723.4 ± 392.7 pmol/g for 2-AG [31]. Analysis of the levels of same myoECs (2-AG and AEA) and the related compounds (OEA and PEA) in the adult rat muscles

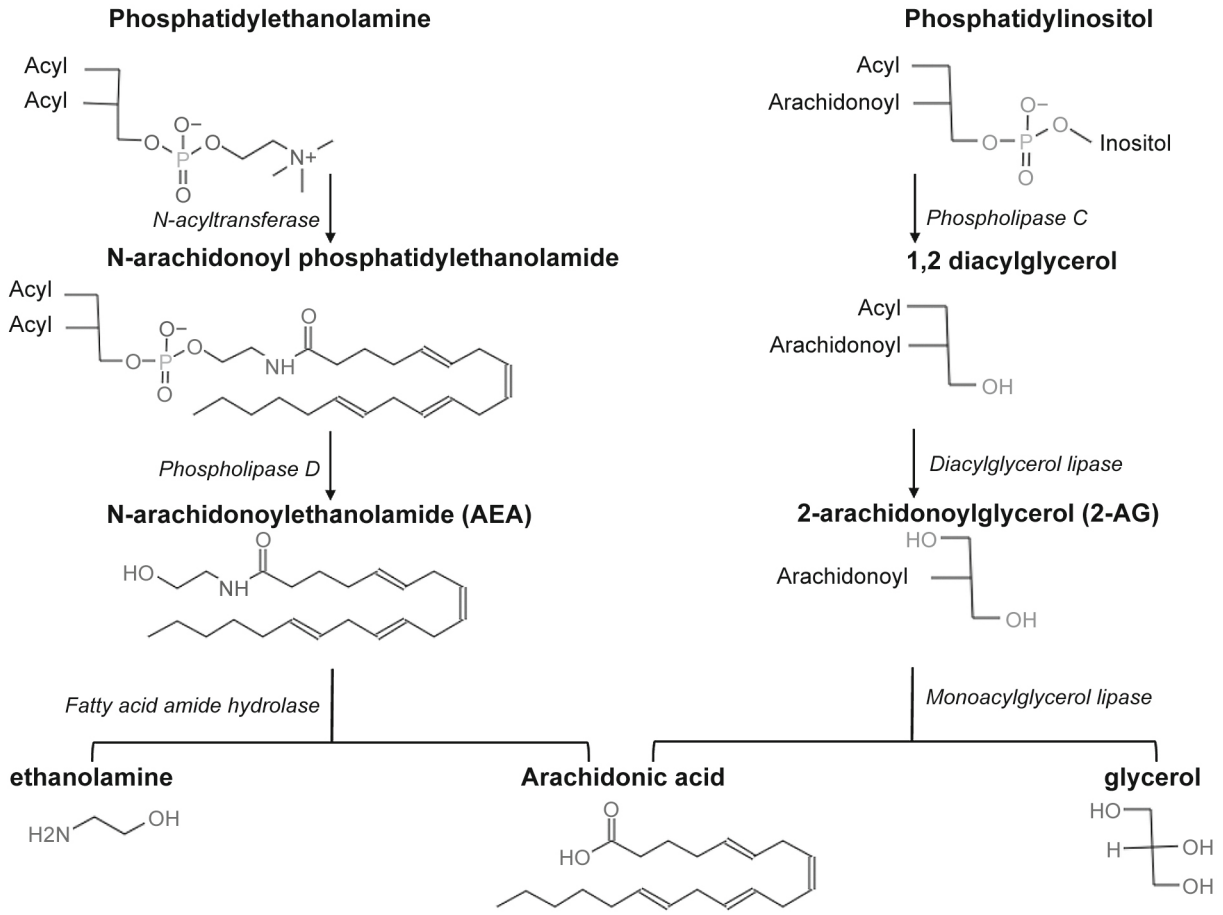

Fig. 1. Scheme of the major pathways of AEA and 2-AG synthesis and degradation.

Table 1. Expression of the mRNA of enzymes responsible for EC synthesis and degradation in different tissues

Tissue	Expression of mRNA, nTPM (normalized transcripts per million)				
	MAGL	DAGLα	DAGLβ	FAAH	NAPE-PLD
Nervous tissue (cerebral cortex)	121.0	17.3	12.3	20.4	11.7
Adipose tissue	165.9	1.7	7	7.3	1.9
Cardiac muscle	98.2	1.8	5.4	4.8	3.8
Skeletal muscle	60.9	1.2	12.8	9.2	2.3

Note. The table is compiled according to The Human Protein Atlas (https://www.proteinatlas.org). DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acylphosphatidylethanolamine-specific phospholipase D.

also showed 2-4-fold difference between the relative levels of 2-AG and AEA in the fast- and slow-twitch muscles, while the levels of the related compounds (PEA and OEA) were considerably lower and differed only slightly in various types of muscles [16].

At present, mRNA expression of the enzymes catalyzing endocannabinoid synthesis and degradation has been determined in quite a number of tissues, primarily adipose tissue and brain tissues (Table 1). However, human and animal skeletal muscles also demonstrate high level of mRNA expression of the enzymes responsible for AEA and 2-AG synthesis (NAPE-PLD and DAGL, respectively) and degradation (FAAH and MAGL, respectively) (Table 1).

It has also been shown that the level of mRNA expression of the enzymes for 2-AG and AEA metabolism

in muscles, as well as the levels of myoECs and related compounds, could vary depending on the regimes of muscle motor activity [19] and particular pathological conditions of skeletal muscles or organism: obesity, other changes in lipid metabolism [16], Duchenne muscular dystrophy-type pathologies [21]. Recently, *in vitro* and *in vivo* experiments in aging rats with the developing age-related sarcopenia have shown changes in the expression of the mRNA of enzymes responsible for 2-AG and AEA synthesis, which varied between different types of muscles, and these differences correlated with the intensity of regulatory effects of ECs on CB1 receptors in the corresponding skeletal muscles. Increase in the expression of mRNA of the enzymes synthesizing both 2-AG and AEA in the case of age-related quadriceps-dominant muscle atrophy was shown to be the most significant. Enhanced synthesis and increased level of myoECs in the muscles prone to sarcopenia correlated with the suppression of transmembrane glucose transport, reduced energy metabolism, and other characteristic metabolic rearrangements in the muscle fibers mediated by the CB1 receptor activity. Thus, it can be concluded that the muscle weakness in the case of muscle atrophy and hyperlipidemia could be related not only to the increase in the systemic level of ECs and lipids, but also to the higher level of the enzymes catalyzing EC synthesis in the skeletal muscles and to the enhanced autocrine effects of myoECs on the CB receptors [16].

An important peculiarity of regulation of the expression and activity of the enzymes responsible for myoEC synthesis and degradation in skeletal muscle is their dependence on the contractile activity of muscles. Correlation between the physical loads and increasing EC level in the blood, which was revealed for the first time 20 years ago [32], made it necessary to elucidate the role of contractile activity in the regulation of myoEC synthesis and release. Analysis of the dependence of activity of endocannabinoid system in human skeletal muscles on the regimes of motor activity (aerobic or with resistance) led to the conclusion that expression of the EC system components responsible for myoEC synthesis, degradation, and signaling depends on physical exercise, and these components differently respond to various types of physical loads [19]. The necessity of contractile activity of a muscle for the release of myoECs and manifestation of their effects on motor synapses (transmitter secretion) has also been revealed in the model of an intensively working isolated nerve–muscle preparation of the mouse extensor digitorum longus [33].

Along with determining the level of mRNA expression of the enzymes for EC synthesis and degradation, the techniques for pharmacological blockade of the activity of such enzymes are widely used nowadays to confirm and describe regulatory activity of ECs in the CNS synapses [34-37] and in skeletal muscles [38]. For example, inhibition of FAAH (the AEA degradation enzyme) by URB597 was accompanied by increase in the amplitude of miniature end-plate potentials (MEPP) and acetylcholine (ACh) quantal sizes in the synapses of the isolated mouse diaphragm. The effect was prevented by blocking CB1 receptors and, in the authors' opinion, was related to accumulation of myogenic AEA and its increased leakage from the muscle, followed by the presynaptic effect stimulating increase in the ACh quantal size in motor terminals [38].

Thus, now there is compelling evidence of expression and activity of the specific enzymes responsible for EC metabolism in skeletal muscles. It has also been confirmed that myoECs and the related compounds are synthesized in muscles and, after being released, could contribute to the tonic level of EC in the body, their regional and systemic activities. Canonical concepts concerning the role of enzymes of EC metabolism in the control of activities of these compounds in brain structures [1] have also been confirmed for skeletal muscles, and it has been shown that the myoEC synthesis, release, and activity depend on the expression of analogous enzymes in muscles. At the same time, the following specific feature has been revealed: involvement of contractile activity of muscles in the regulation of enzyme expression and in manifestation of myoEC effects at the regional and systemic levels of organism. Taken together, these data confirm the ability of skeletal muscles to synthesize myoECs in amounts sufficient for providing their independent regulatory effects, along with the known activity of ECs formed in the nervous, adipose, and other tissues.

CONDITIONS OF MYOENDOCANNABINOIDS RELEASE

Release of neurogenic ECs from postsynaptic structures in the CNS is considered to occur mainly "*on demand*", i.e., under the effect of transmitters on postsynaptic receptors. This is accompanied by the increase in intracellular Ca^{2+} level as a result of depolarization of the postsynaptic membrane or activation of G protein-coupled metabotropic receptors that trigger intracellular cascades accompanied by increase in the level of intracellular Ca^{2+}, which later leads to enzymatic Ca^{2+}-dependent EC synthesis from phospholipids of the postsynaptic membranes of neurons [1]. The possibility of tonic release and activity of ECs in the nervous tissue in the case of suppressed expression or pharmacological blockade of the EC degradation enzymes (MAGL or FAAH) has also been described [35-37, 39]. Accumulation of a constitutively synthesized pool of ECs under these conditions results in their tonic release from postsynaptic

neurons in the CNS and realization of the effects analogous to those observed during their induced release "*on demand*" or under the action of exogenously applied ECs [40].

Despite insufficient knowledge, it has been reported that the transmembrane EC transport is possible with involvement of the membrane-associated fatty acid-binding proteins (chaperones), the so-called FABPs expressed in neurons [41] and capable of binding hydrophobic EC molecules, thereby facilitating their transport across the membrane or to the sites of intracellular localization of EC degradation enzymes [42-45]. The examples are known when the genetic knockout or blockade of the FABP-type proteins by inhibitors results in prolongation of the presence of ECs in the intra- or extracellular environment and potentiation of their effects in the CNS synapses [43, 45, 46].

With regard to peripheral synapses, it has also been shown in mouse motor synapses under the conditions of inhibition of EC degradation enzymes that there is a possibility of tonic release of endogenous myoECs to the synaptic cleft and their further effects similar to the effects of exogenous ECs [15, 33, 38].

In addition, it was shown in our recent studies that the contractile muscle activity could also be an independent factor contributing to the release of myoECs and manifestation of their retrograde presynaptic effects [33]. The studies at the whole organism level also demonstrate that the regimes of acute and chronic loads on skeletal muscles could suppress expression of the AEA (and less frequently 2-AG) degradation enzymes in the subjects, contributing to accumulation and release of myoECs from the muscles and to increase in the EC systemic level in blood. This could affect both the whole organism [24] and the state of muscles [15, 19, 47-49]. Although these concepts have not yet been unambiguously confirmed, it has been undeniably proven using other myokines [such as interleukin 6 (IL-6), brain-derived neurotrophic factor (BDNF), irisin, fibroblast growth factor 2 (FGF2), insulin-like growth factor 1 (IGF1), etc.] that contractile activity of the skeletal muscles and increased level of Ca^{2+} in the myoplasm are precisely the factors that trigger release of myokines and increase in their level in circulating blood, where they can perform hormone-like and other functions [14, 19, 50, 51]. In addition, various signaling molecules that enter bloodstream from different sources during physical exercise (such as BDNF, IL-6, etc.) could also affect the CB1 receptors in muscles (due to heteroreceptor interactions) and stimulate release of myoECs [51-54].

Thus, conditions and mechanisms facilitating release of myoECs from the skeletal muscles are apparently similar to those in other cells, though in this case there is also an important special regulatory factor, namely, contractile muscle activity.

TARGETS OF ENDOCANNABINOIDS IN MUSCLE FIBERS

The first and closest targets of myoECs released from skeletal muscles are obviously muscle *per se*, its membrane and intracellular receptors. Diverse broad-spectrum targets and EC receptors have been described in skeletal muscle fibers. These include specific G protein-coupled metabotropic CB receptors type-1 and -2, TRPV1, nuclear receptors type PPAR and NR4, orphan receptors GPR55 and GPR18 [19, 55, 56] (Fig. 2).

In muscles, the best-known and most abundant receptors are CB1 and CB2 receptors that are also present in other tissues (nervous, adipose, liver, etc.) [57]. These two types of receptors can be activated by both two major (2-AG and AEA) and some other cannabinoids, or by structurally similar lipids.

Along with the initial idea of CB receptor coupling with the $G_{i/0}$ type proteins, the possibility of their interaction in neurons with other G proteins (G_s and G_q) [58, 59], as well as with β-arrestins [40, 60] has been suggested in recent years. Similar polyfunctionality of the CB receptors proved to be inherent also in the muscular CB receptors [61-63] that can trigger numerous intracellular cascades in the muscle fibers (Fig. 2).

At present, CB1 and CB2 receptors have been described on the sarcolemma of developing myoblasts [13] and in different types of functionally mature muscle fibers [17, 18, 64]. Depending on the experimental objects and conditions, the CB1 receptors were shown to prevail in the fast-twitch skeletal muscles (*m. gastrocnemius*, *m. tibialis anterior*) compared to the slow-twitch ones (*m. soleus*) [65-67], or *vice versa* [68].

It has been shown that localization of the CB1 receptors within a single sarcomere is confined to the central I-line and does not correlate with the positions of ryanodine receptors (RyR) identified at the edges of sarcomere, in the sites of location of T-tubules and Z-lines [69].

Expression and density of the CB1 receptors on skeletal muscle fibers, as well as myoEC content in the latter change during the embryonic and postnatal maturation of the muscles and in the case of such pathologies as sarcopenia and muscle dystrophy [16, 70]. During the maturation of myoblasts, local release of myogenic 2-AG inhibits further myogenesis through activation of the CB1 receptors and of the G_q/PLC (phospholipase C) cascade that inhibits Kv7 channels involved in maturation of myotubules [71].

The possibility of activation of muscular CB1 receptors by exogenous 2-AG and AEA has been described, with subsequent decrease in the cAMP level, suppression of the activities of PKA and dihydropyridine receptors of the T-tubules of fibers [69] or the mitochondrial Ca^{2+}-uniporter [20]. The effect of ECs on muscular CB1 receptors could trigger both the PI3-PKB/Akt kinase

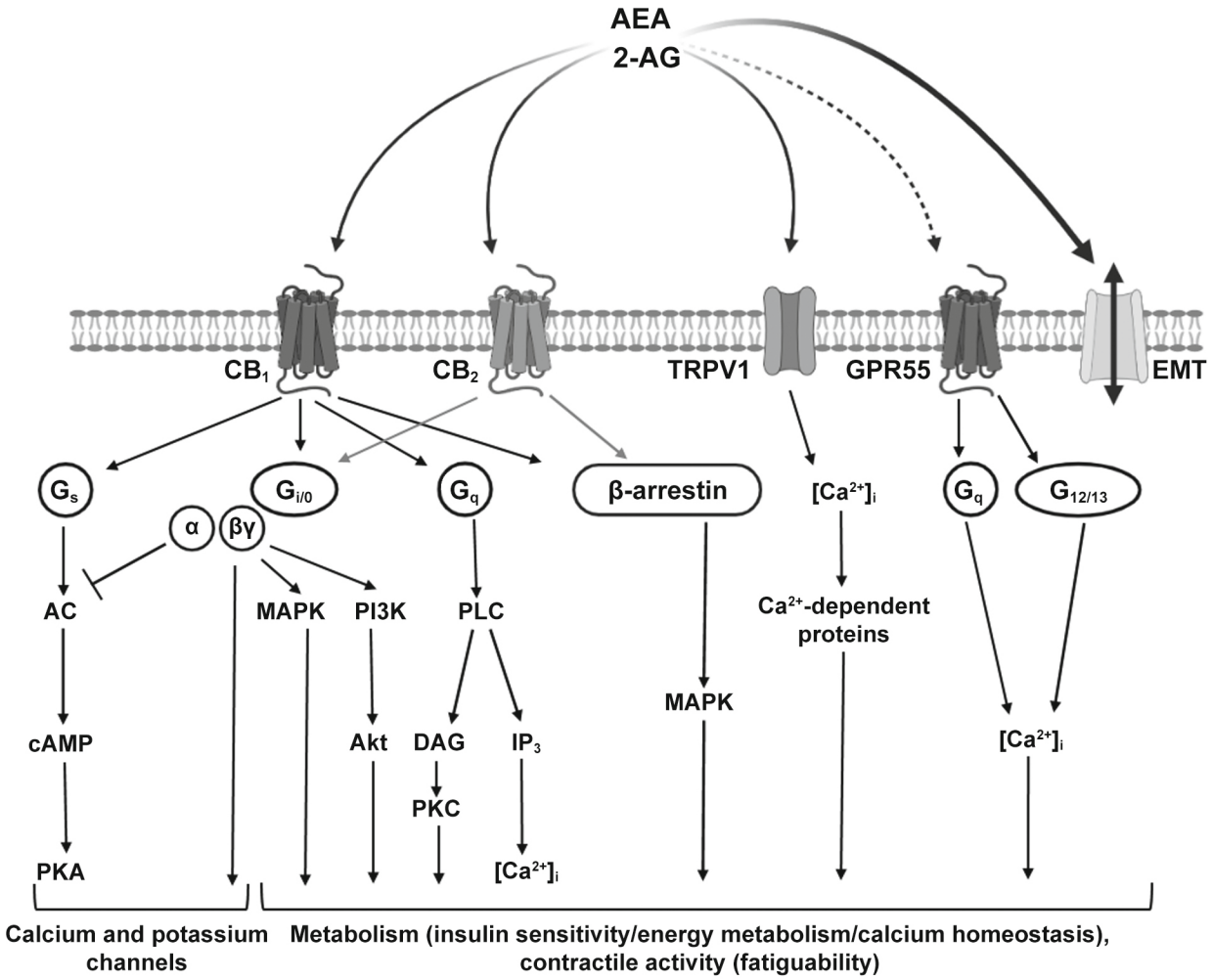

Fig. 2. Targets and molecular cascades triggered by arachidonoyl ethanolamide (AEA) and 2-arachidonoylglycerol (2-AG) in muscle fibers. AC, adenylyl cyclase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; PLC, phospholipase C; DAG, diacylglycerol; IP_3, inositol-1,4,5-triphosphate; $[Ca^{2+}]_i$, intracellular calcium concentration;; CB1 and CB2, cannabinoid receptors type-1 and -2, respectively; TRPV1, transient receptor potential vanilloid 1 channels; GPR55, G protein-coupled orphan receptor type 55; EMT, endocannabinoid membrane transporter.

and Raf-MEK1/2-ERK1/2 kinase pathways [72, 73]. The final target of MAP kinase cascades could be transcription of the genes for some muscle proteins, in particular, myostatin and interleukin 6 [74]. In addition, these pathways are assumed to inhibit the insulin-mediated glucose uptake by muscle fibers and the PKB/Akt enzyme activation in the myoplasm during activation of the muscular CB1 receptors [75]. Testing of the effects of CB1 receptor agonists and antagonists has led to the conclusion widely recognized in the literature that ECs are able to cause insulin resistance of the muscle fibers and to exert negative effect on the functional and metabolic status of muscles [13, 75-77]. However, such manifestations are usually pronounced in the case of persistently elevated EC level in blood, i.e., under conditions of systemic hyperactivity of the EC system, as well as enhanced expression of CB receptors in the muscles, which are observed, e.g., in the case of obesity and other pathologies accompanied by elevated lipid levels, in particular, in sarcopenia [16], dexamethasone-induced dystrophy [78], and Duchenne muscular dystrophy [22, 70]. Such cases are indeed characterized by the muscle attenuation, which is mediated, at least partially, by the excess of myoECs.

It should be noted, however, that insulin resistance and suppression of glucose uptake, which are most pronounced in the cases of elevated EC tone and hyperlipidemia of organs and tissues, as well as excessive expression of CB receptors, is observed not only in skeletal muscles but also in other insulin-dependent organs (liver, adipose tissue) [65, 77]. Thus, the effects of systemic ECs not only on muscular [74] but also on CB receptors of other insulin-dependent organs seem to be aimed primarily at regulating the balance

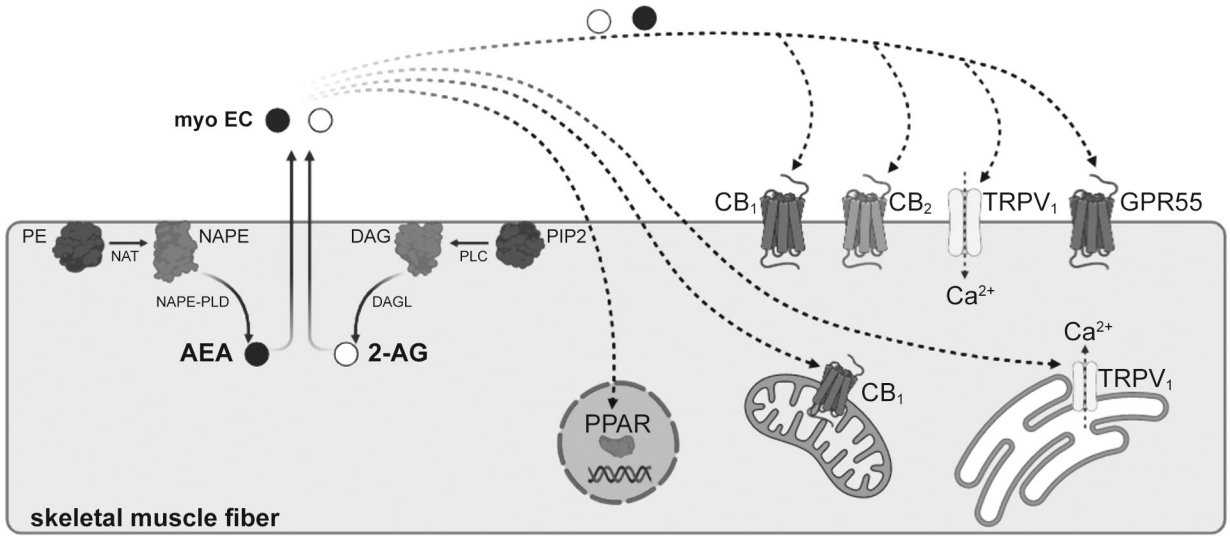

Fig. 3. Scheme of myoEC synthesis and effects in skeletal muscle fibers. The membrane phosphatidylethanolamines (PE) are transformed into N-acyl-phosphatidylethanolamine (NAPE) with involvement of N-acetyltransferase (NAT) and next into AEA with involvement of NAPE phospholipase D (NAPE-PLD). The membrane phosphatidylinositol-4,5-bisphosphate (PIP2) is transformed into diacylglycerol (DAG) with involvement of phospholipase C (PLC) and next into 2-AG with involvement of diacylglycerol lipase (DAGL). CB1 and CB2, cannabinoid receptors type 1 and 2; TRPV1, transient receptor potential vanilloid 1 channel; GPR55, orphan G protein-coupled receptor 55; PPAR, peroxisome proliferator-activated receptors (according to Ge et al. [15], with modifications).

of systemic energy metabolism in the whole organism, but not at selective suppression of energetic and contractile activity of the muscles under normal conditions [13, 65, 79].

It should be emphasized that the widespread opinion on the primary function of muscular CB1 receptors in the development of insulin resistance, suppression of energy metabolism and muscle activity does not reflect all possible manifestations of their regulatory activity.

Some of the revealed effects did not fit into the framework of the generally accepted concept of negative effect of the EC system on skeletal muscles, when either activation or blockade of the muscular CB receptors caused either suppression or stimulation of glucose metabolism and functional status of muscles depending on the conditions of selected experimental models, i.e., depending on concentration, quality, conditions, and time of incubation of various CB1 agonists and antagonists [22]. It was demonstrated using selective genetic knockdown of the muscular CB1 receptors that the presence of this pool in the muscles is necessary for maintaining normal structural and functional status of the muscle fibers (glucose uptake, mitochondrial microstructure, and contractions) under conditions of their activation by the endogenous ECs *in vivo* [20]. One more example of positive shifts in contractile activity and muscle metabolism under the effect of ECs is the EC-induced activation of Ca^{2+}-permeable TRPV1 channels present in the plasma membranes and in the sarcoplasmic reticulum of skeletal muscles (Fig. 3) [80]. It has been noted that AEA at a certain dose can improve glucose uptake by the muscles and activate some of the key molecules of mitochondrial biogenesis and insulin-triggered signaling cascades [13, 81]. The possibility of dual (stimulatory or inhibitory) dose-dependent effects of AEA in the regulation of metabolic and mitochondrial processes has been described recently in the C2C12 myocytes under simultaneous effects of AEA on CB1 receptors and TRPV1 channels [56].

Among the targets of ECs in muscle fibers, the pool of CB1 receptors incorporated in the outer mitochondrial membrane plays an extremely important role (Fig. 3), as their density can exceed the CB1 pool of the outer plasma membrane [82, 83]. ECs and synthetic or plant-derived agonists reaching mitochondrial CB1 receptors can inhibit activity of mitochondrial dehydrogenases, Krebs cycle, and energy metabolism of the muscle fibers [61, 62, 84]. At the same time, recently it has been shown that activation of the muscular CB1 receptors by endogenous ECs *in vivo* is aimed at maintaining normal microstructure and enzymatic activity of mitochondria [20].

It is known that not only CB1 but also CB2 receptors are able, though to a lesser extent, to participate in regulation of metabolic processes in muscles [65]. In particular, they have been shown to play a role in stimulation of glucose metabolism, as well as in regulation of the activity of mitochondrial electron transport chain and ATP synthesis in the C2C12 myocytes [85], stimulation of fiber regeneration [65], and prevention

of myotubule degeneration in the cancer-mediated muscle cachexia [86].

Difficulties of assessing consequences of activation and functional role of muscular CB receptors are complicated even more by the possibility of their heteroreceptor interaction with other metabotropic receptors, which form oligomeric complexes with CB receptors and thereby are able to modify the effects of CB1 receptors under conditions of joint activation by the appropriate ligands. Despite the limited data, the possibility of interaction of muscular CB1 receptors with the A_{2A} and A_{2B} adenosine receptors, as well as with β2 adrenergic receptors in regulation of metabolic processes in muscles has already been shown [65], as well as their interaction with the muscarinic cholinoreceptors in regulation of activity of motor synapses [64].

Along with the "classical" receptors (CB1 and CB2), "nonclassical" EC receptors such as GPR18 [87] and GPR55 [62, 65] have also been found in muscles; their activity has been studied much less. GPR18 are expressed by myoblasts during differentiation; they could participate in the fusion of myoblasts and facilitate muscle regeneration when activated by their endogenous agonists, e.g., N-arachidonoylglycine [87]. GPR55 that could interact not only with AEA and 2-AG as ligands, but also with lysophosphatidylinositol and arachidonoyldopamine, are also involved in regulation of metabolic processes in muscle (in particular, in regulation of insulin resistance in muscle fibers) [62].

Finally, PPAR, which are nuclear receptors functioning as transcription factors, have been described in muscles. Free fatty acids, eicosanoids, ECs, and related compounds are endogenous ligands of PPAR [88]. All three known PPAR isoforms (PPARα, PPARβ/δ, PPARγ) are expressed in muscles. At the same time, PPARα and PPARβ/δ play the key role in regulation of lipid metabolism, while PPARγ promotes insulin-dependent glucose transport [89]. When activated by endogenous ligands, including ECs (Fig. 3), PPAR stimulate expression of the genes regulating energy metabolism, lipid and lipoprotein metabolism, as well as the genes responsible for proliferation and inflammation [90].

Along with PPAR, the muscle fiber NR4 nuclear receptors activated by one of the synthetic analogs of EC, arachidonyl-2′-chloroethylamide (ACEA), have also been found recently. It is considered that the NR4 receptor gene, when activated by ACEA, could be involved in the expression of different mitogenic and inflammatory factors, as well as factors regulating muscle metabolism [55].

It should be emphasized that the current list of potential myoEC targets at the level of skeletal muscles is still incomplete and is continuously extended. To date, it already includes, in addition to the above, Na^+ channels, β-arrestins, satellite cell receptors, and other yet poorly studied targets, which have been discussed in more detail in the recent review by Dalle et al. [65].

The presented data on numerous potential targets of myoECs obviously show that the range of their regulatory effects on muscle fibers could be extremely wide, encompassing metabolic, functional, and genomic processes. At the same time, it becomes quite clear that, depending on the conditions of activation of particular targets by various endogenous ECs and their analogs, as well as on the state of muscles and whole organism, there could be manifestations of both inhibitory and potentiating effects of ECs. As a result, adequate assessment of the regulatory role of myoECs in the skeletal muscles has not yet been completed and requires further detailed investigation, which considers the entire system of EC targets and conditions for their selective activation.

REGULATION OF MUSCLE CONTRACTION WITH INVOLVEMENT OF CB RECEPTORS AND ENDOCANNABINOIDS

Considering the spectrum of EC effects on muscles, the most important issue seems to be their effect on contractile activity. The first observations of the consequences of using cannabis and phytocannabinoids, or systemic effects of the synthetic exogenous endocannabinoids on the body demonstrated, in addition to psychotropic effects, also the syndrome of muscle relaxation [91], or muscle fatigue [92]. In the early works, activation of CB1 receptors during systemic administration of synthetic cannabinoids also decreased locomotor activity of the mice, while administration of CB1 inhibitors increased this activity [93]. However, it remained unclear whether the developing muscle weakness during the systemic increase in the level of ECs was a consequence of their direct effect on muscles and their CB receptors, or it was mediated by the changes in the neurogenic control of voluntary contractions under the influence of ECs and neural CB receptors. In addition, in the light of discovery of myoEC activity, the question arose whether and how the contractile activity of muscles can change with a local EC release and effect on muscles compared to the tonic systemic effect of ECs in the whole body.

The studies of the direct effect of synthetic cannabinoids, including WIN 55,212-2 (WIN), on frog muscle contraction have shown that cannabinoids suppress the caffeine-induced contracture and the processes of electromechanical coupling of muscles, limiting the amount of Ca^{2+} released from the SPR during contraction [66].

Analysis of the direct effects of exogenous ECs in myotubules (C2C12 culture) and in isolated mature

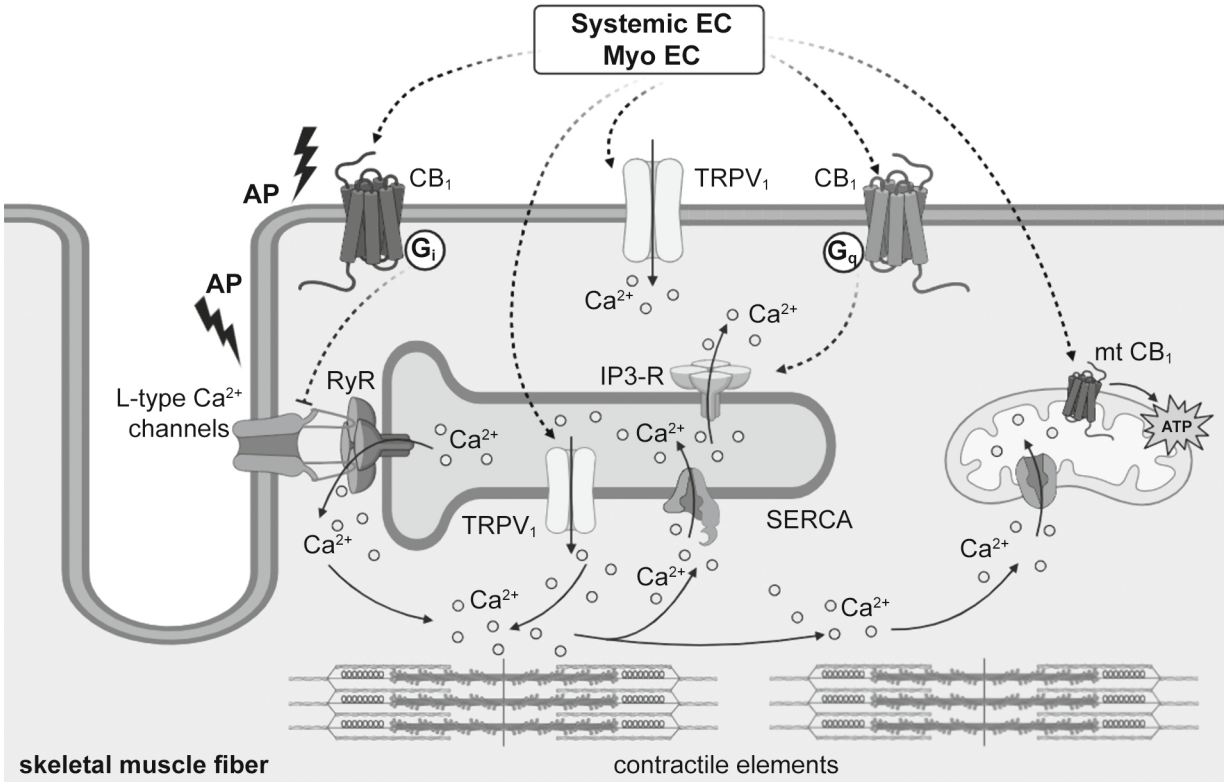

Fig. 4. Effects of myoECs on electromechanical coupling, Ca^{2+} homeostasis and muscle contraction with the involvement of targets such as L-type Ca^{2+}channels (same as dihydropyridine receptors), membrane and mitochondrial CB1 receptors, mitochondrial Ca^{2+} uniporter, TRPV1 (according to Singlár et al. [20] and Oláh et al. [69], with modifications). AP, action potential; mtCB1, mitochondrial CB1 receptors; RYR, ryanodine receptors; IP3-R, inositol-1,4,5-triphosphate receptors; SERCA, Ca^{2+} ATPase of the sarcoplasmic reticulum.

muscles [*m. soleus* and *m. extensor digitorum longus (m. EDL)*] did not reveal any changes in the single or tetanic contractions of muscle fibers, as well as in the values of short-term increases in the level of intracellular Ca^{2+} during its release from the SPR in response to IP3. However, direct activation of CB1 receptors by the exogenous agonists accelerated development of fiber fatigue in response to the long-term tetanic stimulation, decreased total level of intracellular Ca^{2+} and efficiency of electromechanical coupling (EMC) in fatigued muscle fibers [69]. These inhibitory effects of ECs were prevented by the pertussis toxin disrupting the G_i protein-mediated signaling, but persisted in the case of blocking ryanodine receptors. Therefore, the authors suggest that the dihydropyridine receptors (DHPR) of the T-tubules of muscle fibers could be the final target sensitive to the decrease in the cAMP level and PKA activity during the CB1 activation. However, other works demonstrated the possibility of the direct, not CB receptor-mediated effect of cannabinoids on DHPR of the T-tubules of skeletal muscles, which also weakens EMC in the muscle fibers (Fig. 4) [94].

The mice with generalized knockout of the CB receptor genes showed no changes in the strength of contractile responses of muscles to single stimuli, but there was increase in the fatigue resistance, short-term increase in the Ca^{2+} level in the myoplasm, and enhanced efficiency of EMC in the fibers during the generation of contractions. These data, in the authors' opinion, indicate that under normal conditions there are negative constitutive effects of ECs circulating in the body on muscular CB, involving limitation of contractile and metabolic activity of the muscles [69]. At first sight, this fact partially correlates with the data on negative effects of exogenous ECs and synthetic CB receptor agonists on the isolated muscles manifested as limited efficiency of EMC during contraction and enhanced muscle fatigue under long-term loading.

At the same time, recently is has been shown that the selective knockdown of the *Cnr1* gene encoding CB1 receptors in skeletal muscles and, hence, off-switch of the pool of muscular but not all other CB receptors in the mice leads to the opposite pattern of changes in contractile activity, EMC processes, and mitochondrial state [20]. It means that function of the pool of exclusively muscular CB activated by endogenous ECs, which are present in the body under normal conditions, could be targeting not limitation but maintenance of the structural and functional status of the muscles. In this case, the autocrine effect

of myoECs on CB receptors could play the role of a positive feedback supporting muscle activity. This is also supported by our recent data on the stimulatory effects of myogenically released ECs on ACh secretion and on the activity of mouse motor synapses [33].

The data accumulated in recent years do not yet provide an unambiguous answer to the question about the role of ECs in the regulation of muscle contractions, thereby once again proving relevance of the problem of conditions for manifestation of inhibitory or positive EC effects on muscles. It could be suggested that the effects of ECs limiting energy metabolism and functional activity of muscles represent only one of the possible aspects of the EC system functioning, which is manifested mainly when dysregulation of lipid metabolism and/or pathologically high increase in the systemic level of ECs take place. At the same time, under normal conditions and considering autocrine activity of myoECs, the effects of endogenous ECs could involve maintenance of contractile activity, metabolic, structural, and functional status of the muscles.

REGULATION OF ACTIVITY OF MOTOR SYNAPSES WITH INVOLVEMENT OF MYOENDOCANNABINOIDS

Specific activity of ECs (AEA and 2-AG) in the form of their narrowly targeted retrograde action on CB receptors of terminals facilitating inhibition of transmitter secretion, has been described in detail in the CNS synapses [1, 95]. The problem of similar inhibitory (or any other) activity of ECs in the peripheral motor synapses of skeletal muscles has not been studied in sufficient detail, though recently it has been attracting more and more attention [15, 38], particularly due to discovery of the special myoEC pool [15, 33, 38, 96, 97].

The first studies of the effects of exogenous cannabinoids in the motor synapses of different groups of animals yielded contradictory results: together with the inhibitory effects, there were also noncanonical potentiating effects on the MEPP amplitude and frequency, and on the quantal content of the end-plate potentials (EPPs) [64, 98-102]. The exogenous CB receptor agonist WIN (20 μM) caused a significant (more than 50%) increase in the MEPP frequency in synapses of the mouse diaphragm, which depends on the activity of presynaptic CB1 receptors, phospholipase C, protein kinase C, and release of the deposited Ca^{2+} from the ryanodine-sensitive Ca^{2+} depots [97]. In addition, there was a noncanonical presynaptic effect of exogenous AEA (30 μM) in synapses of the mouse diaphragm manifested as higher frequency of spontaneous secretion of ACh and increase in the EPP quantal content. At the same time, these effects of exogenous AEA seem to be realized with involvement of the presynaptic L-type Ca^{2+} channels, since these effects are prevented by blockade of the Ca^{2+} channels with nitrendipine (Fig. 5a) [96, 103].

The recently discovered ability of 2-AG to stimulate increase in the ACh quantal size by acting on the presynaptic CB1 receptors and, thereby, to potentiate neuromuscular transmission has been even more unexpected [33, 96, 103]. The ability of some modulators to stimulate loading of a transmitter into the vesicles and to increase the size of one quantum of neurotransmitter is well-known both for the CNS synapses [104, 105] and for peripheral synapses [106-108], but without the involvement of ECs. In the peripheral motor synapses, such potentiation of ACh quantal size has been described in the case of presynaptic effect of BDNF [109] released from the muscles when they are active, as well as under the effect of calcitonin gene-related peptide (CGRP) secreted from the electron-dense

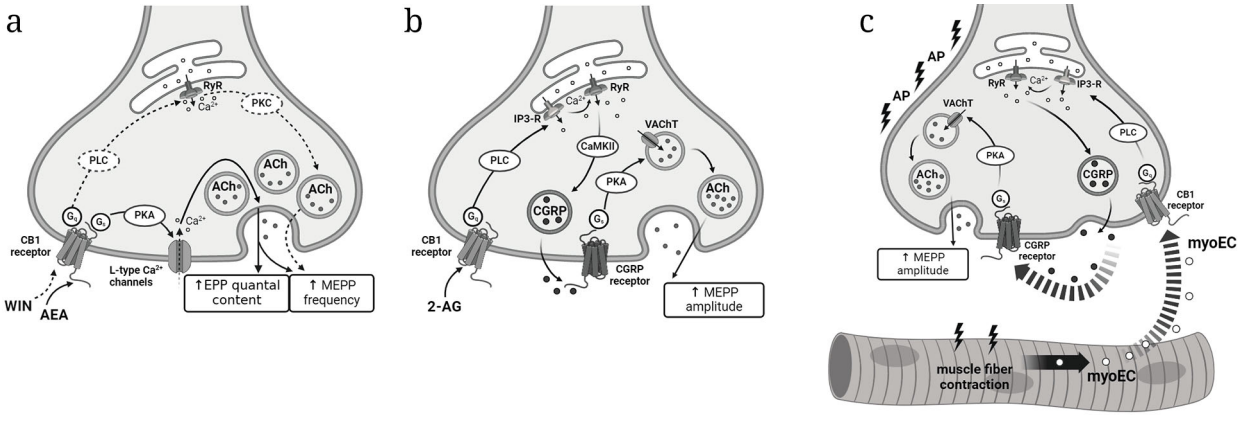

Fig. 5. Summarized diagram of presynaptic effects of exogenous AEA and WIN (a), 2-AG (b) and myogenic ECs (c) facilitating quantal release of ACh in the terminals of motor synapses (for more details, see text of the review). ACh, acetylcholine; CGRP, calcitonin gene-related peptide; myoECs, myogenic endocannabinoids; AP, action potential; RYR, ryanodine receptor; CaMKII, Ca^{2+}/calmodulin-dependent kinase II; CB1 receptor, cannabinoid receptor type 1; IP3-R, inositol-3-phosphate receptor; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; VAChT, vesicular acetylcholine transporter.

vesicles (EDV) of the motor terminals [110, 111]. Our studies have shown that the effects of 2-AG are apparently mediated by the action of CGRP in synapses, since they are completely prevented by blocking CGRP receptors, as well as by blocking CaMKII and RyR, activity of which is required for the CGRP release from EDV of the motor terminals [33, 110, 111]. Thus, it could be assumed that activation of the presynaptic CB1 receptors by exogenous 2-AG triggers the mechanism of EDV exocytosis and release of endogenous CGRP from the terminals into the synaptic cleft. In turn, the subsequent function of CGRP in synapses is to activate presynaptic CGRP receptors and to stimulate the PKA-dependent ACh loading into vesicles, which leads to the increase in the ACh quantal size (Fig. 5b) [33].

It should be emphasized that myoECs could, likely, exert a similar activity. This has been confirmed by the data obtained during tetanic stimulation of the motor nerve (*n. peroneus*) leading to intensive contractions of the extensor digitorum longus muscle (*m. EDL*) in the isolated neuromuscular preparation [33]. Our team has shown that the high-frequency firing activity of synapses and muscle is immediately accompanied by significant (25-30%) increase in the MEPP amplitude, which not only depends on presynaptic CGRP receptors but also requires obligatory activation of CB1 receptors. Generation of a series of action potentials (APs) during rhythmic synaptic activity and muscle contractions is accompanied by the release of myogenic ECs, and their effect on terminals triggers release of CGRP (Fig. 5c). This peptide, in turn, through its autocrine effect on presynaptic CGRP receptors, triggers the cascade leading to stimulation of the ACh loading into vesicles, increase in the ACh quantal size and, thereby, increase of MEPP amplitude in synapses [33].

It is noteworthy that, even in a resting muscle during the long-term (2-4 h) application of the AEA degradation inhibitor (URB597), it was possible to achieve accumulation of the pool of endogenous myoECs and to observe their retrograde presynaptic effect causing increase in the MEPP amplitude, which was prevented by blocking the CB1 receptors and by vesamicol (inhibitor of ACh loading into vesicles) [33].

Thus, in contrast to the central synapses, where ECs retrogradely inhibit transmitter release and secretion by acting on presynaptic terminals, now it has been shown that AEA and 2-AG exhibit a noncanonical regulatory activity in the peripheral motor synapses causing increase in the number of the released quanta of neurotransmitter and in the size of single quanta of ACh, respectively. Importantly, such noncanonical potentiating effects are observed not only under the action of exogenous AEA and 2-AG but also under the action of endogenous ECs, either of those released from skeletal muscles during contractions, or those accumulating in the synaptic cleft at rest under conditions of preliminary inhibition of the EC degradation enzymes. It means that, along with the recently established role of muscular CB receptors in the maintenance of structural and functional status of muscles, there are also effects of myoECs on the CB receptors of motor synapses targeted at increasing synaptic transmission and, consequently, maintaining motor activity of the muscles.

CONTRIBUTION OF MYOENDOCANNABINOIDS TO AUTO-, PARACRINE, AND SYSTEMIC ACTIVITY OF ENDOCANNABINOIDS

The studies of ECs since their discovery in 1990s are still among the most relevant areas of modern physiology and pharmacology [21, 23, 65, 112]. This is due to the numerous biologically important functions and states regulated by ECs. Originally, the main effects of phyto- and systemic endocannabinoids (catalepsy, hypoalgesia, hypothermia, reduced locomotor activity, etc.) were associated with the selective activity of CB receptors in particular types of CNS synapses [113]; however, discovery of the peripheral EC sources such as adipose tissue [114], liver [115], skeletal muscles [74], etc., which also release ECs and have CB receptors and other EC targets, makes it necessary to take peripheral systems into account as the sources and participants of EC activity in the body [2, 11]. In particular, now it has become obvious that there is a possibility of myoEC release and regional auto/paracrine effects at the level of muscles [19, 49] and their synapses [15, 33, 38], which could have their own specificity.

Due to hydrophobic nature of ECs, their formation and accumulation near membranes could be accompanied by the transmembrane transport into the medium via passive diffusion along the concentration gradient [116]. Involvement of heat shock proteins and fatty acid-binding proteins (FABP) in the intracellular and/or transmembrane transport of ECs has also been proven [43]. In bloodstream, ECs can effectively bind to albumins for further transport and systemic effects [117].

According to the currently available data, the circulating ECs can enter bloodstream from many organs and tissues, including brain, muscles, adipose tissues, intestinal epithelium, blood cells, and other organs [11]. Under normal conditions, daily EC fluctuations in the plasma are 1-5 nM for AEA and 10-500 nM for 2-AG [11]. At the same time, the systemic level of ECs in blood demonstrates fluctuations also at rest, especially under particular functional states of the body: stress, physical exercise, overnutrition, as well as inflammation, obesity, and certain pathological conditions [11, 65]. It has been suggested

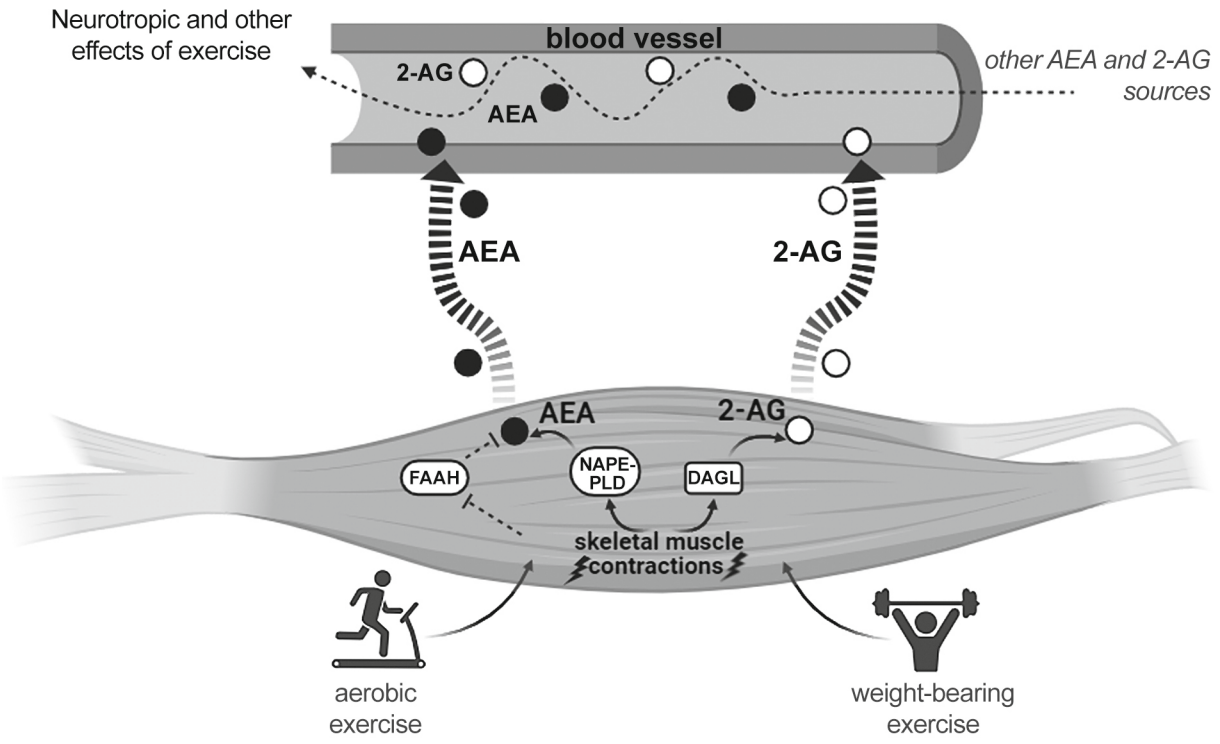

Fig. 6. Scheme of differential shifts in the balance of activities of the myoEC synthesis/degradation enzymes (DAGL, diacylglycerol lipase; NAPE-PLD, N-acylphosphatidylethanolamine-specific phospholipase D; FAAH, fatty acid amide hydrolase) in muscles under certain physical loading regimes, which results in the enhanced release of myogenic AEA and/or 2-AG (according to Schönke et al. [19], with modifications); ←, the activating effect; ⊥, the inhibitory effect.

that one of the causes of diurnal fluctuations in the systemic level of ECs could be, in particular, their high lipophilicity, which allows their unhindered release from the cells in the case of accumulation in cytoplasm and changes in the balance of the intra- and extracellular EC levels in peripheral organs and tissues. In such cases, diurnal EC fluctuations are only an indirect marker of the regionally varying tissue balance of ECs and their local activity in peripheral organs [11].

However, under certain conditions and states of the body, the elevated EC levels in the bloodstream could be of special functional and regulatory significance as an important factor for adaptation of the body to the changing conditions of its functioning. In particular, it is believed that, after physical exercise, the increasing level of ECs could be involved in systemic regulation of energy balance and intake, exerting selective effects on insulin resistance and glucose transport in particular organs and tissues [118].

Recently, the well-known “runner’s high” and the accompanying analgesia and some other important symptoms (slight sedative, antidepressant, anxiolytic, and other effects), which are commonly observed immediately after a run, have been generally associated with the higher level of ECs in blood, but not endorphins as it was considered previously [19, 32, 49, 119]. Indeed, there is increasing evidence that moderate physical loads in animals and humans could be accompanied by the significant increase in the level of AEA and, less frequently, 2-AG [12], and sometimes other ECs in blood [19, 26, 53]. In view of the above, a targeted search is underway for the conditions ensuring relationship between the physical exercise and EC increase, because this could become a tool for correcting some conditions of the body, including neurological diseases (Fig. 6) [24, 26]. The increased interest in the suggested relationship between physical exercise and high EC level in blood and in the possibilities of their practical applications is quite clear. Physical exercise is believed to be a valuable nonpharmacological therapy with an immediate effect, which is economically viable and, moreover, has many health benefits. If one of the consequences of physical loading is elevated level of endogenous cannabinoids in blood, this phenomenon of natural nontoxic increase of ECs in blood could be used for reducing stress and anxiety, for improving well-being [26] and even, as is believed, for potential enhancement of cognitive functions of the brain [53, 120]. However, the mechanisms of possible relationship between these phenomena have yet been poorly studied. It would be important to understand, firstly, what particular physical loading regimes and states of the body lead to the increase in the EC level in blood; secondly, whether the systemic level of ECs

in these cases increases as a result of predominant release of exactly myoECs due to their enhanced synthesis and release under muscle loading; and, thirdly, what are the particular targets of this activity of myogenic ECs at the levels of the CNS and periphery.

Analysis of the state of EC system in the bloodstream and skeletal muscles in different groups of subjects (healthy or with pathologies) shows that the short-term aerobic exercise (as well as weight-bearing exercise) really increases the level of ECs [19, 121]. The pooled meta-analysis of transcriptomic activity of the genes for CB receptors, EC synthesis and degradation enzymes demonstrates absence of significant changes in the level of expression of the CB receptor genes in the muscles of subjects in response to short-term aerobic loading and other types of physical activity. At the same time, expression of the 2-AG synthesis enzymes (DAGL) is downregulated, while expression of the AEA synthesis enzymes (NAPE-PLD) is enhanced (Fig. 6). However, in the majority of other studies it has been shown that the level of expression of the AEA cleavage enzymes (FAAH1) in muscles is inhibited under both short-term and chronic (long-term) regimes of intensive muscle loading. Under the conditions of chronic training loads, activity of NAPE-PLD also increases and, therefore, the level of AEA synthesis in the muscle apparently also increases [13, 122]. It has been established that the EC level in the plasma of subjects significantly increases after considerable aerobic load but is recovered to the initial values within 1 h after the exercise [123]. In opinion of the authors, this is the evidence of adaptive changes in the myoEC level in response to physical loading and possible involvement of myoECs (AEA, 2-AG, etc.) in the increase in the systemic level of ECs under muscle training loads [19, 122]. However, the accumulated experimental data and conclusions from some reviews concerning contribution of the myoEC pool to the increase in the systemic level of ECs under physical loading [11, 24, 26, 122] have not yet been unambiguously confirmed by the data of other currently performed studies [124]. This is mainly due to the difficulty of creating uniform conditions for the analysis of muscular and systemic levels of ECs under different muscle loading regimes in the individuals from different groups.

Large amounts of experimental data accumulated to date are considered in a number of reviews devoted to the relationship between the physical loading and increased level of ECs and the accompanying effects [11, 26, 122]. At the same time, the question remains whether the myoEC pool released under physical loading is capable to make a significant contribution to the increase in the systemic level of ECs and, thereby, to participate in regulation of the subsequent functional shifts in the body at the central and peripheral levels.

Table 2. Examples of regulatory effects of myogenic ECs at different systemic levels

Skeletal muscles	Regulation of: insulin sensitivity; energy metabolism; Ca^{2+} homeostasis; mitochondrial structure and functions; contraction and fatigue; gene expression
Motor synapses	Stimulation of: evoked neurotransmitter release; neurotransmitter quantal size; frequency of spontaneous release of neurotransmitter
Systemic effects	Involvement in: runner's high; analgesia; anti-stress

CONCLUSIONS

In the literature skeletal muscles are considered as an organ with yet little-known function: synthesis, degradation, and release of an independent myoEC pool targeting the muscles *per se*, their synapses, and the whole body (Table 2).

To date, the spectrum of local regulatory effects of myoECs on muscle fibers has been most thoroughly described. MyoECs provide control over embryonic development of myocytes, Ca^{2+} homeostasis in EMC, mitochondrial, metabolic, and contractile activities of mature fibers, and regulation of muscle genome activity. Together with the best-known ability of ECs, through their effect on CB1 receptors, to induce insulin resistance, to inhibit glucose transport and energy metabolism, and to increase muscle fatigue, the review summarizes the facts demonstrating that activation of muscular CB1 receptors and other targets (TRPV1, GPR55) could also provide opposite, i.e., positive effects of myoECs on muscle status. The review presents significant data on involvement of the pool of exactly muscular CB receptors in maintenance of energy metabolism, contractile activity, and fatigue resistance of skeletal muscles. In the context of positive effects of myoECs on muscles, the review presents the data on the unique regulatory activity of myoECs in neuromuscular synapses targeted at enhancement of transmitter secretion, which has no analogs in the CNS.

Finally, the myoEC pool has now attracted great interest also due to its potential involvement in the increase and maintenance of the systemic level of ECs in blood under moderate physical loading, which is accompanied by the effects important for the state

of health of subjects: improved mood, analgesia, stress reduction, etc. This fascinating aspect of the potential systemic activation of myoECs is of special importance in the light of the search of algorithms of nontoxic effects of ECs aimed at improving well-being of the patients with various types of clinical disorders.

In the present review, special attention has been focused for the first time on the yet little-known pool of myoECs; it gives us a new perspective on the targets, mechanisms of action, and significance of this pool, demonstrates its independent and nontrivial patterns of regulatory activity at different levels of organism. Recording and further consideration of specific activity of myoECs could expand modern notions on the regulatory abilities of ECs and the prospects of using them in clinical practice and beyond.

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Contributions. O.P.B. the concept, writing the manuscript; P.O.B. creating the figures and writing the manuscript; E.O.T. writing and editing the manuscript.

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